May 26, 2015

Test Results of
Second Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for

Control No. 190377-1

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
106 East Main Street
Trumann, AR 72472

Prepared by:

AMERICAN INTERPLEX CORPORATION
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AMERICAN INTERPLEX CORPORATION
LABORATORIES

Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
106 East Main Street
Trumann, AR 72472

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
NPDES Permit No. AR0035602 AFIN 56-00047

Dear Mr. Scotty Jones:

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 laboratory director 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:

**Native organisms were present in this sample and may have contributed to the deaths noted in the *Ceriodaphnia dubia* test.**

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 12 % effluent, which is above the critical dilution of 9 %. The NOEC for growth occurred at 12 % effluent, which is above the critical dilution of 9 %. **The sample, therefore, PASSED 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 9 % effluent, which is equal to the critical dilution of 9 %. The NOEC for reproduction occurred at 9 % effluent, which is equal to the critical dilution of 9 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

**AMERICAN INTERPLEX CORPORATION**

John Overbey
Laboratory Director

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I. Control Acceptance Criteria

*Pimephales promelas* (Fathead minnow) Method 1000.0

| CRITERIA | RESULTS | PASS/FAIL |
|---|---|---|
| Control Survival > or = 80% | 95.0 | PASS |
| Control Growth > or = 0.25 mg per Surviving minnow | 0.425 | PASS |
| Control Growth CV < or = 40% | 17.5 | PASS |
| Growth Minimum Significant Difference 12 to 30% | 17.2 | PASS |
| Critical Dilution CV < or = 40% | 9.73 | PASS |

*Ceriodaphnia dubia* Method 1002.0

| CRITERIA | RESULTS | PASS/FAIL |
|---|---|---|
| Control Survival > or = 80% | 100 | PASS |
| Control Reproduction > or = 15 per Surviving Female | 30.1 | PASS |
| Control CV < or = 40% per Surviving Female | 6.91 | PASS |
| Reproduction Minimum Significant Difference 13 to 47% | 18.7 | PASS |
| Critical Dilution CV < or = 40% | 37.6 | PASS |

II. Outlined Report

A. Introduction

1. Permit Number: AR0035602 AFIN 56-00047
2. Test Requirements:
   Test Methods 1000.0 and 1002.0
3. Receiving Stream:

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point:
   b. Chemical Data:

| Analysis | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Dissolved oxygen (mg/l) | 7.0 | 7.3 | 7.7 |
| pH (standard units) | 7.3 | 7.3 | 7.6 |
| Alkalinity (mg/l as CaCO3) | 85 | 84 | 80 |
| Hardness (mg/l as CaCO3) | 30 | 29 | 30 |
| Conductivity (umhos/cm) | 380 | 360 | 380 |
| Residual Chlorine (mg/l) | <0.05 | <0.05 | <0.05 |
| Ammonia as N (mg/l) | 1.8 | <0.1 | 0.24 |

2. Dilution Water Samples: Synthetic Moderately Hard Water #4212
   a. Dates Prepared: May 7 through May 21, 2015
   b. Chemical Data:

| Analysis | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Dissolved oxygen (mg/l) | 7.5 | 7.3 | 7.8 |
| pH (standard units) | 7.8 | 7.6 | 7.9 |
| Alkalinity (mg/l as CaCO3) | 57 | 57 | 57 |
| Hardness (mg/l as CaCO3) | 84 | 84 | 84 |
| Conductivity (umhos/cm) | 280 | 260 | 260 |
| Residual Chlorine (mg/l) | <0.05 | <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 * ! ( ) +$"#, $'+-. $ 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: | May 12, 2015 at 1515 |
| Date & Time Test Terminated: | May 19, 2015 at 1535 |
| 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 Growth Method 1002.0

| | |
|---|---|
| Date & Time Test Initiated: | May 12, 2015 at 1500 |
| Date & Time Test Terminated: | May 19, 2015 at 1415 |
| 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. Acclimation of test organisms: Obtained from in-house cultures
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.

*Pimephales promelas* (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank 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 Shapiro-Wilk's and analyzed with Wilcoxon's Rank Sum with Bonferroni Adjustment to determine the No Observable Effects Concentration (NOEC) for reproduction. Dunnett's Test was used to calculate the PMSD.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

*Pimephales promelas* (Fathead minnow)

Chronic reference tests are performed monthly.
A chronic reference test was performed on April 7, 2015 at 1040 to April 14, 2015 at 0910
The results were as follows: (Control No. 189181-1.)
Survival LC-50: 3291 mg/l
Growth IC-25: 2407 mg/l
Growth PMSD: 21.1

*Ceriodaphnia dubia*

Chronic reference tests are performed monthly.
A chronic reference test was performed on April 7, 2015 at 1115 to April 14, 2015 at 1100
The results were as follows: (Control No. 189181-2.)
Survival LC-50: 1616 mg/l
Growth IC-25: 1387 mg/l
Growth PMSD: 12.3

V. Chemical Analysis/Quality Control

| Parameter | Method | % Recovery | Relative % Difference |
|---|---|---|---|
| Alkalinity | SM 2320 B | NA | 0.812 |
| Hardness | EPA 200.7 | 100 | 2.38 |
| pH | SM 4500-H+ B | 101 | 0.00 |
| Conductivity | EPA 120.1 | 105 | 2.63 |

VI. Organism History

*Pimephales promelas* (Fathead minnow)

Date: May 12, 2015
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

*Ceriodaphnia dubia*

Date: May 12, 2015
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

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

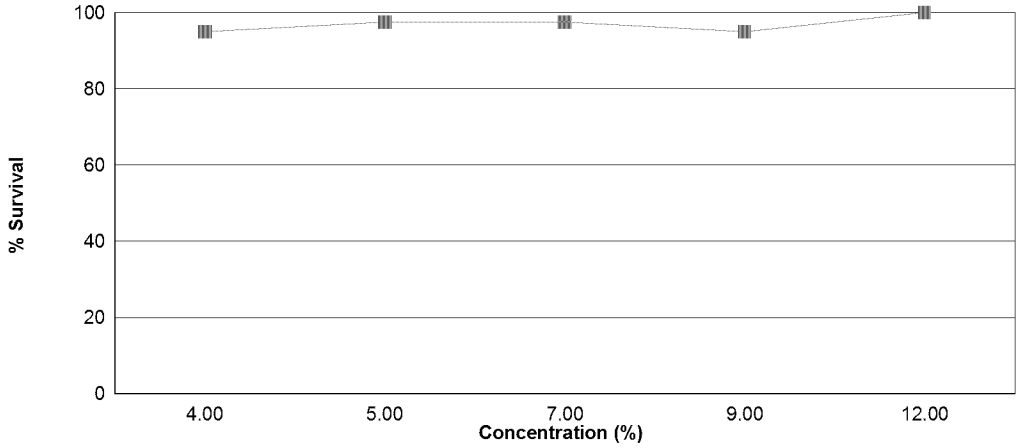
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 9 % effluent.

