

Protecting Our Water Environment



Metropolitan Water Reclamation District of Greater Chicago

***MONITORING AND RESEARCH
DEPARTMENT***

REPORT NO. 21-10

*RESULTS OF ACUTE TOXICITY TESTING WITH Ceriodaphnia dubia
AND Pimephales promelas ON A FEBRUARY 2021 EFFLUENT
SAMPLE FROM METROPOLITAN WATER RECLAMATION DISTRICT
(MWRD)*

March 2021

Metropolitan Water Reclamation District of Greater Chicago
100 East Erie Street Chicago, Illinois 60611-2803 (312) 751-5600

RESULTS OF ACUTE TOXICITY TESTING WITH *Ceriodaphnia dubia* AND *Pimephales promelas* ON A FEBRUARY 2021 EFFLUENT SAMPLE FROM METROPOLITAN WATER RECLAMATION DISTRICT (MWRD)

By

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CECIL LUE-HING RESEARCH AND DEVELOPMENT COMPLEX
6001 WEST PERSHING ROAD CICERO, ILLINOIS 60804-4112

March 23, 2021

Illinois Environmental Protection Agency
Compliance Assurance Section CAS # 19
1021 North Grand Avenue
P.O. Box 19276
Springfield, IL 62794-9276

Subject: Biomonitoring Report for 2021 – Acute Toxicity Test Results for the Calumet Water Reclamation Plant, National Pollutant Discharge Elimination System Permit Number IL0028061

The subject Biomonitoring Report including Acute Whole Effluent Toxicity test results for *Pimephales promelas* and *Ceriodaphnia dubia* is submitted in compliance with National Pollutant Discharge Elimination System Permit Number IL0028061, Special Condition 10. The report covers the monitoring done for samples collected in the seventeenth month before the expiration of the permit.

The subject report prepared by EA Engineering, Science, and Technology, Inc., PBC includes copies of all bench sheets, chain-of-custody forms, sample receipt, preparation forms, summary of final results and test information, and quality assurance record.

If you have any questions concerning this report, please contact Mr. Thomas Minarik, Principal Environmental Scientist, at (708) 588-4223.

Very truly yours,



Albert Cox
Environmental Monitoring
and Research Manager
Monitoring and Research Department

AC:TM:NK:lf

Enclosure

cc: E. Podczerwinski/J. Murray
P. Connolly/H. Zhang
T. Minarik/N. Kollias

Via electronic mail



RESULTS OF ACUTE TOXICITY TESTING
WITH *Ceriodaphnia dubia* AND *Pimephales promelas*
ON A FEBRUARY 2021 EFFLUENT SAMPLE FROM
METROPOLITAN WATER RECLAMATION DISTRICT (MWRD)

Prepared for:

Metropolitan Water Reclamation District of Greater Chicago
6001 W. Pershing Road
Cicero, Illinois 60804

Prepared by:

EA Engineering, Science, and Technology, Inc., PBC
231 Schilling Circle
Hunt Valley, Maryland 21031
For questions, please contact Michael Chanov
ph: 410-584-7000

Results relate only to the items tested or to the samples as received by the laboratory.

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EA Engineering, Science, and Technology, Inc., PBC*

This report contains 8 pages plus 2 attachments

Michael K. Chanov II
Laboratory Director

15 March 2021

Date

EA Project Number 70019.TOX



EA Report Number 8505

INTRODUCTION

At the request of Metropolitan Water Reclamation District (MWRD), EA Engineering, Science, and Technology performed acute toxicity testing on a composite sample of Outfall 001 final effluent from MWRD's Calumet Water Reclamation Plant in Chicago, Illinois. The effluent composite sample was collected on 24-25 February 2021. The test organisms, *Ceriodaphnia dubia* (water flea) and *Pimephales promelas* (fathead minnow), were exposed to 100, 50, 25, 12.5 and 6.25 percent effluent, and a laboratory water control. The objective of this study was to assess the acute lethality of the effluent sample to the test species, expressed as a 48-hour (*C. dubia*), or 96-hour (*P. promelas*) median lethal concentration (LC50). This toxicity testing was conducted under the Section 10 biomonitoring requirements of Metropolitan Water Reclamation District's discharge permit number IL0028061.

This toxicity testing was conducted following EA's standard operating procedures (EA 2018) which are in accordance with US EPA guidance (2002). The results of the acute toxicity tests were analyzed using the ToxCalc statistical software package (Version 5.0, Tidepool Scientific Software) and followed US EPA guidance (2002). Summaries of sample and test information are presented on pages 5-6 for *C. dubia* and on pages 7-8 for *P. promelas*. Copies of raw data sheets and statistics are included in Attachment I. The Report Quality Assurance Record is included as Attachment II.

