

Protecting Our Water Environment



Metropolitan Water Reclamation District of Greater Chicago

***MONITORING AND RESEARCH
DEPARTMENT***

REPORT NO. 21-11

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

May 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 AN APRIL 2021 EFFLUENT SAMPLE FROM
METROPOLITAN WATER RECLAMATION DISTRICT (MWRD)**

By

**EA Engineering, Science, and Technology, Inc., PBC
231 Schilling Circle
Hunt Valley, Maryland 21031**

Kari K. Steele
President
Barbara J. McGowan
Vice President
Marcelino Garcia
Chairman of Finance
Cameron Davis
Kimberly Du Buclet
Josina Morita
Eira L. Corral Sepúlveda
Debra Shore
Mariyana T. Spyropoulos

Metropolitan Water Reclamation District of Greater Chicago

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

May 4, 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 O’Brien Water Reclamation Plant, National Pollutant Discharge Elimination System Permit Number IL0028088

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 IL0028088, Special Condition 9. The report covers the monitoring done for samples collected in the fifteenth 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

Enclosures

cc: E. Podczerwinski/J. Murray
A. Poonsapaya/H. Zhang
T. Minarik/N. Kollias

Via electronic mail



RESULTS OF ACUTE TOXICITY TESTING
WITH *Ceriodaphnia dubia* AND *Pimephales promelas*
ON AN APRIL 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

A handwritten signature in black ink, appearing to read 'Michael K. Chanov II', is positioned above a horizontal line.

Michael K. Chanov II
Laboratory Director

26 April 2021

Date

EA Project Number 70019.TOX



EA Report Number 8542

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 O'Brien Water Reclamation Plant in Skokie, Illinois. The effluent composite sample was collected on 12-13 April 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 IL0028088.

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 12-13 April 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 100 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 80 percent survival in all of the effluent concentrations. The laboratory control had 95 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 2,050 mg/L NaCl, and acceptable control chart limits of 1,608-2,259 mg/L NaCl. The results of the *P. promelas* reference toxicant test was acceptable, with a 48-hour LC50 of 658 mg/L KCl, and acceptable control chart limits of 626-1,237 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: **IL0028088**

Receiving Water: **North Shore Channel**

Sample Description: **Outfall 001 Final Effluent**

EA Accession Number: **AT1-214**

Collection Time and Date: 0600, 12 April 2021 to 0600, 13 April 2021

Receipt Time and Date: 0933, 14 April 2021

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

EA Test Number: **TN-21-214**

Test Initiation Time and Date: 1050, 14 April 2021

Test Completion Time and Date: 1040, 16 April 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-21-055

Test Date and Time: 1009, 12 April 2021 to 1040, 14 April 2021

Dilution Water: Moderately hard synthetic freshwater

48-hour LC50: 2,050 mg/L NaCl

Laboratory control chart acceptability range for 48-hour LC50: 1,608-2,259 mg/L NaCl

SUMMARY OF SAMPLE/TEST INFORMATION (continued)

Test Species: ***Ceriodaphnia dubia* (water flea)**
 Sample Description: Outfall 001 Final Effluent – MWRD
 Sample Date: 12-13 April 2021
 EA Test Number: TN-21-214

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

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

| <u>Water Quality Parameters on Test Solutions</u> | <u>Range</u> |
|---|--------------|
| Temperature (°C): | 24.0 – 25.4 |
| pH: | 7.7 – 8.3 |
| Dissolved Oxygen (mg/L): | 8.2 – 9.0 |
| Conductivity (µS/cm): | 317 – 1,373 |

| <u>Water Quality Parameters Measured on Sample Upon Receipt</u> | <u>Outfall 001 (AT1-214)</u> |
|---|----------------------------------|
| Temperature (°C): | 1.1 |
| pH: | 7.6 |
| Total Residual Chlorine (mg/L): | <0.01 |
| Alkalinity (mg/L as CaCO ₃): | 156 |
| Hardness (mg/L as CaCO ₃): | 240 |
| Conductivity (µS/cm): | 1,155 |