The test was initiated on May 12, 2015 at 1515 and continued through May 19, 2015 at 1535. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 12 % effluent
b.) NOEC growth = 12 % effluent




| Summary of the 7-day Fathead Minnow Survival and Growth | | |
|---|---|---|
| Concentration | Percent Survival | Mean Growth (mg) |
| Control | 95.0 | 0.404 |
| 4 % | 95.0 | 0.403 |
| 5 % | 97.5 | 0.373 |
| 7 % | 97.5 | 0.379 |
| 9 % | 95.0 | 0.421 |
| 12 % | 100 | 0.367 |

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

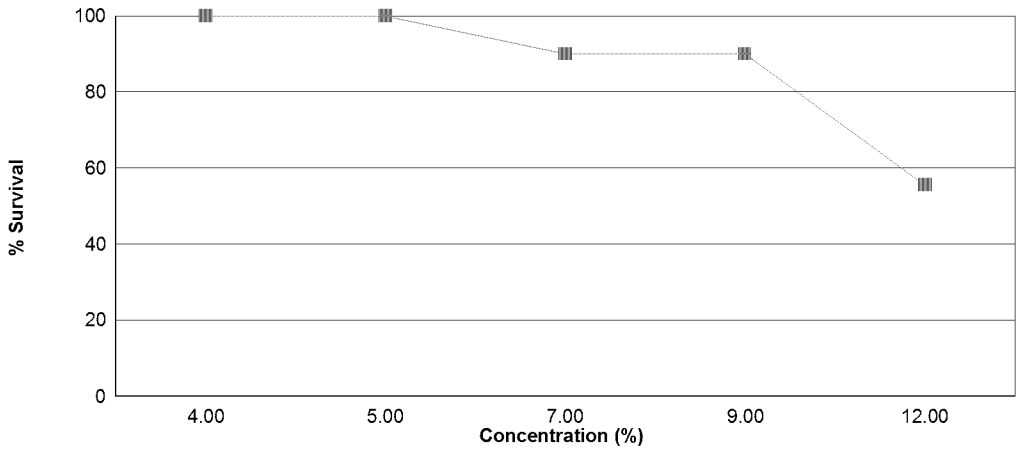
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 9 % effluent.

The test was initiated on May 12, 2015 at 1500 and continued through May 19, 2015 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 9 % effluent
b.) NOEC reproduction = 9 % effluent




| Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data | | |
|---|---|---|
| Concentration | Percent Survival | Mean Reproduction |
| Control | 100 | 30.1 |
| 4 % | 100 | 31.7 |
| 5 % | 100 | 32.9 |
| 7 % | 90.0 | 29.4 |
| 9 % | 90.0 | 23.0 |
| 12 % | 55.6 * | -- |

*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: May 12, 2015 at 1515
Date and Time Test Terminated: May 19, 2015 at 1535

| 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 | 7 |
| | D | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | E | 8 | 7 | 7 | 7 | 7 | 7 | 7 |
| 4 % | A | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | B | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | C | 8 | 7 | 7 | 7 | 7 | 7 | 7 |
| | D | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 7 |
| 5 % | 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 | 8 | 8 | 8 | 8 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 7 |
| 7 % | A | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | B | 8 | 8 | 8 | 8 | 8 | 8 | 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 |
| 9 % | A | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | B | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | C | 8 | 8 | 6 | 6 | 6 | 6 | 6 |
| | D | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 12 % | 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 | 8 | 8 | 8 | 8 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Growth

Test Initiated: May 12, 2015 at 1515
Test Terminated: May 19, 2015 at 1535

Drying Started: May 18, 2015 at 1620
Drying Ended: May 20, 2015 at 1040

| Concentration | Replicate | Weight of pan | Weight of pan + fish | Total weight of fish (g) | Original # of fish | Mean dry weight (mg) |
|---|---|---|---|---|---|---|
| Control | A | .92858 | .93222 | 0.00364 | 8 | 0.455 |
| | B | .93668 | .94039 | 0.00371 | 8 | 0.464 |
| | C | .92876 | .93147 | 0.00271 | 8 | 0.339 |
| | D | .93478 | .93834 | 0.00356 | 8 | 0.445 |
| | E | .93584 | .93836 | 0.00252 | 8 | 0.315 |
| 4 % | A | .92977 | .93282 | 0.00305 | 8 | 0.381 |
| | B | .93088 | .93370 | 0.00282 | 8 | 0.352 |
| | C | .93318 | .93614 | 0.00296 | 8 | 0.370 |
| | D | .93290 | .93636 | 0.00346 | 8 | 0.432 |
| | E | .93373 | .93759 | 0.00386 | 8 | 0.482 |
| 5 % | A | .92652 | .92929 | 0.00277 | 8 | 0.346 |
| | B | .93073 | .93408 | 0.00335 | 8 | 0.419 |
| | C | .93298 | .93577 | 0.00279 | 8 | 0.349 |
| | D | .93713 | .93994 | 0.00281 | 8 | 0.351 |
| | E | .93763 | .94081 | 0.00318 | 8 | 0.398 |
| 7 % | A | .93699 | .93986 | 0.00287 | 8 | 0.359 |
| | B | .93805 | .94097 | 0.00292 | 8 | 0.365 |
| | C | .93916 | .94237 | 0.00321 | 8 | 0.401 |
| | D | .94299 | .94648 | 0.00349 | 8 | 0.436 |
| | E | .94433 | .94702 | 0.00269 | 8 | 0.336 |
| 9 % | A | .94366 | .94680 | 0.00314 | 8 | 0.392 |
| | B | .93910 | .94269 | 0.00359 | 8 | 0.449 |
| | C | .93620 | .93911 | 0.00291 | 8 | 0.364 |
| | D | .93197 | .93562 | 0.00365 | 8 | 0.456 |
| | E | .93166 | .93523 | 0.00357 | 8 | 0.446 |
| 12 % | A | .93159 | .93427 | 0.00268 | 8 | 0.335 |
| | B | .93222 | .93503 | 0.00281 | 8 | 0.351 |
| | C | .93835 | .94144 | 0.00309 | 8 | 0.386 |
| | D | .93880 | .94165 | 0.00285 | 8 | 0.356 |
| | E | .93915 | .94240 | 0.00325 | 8 | 0.406 |

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: May 12, 2015 at 1500
Date and Time Test Terminated: May 19, 2015 at 1415

| 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 | 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 | 5 | 3 | 6 | 3 | 4 | 5 | 4 | 4 | 5 | 4 | 43 | 10 | 4.30 |
| 5 | 11 | 10 | 8 | 12 | 9 | 12 | 11 | 12 | 8 | 11 | 104 | 10 | 10.4 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 10 | 0.100 |
| 7 | 16 | 15 | 16 | 15 | 16 | 16 | 17 | 14 | 13 | 15 | 153 | 10 | 15.3 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 32 | 28 | 30 | 30 | 29 | 33 | 32 | 30 | 26 | 31 | 301 | 10 | 30.1 |

| Concentration: 4 % | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | LIA | 0 | 0 | 0 | 0 | 0 | 9 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | LIA | 0 | 0 | 0 | 0 | 0 | 9 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | LIA | 0 | 0 | 0 | 0 | 0 | 9 | 0.00 |
| 4 | 3 | 5 | 5 | 4 | 4 | LIA | 6 | 5 | 4 | 5 | 41 | 9 | 4.56 |
| 5 | 12 | 11 | 11 | 9 | 10 | LIA | 11 | 9 | 11 | 9 | 93 | 9 | 10.3 |
| 6 | 0 | 0 | 1 | 0 | 0 | LIA | 0 | 0 | 0 | 0 | 1 | 9 | 0.111 |
| 7 | 16 | 16 | 19 | 18 | 16 | LIA | 16 | 17 | 18 | 14 | 150 | 9 | 16.7 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 31 | 32 | 36 | 31 | 30 | | 33 | 31 | 33 | 28 | 285 | 9 | 31.7 |

LIA = Lost in Analysis

| Concentration: 5 % | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 4 | 6 | 4 | 3 | 4 | 3 | 4 | 6 | 3 | 5 | 42 | 10 | 4.20 |
| 5 | 10 | 11 | 10 | 8 | 8 | 11 | 9 | 8 | 12 | 12 | 99 | 10 | 9.90 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 7 | 19 | 16 | 16 | 19 | 18 | 20 | 18 | 21 | 22 | 19 | 188 | 10 | 18.8 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 33 | 33 | 30 | 30 | 30 | 34 | 31 | 35 | 37 | 36 | 329 | 10 | 32.9 |

