AMERICAN INTERPLEX CORPORATION
LABORATORIES



February 23, 2015

Test Results of
First Quarter
Acute 48 hour Renewal
Biomonitoring Testing
for
Outfall 001
City of Alma

Control No. 187490-1



Prepared for:

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Prepared by:

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Data Testing, Inc.
ATTN: Ms. Dolores Shelby
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Re: Acute 48 hour Renewal Biomonitoring utilizing *Pimephales promelas* (Fathead Minnow) and *Daphnia pulex*
Outfall 001 - City of Alma
Client NPDES Permit No. AR0021466 AFIN#17-00059

Dear Ms. Dolores Shelby:

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 appropriate laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" EPA-821-R-02-012, Fifth Edition, October 2002. Test results are summarized below:

Acute *Pimephales promelas* (Fathead Minnow) Survival Test: The No Observable Effects Concentration (NOEC) for survival was 15% effluent, and the LC-50 value was >15% effluent; the sample, therefore, **PASSED** at low flow of 11% effluent for lethal effects.

Acute *Daphnia pulex* Survival Test: The No Observable Effects Concentration (NOEC) for survival was 15% effluent, and the LC-50 value was >15% effluent; the sample, therefore, **PASSED** at low flow of 11% effluent for lethal effects.

**AMERICAN INTERPLEX CORPORATION**

John Overbey
Laboratory Director

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I. Introduction and Summary

Biomonitoring testing of 48-hour renewal definitive toxicity tests using *Daphnia pulex* and *Pimephales promelas* were performed.

The *Daphnia pulex* test was conducted from February 10, 2015 at 1540 to February 12, 2015 at 1415.

The *Pimephales promelas* test was conducted from February 10, 2015 at 1835 to February 12, 2015 at 1635.

The tests were performed in accordance with EPA-821-R-02-012. Statistical analyses were performed on the observed data.

The tests were conducted in temperature and light cycle controlled environmental chamber. The test temperature was 25 degrees C +/- 1 degree for the *Daphnia pulex* and 25 degrees C +/- 1 degree for the *Pimephales promelas*.

II. Control Acceptance Criteria

| ORGANISM | CRITERIA | RESULTS | PASS/FAIL |
|---|---|---|---|
| *Daphnia pulex* | Control Survival >= 90% | 100 | PASS |
| *Pimephales promelas* | Control Survival >= 90% | 100 | PASS |

III. Outlined Report

A. Introduction

1. Permit Number: AR0021466 AFIN#17-00059
2. Test Requirements: 48-hour renewal definitive toxicity test using:
   *Daphnia pulex*
   *Pimephales promelas*

B. Source of Effluent/Dilution Water

1. Effluent Samples:
   a. Sampling Point: Outfall 001
      February 9 to February 10
   b. Chemical Data:

| Analysis | Sample 1 | Sample 2 |
|---|---|---|
| Dissolved oxygen (mg/l) | 8.3 | 7.8 |
| pH (standard units) | 7.6 | 7.4 |
| Alkalinity (mg/l as CaCO3) | 100 | 100 |
| Hardness (mg/l as CaCO3) | 46 | 45 |
| Conductivity (umhos/cm) | 380 | 290 |
| Residual Chlorine (mg/l) | 0.15 | 0.15 |

2. Dilution Water Samples: Natural Receiving Water
   a. Dates Collected/Prepared: Feb 9, 2015 at 0915 & Feb 10, 2015 at 0900
   b. Chemical Data:

| Analysis | Sample 1 | Sample 2 |
|---|---|---|
| Dissolved oxygen (mg/l) | 8.2 | 8.0 |
| pH (standard units) | 6.7 | 6.6 |
| Alkalinity (mg/l as CaCO3) | 17 | 23 |
| Hardness (mg/l as CaCO3) | 23 | 24 |
| Conductivity (umhos/cm) | 62 | 63 |
| Residual Chlorine (mg/l) | <0.05 | <0.05 |

