MONITORING AND RESEARCH DEPARTMENT

REPORT NO. 21-23

RESULTS OF ACUTE TOXICITY TESTING WITH CERIODAPHNIA DUBIA AND PIMEPHALES PROMELAS ON A MAY 2021 SAMPLE FROM METROPOLITAN WATER RECLAMATION DISTRICT (MWRD)

June 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 MAY 2021 SAMPLE FROM METROPOLITAN WATER RECLAMATION DISTRICT (MWRD)

By

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

Monitoring and Research Department
Edward W. Podczerwinski, Director

June 2021

Protecting Our Water Environment

Metropolitan Water Reclamation District of Greater Chicago

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

June 11, 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 fourteenth 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,

John Mirl for AC

Albert Cox
Environmental Monitoring
and Research Manager
Monitoring and Research Department

AC:TM:NK:lf
Enclosure
cc: E. Podczerwinski/P. Connolly
J. Skawski/Ann Ko
H. Zhang/T. Minarik
N. Kollias
Via electronic mail

RESULTS OF ACUTE TOXICITY TESTING WITH *Ceriodaphnia dubia* AND *Pimephales promelas* ON A MAY 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.

This report contains 8 pages plus 2 attachments

Michael K. Chanov II
Laboratory Director

4 June 2021
Date

EA Project Number 70019.TOX

ACCREDITED IN ACCORDANCE WITH nelac ID# E87550

EA Report Number 8559

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 10-11 May 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 10-11 May 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 85 percent survival in all of the effluent concentrations. The laboratory control had 90 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 1,912 mg/L NaCl, and acceptable control chart limits of 1,617-2,270 mg/L NaCl. The results of the *P. promelas* reference toxicant test was acceptable, with a 48-hour LC50 of 810 mg/L KCl, and acceptable control chart limits of 594-1,249 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: **AT1-280**

Collection Time and Date: 0600, 10 May 2021 to 0600, 11 May 2021
Receipt Time and Date: 0930, 12 May 2021

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

EA Test Number: **TN-21-281**

Test Initiation Time and Date: 1038, 12 May 2021
Test Completion Time and Date: 1022, 14 May 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-21-068
Test Date and Time: 0955, 6 May 2021 to 0902, 8 May 2021
Dilution Water: Moderately hard synthetic freshwater
48-hour LC50: 1,912 mg/L NaCl
Laboratory control chart acceptability range for 48-hour LC50: 1,617-2,270 mg/L NaCl

Test Species:	***Ceriodaphnia dubia* (water flea)**
Sample Description:	Outfall 001 Final Effluent – MWRD
Sample Date:	10-11 May 2021
EA Test Number:	TN-21-281

Test Concentration (percent effluent)	48-Hour Survival (percent)
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)**

Water Quality Parameters on Test Solutions	Range
Temperature (°C):	24.0 – 25.7
pH:	7.7 – 8.5
Dissolved Oxygen (mg/L):	8.0 – 9.1
Conductivity (µS/cm):	324 – 1,197

Water Quality Parameters Measured on Sample Upon Receipt	Outfall 001 (AT1-280)
Temperature (°C):	2.3
pH:	7.8
Total Residual Chlorine (mg/L):	<0.01
Alkalinity (mg/L as $CaCO_3$):	164
Hardness (mg/L as $CaCO_3$):	248
Conductivity (µS/cm):	1,153

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: **AT1-280**

Collection Time and Date: 0600, 10 May 2021 to 0600, 11 May 2021
Receipt Time and Date: 0930, 12 May 2021

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

EA Test Number: **TN-21-282**

Test Initiation Time and Date: 1055, 12 May 2021
Test Completion Time and Date: 1014, 16 May 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: FH1-5/8-9
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 Chemicals Lot#19430079 (Received 10/20/20)
EA Test Number: RT-21-070
Test Date and Time: 1025, 6 May 2021 to 0932, 8 May 2021
Dilution Water: Moderately hard synthetic freshwater
48-hour LC50: 810 mg/L KCl
Laboratory control chart acceptability range for 48-hour LC50: 594-1,249 mg/L KCl

Test Species: ***Pimephales promelas*** **(fathead minnow)**

Sample Description: Outfall 001 Final Effluent – MWRD

Sample Date: 10-11 May 2021

EA Test Number: TN-21-282

Test Concentration (percent effluent)	48-Hour Survival (percent)	96-Hour Survival (percent)
Lab Control	95	90
6.25	100	100
12.5	100	100
25	100	100
50	95	85
100	95	95

96-Hour LC50 (percent effluent): **>100 (TU$_a$ <1.0)**

Water Quality Parameters on Test Solutions	Range
Temperature (°C):	24.0 – 25.5
pH:	7.7 – 8.7
Dissolved Oxygen (mg/L):	7.6 – 8.6
Conductivity (µS/cm):	324 – 1,176

ATTACHMENT I

Data Sheets
(18 pages)

Chain-of-Custody Record

EA Engineering, Science, and Technology

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 #: 12274 4748 4950 8033 7

Client: MWRDGC Project No.: 4652-126-1
NPDES Number: IL0028761 Client Purchase Order Number: 3111991
City/State Collected: Chicago, IL

PLEASE READ SAMPLING INSTRUCTIONS ON BACK OF FORM

Accession Number (office use only)	Grab	Composite	Collection Start Date/Time	Collection End Date/Time	Sample Description (including Site, Station Number, and Outfall Number)	Number/Volume of Container
ATI-280		✓	5/10/21 0600	5/11/21 0600	Calumet WRP Final Effluent Discharge #001	1 gallon