SUMMARY OF RESULTS

The results of the acute toxicity tests indicated that the 24-25 February 2021 Outfall 001 final effluent sample was not acutely toxic to *Ceriodaphnia dubia* or *Pimephales promelas*. The results of these toxicity tests comply with current NELAC standards.

The results of the *C. dubia* acute toxicity test are presented on page 6. After 48 hours, there was a minimum of 95 percent survival in all of the effluent concentrations and 100 percent survival in the dilution water control. The 48-hour *C. dubia* LC50 for this test was >100 percent effluent (<1.0 TU_a). In the *P. promelas* acute toxicity test (page 8), at the end of 96 hours there was a minimum of 90 percent survival in all of the effluent concentrations. The laboratory control had 100 percent survival. The resulting 96-hour LC50 for *P. promelas* was >100 percent effluent (<1.0 TU_a).

In conformance with EA's quality assurance/quality control program, monthly reference toxicant tests using sodium chloride (NaCl) and potassium chloride (KCl) were performed on the in-house cultured test species. The results of the *C. dubia* reference toxicant test was acceptable, with a 48-hour LC50 of 1,980 mg/L NaCl, and acceptable control chart limits of 1,684-2,156 mg/L NaCl. The results of the *P. promelas* reference toxicant test was acceptable, with a 48-hour LC50 of 866 mg/L KCl, and acceptable control chart limits of 626-1,253 mg/L KCl.

REFERENCES

- EA. 2018. EA Ecotoxicology Laboratory Quality Assurance and Standard Operating Procedures Manual. EA Manual ATS-102. Internal document prepared by EA's Ecotoxicology Laboratory, EA Engineering, Science, and Technology, Inc., PBC, Hunt Valley, Maryland.
- US EPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. EPA-821-R-02-012. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

SUMMARY OF SAMPLE/TEST INFORMATION

Test: ***Ceriodaphnia dubia* 48-hour static acute toxicity test**

Test Procedure: **EA Protocol CD-AC-05**

Acute assay with water flea (*Ceriodaphnia dubia*)

Client Name: **Metropolitan Water Reclamation District (MWRD)**

Permit Number: **IL0028061**

Receiving Water: **Little Calumet River**

Sample Description: **Outfall 001 Final Effluent**

EA Accession Number: **AT1-118**

Collection Time and Date: 0600, 24 February 2021 to 0600, 25 February 2021

Receipt Time and Date: 0953, 26 February 2021

Dilution Water Description: **Moderately hard synthetic freshwater**

EA Test Number: **TN-21-127**

Test Initiation Time and Date: 1400, 26 February 2021

Test Completion Time and Date: 1403, 28 February 2021

Number of Replicates: **4**

Number of Organisms Per Replicate: **5**

Test Chamber: **30 ml cup**

Volume per Test Chamber: **15 ml**

Feeding: **None**

Organism Lot Information

Lot Number: N/A

Source: EA's Culture Facility (Hunt Valley, Maryland)

Age: <24 hours old

Reference Toxicant Test Information

Reference Toxicant: Sodium chloride (NaCl)

Reference Toxicant Information: Lab Chem Lot#F214-24 (Received 9/7/16)

EA Test Number: RT-21-023

Test Date and Time: 1359, 4 February 2021 to 1322, 6 February 2021

Dilution Water: Moderately hard synthetic freshwater

48-hour LC50: 1,980 mg/L NaCl

Laboratory control chart acceptability range for 48-hour LC50: 1,684-2,156 mg/L NaCl

SUMMARY OF SAMPLE/TEST INFORMATION (continued)

Test Species: *Ceriodaphnia dubia* (water flea)
 Sample Description: Outfall 001 Final Effluent – MWRD
 Sample Date: 24-25 February 2021
 EA Test Number: TN-21-127

| Test Concentration (percent effluent) | 48-Hour Survival (percent) |
|--|-------------------------------|
| Lab Control | 100 |
| 6.25 | 100 |
| 12.5 | 100 |
| 25 | 100 |
| 50 | 95 |
| 100 | 100 |

48-Hour LC50 (percent effluent): **>100 (TU_a <1.0)**

| Water Quality Parameters on Test Solutions | Range |
|--|-------------|
| Temperature (°C): | 24.0 – 25.8 |
| pH: | 7.6 – 8.3 |
| Dissolved Oxygen (mg/L): | 8.2 – 8.6 |
| Conductivity (µS/cm): | 343 – 3,931 |

| Water Quality Parameters Measured on Sample Upon Receipt | Outfall 001 (AT1-118) |
|--|--------------------------|
| Temperature (°C): | 0.8 |
| pH: | 7.6 |
| Total Residual Chlorine (mg/L): | <0.01 |
| Alkalinity (mg/L as CaCO ₃): | 144 |
| Hardness (mg/L as CaCO ₃): | 304 |
| Conductivity (µS/cm): | 3,375 |