C. Test Methods
1. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, (Fifth Ed.), EPA-821-R-02-012, 48-hour acute definitive test.
   a. Endpoints:
      Death; the criteria employed to establish death are:
      i. No movement
      ii. No reaction to gentle prodding

| Criteria | *Pimephales promelas* | *Daphnia pulex* |
|---|---|---|
| Type and Volume of Test Chamber | 500 ml disposable beaker | 30 ml disposable beaker |
| Volume of Sample | 250 ml | 25 ml |
| Organisms per chamber | 8 | 8 |
| Replicates per dilution | 5 | 5 |
| Test Temperature | 25 deg. C | 25 deg. C |
| Test Initiated | February 10, 2015 at 1835 | February 10, 2015 at 1540 |
| Test Terminated | February 12, 2015 at 1635 | February 12, 2015 at 1415 |
| Feeding | None required | None required |
| Age of Test Organisms | 1 day | <24 hours |

2. Chemical Methods Employed:

| Analysis | Method |
|---|---|
| Dissolved oxygen (mg/l) | SM 4500-O C |
| pH (standard units) | SM 4500-H+ B |
| Alkalinity (mg/l as CaCO3) | SM 2320 B |
| Hardness (mg/l as CaCO3) | EPA 200.7 |
| Conductivity (umhos/cm) | EPA 120.1 |
| Residual Chlorine (mg/l) | SM 4500-CL- F |
| Temperature (deg.C) | EPA 170.1 |

D. Test Organisms
1. Scientific Name
*Daphnia pulex*
*Pimephales promelas*

2. Acclimation of test organisms:
*Daphnia pulex*
Organisms were obtained from in-house cultures. The organisms were raised in moderately hard reconstituted water.
*Pimephales promelas*
Organisms were obtained from in-house cultures. The organisms were raised in moderately hard reconstituted water.

E. Quality Assurance
1. Toxicity Tests
a. Reference Toxicant: Sodium Chloride
b. Date of test:
*Daphnia pulex*: February 9, 2015 at 1610 to February 11, 2015 at 1635
*Pimephales promelas*: February 4, 2015 at 1300 to February 6, 2015 at 1145
c. Synthetic moderately hard dilution water used

| Organism | LC50 | Warning Limits |
|---|---|---|
| *Daphnia pulex* | 1.83 g/l | 1.38-2.39 g/l |
| *Pimephales promelas* | 7.72 g/l | 5.27-9.12 g/l |

2. Chemical and Physical Analyses

| Analysis | % Recovery | Relative % Difference |
|---|---|---|
| Alkalinity | NA | 2.17 |
| Hardness | 98.9 | 0.767 |
| pH | 101 | 0.134 |
| Conductivity | 104 | 5.37 |

F. Organism History
*Daphnia pulex*
Date: February 10, 2015 at 1540
Age: <24 hours
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C
*Pimephales promelas* (Fathead minnow)
Date: February 10, 2015 at 1835
Age: 1 day
Source: In-house culture
Water Chemistry Record:
Alkalinity: 57-64 mg/l
Hardness: 80-100 mg/l
Temperature: 25 deg.C

IV. Results Summary

*Daphnia pulex* and *Pimephales promelas* are exposed in a static renewal system to different concentrations of effluent and dilution water. Effluent dilutions for this test were 5%, 6%, 8%, 11%, 15%, Control%. The low-flow concentration was 11%. Test results were based on survival.

*Daphnia pulex*

The *Daphnia pulex* test was conducted from February 10, 2015 at 1540 to February 12, 2015 at 1415.

Statistical analyses:

NOEC = 15%
LC50 = >15%

| Concentration | 24 hour % Survival | 48 hour % Survival |
|---|---|---|
| Receiving | 100 | 100 |
| 5% | 100 | 100 |
| 6% | 100 | 100 |
| 8% | 100 | 100 |
| 11% | 100 | 100 |
| 15% | 100 | 100 |

*Pimephales promelas*

The *Pimephales promelas* test was conducted from February 10, 2015 at 1835 to February 12, 2015 at 1635.

Statistical analyses:

NOEC = 15%
LC50 = >15%

| Concentration | 24 hour % Survival | 48 hour % Survival |
|---|---|---|
| Receiving | 100 | 100 |
| 5% | 100 | 100 |
| 6% | 100 | 100 |
| 8% | 100 | 100 |
| 11% | 100 | 100 |
| 15% | 100 | 100 |

Appendix: A1

*Daphnia pulex*
Survival Data

Number of organisms per chamber: 8
Volume of test chamber: 30 ml

Age of organisms: <24 hours
Volume of test solution: 25 ml

| Effluent Concentration | | Number of Survivors 24 Hours | Number of Survivors 48 Hours | % Survival | CV % |
|---|---|---|---|---|---|
| Receiving | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 5% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 6% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 8% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 11% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 15% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |