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: May 12, 2015 at 1500
Date and Time Test Terminated: May 19, 2015 at 1415

| Concentration: 7 % | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | X | 0 | 0 | 9 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 9 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 9 | 0.00 |
| 4 | 4 | 5 | 5 | 4 | 6 | 4 | 4 | 4 | X | 5 | 41 | 9 | 4.56 |
| 5 | 9 | 0 | 13 | 14 | 9 | 9 | 10 | 11 | X | 12 | 87 | 9 | 9.67 |
| 6 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | X | 0 | 1 | 9 | 0.111 |
| 7 | 16 | 12 | 23 | 17 | 18 | 19 | 21 | 17 | X | 22 | 165 | 9 | 18.3 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 29 | 17 | 41 | 36 | 33 | 32 | 35 | 32 | 0 | 39 | 294 | 10 | 29.4 |

| Concentration: 9 % | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | X | 0 | 0 | 0 | 9 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | 9 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | 9 | 0.00 |
| 4 | 4 | 5 | 3 | 3 | 6 | 3 | 2 | X | 4 | 4 | 34 | 9 | 3.78 |
| 5 | 11 | 0 | 11 | 0 | 0 | 0 | 0 | X | 0 | 11 | 33 | 9 | 3.67 |
| 6 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | X | 0 | 0 | 10 | 9 | 1.11 |
| 7 | 20 | 13 | 23 | 19 | 23 | 4 | 16 | X | 12 | 23 | 153 | 9 | 17.0 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 35 | 18 | 37 | 22 | 29 | 12 | 23 | 0 | 16 | 38 | 230 | 10 | 23.0 |

| Concentration: 12 % | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
| 1 | LIA | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | X | 0 | 7 | 0.00 |
| 2 | LIA | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | X | 0 | 7 | 0.00 |
| 3 | LIA | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | X | 0 | 7 | 0.00 |
| 4 | LIA | 3 | 4 | X | 5 | X | 9 | 4 | 2 | X | 27 | 6 | 4.50 |
| 5 | LIA | X | 10 | X | 9 | X | 11 | 15 | 0 | X | 45 | 5 | 9.00 |
| 6 | LIA | X | 0 | X | 0 | X | 0 | 0 | 1 | X | 1 | 5 | 0.200 |
| 7 | LIA | X | 21 | X | 13 | X | 18 | 22 | 10 | X | 84 | 5 | 16.8 |
| 8 | | | | | | | | | | | | | |
| TOTAL | | 3 | 35 | 0 | 27 | 0 | 38 | 41 | 13 | 0 | 157 | 9 | 17.4 |

LIA = Lost in Analysis X = Death of Mother

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 | 0.87500 | 1.20940 |
| 1 | Control | 4 | 1.00000 | 1.39310 |
| 1 | Control | 5 | 0.87500 | 1.20940 |
| 2 | 4 % | 1 | 1.00000 | 1.39310 |
| 2 | 4 % | 2 | 1.00000 | 1.39310 |
| 2 | 4 % | 3 | 0.87500 | 1.20940 |
| 2 | 4 % | 4 | 1.00000 | 1.39310 |
| 2 | 4 % | 5 | 0.87500 | 1.20940 |
| 3 | 5 % | 1 | 1.00000 | 1.39310 |
| 3 | 5 % | 2 | 1.00000 | 1.39310 |
| 3 | 5 % | 3 | 1.00000 | 1.39310 |
| 3 | 5 % | 4 | 1.00000 | 1.39310 |
| 3 | 5 % | 5 | 0.87500 | 1.20940 |
| 4 | 7 % | 1 | 1.00000 | 1.39310 |
| 4 | 7 % | 2 | 0.87500 | 1.20940 |
| 4 | 7 % | 3 | 1.00000 | 1.39310 |
| 4 | 7 % | 4 | 1.00000 | 1.39310 |
| 4 | 7 % | 5 | 1.00000 | 1.39310 |
| 5 | 9 % | 1 | 1.00000 | 1.39310 |
| 5 | 9 % | 2 | 1.00000 | 1.39310 |
| 5 | 9 % | 3 | 0.75000 | 1.04720 |
| 5 | 9 % | 4 | 1.00000 | 1.39310 |
| 5 | 9 % | 5 | 1.00000 | 1.39310 |
| 6 | 12 % | 1 | 1.00000 | 1.39310 |
| 6 | 12 % | 2 | 1.00000 | 1.39310 |
| 6 | 12 % | 3 | 1.00000 | 1.39310 |
| 6 | 12 % | 4 | 1.00000 | 1.39310 |
| 6 | 12 % | 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 = 0.2307 | | |
| W = 0.779 | | |
| Critical W = 0.9 | (alpha = 0.01, N = 30) | |
| Critical W = 0.927 | (alpha = 0.05, N = 30) | |

Data FAIL normality test (alpha = 0.01).

| Steel's Many-One Rank Test | | | | | Transform: Arc Sin(Square Root(Y)) |
|---|---|---|---|---|---|
| Ho:Control<Treatment | | | | | |
| Group | Identification | Rank Sum | Critical Value | DF | Sig 0.05 |
| 1 | Control | | | | |
| 2 | 4 % | 27.50 | 16.00 | 5.00 | |
| 3 | 5 % | 30.00 | 16.00 | 5.00 | |
| 4 | 7 % | 30.00 | 16.00 | 5.00 | |
| 5 | 9 % | 29.00 | 16.00 | 5.00 | |
| 6 | 12 % | 32.50 | 16.00 | 5.00 | |

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Growth

| Shapiro - Wilk's Test for Normality | | No Transformation |
|---|---|---|
| D = 0.05199 | | |
| W = 0.9612 | | |
| 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 | | No Transformation |
|---|---|---|
| Calculated B1 statistic = 4.057 | | |
| 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) Growth

| ANOVA Table | | | | No Transformation |
|---|---|---|---|---|
| SOURCE | DF | SS | MS | F |
| Between | 5 | 0.01148 | 0.002296 | 1.06 |
| Within (Error) | 24 | 0.05199 | 0.002166 | |
| Total | 29 | 0.06347 | | |

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 | | | | | No Transformation |
|---|---|---|---|---|---|
| Ho:Control<Treatment | | | | | |
| Group | Identification | Transformed Mean | Mean In Original Units | T Stat | Sig 0.05 |
| 1 | Control | 0.4036 | 0.4036 | | |
| 2 | 4 % | 0.4034 | 0.4034 | 0.006795 | |
| 3 | 5 % | 0.3726 | 0.3726 | 1.053 | |
| 4 | 7 % | 0.3794 | 0.3794 | 0.8222 | |
| 5 | 9 % | 0.4214 | 0.4214 | -0.6047 | |
| 6 | 12 % | 0.3668 | 0.3668 | 1.25 | |

Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)

| 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 | 4 % | 5 | 0.06947 | 17.2 | 0.0002 |
| 3 | 5 % | 5 | 0.06947 | 17.2 | 0.031 |
| 4 | 7 % | 5 | 0.06947 | 17.2 | 0.0242 |
| 5 | 9 % | 5 | 0.06947 | 17.2 | -0.0178 |
| 6 | 12 % | 5 | 0.06947 | 17.2 | 0.0368 |

Appendix A2: Statistics

*Ceriodaphnia dubia* Survival

Fisher's Exact Test

| Identification | Alive | Dead | Total Animals |
|---|---|---|---|
| Control | 10 | 0 | 10 |
| 4 % | 9 | 0 | 9 |
| Total | 19 | 0 | 19 |

Critical Fisher's value (10,9,10) (alpha=0.05) is 5. b value is 9. Since b is greater than 5 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 |
| 5 % | 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 |
| 7 % | 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.