SUMMARY OF SAMPLE/TEST INFORMATION

Test: ***Pimephales promelas* 96-hour static renewal acute toxicity test**

Test Procedure: **EA Protocol FH-AC-05**

Acute assay with fathead minnows (*Pimephales promelas*)

Client Name: **Metropolitan Water Reclamation District (MWRD)**

Permit Number: **IL0028061**

Receiving Water: **Little Calumet River**

Sample Description: **Outfall 001 Final Effluent**

EA Accession Number: **AT1-118**

Collection Time and Date: 0600, 24 February 2021 to 0600, 25 February 2021

Receipt Time and Date: 0953, 26 February 2021

Dilution Water Description: **Moderately hard synthetic freshwater**

EA Test Number: **TN-21-126**

Test Initiation Time and Date: 1525, 26 February 2021

Test Completion Time and Date: 1430, 2 March 2021

Number of Replicates: **2**

Number of Organisms Per Replicate: **10**

Test Chamber: **1-L beaker**

Volume per Test Chamber: **250 ml**

Feeding: **0.2 mL *Artemia* nauplii Daily**

Organism Lot Information

Lot Number: FH1-2/20-21

Source: EA's Culture Facility (Hunt Valley, Maryland)

Age: 5-6 days old (hatched within a 24-hour period)

Reference Toxicant Test Information

Reference Toxicant: Potassium chloride (KCl)

Reference Toxicant Information: LabChem #K085-18 (Received 10/20/20)

EA Test Number: RT-21-026

Test Date and Time: 1329, 3 February 2021 to 1338, 5 February 2021

Dilution Water: Moderately hard synthetic freshwater

48-hour LC50: 866 mg/L KCl

Laboratory control chart acceptability range for 48-hour LC50: 626-1,253 mg/L KCl

SUMMARY OF SAMPLE/TEST INFORMATION (continued)

Test Species: *Pimephales promelas* (fathead minnow)
 Sample Description: Outfall 001 Final Effluent – MWRD
 Sample Date: 24-25 February 2021
 EA Test Number: TN-21-126

| <u>Test Concentration (percent effluent)</u> | <u>48-Hour Survival (percent)</u> | <u>96-Hour Survival (percent)</u> |
|--|---------------------------------------|---------------------------------------|
| Lab Control | 100 | 100 |
| 6.25 | 90 | 90 |
| 12.5 | 100 | 100 |
| 25 | 100 | 100 |
| 50 | 95 | 95 |
| 100 | 100 | 95 |

96-Hour LC50 (percent effluent): >100 (TU_a <1.0)

| <u>Water Quality Parameters on Test Solutions</u> | <u>Range</u> |
|---|--------------|
| Temperature (°C): | 24.0 – 26.0 |
| pH: | 7.6 – 8.3 |
| Dissolved Oxygen (mg/L): | 6.6 – 8.6 |
| Conductivity (µS/cm): | 311 – 3,433 |

ATTACHMENT I

Data Sheets
(18 pages)



EA Ecotoxicology Laboratory
 231 Schilling Circle
 Hunt Valley, Maryland 21031
 Telephone: 410-584-7000
 Fax: 410-584-1057



Sample Shipped By: (circle)
 Fed. Ex. UPS Other: _____
 Tracking #: 122744748495610273

Client: MWRDGC Project No.: 4652-126-1
 NPDES Number: IL0028061 Client Purchase Order Number: 3111991
 City/State Collected: Calumet, IL Chicago, IL

PLEASE READ SAMPLING INSTRUCTIONS ON BACK OF FORM

| Accession Number (office use only) | Grab | Composite | Collection | | Sample Description (including Site, Station Number, and Outfall Number) | Number/Volume of Container |
|---------------------------------------|------|-----------|-----------------|-----------------|--|----------------------------|
| | | | Start Date/Time | End Date/Time | | |
| ATI-118 | | ✓ | 2/24/21 0600 | 2/25/21 0600 | Calumet WRP Final Effluent Discharge # 001 | 1 gal |
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|---|-----------------------------|---|---------------------------|
| Sampled By: <i>Nick Kellios</i> | Date/Time 2/25/21 | Received By: | Date/Time |
| Sampler's Printed Name: Nick Kellios | Title: Aquatic Biologist | Relinquished By: | Date/Time |
| Relinquished By: Nick Kellios | Date/Time 2/25/21 0930 | Received By Laboratory: <i>[Signature]</i> | Date/Time 2/26/21 0953 |