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: **IL0028088**

Receiving Water: **North Shore Channel**

Sample Description: **Outfall 001 Final Effluent**

EA Accession Number: **AT1-214**

Collection Time and Date: 0600, 12 April 2021 to 0600, 13 April 2021

Receipt Time and Date: 0933, 14 April 2021

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

EA Test Number: **TN-21-213**

Test Initiation Time and Date: 1100, 14 April 2021

Test Completion Time and Date: 1150, 18 April 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 at 48 hours**

Organism Lot Information

Lot Number: FH1-4/10-11

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

Age: 3-4 days old (hatched within a 24-hour period)

Reference Toxicant Test Information

Reference Toxicant: Potassium chloride (KCl)

Reference Toxicant Information: GFS Lot #19430079 (Received 10/20/20)

EA Test Number: RT-21-052

Test Date and Time: 1204, 6 April 2021 to 1115, 8 April 2021

Dilution Water: Moderately hard synthetic freshwater

48-hour LC50: 658 mg/L KCl

Laboratory control chart acceptability range for 48-hour LC50: 626-1,237 mg/L KCl

SUMMARY OF SAMPLE/TEST INFORMATION (continued)

Test Species: ***Pimephales promelas* (fathead minnow)**
 Sample Description: Outfall 001 Final Effluent – MWRD
 Sample Date: 12-13 April 2021
 EA Test Number: TN-21-213

| <u>Test Concentration (percent effluent)</u> | <u>48-Hour Survival (percent)</u> | <u>96-Hour Survival (percent)</u> |
|--|---------------------------------------|---------------------------------------|
| Lab Control | 100 | 95 |
| 6.25 | 85 | 80 |
| 12.5 | 100 | 100 |
| 25 | 100 | 100 |
| 50 | 95 | 85 |
| 100 | 90 | 90 |

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): | 6.3 – 8.5 |
| Conductivity (µS/cm): | 315 – 1,179 |

ATTACHMENT I

Data Sheets and Statistical Analysis
(18 pages)



**SAMPLE CHECK-IN
FOR TESTING.**

Client: MWRD

EA Accession Number: AT1-214

| Parameter | Acceptable Range | Measurement * | Meter | Date | Time | Initials |
|--------------------|------------------|---------------|-------|---------|-------------------|----------|
| Temperature (°C) | ≤4 | 1.1 | T-21 | 4/14/21 | 0934 ³ | TP |
| Is ice present? | — | yes | N/A | ↓ | ↓ | ↓ |
| pH | 6.0-9.0 | 7.6 | 681 | ↓ | ↓ | ↓ |
| TRC (mg/L) | <0.01 | Lo.01 | AT-01 | ↓ | ↓ | ↓ |
| Visual Description | — | Clear | N/A | ↓ | ↓ | ↓ |

*If outside acceptable range, contact project manager.

Ⓟ ISO 4/20/21
for TP

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-21-213

TEST ORGANISM INFORMATION

Common Name: Fathead minnow Adults Isolated (Time, Date): _____
 Scientific Name: P. promelas Neonates Pulled & Fed (Time, Date): _____
 Lot Number: FH1-9110-11 Acclimation: <24 hrs Age: 3-4 days
 Source: EA Culture Water (T/S): 25-0 °C 0 ppt

TEST SET-UP

| TEST INITIATION | | | | CONCENTRATION SERIES | | |
|-----------------|------|----------|-----------------------|----------------------|----------------------|--------------|
| Date | Time | Initials | Activity | Test Concentration | Volume Test Material | Final Volume |
| 4/14/21 | 1021 | LAD | Dilutions Made | Control | 0 ml | 500 ml |
| | | | | 6.25% | 31.25 ml | |
| | | | | 12.5% | 62.5 ml | |
| | 1055 | TP | Test Vessels Filled | 25% | 125 ml | |
| | | | | 50% | 250 ml | |
| | 1100 | ↓ | Organisms Transferred | 100% | 500 ml | |
| | 1141 | LAD | Head Counts | | | |