Fisher's Exact Test

| Identification | Alive | Dead | Total Animals |
|---|---|---|---|
| Control | 10 | 0 | 10 |
| 9 % | 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.

Appendix A2: Statistics

*Ceriodaphnia dubia* Survival

| Fisher's Exact Test | | | |
|---|---|---|---|
| Identification | Alive | Dead | Total Animals |
| Control | 10 | 0 | 10 |
| 12 % | 5 | 4 | 9 |
| Total | 15 | 4 | 19 |

Critical Fisher's value (10,9,10) (alpha=0.05) is 5. b value is 5. Since b is less than or equal to 5 there is A 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 | 4 % | 9 | 0 | |
| 2 | 5 % | 10 | 0 | |
| 3 | 7 % | 10 | 1 | |
| 4 | 9 % | 10 | 1 | |
| 5 | 12 % | 9 | 4 | * |

Appendix A2: Statistics

*Ceriodaphnia dubia* Reproduction

| Shapiro - Wilk's Test for Normality | | No Transformation |
|---|---|---|
| D = 2812 | | |
| W = 0.8608 | | |
| Critical W = 0.929 | (alpha = 0.01, N = 49) | |
| Critical W = 0.947 | (alpha = 0.05, N = 49) | |

Data FAIL normality test (alpha = 0.01).

| Wilcoxon's Rank Sum w/ Bonferroni Adjustment | | | | | No Transformation |
|---|---|---|---|---|---|
| Ho:Control<Treatment | | | | | |
| Group | Identification | Rank Sum | Critical Value | Reps | Sig 0.05 |
| 1 | Control | | | | |
| 2 | 4 % | 107.50 | 62.00 | 9 | |
| 3 | 5 % | 134.00 | 75.00 | 10 | |
| 4 | 7 % | 123.00 | 75.00 | 10 | |
| 5 | 9 % | 87.50 | 75.00 | 10 | |

Critical values are 1 tailed (k=4)

Appendix A2: Statistics

*Ceriodaphnia dubia* Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table — No Transformation

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 4 | 333.1 | 83.28 | 2.767 |
| Within (Error) | 42 | 1264 | 30.1 | |
| Total | 46 | 1597 | | |

Critical F = 3.8 (alpha = 0.01, df = 4,42)
2.59 (alpha = 0.05, df = 4,42)

Since F > Critical F 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 | 30.1 | 30.1 | | |
| 2 | 4 % | 31.667 | 31.667 | -0.6216 | |
| 3 | 5 % | 32.9 | 32.9 | -1.141 | |
| 4 | 7 % | 32.667 | 32.667 | -1.018 | |
| 5 | 9 % | 25.556 | 25.556 | 1.803 | |

Dunnett's critical value = 2.23 (1 Tailed, alpha = 0.05, df [used] = 4,40) (Actual df = 4,42)
WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.

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 | 4 % | 9 | 5.621 | 18.7 | -1.567 |
| 3 | 5 % | 10 | 5.471 | 18.2 | -2.8 |
| 4 | 7 % | 9 | 5.621 | 18.7 | -2.567 |
| 5 | 9 % | 9 | 5.621 | 18.7 | 4.544 |

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: May 12, 2015 at 1033
Date and Time Test Terminated: May 19, 2015 at 1535

| Effluent Conc.: Control | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 7.5 | 7.6 | 7.3 | 7.8 | 7.8 | 7.2 | 7.8 |
| | Final *1 | 7.7 | 6.6 | 6.2 | 6.3 | 7.1 | 6.1 | 6.6 |
| | Final *2 | 7.5 | 7.5 | 7.1 | 7.5 | 7.8 | 7.4 | 7.8 |
| pH, units | Initial | 7.8 | 7.8 | 7.6 | 8.1 | 7.9 | 7.9 | 7.9 |
| | Final *1 | 8.0 | 7.3 | 7.4 | 7.7 | 8.1 | 7.6 | 7.7 |
| | Final *2 | 7.8 | 7.8 | 7.9 | 7.9 | 7.9 | 7.9 | 7.6 |
| Alkalinity, mg CaCO3/l | | 57 | NA | 57 | NA | 57 | NA | NA |
| Hardness, mg CaCO3/l | | 84 | NA | 84 | NA | 84 | NA | NA |
| Conductivity, umhos/cm | | 280 | 280 | 260 | 320 | 260 | 270 | 270 |
| Res. Chlorine, mg/l | | <0.05 | NA | <0.05 | NA | <0.05 | NA | NA |

| Effluent Conc.: 4 % | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 7.5 | 7.6 | 7.5 | 7.7 | 7.3 | 7.6 | 7.8 |
| | Final *1 | 7.4 | 6.8 | 6.2 | 6.6 | 7.5 | 6.4 | 6.9 |
| | Final *2 | 7.6 | 7.3 | 7.5 | 7.8 | 7.8 | 7.4 | 7.5 |
| pH, units | Initial | 7.7 | 7.7 | 7.6 | 8.0 | 7.9 | 7.9 | 7.9 |
| | Final *1 | 7.9 | 7.4 | 7.3 | 7.7 | 8.1 | 7.8 | 7.8 |
| | Final *2 | 7.9 | 7.6 | 7.8 | 7.9 | 8.0 | 7.8 | 7.6 |

| Effluent Conc.: 5 % | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 7.5 | 7.8 | 7.6 | 7.7 | 7.7 | 7.9 | 8.0 |
| | Final *1 | 7.4 | 7.0 | 5.7 | 6.6 | 7.3 | 6.4 | 6.8 |
| | Final *2 | 7.6 | 7.4 | 7.6 | 7.8 | 8.3 | 7.7 | 7.3 |
| pH, units | Initial | 7.8 | 7.6 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Final *1 | 7.9 | 7.3 | 7.2 | 7.6 | 8.1 | 7.8 | 7.8 |
| | Final *2 | 7.9 | 7.6 | 7.9 | 7.9 | 7.9 | 7.8 | 7.6 |

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: May 12, 2015 at 1033
Date and Time Test Terminated: May 19, 2015 at 1535

| Effluent Conc.: 7 % | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 7.7 | 7.4 | 7.5 | 7.8 | 7.4 | 7.5 | 7.9 |
| | Final *1 | 7.4 | 6.7 | 5.8 | 6.6 | 7.3 | 6.8 | 6.7 |
| | Final *2 | 7.7 | 7.3 | 7.4 | 7.1 | 8.0 | 7.1 | 7.6 |
| pH, units | Initial | 7.7 | 7.7 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Final *1 | 7.8 | 7.4 | 7.3 | 7.7 | 8.1 | 7.9 | 7.8 |
| | Final *2 | 7.9 | 7.8 | 7.9 | 7.9 | 7.9 | 7.8 | 7.6 |

| Effluent Conc.: 9 % | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 7.6 | 7.4 | 7.5 | 7.7 | 7.7 | 8.0 | 7.8 |
| | Final *1 | 7.5 | 6.6 | 6.0 | 6.0 | 6.9 | 6.7 | 7.0 |
| | Final *2 | 7.6 | 7.7 | 7.3 | 7.7 | 8.1 | 6.9 | 7.7 |
| pH, units | Initial | 7.7 | 7.6 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Final *1 | 7.9 | 7.3 | 7.2 | 7.6 | 8.0 | 7.8 | 7.8 |
| | Final *2 | 7.8 | 7.8 | 7.9 | 7.8 | 7.9 | 7.8 | 7.6 |
| Alkalinity, mg CaCO3/l | | 65 | NA | 66 | NA | 63 | NA | NA |
| Hardness, mg CaCO3/l | | 86 | NA | 82 | NA | 78 | NA | NA |
| Conductivity, umhos/cm | | 280 | 290 | 270 | 330 | 280 | 280 | 270 |
| Res. Chlorine, mg/l | | <0.05 | NA | <0.05 | NA | <0.05 | NA | NA |