CV = Coefficient of variance = standard deviation X 100/mean

Appendix: A1

*Pimephales promelas*
Survival Data

Number of organisms per chamber: 8
Volume of test chamber: 500 ml

Age of organisms: 1 day
Volume of test solution: 250 ml

| Effluent Concentration | | Number of Survivors 24 Hours | Number of Survivors 48 Hours | % Survival | CV % |
|---|---|---|---|---|---|
| Receiving | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 5% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 6% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 8% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 11% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |
| 15% | rep. A | 8 | 8 | 100 | 0.00 |
| | rep. B | 8 | 8 | | |
| | rep. C | 8 | 8 | | |
| | rep. D | 8 | 8 | | |
| | rep. E | 8 | 8 | | |

CV = Coefficient of variance = standard deviation X 100/mean

Appendix A2: Statistics

*Daphnia pulex*

| | | Transformation of Data | | Transform: Arc Sin(Square Root(Y)) |
|---|---|---|---|---|
| Group | Identification | Rep | Value | Transformed |
| 1 | Receiving | 1 | 1.00000 | 1.39310 |
| 1 | Receiving | 2 | 1.00000 | 1.39310 |
| 1 | Receiving | 3 | 1.00000 | 1.39310 |
| 1 | Receiving | 4 | 1.00000 | 1.39310 |
| 1 | Receiving | 5 | 1.00000 | 1.39310 |
| 2 | 5% | 1 | 1.00000 | 1.39310 |
| 2 | 5% | 2 | 1.00000 | 1.39310 |
| 2 | 5% | 3 | 1.00000 | 1.39310 |
| 2 | 5% | 4 | 1.00000 | 1.39310 |
| 2 | 5% | 5 | 1.00000 | 1.39310 |
| 3 | 6% | 1 | 1.00000 | 1.39310 |
| 3 | 6% | 2 | 1.00000 | 1.39310 |
| 3 | 6% | 3 | 1.00000 | 1.39310 |
| 3 | 6% | 4 | 1.00000 | 1.39310 |
| 3 | 6% | 5 | 1.00000 | 1.39310 |
| 4 | 8% | 1 | 1.00000 | 1.39310 |
| 4 | 8% | 2 | 1.00000 | 1.39310 |
| 4 | 8% | 3 | 1.00000 | 1.39310 |
| 4 | 8% | 4 | 1.00000 | 1.39310 |
| 4 | 8% | 5 | 1.00000 | 1.39310 |
| 5 | 11% | 1 | 1.00000 | 1.39310 |
| 5 | 11% | 2 | 1.00000 | 1.39310 |
| 5 | 11% | 3 | 1.00000 | 1.39310 |
| 5 | 11% | 4 | 1.00000 | 1.39310 |
| 5 | 11% | 5 | 1.00000 | 1.39310 |
| 6 | 15% | 1 | 1.00000 | 1.39310 |
| 6 | 15% | 2 | 1.00000 | 1.39310 |
| 6 | 15% | 3 | 1.00000 | 1.39310 |
| 6 | 15% | 4 | 1.00000 | 1.39310 |
| 6 | 15% | 5 | 1.00000 | 1.39310 |

Appendix A2: Statistics

*Daphnia pulex*

| Shapiro - Wilk's Test for Normality | | Transform: Arc Sin(Square Root(Y)) |
|---|---|---|
| D = 0 | | |
| W = 0 | | |
| 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 | 5% | 27.50 | 16.00 | 5.00 | |
| 3 | 6% | 27.50 | 16.00 | 5.00 | |
| 4 | 8% | 27.50 | 16.00 | 5.00 | |
| 5 | 11% | 27.50 | 16.00 | 5.00 | |
| 6 | 15% | 27.50 | 16.00 | 5.00 | |
| Critical values are 1 tailed (k=5) | | | | | |