Was Sample Chilled During Collection? (Yes) No

Comments:

Sample Collection Parameters

Visual Description: clear, green
 Temperature (°C): 6.0
 pH: 6.715
 TRC (mg/L): 0
 Other:



**SAMPLE CHECK-IN
FOR TESTING.**

Client: MWRD

EA Accession Number: AT1-118

| Parameter | Acceptable Range | Measurement * | Meter | Date | Time | Initials |
|--------------------|------------------|---------------|-------|---------|------|----------|
| Temperature (°C) | ≤4 | 0.8 | 680 | 2/26/21 | 0953 | RSB |
| Is ice present? | -- | yes | N/A | ↓ | ↓ | ↓ |
| pH | 6.0-9.0 | 7.6 | 680 | | | |
| TRC (mg/L) | <0.01 | 0.01 | AT-01 | | | |
| Visual Description | -- | clear | N/A | | | |

*If outside acceptable range, contact project manager.

OTHER PARAMETERS IF REQUIRED (SEE STUDY PLAN):

| Parameter | Acceptable Range | (✓) | Meter | Date | Time | Initials |
|----------------------------|------------------|---------------|-------|------|------|----------|
| Ammonia (preserve aliquot) | -- | | N/A | | | |
| Parameter | Acceptable Range | Measurement * | Meter | Date | Time | Initials |
| Salinity (ppt) | -- | | | | | |



TOXICITY TEST SET-UP BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-127

| TEST ORGANISM INFORMATION | | | |
|----------------------------------|---|------------------------|-----|
| Common Name: <u>Water flea</u> | Adults Isolated (Time, Date): <u>2/25/21 1603</u> | | |
| Scientific Name: <u>C. dubia</u> | Neonates Pulled & Fed (Time, Date): <u>2/26/21 1025</u> | | |
| Lot Number: <u>N/A</u> | Acclimation: <u><24hrs</u> | Age: <u><24 hrs</u> | |
| Source: <u>EA</u> | Culture Water (T/S): <u>24.7</u> °C | <u>0</u> | ppt |

| TEST INITIATION | | | |
|-----------------|-------------|------------|-----------------------|
| Date | Time | Initials | Activity |
| <u>2/26/21</u> | <u>1330</u> | <u>TP</u> | Dilutions Made |
| ↓ | ↓ | ↓ | Test Vessels Filled |
| | <u>1400</u> | | Organisms Transferred |
| | <u>1648</u> | <u>UAD</u> | Head Counts |

| TEST SET-UP | | |
|---------------------------------|----------------------|--------------|
| Sample Number: <u>AT1-118</u> | | |
| Dilution Number: <u>AD1-119</u> | | |
| Test Concentration | Volume Test Material | Final Volume |
| Control | 0 ml | 200 ml |
| 6.25% | 12.5 ml | ↓ |
| 12.5% | 25 ml | |
| 25% | 50 ml | |
| 50% | 100 ml | |
| 100% | 200 ml | |



ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019-TOX TEST ORGANISM: Water flea Beginning Date: 2/26/21 Time: 1400
 Client: MWRD Common Name: Water flea Ending Date: 2/28/21 Time: 1403
 QC Test Number: TN-21-127 Scientific Name: C. dubia TEST TYPE: Static Flowthrough
 Test Material: Effluent TARGET VALUES: Renewal / Non-renewal
 Accession Number: AT1-118 Temp: 25±1 °C DO: >4.0 mg/L Test Container: 30 ml cup
 Dilution Water: Mod Hard pH: 6.0 - 9.0 Salinity: 0 ppt Test Volume: 15 ml
 Accession Number: LD1-119 Photoperiod: 16 L, 8 d Light Intensity: 50 - 100 fc Test Duration: 48 hrs