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 | 4/14/21 | 1021 | LAD | AT1-214 TD1-218 | 0 | | | |
| 1 | | | | | 1 | | | |
| 2 | 4/14/21 | 0851 | LAD | AT1-214 TD-229 | 2 | | LAD 0746 3 drops | |
| 3 | | | | | 3 | | | |
| 4 | | | | | 4 | | | |
| 5 | | | | | 5 | | | |
| 6 | | | | | 6 | | | |



ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019_TOX Beginning Date: 4/14/01 Time: 1100
 Client: MWRD Common Name: Fathead minnow Ending Date: 4/18/01 Time: 1150
 QC Test Number: IN-21-213 Scientific Name: P. promelas TEST TYPE: Static / Flowthrough
 Test Material: Effluent TARGET VALUES: Renewal / Non-renewal

Accession Number: AT-214 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: LD-218 Photoperiod: 16L, 8d 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 | 21.6 | | | | | 7.9 | 8.0 | | | | 8.5 | | | | | 8.3 | | | | | 315 |
| | B | 10 | 10 | 10 | 10 | 9 | 21.6 | | | | | | | | | | | | | | | | | | | | |
| 6.25% | A | 10 | 8 | 8 | 7 | 7 | 21.5 | | | | | 7.9 | 8.0 | | | | 8.3 | | | | | 8.3 | | | | 369 | |
| | B | 10 | 10 | 9 | 9 | 9 | 21.7 | | | | | | | | | | | | | | | | | | | | |
| 12.5% | A | 10 | 10 | 10 | 10 | 10 | 21.5 | | | | | 7.9 | 8.0 | | | | 8.3 | | | | | 8.3 | | | | 410 | |
| | B | 10 | 10 | 10 | 10 | 10 | 21.7 | | | | | | | | | | | | | | | | | | | | |
| 25% | A | 10 | 10 | 10 | 10 | 10 | 21.4 | | | | | 7.8 | 8.0 | | | | 8.3 | | | | | 8.3 | | | | 519 | |
| | B | 10 | 10 | 10 | 10 | 10 | 21.7 | | | | | | | | | | | | | | | | | | | | |
| 50% | A | 10 | 10 | 9 | 7 | 7 | 21.2 | | | | | 7.8 | 8.0 | | | | 8.3 | | | | | 8.3 | | | | 741 | |
| | B | 10 | 10 | 10 | 10 | 10 | 21.7 | | | | | | | | | | | | | | | | | | | | |
| 100% | A | 10 | 9 | 9 | 9 | 9 | 21.0 | | | | | 7.7 | 8.0 | | | | 8.4 | | | | | 8.2 | | | | 1153 | |
| | B | 10 | 9 | 9 | 9 | 9 | 21.7 | | | | | | | | | | | | | | | | | | | | |
| Meter Number | | | | | | | 660 | | | | | 680 | 681 | | | | 680 | | | | | 681 | | | | 681 | |
| Time | | 1141 | 1057 | 1126 | 1000 | 1150 | 1027 | | | | 1059 | 1057 | | | | | 1057 | | | | | 1059 | | | | 1059 | |
| Initials | | WFD | WFD | WFD | WFD | WFD | WFD | | | | WFD | WFD | | | | | WFD | | | | | WFD | | | | WFD | |