Appendix A2: Statistics

*Pimephales promelas*

| | | Transformation of Data | | Transform: Arc Sin(Square Root(Y)) |
|---|---|---|---|---|
| Group | Identification | Rep | Value | Transformed |
| 1 | Receiving | 1 | 1.00000 | 1.39310 |
| 1 | Receiving | 2 | 1.00000 | 1.39310 |
| 1 | Receiving | 3 | 1.00000 | 1.39310 |
| 1 | Receiving | 4 | 1.00000 | 1.39310 |
| 1 | Receiving | 5 | 1.00000 | 1.39310 |
| 2 | 5% | 1 | 1.00000 | 1.39310 |
| 2 | 5% | 2 | 1.00000 | 1.39310 |
| 2 | 5% | 3 | 1.00000 | 1.39310 |
| 2 | 5% | 4 | 1.00000 | 1.39310 |
| 2 | 5% | 5 | 1.00000 | 1.39310 |
| 3 | 6% | 1 | 1.00000 | 1.39310 |
| 3 | 6% | 2 | 1.00000 | 1.39310 |
| 3 | 6% | 3 | 1.00000 | 1.39310 |
| 3 | 6% | 4 | 1.00000 | 1.39310 |
| 3 | 6% | 5 | 1.00000 | 1.39310 |
| 4 | 8% | 1 | 1.00000 | 1.39310 |
| 4 | 8% | 2 | 1.00000 | 1.39310 |
| 4 | 8% | 3 | 1.00000 | 1.39310 |
| 4 | 8% | 4 | 1.00000 | 1.39310 |
| 4 | 8% | 5 | 1.00000 | 1.39310 |
| 5 | 11% | 1 | 1.00000 | 1.39310 |
| 5 | 11% | 2 | 1.00000 | 1.39310 |
| 5 | 11% | 3 | 1.00000 | 1.39310 |
| 5 | 11% | 4 | 1.00000 | 1.39310 |
| 5 | 11% | 5 | 1.00000 | 1.39310 |
| 6 | 15% | 1 | 1.00000 | 1.39310 |
| 6 | 15% | 2 | 1.00000 | 1.39310 |
| 6 | 15% | 3 | 1.00000 | 1.39310 |
| 6 | 15% | 4 | 1.00000 | 1.39310 |
| 6 | 15% | 5 | 1.00000 | 1.39310 |

Appendix A2: Statistics

*Pimephales promelas*

| Shapiro - Wilk's Test for Normality | | Transform: Arc Sin(Square Root(Y)) |
|---|---|---|
| D = 0 | | |
| W = 0 | | |
| 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).

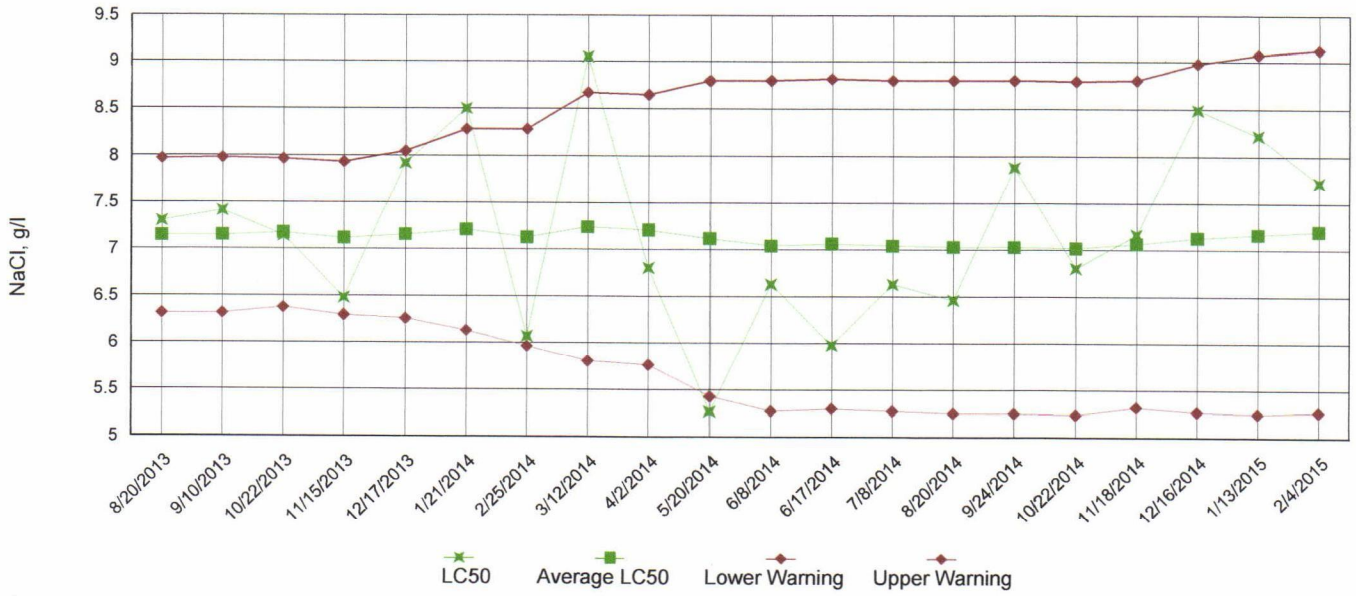
| Steel's Many-One Rank Test | | | | | Transform: Arc Sin(Square Root(Y)) |
|---|---|---|---|---|---|
| Ho:Control<Treatment | | | | | |
| Group | Identification | Rank Sum | Critical Value | DF | Sig 0.05 |
| 1 | Control | | | | |
| 2 | 5% | 27.50 | 16.00 | 5.00 | |
| 3 | 6% | 27.50 | 16.00 | 5.00 | |
| 4 | 8% | 27.50 | 16.00 | 5.00 | |
| 5 | 11% | 27.50 | 16.00 | 5.00 | |
| 6 | 15% | 27.50 | 16.00 | 5.00 | |