| Concentration | Rep | Number of Live Organisms | | | | Temperature (°C) | | | | pH | | | | Dissolved Oxygen (mg/L) | | | | Conductivity (µS/cm) | | | |
|---------------|-----|--------------------------|------|------|----|------------------|------|------|-----|-----|-----|-----|-----|-------------------------|-----|-----|-----|----------------------|-----|-----|----|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 |
| Control | A | 5 | 5 | 5 | 5 | 24.0 | 25.4 | 24.0 | 8.3 | 8.1 | 8.1 | 8.5 | 8.3 | 8.6 | 343 | 358 | 388 | | | | |
| | B | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| 6.25% | A | 5 | 5 | 5 | 5 | 24.0 | 25.4 | 24.0 | 8.3 | 8.0 | 8.1 | 8.6 | 8.4 | 484 | 499 | 527 | | | | | |
| | B | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| 12.5% | A | 5 | 5 | 5 | 5 | 24.0 | 25.7 | 24.5 | 8.1 | 8.0 | 8.1 | 8.5 | 8.4 | 704 | 715 | 780 | | | | | |
| | B | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| Meter Number | | | | | | | | | | | | | | | | | | | | | |
| Time | | 1049 | 1315 | 1403 | | 679 | 681 | 680 | 679 | 681 | 680 | 679 | 681 | 680 | 679 | 681 | 680 | 679 | 681 | 680 | |
| Initials | | UD | TP | M | | TP | TP | AT | TP | TP | AT | TP | TP | AT | TP | TP | AT | TP | TP | AT | |



ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019_TOX
 Client: MWRD
 QC Test Number: IN-21-127
 Test Material: Effluent
 Accession Number: AT1-118
 Dilution Water: Mod Hard
 Accession Number: Q1-119

TEST ORGANISM
 Common Name: Water flea
 Scientific Name: C. dubia
 TARGET VALUES
 Temp: 25±1 °C DO: >4.0 mg/L
 pH: 6.0-9.0 Salinity: 0 ppt
 Photoperiod: 16 L, 8 d Light Intensity: 50-100 fc
 Test Duration: 48 hrs

Beginning Date: 2/26/21 Time: 1400
 Ending Date: 2/28/21 Time: 1403

TEST TYPE: Static Flowthrough
 Renewal / Non-renewal

Test Container: 30 ml cup
 Test Volume: 15 ml
 Test Duration: 48 hrs

| Concentration | Rep | Number of Live Organisms | | | | Temperature (°C) | | | | pH | | | | Dissolved Oxygen (mg/L) | | | | Conductivity (µS/cm) | | | | | |
|---------------|------|--------------------------|------|----|----|------------------|------|------|------|-----|-----|-----|-----|-------------------------|-----|-----|-----|----------------------|------|------|------|------|--|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | | |
| 25% | A | 5 | 5 | 5 | 5 | 5 | 24.0 | 25.7 | 24.5 | 8.0 | 8.0 | 8.0 | 8.4 | 8.4 | 8.4 | 8.6 | 8.4 | 8.4 | 8.4 | 1134 | 1161 | 1203 | |
| | B | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| 50% | A | 5 | 5 | 5 | 5 | 5 | 24.0 | 25.8 | 24.5 | 7.8 | 8.6 | 8.0 | 8.4 | 8.7 | 8.5 | 8.5 | 8.4 | 8.7 | 1916 | 1929 | 2076 | | |
| | B | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| 100% | A | 5 | 5 | 5 | 5 | 5 | 25.3 | 25.8 | 24.7 | 7.6 | 7.9 | 8.0 | 8.4 | 8.7 | 8.5 | 8.4 | 8.7 | 8.7 | 3433 | 3566 | 3731 | | |
| | B | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | | |
| Meter Number | | | | | | | | | | | | | | | | | | | | | | | |
| Time | 1046 | 1315 | 1403 | | | 679 | 681 | 680 | | | | | | | | 679 | 681 | 680 | | | | | |
| Initials | UPD | RP | BT | | | RP | RP | BT | | | | | | | | RP | RP | BT | | | | | |



TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-127

Date/Time/Initials

Comments/Activity



RANDOMIZATION CHART

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-127

| | | | | | |
|---|---|---|---|---|---|
| 5 | 4 | 1 | 3 | 6 | 2 |
| 1 | 5 | 3 | 2 | 4 | 6 |
| 6 | 2 | 4 | 1 | 5 | 3 |
| 4 | 1 | 2 | 6 | 3 | 5 |



TOXICOLOGY LABORATORY BENCH SHEET - TESTING LOCATION

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-67

| Day | Testing Location | Date | Time | Initials |
|-----|------------------|---------|------|----------|
| 0 | 17B | 2/26/21 | 1649 | UAD |
| 1 | 17B | 2/27/21 | 1316 | UAD |
| 2 | 17B | 2/28/21 | 1400 | UAD |
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TOXICOLOGY LABORATORY CORRECTION BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-127

Correction Explanations

- (a) Technician Error-Mathematical
- (b) Technician Error-Manual Data Recording
- (c) Technician Error-Head Count Observation
- (d) Technician Error-Overwrite
- (e) Technician Error-Missing Data
- (f) Technician Error-Lost Organism
- (g) Technician Error-Transcription Error
- (h) Technician Error-Other:
- (i) Meter Malfunction