ACUTE TOXICITY TEST DATA SHEET - OLD SOLUTIONS

Project Number: 70019_TOX TEST ORGANISM: 4/14/21 Beginning Date: 4/14/21 Time: 1100
 Client: MWRD Common Name: Fathead minnow Ending Date: 4/18/21 Time: 1150
 QC Test Number: TN-21-213 Scientific Name: P. promelas TEST TYPE: Static Flowthrough
 Test Material: Effluent TARGET VALUES: Renewal Non-renewal
 Accession Number: AT-214 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: 01-218 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) | | | | | |
|---------------|-----|--------------------------|------------------|----|----|-----|-----|-----|-------------------------|-----|-----|----------------------|-----|------|------|------|------|
| | | | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 | | | |
| Control | A | | | | | | | | | | | | | | | | |
| | B | | | | | 8.2 | 8.1 | 7.9 | 8.1 | 8.3 | 7.6 | 7.7 | 8.0 | 335 | 334 | 341 | 343 |
| 6.25% | A | | | | | | | | | | | | | | | | |
| | B | | | | | 8.2 | 8.0 | 7.9 | 8.0 | 8.2 | 7.6 | 6.8 | 7.8 | 382 | 380 | 390 | 392 |
| 12.5% | A | | | | | | | | | | | | | | | | |
| | B | | | | | 8.1 | 8.0 | 7.8 | 7.9 | 7.7 | 7.6 | 6.5 | 7.2 | 429 | 408 | 437 | 435 |
| 25% | A | | | | | | | | | | | | | | | | |
| | B | | | | | 8.1 | 8.1 | 7.8 | 7.9 | 7.7 | 7.6 | 6.3 | 7.1 | 530 | 528 | 540 | 537 |
| 50% | A | | | | | | | | | | | | | | | | |
| | B | | | | | 8.1 | 8.0 | 7.7 | 8.0 | 7.7 | 7.3 | 6.7 | 7.1 | 741 | 739 | 765 | 759 |
| 100% | A | | | | | | | | | | | | | | | | |
| | B | | | | | 8.2 | 8.3 | 7.6 | 8.0 | 7.4 | 7.2 | 6.3 | 7.0 | 1135 | 1132 | 1179 | 1163 |
| Meter Number | | | | | | | | | | | | | | | | | |
| Time | | | | | | | | | | | | | | | | | |
| Initials | | | | | | | | | | | | | | | | | |



TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21213

Date/Time/Initials

Comments/Activity



RANDOMIZATION CHART

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-213

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



TOXICOLOGY LABORATORY BENCH SHEET - TESTING LOCATION

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-213

| Day | Testing Location | Date | Time | Initials |
|-----|------------------|---------|------|----------|
| 0 | 16 | 4/14/21 | 1100 | TD |
| 1 | 16 | 4/15/21 | 1057 | AT |
| 2 | 16 | 4/16/21 | 1110 | CAF |
| 3 | 16 | 4/17/21 | 1020 | TD |
| 4 | 16 | 4/18/21 | 1145 | AT |
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TOXICOLOGY LABORATORY CORRECTION BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-213

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-21-214

TEST ORGANISM INFORMATION

Common Name: Water flea Adults Isolated (Time, Date): 4/13/21 1555
 Scientific Name: C. dubia Neonates Pulled & Fed (Time, Date): 4/14/21 0838
 Lot Number: N/A Acclimation: <24hrs Age: <24 hrs
 Source: EA Culture Water (T/S): 24.7 °C 0 ppt

TEST INITIATION

| Date | Time | Initials | Activity |
|---------|------|----------|-----------------------|
| 4/14/21 | 1021 | UAD | Dilutions Made |
| ↓ | L | ↓ | Test Vessels Filled |
| | 1050 | TP | Organisms Transferred |
| | 1158 | UAD | Head Counts |

TEST SET-UP

Sample Number: ATI-214

Dilution Number: CD1-218

| 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 Beginning Date: 4/11/21 Time: 1050
 Client: MWRD Common Name: Water flea Ending Date: 4/16/21 Time: 1040
 QC Test Number: TN-21-214 Scientific Name: C. dubia TEST TYPE: Static Flowthrough
 Test Material: Effluent TARGET VALUES: Renewal / Non-renewal
 Accession Number: AT1-214 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: WT-212 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 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | |
| Control | A | 5 | 5 | 5 | 5 | 24.6 | 24.5 | 24.0 | | 7.9 | 7.8 | 7.8 | | 8.5 | 8.5 | 8.7 | | 317 | 353 | 407 | |
| | B | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| 6.25% | A | 5 | 5 | 5 | 5 | 24.5 | 25.0 | 24.0 | | 7.9 | 8.0 | 7.9 | | 8.3 | 8.5 | 9.0 | | 370 | 395 | 409 | |
| | B | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| 12.5% | A | 5 | 5 | 5 | 5 | 24.5 | 25.2 | 24.4 | | 7.9 | 8.0 | 8.0 | | 8.3 | 8.4 | 8.9 | | 417 | 443 | 484 | |
| | B | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| Meter Number | | | | | | | | | | | | | | | | | | | | | |
| Time | | 1150 | 1100 | 1040 | | | | | | | | | | | | | | | | | |
| Initials | | UAD | JA | UAD | | | | | | | | | | | | | | | | | |



ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019.TOX Beginning Date: 4/14/21 Time: 1050
 Client: MWRD Ending Date: 4/16/21 Time: 1040
 Common Name: Water flea
 Scientific Name: C. dubia TEST TYPE: Static / Flowthrough
 Test Material: Effluent Renewal / Non-renewal
 Accession Number: AT1-214 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: W1-210 Photoperiod: 16L, 8d Light Intensity: 50 - 100 fc Test Duration: 48 hrs

TARGET VALUES

| 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 | 7.0 | 8.1 | 8.0 | 8.0 | 8.3 | 8.4 | 8.9 | 520 | 549 | 598 | | | | | |
| | 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 | 7.8 | 8.2 | 8.0 | 8.0 | 8.3 | 8.3 | 8.9 | 707 | 770 | 813 | | | | | |
| | 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 | 7.7 | 8.3 | 8.0 | 8.0 | 8.4 | 8.2 | 8.8 | 1135 | 1224 | 1373 | | | | | |
| | B | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | |
| | C | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | |
| | D | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | |
| Meter Number | | | | | | | | | | | | | | | | | | | | | |
| Time | 1150 | 1100 | 1040 | | | | | | | | | | | | | | | | | | |
| Initials | UAD | K | UAD | | | | | | | | | | | | | | | | | | |



TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-214

Date/Time/Initials

Comments/Activity



RANDOMIZATION CHART

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-214

| | | | | | |
|---|---|---|---|---|---|
| 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-21-214

| Day | Testing Location | Date | Time | Initials |
|-----|------------------|---------|------|----------|
| 0 | 16 | 4/14/21 | 1050 | TP |
| 1 | 16 | 4/15/21 | 1102 | AT |
| 2 | 16 | 4/16/21 | 1042 | VFD |
| 3 | | | | |
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| 30 | | | | |



TOXICOLOGY LABORATORY CORRECTION BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-214

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

ATTACHMENT II

Report Quality Assurance Record
(2 pages)



REPORT QUALITY ASSURANCE RECORD

Client: MWRD Project Number: 70019.TOX
 Author: Rachael Brooks EA Report Number: 8542

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>[Signature]</u> | <u>4/19/2021</u> |
| 2. Samples prepared and processed according to study plan requirements. | <u>[Signature]</u> | <u>4/19/2021</u> |
| 3. Data collected using calibrated instruments and equipment. | <u>[Signature]</u> | <u>4/19/2021</u> |
| 4. Calculations checked: - Hand calculations checked - Documented and verified statistical procedure used. | <u>[Signature]</u> | <u>4/19/2021</u> |
| | <u>[Signature]</u> | <u>4/19/2021</u> |
| 5. Data input/statistical analyses complete and correct. | <u>[Signature]</u> | <u>4/25/2021</u> |
| 6. Reported results and facts checked against original sources. | <u>[Signature]</u> | <u>4/25/2021</u> |
| 7. Data presented in figures and tables correct and in agreement with text. | <u>[Signature]</u> | <u>4/25/2021</u> |
| 8. Results reviewed for compliance with study plan requirements. | <u>[Signature]</u> | <u>4/19/2021</u> |

| | <u>AUTHOR</u> | <u>DATE</u> |
|---|--------------------------|------------------|
| 9. Commentary reviewed and resolved. | <u>[Signature]</u> | <u>4/26/2021</u> |
| 10. All study plan and quality assurance/control requirements have been met and the report is approved: | <u>[Signature]</u> | <u>4/26/21</u> |
| | PROJECT MANAGER | DATE |
| | <u>[Signature]</u> | <u>4/25/2021</u> |
| | QUALITY CONTROL OFFICER | DATE |
| | <u>[Signature]</u> | <u>4/26/21</u> |
| | SENIOR TECHNICAL OFFICER | DATE |