Critical values are 1 tailed (k=5)

Appendix: A3

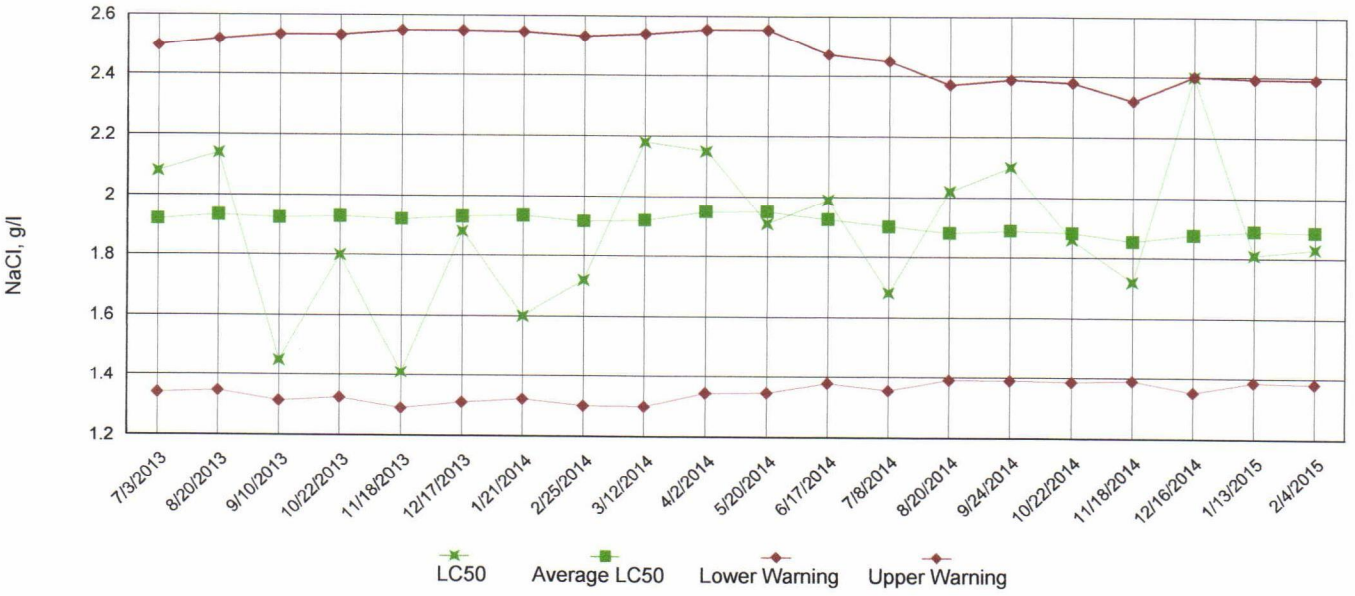
Acute Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data