TOXICITY TEST SET-UP BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-126

| TEST ORGANISM INFORMATION | | | |
|-------------------------------------|--|----------------------|--|
| Common Name: <u>Fathead minnow</u> | Adults Isolated (Time, Date): _____ | | |
| Scientific Name: <u>P. promelas</u> | Neonates Pulled & Fed (Time, Date): _____ | | |
| Lot Number: <u>FH1-2120-21</u> | Acclimation: <u><24 hrs</u> | Age: <u>5-6 days</u> | |
| Source: <u>EA</u> | Culture Water (T/S): <u>25.7</u> °C <u>0</u> ppt | | |

| TEST SET-UP | | | | | | |
|-----------------|-------------|------------|-----------------------|----------------------|----------------------|--------------|
| TEST INITIATION | | | | CONCENTRATION SERIES | | |
| Date | Time | Initials | Activity | Test Concentration | Volume Test Material | Final Volume |
| <u>2/26/21</u> | <u>1330</u> | <u>TP</u> | Dilutions Made | Control | 0 ml | 500 ml |
| ↓ | ↓ | ↓ | Test Vessels Filled | 6.25% | 31.25 ml | ↓ |
| ↓ | ↓ | ↓ | Organisms Transferred | 12.5% | 62.5 ml | ↓ |
| ↓ | <u>1525</u> | ↓ | Head Counts | 25% | 125 ml | ↓ |
| ↓ | <u>1640</u> | <u>LAO</u> | | 50% | 250 ml | ↓ |
| | | | | 100% | 500 ml | ↓ |
| Comments: | | | | | | |

| INTERMEDIATE DILUTION PREPARATION AND FEEDING | | | | | | | | |
|---|----------------|-------------|-----------|----------------------------------|----------------------|------------------------|------------------------|---------------------------|
| DILUTION PREPARATION | | | | | FEEDING | | | |
| Day | Date | Time | Initials | Sample / Diluent | Food: <i>Artemia</i> | | | |
| | | | | | Day | Time, Initials, Amount | Time, Initials, Amount | Time, Initials, Amount |
| 0 | <u>2/26/21</u> | <u>1330</u> | <u>TP</u> | <u>AT1-118</u> <u>LD1-119</u> | 0 | | | 3 drops <u>1530 TP</u> |
| 1 | | | | | 1 | | | 3 drops <u>1653 TP</u> |
| 2 | <u>2/28/21</u> | <u>1430</u> | <u>AT</u> | <u>AT1-118</u> <u>LD1-123</u> | 2 | | | 3 drops <u>1545 AT</u> |
| 3 | | | | | 3 | | | 3 drops <u>1600 AT</u> |
| 4 | | | | | 4 | | | |
| 5 | | | | | 5 | | | |
| 6 | | | | | 6 | | | |



ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019_TOX TEST ORGANISM: Fathead minnow Beginning Date: 2/26/21 Time: 1525
 Client: MWRD Common Name: Fathead minnow Ending Date: 3/2/21 Time: 1430
 QC Test Number: TN-21-126 Scientific Name: P. promelas TEST TYPE: Static Flowthrough
 Test Material: Effluent TARGET VALUES: Renewal Non-renewal
 Accession Number: AT1-118 Temp: 25±1 °C DO: >4.0 mg/L Test Container: 1 L Beaker
 Dilution Water: Mod Hard pH: 6.0-9.0 Salinity: 0 ppt Test Volume: 250 ml
 Accession Number: LOT-119 Photoperiod: 16 L, 8 d Light Intensity: 50-100 fc Test Duration: 96 hrs