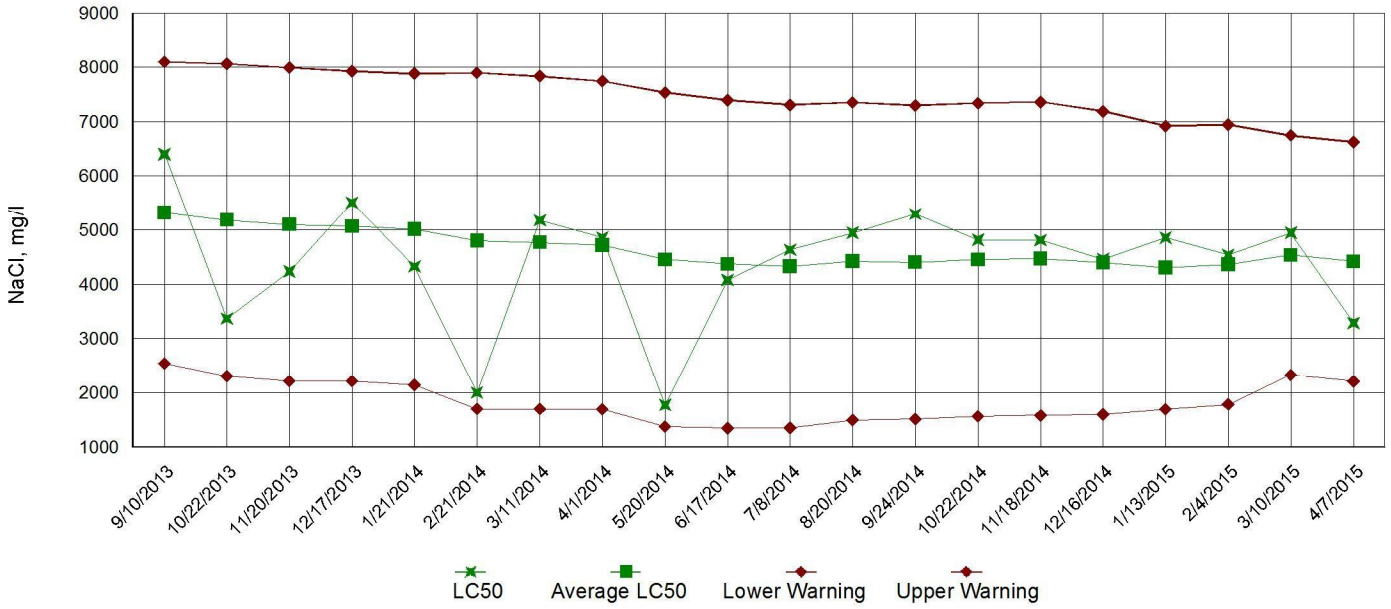
| Effluent Conc.: 12 % | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 7.7 | 7.5 | 7.3 | 7.5 | 7.2 | 7.5 | 7.7 |
| | Final *1 | 7.2 | 6.8 | 6.2 | 6.7 | 7.4 | 6.4 | 7.1 |
| | Final *2 | 7.6 | 7.5 | 7.4 | 7.6 | 8.1 | 7.7 | 7.7 |
| pH, units | Initial | 7.7 | 7.6 | 7.6 | 7.9 | 7.8 | 7.9 | 7.8 |
| | Final *1 | 7.9 | 7.4 | 7.3 | 7.7 | 8.0 | 7.8 | 7.8 |
| | Final *2 | 7.9 | 7.8 | 8.0 | 8.0 | 8.0 | 8.0 | 7.6 |

*1 = data from the *Pimephales promelas* (Fathead Minnow) test *2 = data from the *Ceriodaphnia dubia* test