Acute Reference Toxicant, *Daphnia pulex*

LC50 Survival Data



Appendix: A4

Chemical Data for
*Pimephales promelas*
and
*Daphnia pulex*

| Day 1 | | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 8.2 | 8.8 | 8.1 | 9.0 | 7.9 | 9.0 |
| DO, mg/l | Final 1* | 7.3 | 7.6 | 7.5 | 7.3 | 7.4 | 7.3 |
| DO, mg/l | Final 2* | 7.7 | 7.4 | 7.7 | 7.4 | 7.5 | 7.5 |
| pH, su | Initial | 6.7 | 6.7 | 6.7 | 6.8 | 6.8 | 7.0 |
| pH, su | Final 1* | 7.0 | 7.0 | 7.0 | 7.1 | 7.1 | 7.2 |
| pH, su | Final 2* | 7.2 | 7.0 | 7.0 | 7.0 | 7.1 | 7.1 |
| Alkalinity, mg/l | | 17 | NA | NA | NA | 25 | NA |
| Hardness, mg/l | | 23 | NA | NA | NA | 25 | NA |
| Conductivity, umho/cm | | 62 | 77 | 98 | 80 | 110 | 100 |
| Residual Chlorine, mg/l | | <0.05 | NA | NA | NA | <0.05 | NA |

| Day 2 | | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 8.0 | 8.0 | 8.2 | 7.8 | 7.8 | 7.7 |
| DO, mg/l | Final 1* | 8.3 | 7.5 | 7.3 | 7.5 | 7.7 | 7.4 |
| DO, mg/l | Final 2* | 7.8 | 7.5 | 7.7 | 7.6 | 7.6 | 7.5 |
| pH, su | Initial | 6.6 | 6.7 | 6.8 | 6.8 | 6.9 | 6.9 |
| pH, su | Final 1* | 7.0 | 6.8 | 6.7 | 6.9 | 6.8 | 6.8 |
| pH, su | Final 2* | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| Alkalinity, mg/l | | 23 | NA | NA | NA | 33 | NA |
| Hardness, mg/l | | 24 | NA | NA | NA | 25 | NA |
| Conductivity, umho/cm | | 63 | 78 | 82 | 84 | 96 | 110 |
| Residual Chlorine, mg/l | | <0.05 | NA | NA | NA | <0.05 | NA |

*1 data from *Pimephales promelas*
*2 data from *Daphnia pulex*

Appendix: B

*Daphnia pulex* Survival Data

| | | | |
|---|---|---|---|
| Permitee: | Data Testing, Inc. | Critical Dilution: | 11% |
| NPDES No: | AR0021466 AFIN#17-00059 | Sample Source: | Outfall 001 |
| Contact: | Ms. Dolores Shelby | Species Age: | <24 hours |
| Test Type: | 48-hour renewal definitive toxicity test | Analysts: | 280, 304, 310 |
| Dilution Water: | Natural Receiving Water | | |
| Test Initiated: | February 10, 2015 at 1540 | | |
| Test Terminated: | February 12, 2015 at 1415 | | |

PERCENT SURVIVAL

| 24 hours | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|
| Rep. A | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. B | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. C | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. D | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. E | 100 | 100 | 100 | 100 | 100 | 100 |

| 48 hours | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|
| Rep. A | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. B | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. C | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. D | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. E | 100 | 100 | 100 | 100 | 100 | 100 |

Dunnett's Procedure or Steel's Many-One Rank Test as appropriate. Is the mean survival at 48 hours significantly different (p=0.05) than the control survival for the % effluent corresponding to:

a) Low Flow 11%: ____ Yes X No
b) 1/2 Low Flow (NA): ____ Yes ____ No

Pass/Fail #TEM3D. 0

NOEL *Daphnia pulex* lethality #TOM3D: 15%

Coefficient of variation for *Daphnia pulex* survival #TQM3D: 0

Enter percent effluent corresponding to LC-50 below.
LC-50 effluent: >15%
Method of LC-50 calculation: NA

Reference Toxicity Test Performed on February 9, 2015 at 1610 to February 11, 2015 at 1635:
LC-50 effluent: 1.83 g/l
Warning Limits: 1.38 to 2.39 g/l