| Concentration | Rep | Number of Live Organisms | | | | | Temperature (°C) | | | | | pH | | | | | Dissolved Oxygen (mg/L) | | | | | Conductivity (µS/cm) | | | | | |
|---------------|-----|--------------------------|------|------|------|------|------------------|----|----|----|----|------|----|----|----|----|-------------------------|----|----|----|----|----------------------|----|----|----|----|--|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | |
| Control | A | 10 | 10 | 10 | 10 | 10 | 24.0 | | | | | 8.3 | | | | | 8.5 | | | | | | | | | | |
| | B | 10 | 10 | 10 | 10 | 10 | 24.2 | | | | | 8.3 | | | | | 8.6 | | | | | | | | | | |
| 6.25% | A | 10 | 10 | 10 | 10 | 10 | 24.0 | | | | | 8.2 | | | | | 8.5 | | | | | | | | | | |
| | B | 10 | 10 | 8 | 8 | 8 | 24.2 | | | | | 8.2 | | | | | 8.5 | | | | | | | | | | |
| 12.5% | A | 10 | 10 | 10 | 10 | 10 | 24.0 | | | | | 8.1 | | | | | 8.5 | | | | | | | | | | |
| | B | 10 | 10 | 10 | 10 | 10 | 24.1 | | | | | 8.1 | | | | | 8.6 | | | | | | | | | | |
| 25% | A | 10 | 10 | 10 | 10 | 10 | 24.0 | | | | | 8.0 | | | | | 8.5 | | | | | | | | | | |
| | B | 10 | 10 | 10 | 10 | 10 | 24.1 | | | | | 8.1 | | | | | 8.6 | | | | | | | | | | |
| 50% | A | 10 | 10 | 10 | 10 | 10 | 24.0 | | | | | 7.8 | | | | | 8.5 | | | | | | | | | | |
| | B | 10 | 10 | 9 | 9 | 9 | 24.5 | | | | | 8.0 | | | | | 8.5 | | | | | | | | | | |
| 100% | A | 10 | 10 | 10 | 10 | 9 | 25.3 | | | | | 7.6 | | | | | 8.5 | | | | | | | | | | |
| | B | 10 | 10 | 10 | 10 | 10 | | | | | | 7.9 | | | | | 8.5 | | | | | | | | | | |
| Meter Number | | | | | | | 679 | | | | | 680 | | | | | 679 | | | | | | | | | | |
| Time | | 1640 | 1506 | 1440 | 1430 | 1430 | 1337 | | | | | 1433 | | | | | 1337 | | | | | | | | | | |
| Initials | | UPD | UPD | KA | KA | KA | TP | | | | | AT | | | | | TP | | | | | | | | | | |



ACUTE TOXICITY TEST DATA SHEET - OLD SOLUTIONS

Project Number: 70019.TOX TEST ORGANISM: Fathead minnow Beginning Date: 2/26/21 Time: 1525
 Client: MWRD Common Name: P. promelas Ending Date: 3/2/21 Time: 1430
 QC Test Number: TN-21-126 Scientific Name: P. promelas TEST TYPE: Static Flowthrough
 Test Material: Effluent TARGET VALUES: Renewal / Non-renewal
 Accession Number: A11-116 Temp: 25±1 °C DO: >4.0 mg/L Test Container: 1 L Beaker
 Dilution Water: Mod Hard pH: 6.0 - 9.0 Salinity: 0 ppt Test Volume: 250 ml
 Accession Number: LD1-119 Photoperiod: 16 L 8 d Light Intensity: 50 - 100 fc Test Duration: 96 hrs

| Concentration | Rep | Number of Live Organisms | | | | | | Temperature (°C) | | | pH | | | Dissolved Oxygen (mg/L) | | | Conductivity (µS/cm) | | | | |
|---------------|-----|--------------------------|------|------|------|------|---|------------------|------|------|------|---|------|-------------------------|------|------|----------------------|------|------|------|------|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 |
| Control | A | | 249 | 260 | 260 | 243 | | 8.3 | 8.3 | 8.1 | 8.3 | | 8.2 | 8.2 | 7.7 | 8.0 | | 363 | 353 | 359 | 359 |
| | B | | | 249 | | | | | | | | | | | | | | | | | |
| 6.25% | A | | 251 | 25.2 | 26.0 | 25.2 | | 8.1 | 8.2 | 7.9 | 8.2 | | 8.1 | 8.2 | 7.0 | 7.9 | | 481 | 486 | 515 | 510 |
| | B | | | 26.0 | | | | | | | | | | | | | | | | | |
| 12.5% | A | | 26.0 | 25.9 | 26.0 | 25.5 | | 8.0 | 8.1 | 7.9 | 8.1 | | 7.9 | 7.8 | 7.0 | 7.0 | | 698 | 695 | 723 | 706 |
| | B | | | | | | | | | | | | | | | | | | | | |
| 25% | A | | 26.0 | 26.0 | 26.0 | 25.7 | | 8.0 | 8.0 | 7.9 | 8.0 | | 7.8 | 8.0 | 6.8 | 6.8 | | 1105 | 1117 | 1131 | 1110 |
| | B | | | | | | | | | | | | | | | | | | | | |
| 50% | A | | 26.0 | 26.0 | 26.0 | 25.5 | | 7.9 | 7.9 | 7.8 | 8.0 | | 7.8 | 8.0 | 6.6 | 6.9 | | 1863 | 1871 | 1917 | 1879 |
| | B | | | | | | | | | | | | | | | | | | | | |
| 100% | A | | 26.0 | 26.0 | 26.0 | 25.7 | | 7.8 | 7.9 | 7.7 | 7.8 | | 7.8 | 7.8 | 6.7 | 6.7 | | 5244 | 3201 | 3413 | 3319 |
| | B | | | | | | | | | | | | | | | | | | | | |
| Meter Number | | | 681 | 100 | 100 | 680 | | 681 | 680 | 680 | 680 | | 681 | 680 | 680 | 680 | | 681 | 680 | 680 | 680 |
| Time | | | 1300 | 1410 | 1500 | 0907 | | 1300 | 1410 | 1500 | 0907 | | 1300 | 1410 | 1500 | 0907 | | 1300 | 1410 | 1500 | 0907 |
| Initials | | | TP | AT | AT | AT | | TP | AT | AT | AT | | TP | AT | AT | AT | | TP | AT | AT | AT |