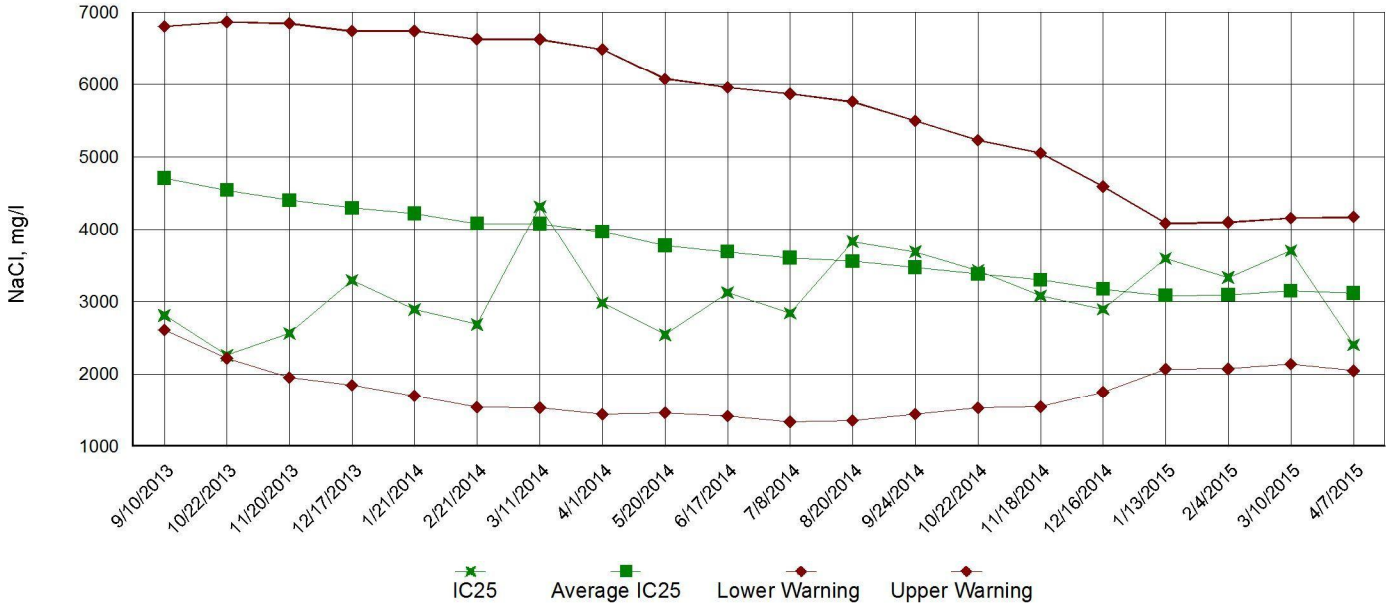
Appendix A4: Test 1000.0

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

LC50 Survival Data



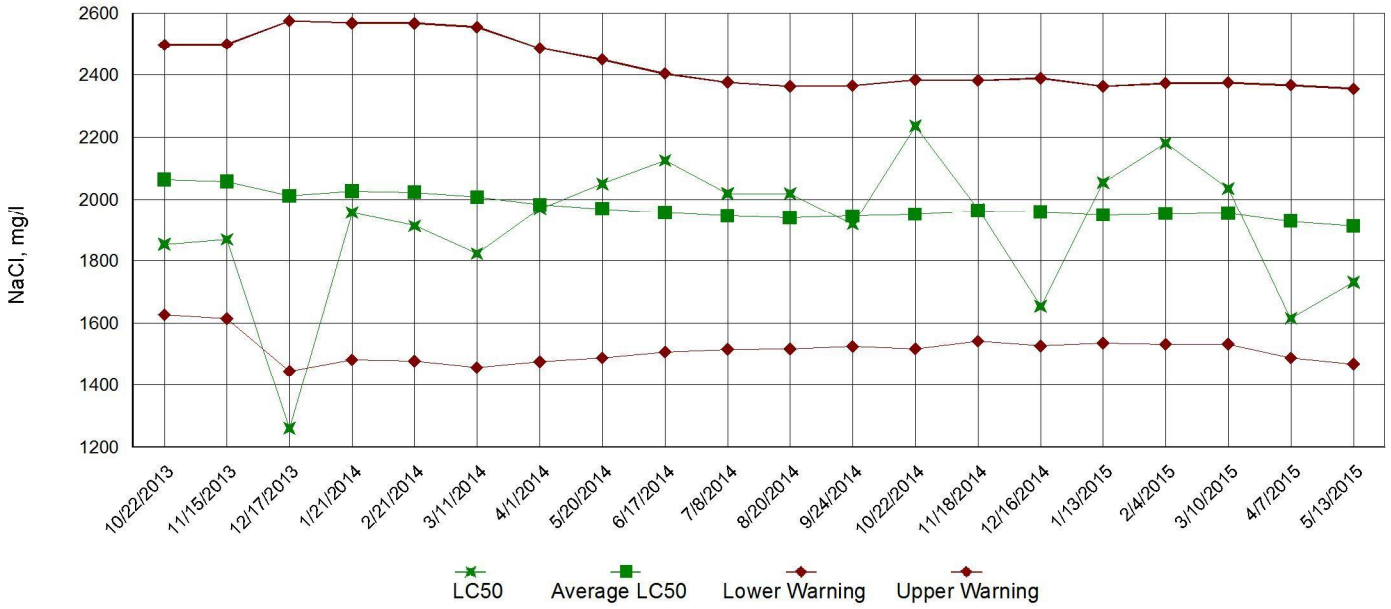

IC25 Growth Data



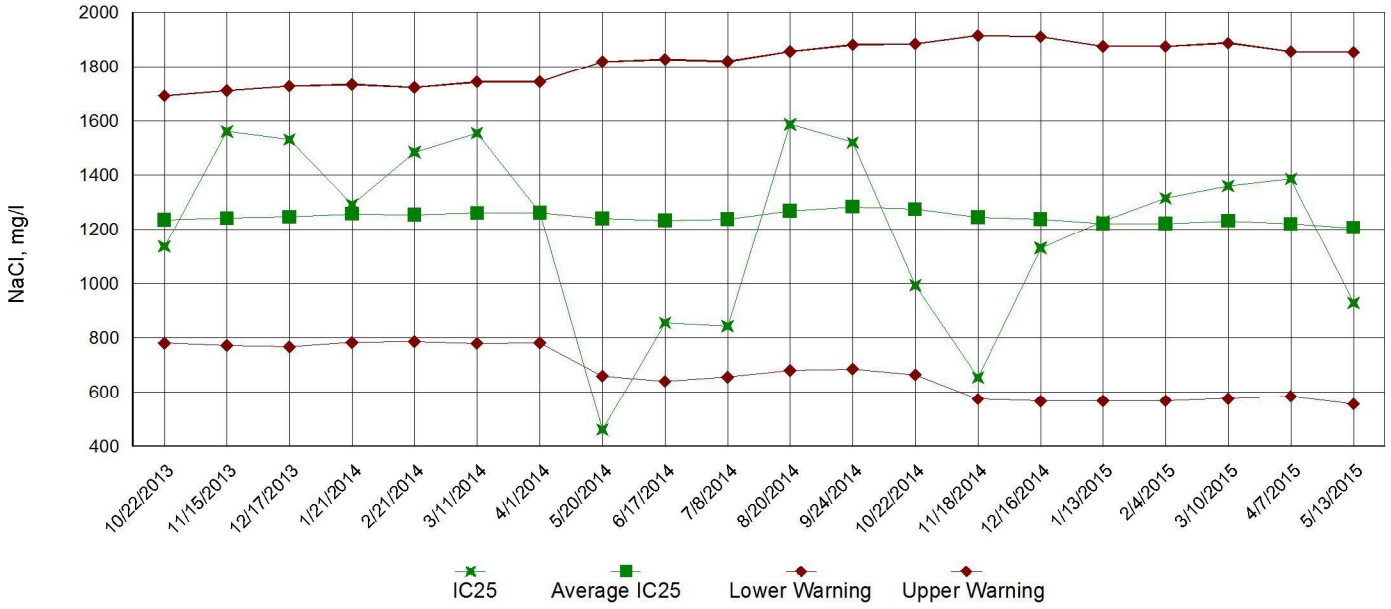
Appendix A4: Test 1002.0

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: Trumann Water and Sewer Commission

NPDES No.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: May 12, 2015 at 1515
Date and Time Test Terminated: May 19, 2015 at 1535
Dilution water used: Synthetic Moderately Hard Water #4212

DATA TABLE FOR SURVIVAL

| Effluent Conc. % | Percent Survival in replicate chambers A | B | C | D | E | Mean percent survival 24 hr | 48 hr | 7 days | CV% |
|---|---|---|---|---|---|---|---|---|---|
| Control | 100 | 100 | 87.5 | 100 | 87.5 | 100 | 97.5 | 95.0 | 7.21 |
| 4 % | 100 | 100 | 87.5 | 100 | 87.5 | 100 | 97.5 | 95.0 | 7.21 |
| 5 % | 100 | 100 | 100 | 100 | 87.5 | 100 | 100 | 97.5 | 5.73 |
| 7 % | 100 | 87.5 | 100 | 100 | 100 | 100 | 100 | 97.5 | 5.73 |
| 9 % | 100 | 100 | 75.0 | 100 | 100 | 100 | 100 | 95.0 | 11.8 |
| 12 % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.00 |

DATA TABLE FOR GROWTH

| Effluent Conc. % | Average dry weight, mg replicate chambers A | B | C | D | E | Mean dry weight, mg | CV% |
|---|---|---|---|---|---|---|---|
| Control | 0.455 | 0.464 | 0.339 | 0.445 | 0.315 | 0.404 | 17.5 |
| 4 % | 0.381 | 0.352 | 0.370 | 0.432 | 0.482 | 0.403 | 13.1 |
| 5 % | 0.346 | 0.419 | 0.349 | 0.351 | 0.398 | 0.373 | 9.03 |
| 7 % | 0.359 | 0.365 | 0.401 | 0.436 | 0.336 | 0.379 | 10.4 |
| 9 % | 0.392 | 0.449 | 0.364 | 0.456 | 0.446 | 0.421 | 9.73 |
| 12 % | 0.335 | 0.351 | 0.386 | 0.356 | 0.406 | 0.367 | 7.81 |

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. Steel's Many-One Rank 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 | (9 %) | ____YES | X NO |
   | b.) 1/2 LOW FLOW DILUTION | (NA) | ____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 | (9 %) | ____YES | X NO |
   | b.) 1/2 LOW FLOW DILUTION | (NA) | ____YES | ____NO |

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP6C)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP6C)

5. NOEC Pimephales Lethality: 12 % (TOP6C)

6. LOEC Pimephales Lethality: 12 % (TXP6C)

7. NOEC Pimephales Sublethality: 12 % (TPP6C)

8. LOEC Pimephales Sublethality: 12 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 17.5 (TQP6C)

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
*Pimephales promelas* (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AR0035602 AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 304, 310, 314