Appendix: B

*Daphnia pulex* Chemical Parameters Chart

| | | | |
|---|---|---|---|
| Permitee: | Data Testing, Inc. | Critical Dilution: | 11% |
| NPDES No: | AR0021466 AFIN#17-00059 | Sample Source: | Outfall 001 |
| Contact: | Ms. Dolores Shelby | Species Age: | <24 hours |
| Test Type: | 48-hour renewal definitive toxicity test | Analysts: | 280, 304, 310 |
| Dilution Water: | Natural Receiving Water | | |
| Test Initiated: | February 10, 2015 at 1540 | | |
| Test Terminated: | February 12, 2015 at 1415 | | |

| Day 1 | | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 8.2 | 8.8 | 8.1 | 9.0 | 7.9 | 9.0 |
| DO, mg/l | Final | 7.7 | 7.4 | 7.7 | 7.4 | 7.5 | 7.5 |
| pH, su | Initial | 6.7 | 6.7 | 6.7 | 6.8 | 6.8 | 7.0 |
| pH, su | Final | 7.2 | 7.0 | 7.0 | 7.0 | 7.1 | 7.1 |
| Alkalinity, mg/l | | 17 | NA | NA | NA | 25 | NA |
| Hardness, mg/l | | 23 | NA | NA | NA | 25 | NA |
| Conductivity, umho/cm | | 62 | 77 | 98 | 80 | 110 | 100 |
| Residual Chlorine, mg/l | | <0.05 | NA | NA | NA | <0.05 | NA |

| Day 2 | | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 8.0 | 8.0 | 8.2 | 7.8 | 7.8 | 7.7 |
| DO, mg/l | Final | 7.8 | 7.5 | 7.7 | 7.6 | 7.6 | 7.5 |
| pH, su | Initial | 6.6 | 6.7 | 6.8 | 6.8 | 6.9 | 6.9 |
| pH, su | Final | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| Alkalinity, mg/l | | 23 | NA | NA | NA | 33 | NA |
| Hardness, mg/l | | 24 | NA | NA | NA | 25 | NA |
| Conductivity, umho/cm | | 63 | 78 | 82 | 84 | 96 | 110 |
| Residual Chlorine, mg/l | | <0.05 | NA | NA | NA | <0.05 | NA |

Appendix: B

*Pimephales promelas* Survival Data

| | | | |
|---|---|---|---|
| Permitee: | Data Testing, Inc. | Critical Dilution: | 11% |
| NPDES No: | AR0021466 AFIN#17-00059 | Sample Source: | Outfall 001 |
| Contact: | Ms. Dolores Shelby | Species Age: | 1 day |
| Test Type: | 48-hour renewal definitive toxicity test | Analysts: | 280, 304, 310 |
| Dilution Water: | Natural Receiving Water | | |
| Test Initiated: | February 10, 2015 at 1835 | | |
| Test Terminated: | February 12, 2015 at 1635 | | |

PERCENT SURVIVAL

| 24 hours | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|
| Rep. A | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. B | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. C | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. D | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. E | 100 | 100 | 100 | 100 | 100 | 100 |

| 48 hours | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|
| Rep. A | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. B | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. C | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. D | 100 | 100 | 100 | 100 | 100 | 100 |
| Rep. E | 100 | 100 | 100 | 100 | 100 | 100 |

Dunnett's Procedure or Steel's Many-One Rank Test as appropriate. Is the mean survival at 48 hours significantly different (p=0.05) than the control survival for the % effluent corresponding to:

a) Low Flow 11%: _____ Yes X No
b) 1/2 Low Flow (NA): _____ Yes _____ No

Pass/Fail #TEM6C. 0

NOEL *Pimephales promelas* lethality #TOM6C: 15%

Coefficient of variation for *Pimephales promelas* survival #TQM6C: 0

Enter percent effluent corresponding to LC-50 below.
LC-50 effluent: >15%
Method of LC-50 calculation: NA

Reference Toxicity Test Performed on February 4, 2015 at 1300 to February 6, 2015 at 1145:
LC-50 effluent: 7.72 g/l
Warning Limits: 5.27 to 9.12 g/l

Appendix: B

*Pimephales promelas* Chemical Parameters Chart

Permitee: Data Testing, Inc.
NPDES No: AR0021466 AFIN#17-00059
Contact: Ms. Dolores Shelby
Test Type: 48-hour renewal definitive toxicity test
Dilution Water: Natural Receiving Water
Test Initiated: February 10, 2015 at 1835
Test Terminated: February 12, 2015 at 1635