TOXICOLOGY LABORATORY BENCH SHEET - TESTING LOCATION

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-126

| Day | Testing Location | Date | Time | Initials |
|-----|------------------|---------|------|----------|
| 0 | 17B | 2/26/21 | 1641 | LAD |
| 1 | 17B | 2/27/21 | 1515 | AD |
| 2 | 17B | 2/28/21 | 1546 | AJ |
| 3 | 17B | 3/1/21 | 1503 | AJ |
| 4 | 17B | 3/2/21 | 0927 | AJ |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | | | | |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | | | | |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | | | | |
| 23 | | | | |
| 24 | | | | |
| 25 | | | | |
| 26 | | | | |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | | | | |



TOXICOLOGY LABORATORY CORRECTION BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-126

Correction Explanations

- (a) Technician Error-Mathematical
- (b) Technician Error-Manual Data Recording
- (c) Technician Error-Head Count Observation
- (d) Technician Error-Overwrite
- (e) Technician Error-Missing Data
- (f) Technician Error-Lost Organism
- (g) Technician Error-Transcription Error
- (h) Technician Error-Other:
- (i) Meter Malfunction



TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-126

Date/Time/Initials

Comments/Activity



RANDOMIZATION CHART

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-126

| | | | | | |
|---|---|---|---|---|---|
| 5 | 4 | 1 | 3 | 6 | 2 |
| 1 | 5 | 3 | 2 | 4 | 6 |



TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-126,127

Aliquot of sample warmed to test temperature, then aerated if supersaturated:

| Date | Sample # | ON AIR | | | OFF AIR | | |
|---------|----------|-------------------|------|----------|-----------------|------|----------|
| | | Initial DO (mg/L) | Time | Initials | Final DO (mg/L) | Time | Initials |
| 2/26/21 | AT1-118 | 9.7 | 1008 | RSB | 8.5 | 1018 | RSB |
| 2/28/21 | AT1-118 | 9.3 | 1413 | AT | 8.3 | 1423 | AT |

ATTACHMENT II

Report Quality Assurance Record
(2 pages)



REPORT QUALITY ASSURANCE RECORD

Client: MWRD Project Number: 70019.TOX
 Author: Rachael Brooks EA Report Number: 8505

REPORT CHECKLIST

| <u>QA/OC ITEM</u> | <u>REVIEWER</u> | <u>DATE</u> |
|---|-----------------|------------------|
| 1. Samples collected, transported, and received according to study plan requirements. | | <u>3/2/2021</u> |
| 2. Samples prepared and processed according to study plan requirements. | | <u>3/2/2021</u> |
| 3. Data collected using calibrated instruments and equipment. | | <u>3/2/2021</u> |
| 4. Calculations checked: | | |
| - Hand calculations checked | | <u>3/2/2021</u> |
| - Documented and verified statistical procedure used. | | <u>3/2/2021</u> |
| 5. Data input/statistical analyses complete and correct. | | <u>3/10/2021</u> |
| 6. Reported results and facts checked against original sources. | | <u>3/10/2021</u> |
| 7. Data presented in figures and tables correct and in agreement with text. | | <u>3/10/2021</u> |
| 8. Results reviewed for compliance with study plan requirements. | | <u>3/2/2021</u> |

| | <u>AUTHOR</u> | <u>DATE</u> |
|---|--------------------------|------------------|
| 9. Commentary reviewed and resolved. | | <u>3/15/2021</u> |
| 10. All study plan and quality assurance/control requirements have been met and the report is approved: | | |
| | | <u>3/15/21</u> |
| | PROJECT MANAGER | DATE |
| | | <u>3/10/2021</u> |
| | QUALITY CONTROL OFFICER | DATE |
| | | <u>3/15/21</u> |
| | SENIOR TECHNICAL OFFICER | DATE |