SAMPLE No. 1 COLLECTED ending: DATE: May 11, 2015 TIME: 0800
SAMPLE No. 2 COLLECTED ending: DATE: May 13, 2015 TIME: 0800
SAMPLE No. 3 COLLECTED ending: DATE: May 15, 2015 TIME: 0800
Test Initiated: DATE: May 12, 2015 TIME: 1515
Test Terminated: DATE: May 19, 2015 TIME: 1535

| DILUTION Control | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.5 | 7.6 | 7.3 | 7.8 | 7.8 | 7.2 | 7.8 |
| Final | 7.7 | 6.6 | 6.2 | 6.3 | 7.1 | 6.1 | 6.6 |
| pH Initial | 7.8 | 7.8 | 7.6 | 8.1 | 7.9 | 7.9 | 7.9 |
| Final | 8.0 | 7.3 | 7.4 | 7.7 | 8.1 | 7.6 | 7.7 |
| Alkalinity | 57 | NA | 57 | NA | 57 | NA | NA |
| Hardness | 84 | NA | 84 | NA | 84 | NA | NA |
| Conductivity | 280 | 280 | 260 | 320 | 260 | 270 | 270 |
| Chlorine | <0.05 | NA | <0.05 | NA | <0.05 | NA | NA |

| DILUTION 4 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.5 | 7.6 | 7.5 | 7.7 | 7.3 | 7.6 | 7.8 |
| Final | 7.4 | 6.8 | 6.2 | 6.6 | 7.5 | 6.4 | 6.9 |
| pH Initial | 7.7 | 7.7 | 7.6 | 8.0 | 7.9 | 7.9 | 7.9 |
| Final | 7.9 | 7.4 | 7.3 | 7.7 | 8.1 | 7.8 | 7.8 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 280 | 280 | 260 | 320 | 270 | 270 | 270 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

| DILUTION 5 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.5 | 7.8 | 7.6 | 7.7 | 7.7 | 7.9 | 8.0 |
| Final | 7.4 | 7.0 | 5.7 | 6.6 | 7.3 | 6.4 | 6.8 |
| pH Initial | 7.8 | 7.6 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| Final | 7.9 | 7.3 | 7.2 | 7.6 | 8.1 | 7.8 | 7.8 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 280 | 290 | 260 | 320 | 270 | 280 | 270 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

| DILUTION 7 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.7 | 7.4 | 7.5 | 7.8 | 7.4 | 7.5 | 7.9 |
| Final | 7.4 | 6.7 | 5.8 | 6.6 | 7.3 | 6.8 | 6.7 |
| pH Initial | 7.7 | 7.7 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| Final | 7.8 | 7.4 | 7.3 | 7.7 | 8.1 | 7.9 | 7.8 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 280 | 290 | 270 | 330 | 280 | 280 | 280 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

| DILUTION 9 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.6 | 7.4 | 7.5 | 7.7 | 7.7 | 8.0 | 7.8 |
| Final | 7.5 | 6.6 | 6.0 | 6.0 | 6.9 | 6.7 | 7.0 |
| pH Initial | 7.7 | 7.6 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| Final | 7.9 | 7.3 | 7.2 | 7.6 | 8.0 | 7.8 | 7.8 |
| Alkalinity | 65 | NA | 66 | NA | 63 | NA | NA |
| Hardness | 86 | NA | 82 | NA | 78 | NA | NA |
| Conductivity | 280 | 290 | 270 | 330 | 280 | 280 | 270 |
| Chlorine | <0.05 | NA | <0.05 | NA | <0.05 | NA | NA |

| DILUTION 12 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.7 | 7.5 | 7.3 | 7.5 | 7.2 | 7.5 | 7.7 |
| Final | 7.2 | 6.8 | 6.2 | 6.7 | 7.4 | 6.4 | 7.1 |
| pH Initial | 7.7 | 7.6 | 7.6 | 7.9 | 7.8 | 7.9 | 7.8 |
| Final | 7.9 | 7.4 | 7.3 | 7.7 | 8.0 | 7.8 | 7.8 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 290 | 290 | 270 | 330 | 280 | 280 | 280 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
*Ceriodaphnia dubia*
SURVIVAL AND REPRODUCTION

Permittee: Trumann Water and Sewer Commission

NPDES No.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: May 12, 2015 at 1500
Date and Time Test Terminated: May 19, 2015 at 1415
Dilution water used: Synthetic Moderately Hard Water #4212

PERCENT SURVIVAL

| | | Percent Effluent | | | | |
|---|---|---|---|---|---|---|
| Time of Reading | Control | 4 % | 5 % | 7 % | 9 % | 12 % |
| 24 hour | 100 | 100 | 100 | 90.0 | 90.0 | 77.8 |
| 48 hour | 100 | 100 | 100 | 90.0 | 90.0 | 77.8 |
| 7 day | 100 | 100 | 100 | 90.0 | 90.0 | 55.6 |

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

| | | Percent Effluent | | | | |
|---|---|---|---|---|---|---|
| Replicates | Control | 4 % | 5 % | 7 % | 9 % | 12 % |
| A | 32 | 31 | 33 | 29 | 35 | |
| B | 28 | 32 | 33 | 17 | 18 | 3 |
| C | 30 | 36 | 30 | 41 | 37 | 35 |
| D | 30 | 31 | 30 | 36 | 22 | 0 |
| E | 29 | 30 | 30 | 33 | 29 | 27 |
| F | 33 | | 34 | 32 | 12 | 0 |
| G | 32 | 33 | 31 | 35 | 23 | 38 |
| H | 30 | 31 | 35 | 32 | 0 | 41 |
| I | 26 | 33 | 37 | 0 | 16 | 13 |
| J | 31 | 28 | 36 | 39 | 38 | 0 |
| Mean per Adult | 30.1 | 31.7 | 32.9 | 29.4 | 23.0 | 17.4 |
| Mean per Surviving Adult | 30.1 | 31.7 | 32.9 | 32.7 | 25.6 | 30.8 |
| CV % | 6.91 | 7.06 | 7.91 | 21.3 | 37.6 | 36.5 |

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 | (9 %) | _____YES | __X__ NO |
   | b.) 1/2 LOW FLOW DILUTION | (NA) | _____YES | _____ NO |

2. Wilcoxon's Rank Sum with Bonferroni Adjustment 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 | (9 %) | _____YES | __X__ NO |
   | b.) 1/2 LOW FLOW DILUTION | (NA) | _____YES | _____ 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]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 9 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 12 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 9 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 12 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 37.6 (TQP3B)

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Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
*Ceriodaphnia dubia*
CHEMICAL PARAMETERS CHART

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AR0035602 AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 304, 310, 314

SAMPLE No. 1 COLLECTED ending: DATE: May 11, 2015 TIME: 0800
SAMPLE No. 2 COLLECTED ending: DATE: May 13, 2015 TIME: 0800
SAMPLE No. 3 COLLECTED ending: DATE: May 15, 2015 TIME: 0800
Test Initiated: DATE: May 12, 2015 TIME: 1500
Test Terminated: DATE: May 19, 2015 TIME: 1415

| DILUTION Control | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.5 | 7.6 | 7.3 | 7.8 | 7.8 | 7.2 | 7.8 |
| Final | 7.5 | 7.5 | 7.1 | 7.5 | 7.8 | 7.4 | 7.8 |
| pH Initial | 7.8 | 7.8 | 7.6 | 8.1 | 7.9 | 7.9 | 7.9 |
| Final | 7.8 | 7.8 | 7.9 | 7.9 | 7.9 | 7.9 | 7.6 |
| Alkalinity | 57 | NA | 57 | NA | 57 | NA | NA |
| Hardness | 84 | NA | 84 | NA | 84 | NA | NA |
| Conductivity | 280 | 280 | 260 | 320 | 260 | 270 | 270 |
| Chlorine | <0.05 | NA | <0.05 | NA | <0.05 | NA | NA |