Critical Dilution: 11%
Sample Source: Outfall 001
Species Age: 1 day
Analysts: 280, 304, 310

| Day 1 | | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 8.2 | 8.8 | 8.1 | 9.0 | 7.9 | 9.0 |
| DO, mg/l | Final | 7.3 | 7.6 | 7.5 | 7.3 | 7.4 | 7.3 |
| pH, su | Initial | 6.7 | 6.7 | 6.7 | 6.8 | 6.8 | 7.0 |
| pH, su | Final | 7.0 | 7.0 | 7.0 | 7.1 | 7.1 | 7.2 |
| Alkalinity, mg/l | | 17 | NA | NA | NA | 25 | NA |
| Hardness, mg/l | | 23 | NA | NA | NA | 25 | NA |
| Conductivity, umho/cm | | 62 | 77 | 98 | 80 | 110 | 100 |
| Residual Chlorine, mg/l | | <0.05 | NA | NA | NA | <0.05 | NA |

| Day 2 | | Receiving | 5% | 6% | 8% | 11% | 15% |
|---|---|---|---|---|---|---|---|
| DO, mg/l | Initial | 8.0 | 8.0 | 8.2 | 7.8 | 7.8 | 7.7 |
| DO, mg/l | Final | 8.3 | 7.5 | 7.3 | 7.5 | 7.7 | 7.4 |
| pH, su | Initial | 6.6 | 6.7 | 6.8 | 6.8 | 6.9 | 6.9 |
| pH, su | Final | 7.0 | 6.8 | 6.7 | 6.9 | 6.8 | 6.8 |
| Alkalinity, mg/l | | 23 | NA | NA | NA | 33 | NA |
| Hardness, mg/l | | 24 | NA | NA | NA | 25 | NA |
| Conductivity, umho/cm | | 63 | 78 | 82 | 84 | 96 | 110 |
| Residual Chlorine, mg/l | | <0.05 | NA | NA | NA | <0.05 | NA |

AMERICAN INTERPLEX CORPORATION LABORATORIES

# CHAIN OF CUSTODY / ANALYSIS REQUEST FORM



Client: Data Testing
Project Reference: Almi
Project Manager:
Sampled By: Data Testing

PO No.

MATRIX

| AIC No. | Sample Identification | Date/Time Collected | GRAB | COMP | WATER | SOIL | | NO OF BOTTLES | ANALYSES REQUESTED: Sequence acrylic |
|---|---|---|---|---|---|---|---|---|---|
| 1 | effluent | 2-25-15 800-900 | | ✓ | ✓ | | | 1 | |
| 2 | rec. water | 2-9:15 | ✓ | | ✓ | | | 3 | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | Container Type | | | | | | | |
| | | Preservative | | | | | | | |

AIC CONTROL NO: 187490
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 3.3c
Remarks
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, A=$(NH_4)_2SO_4$, $NH_4OH$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone:_____ Fax:
Report Attention to:
Report Address to:
Email Address:

| Relinquished By: | Date/Time | Received By: | Date/Time |
|---|---|---|---|
| [illegible] Peterson | 2-9-15 150 | | |
| Relinquished By: | Date/Time | Received in Lab By: Jimmy Day | Date/Time 2/10/15 1040 |

Comments:

9/2014

UPS 12 140 RR8 03 9652 7783

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

# CHAIN OF CUSTODY / ANALYSIS REQUEST FORM



Client: Data Testing
Project Reference: Alma
Project Manager:
Sampled By: Data Testing

PO No.

MATRIX

AIC CONTROL NO: 187490
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 21 C

| AIC No. | Sample Identification | Date/Time Collected | GRAB | COMP | WATER | SOIL | | NO OF BOTTLES | ANALYSES REQUESTED (bacteria summary) | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| (3) | effluent | 2-9-10-15 8am-5pm | | ✓ | ✓ | | | 1 | | |
| (4) | rec. water | 2-10 9am | ✓ | | ✓ | | | 3 | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | Container Type | | | | | | | | Field pH calibration |
| | | Preservative | | | | | | | | 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 A=$(NH_4)_2SO_4$, $NH_4OH$

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone:_____ Fax:
Report Attention to:
Report Address to:
Email Address:

| Relinquished By: | Date/Time | Received By: | Date/Time |
|---|---|---|---|
| Cindy Peterson | 2-10-15 200 | | |
| Relinquished By: | Date/Time | Received in Lab By: | Date/Time |
| | | Troy Williams | 9:40 2-11-15 |

Comments:

9/2014

UPS 1Z 140 RR2 03976 9992

FORM 0060