| DILUTION 4 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.5 | 7.6 | 7.5 | 7.7 | 7.3 | 7.6 | 7.8 |
| Final | 7.6 | 7.3 | 7.5 | 7.8 | 7.8 | 7.4 | 7.5 |
| pH Initial | 7.7 | 7.7 | 7.6 | 8.0 | 7.9 | 7.9 | 7.9 |
| Final | 7.9 | 7.6 | 7.8 | 7.9 | 8.0 | 7.8 | 7.6 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 280 | 280 | 260 | 320 | 270 | 270 | 270 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

| DILUTION 5 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.5 | 7.8 | 7.6 | 7.7 | 7.7 | 7.9 | 8.0 |
| Final | 7.6 | 7.4 | 7.6 | 7.8 | 8.3 | 7.7 | 7.3 |
| pH Initial | 7.8 | 7.6 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| Final | 7.9 | 7.6 | 7.9 | 7.9 | 7.9 | 7.8 | 7.6 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 280 | 290 | 260 | 320 | 270 | 280 | 270 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

| DILUTION 7 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.7 | 7.4 | 7.5 | 7.8 | 7.4 | 7.5 | 7.9 |
| Final | 7.7 | 7.3 | 7.4 | 7.1 | 8.0 | 7.1 | 7.6 |
| pH Initial | 7.7 | 7.7 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| Final | 7.9 | 7.8 | 7.9 | 7.9 | 7.9 | 7.8 | 7.6 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 280 | 290 | 270 | 330 | 280 | 280 | 280 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

| DILUTION 9 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.6 | 7.4 | 7.5 | 7.7 | 7.7 | 8.0 | 7.8 |
| Final | 7.6 | 7.7 | 7.3 | 7.7 | 8.1 | 6.9 | 7.7 |
| pH Initial | 7.7 | 7.6 | 7.6 | 8.0 | 7.8 | 7.9 | 7.8 |
| Final | 7.8 | 7.8 | 7.9 | 7.8 | 7.9 | 7.8 | 7.6 |
| Alkalinity | 65 | NA | 66 | NA | 63 | NA | NA |
| Hardness | 86 | NA | 82 | NA | 78 | NA | NA |
| Conductivity | 280 | 290 | 270 | 330 | 280 | 280 | 270 |
| Chlorine | <0.05 | NA | <0.05 | NA | <0.05 | NA | NA |

| DILUTION 12 % | DAY 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| D.O. Initial | 7.7 | 7.5 | 7.3 | 7.5 | 7.2 | 7.5 | 7.7 |
| Final | 7.6 | 7.5 | 7.4 | 7.6 | 8.1 | 7.7 | 7.7 |
| pH Initial | 7.7 | 7.6 | 7.6 | 7.9 | 7.8 | 7.9 | 7.8 |
| Final | 7.9 | 7.8 | 8.0 | 8.0 | 8.0 | 8.0 | 7.6 |
| Alkalinity | NA | NA | NA | NA | NA | NA | NA |
| Hardness | NA | NA | NA | NA | NA | NA | NA |
| Conductivity | 290 | 290 | 270 | 330 | 280 | 280 | 280 |
| Chlorine | NA | NA | NA | NA | NA | NA | NA |

AMERICAN INTERPLEX CORPORATION
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# CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Trumann Water Works
Project Reference: AR0035602
Project Manager: Scotty Jones
Sampled By: Lorre Holt

PO No.

Sample Matrix: WATER, SOIL

No of BOTTLES

Analyses Requested: Biomonitoring-Chronic C0+FH

AIC Control No: 190377
AIC Proposal No:
Carrier: FedEx
Received Temperature °C: 0.1

| AIC No. | Sample Identification | Date/Time Collected | GRAB | COMP | WATER | SOIL | No of BOTTLES | Remarks |
|---|---|---|---|---|---|---|---|---|
| 1 | AR0035602 | 5/10/15 - 5/11/15 8:00AM - 8:00AM | | V | V | | 3 | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | Container Type | P | | | | | Field pH calibration |
| | | Preservative | ND | | | | | on _____ @ _____ |
| | | | | | | | | Buffer: |

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z = Zinc acetate

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: Lorre Holt
Phone: 870-483-2882 Fax: 870-483-6525
Report Attention to: Lorre Holt
Report Address to: 106 E. Main St, Trumann, AR. 72472

| Relinquished By: | Date/Time | Received By: | Date/Time |
|---|---|---|---|
| Lorre Holt | 5/11/15 @ 12:00PM | | |
| Relinquished By: | Date/Time | Received in Lab By: | Date/Time |
| | | D. Brown | 5-12-15 0820 |

Comments: FedEx: 8066 7411 5840

AMERICAN INTERPLEX CORPORATION
LABORATORIES

# CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: TRUMANN WATER WORKS
Project Reference: ARD035602
Project Manager: Scotty Jones
Sampled By: LORRE Holt

PO No.

Sample Matrix: WATER, SOIL

No of BOTTLES

Analyses Requested: BIOMONITORING-CHRONIC CD+FH

AIC Control No: 190377
AIC Proposal No:
Carrier: FED X
Received Temperature °C: 0-1
Remarks

| AIC No. | Sample Identification | Date/Time Collected | GRAB | COMP | WATER | SOIL | No of BOTTLES |
|---|---|---|---|---|---|---|---|
| 2 | ARD035602 | 5/12/15-5/13/15 8:00AM - 8:00AM | | V | V | | 3 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | Container Type | P | | | | |
| | | Preservative | ND | | | | |

Field pH calibration on _____ @ _____ Buffer:

G = Glass, NO = none, P = Plastic, S = Sulfuric acid pH2, V = VOA vials, N = Nitric acid pH2, H = HCl to pH2, B = NaOH to pH12, T = Sodium Thiosulfate, Z = Zinc acetate

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: LORRE Holt
Phone: 870-483-2882 Fax: 870-483-6525
Report Attention to: LORRE HOLT
Report Address to: 106 E. Main St, TRUMANN, AR. 72472

| Relinquished By: | Date/Time | Received By: | Date/Time |
|---|---|---|---|
| LORRE Holt | 5/13/15 @ 9:30 PM | | |
| Relinquished By: | Date/Time | Received in Lab By: | Date/Time |
| | | D. Brown | 5-14-15 : 0840 |

Comments: FED X: 8006 7411 5861

AMERICAN INTERPLEX CORPORATION
LABORATORIES

# CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Truman Water Works
Project Reference: AR0035202
Project Manager: Scotty Jones
Sampled By: Lorre Holt

PO No.

Sample Matrix: WATER, SOIL

No of BOTTLES

Analyses Requested: Biomonitoring-Chronic CDLFH

AIC Control No: 190397
AIC Proposal No:
Carrier: FedEx
Received Temperature °C: 2.6°
Remarks

| AIC No. | Sample Identification | Date/Time Collected | GRAB | COMP | WATER | SOIL | No of BOTTLES |
|---|---|---|---|---|---|---|---|
| 3 | AR0035202 | 5/14/15 - 5/15/15 8:00 AM - 8:00 AM | | V | V | | 3 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | Container Type | P | | | | |
| | | Preservative | NO | | | | |

Field pH calibration on ____ @ ____ Buffer:

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z = Zinc acetate

Turnaround Time Requested: (Please circle)
(NORMAL) or EXPEDITED IN ____ DAYS
Expedited results requested by: ____
Who should AIC contact with questions: Lorre Holt
Phone: 870-483-2882 Fax: 870-483-6525
Report Attention to: Lorre Holt
Report Address to: 106 E. Main St, Truman, AR. 72472

| Relinquished By: | Date/Time | Received By: | Date/Time |
|---|---|---|---|
| Lorre Holt | 5/15/15 @ 10:05 AM | | |
| Relinquished By: | Date/Time | Received in Lab By: | Date/Time |
| | | Steve Worm | 5-16-15 0815 |

Comments: FEDEX: 8066 7411 5850