Sampled By:	Date/Time	Received By:	Date/Time
[signature]	5/11/21 0900		
Sampler's Printed Name: Nick Kollias	Title: Aquatic Biologist	Relinquished By:	Date/Time
Relinquished By: [signature]	Date/Time 5/11/21 0900	Received By Laboratory: [signature]	Date/Time 5-12-21 0930

Was Sample Chilled During Collection? (Yes) / No

Comments:

Sample Collection Parameters

Visual Description: Clear, green
Temperature (°C): 11.7
pH: 7.27
TRC (mg/L): 0
Other:

SAMPLE CHECK-IN FOR TESTING

Client: MWRD

EA Accession Number: ATI-280

Parameter	Acceptable Range	Measurement *	Meter	Date	Time	Initials
Temperature (°C)	≤4	2.3	T-21	5/12/21	0937	LAO
Is ice present?	—	yes	N/A	↓	↓	↓
pH	6.0-9.0	7.8	681			
TRC (mg/L)	<0.01	<0.01	AT-01			
Visual Description	—	turbid + slightly yellow	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-21-281

TEST ORGANISM INFORMATION

Common Name: Water flea

Scientific Name: *C. dubia*

Lot Number: N/A

Source: EA

Adults Isolated (Time, Date): 5/11/21, 1630

Neonates Pulled & Fed (Time, Date): 5/12/21, 0805

Acclimation: <24hrs Age: <24 hrs

Culture Water (T/S): 24.3 °C 0 ppt

TEST INITIATION

Date	Time	Initials	Activity
5/12/21	1021	TP	Dilutions Made
↓	↓	↓	
	1038		Test Vessels Filled
			Organisms Transferred
	1137	DO	Head Counts

TEST SET-UP

Sample Number: ATI-280

Dilution Number: LDI-279

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
Client: MWRD
QC Test Number: TN- 21-281
Test Material: Effluent
Accession Number: PT 1-280
Dilution Water: Mod Hard
Accession Number: LD 1-279

TEST ORGANISM
Common Name: Water flea
Scientific Name: *C.dubia*

TARGET VALUES
Temp: 25±1 °C
pH: 6.0 - 9.0
Photoperiod: 16 l, 8 d
DO: ≥4.0 mg/L
Salinity: 0 ppt
Light Intensity: 50 - 100 fc

Beginning Date: 5/12/21 Time: 1038
Ending Date: 5/14/21 Time: 1022
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	0	24	48	72	96
Control	A	5	5	5			24.0	24.4	24.7			7.8	8.3	8.5			8.0	8.8	9.0			324	338	367		
	B	5	5	5																						
	C	5	5	5																						
	D	5	5	5																						
6.25%	A	5	5	5			24.0	24.8	24.9			7.8	8.2	8.4			8.3	8.7	9.1			378	383	394		
	B	5	5	5																						
	C	5	5	5																						
	D	5	5	5																						
12.5%	A	5	5	5			24.1	25.5	25.6			7.8	8.2	8.3			8.4	8.4	8.9			438	449	449		
	B	5	5	5																						
	C	5	5	5																						
	D	5	5	5																						
Meter Number							680	681	680			680	681	680			680	681	680			680	681	680		
Time		1137	0957	1037			1030	0850	1019			1030	0850	1019			1030	0850	1019			1030	0850	1019		
Initials		LAD	-V	LAD			TP	TP	LAD			TP	TP	LAD			TP	TP	LAD			TP	TP	LAD		

EPA Test Method: EPA 821-R-02-012 (CHECK ONE)

Ceriodaphnia: 2002.0 X
Magna/pulex: 2021.0
Fathead: 2000.0
Trout: 2019.0
Americamysis: 2007.0
Cyprinodon: 2004.0
Menidia: 2006.0
OTHER:

ATS-T01
12/02/08

ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019.TOX
Client: MWRD
QC Test Number: TN- 21-281
Test Material: Effluent
Accession Number: AT1-280
Dilution Water: Mod Hard
Accession Number: WD1-279

TEST ORGANISM
Common Name: Water flea
Scientific Name: *C.dubia*

TARGET VALUES
Temp: 25±1 °C
pH: 6.0 - 9.0
Photoperiod: 16 *l*, 8 *d*
DO: ≥4.0 mg/L
Salinity: 0 ppt
Light Intensity: 50 - 100 fc

Beginning Date: 5/12/21 Time: 1038
Ending Date: 5/14/21 Time: 1022
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	0	24	48	72	96
25%	A	5	5	5			24.3	25.6	25.3			7.8	8.2	8.3			8.4	8.3	8.9			534	540	549		
	B	5	5	5																						
	C	5	5	5																						
	D	5	5	5																						
50%	A	5	5	5			24.6	25.7	25.7			7.8	8.2	8.2			8.4	8.2	8.8			750	748	760		
	B	5	5	5																						
	C	5	5	5																						
	D	5	5	5																						
100%	A	5	5	5			25.4	25.6	25.7			7.7	8.2	8.3			8.3	8.2	8.7			1176	1173	1197		
	B	5	5	5																						
	C	5	5	5																						
	D	5	5	5																						
Meter Number							680	681	680			680	681	680			680	681	680			680	681	680		
Time		1137	0957	1022			1030	0850	1019			1030	0850	1019			1030	0850	1019			1030	0850	1019		
Initials		WD	TP	WD			TP	TP	WD			TP	TP	WD			TP	TP	WD			TP	TP	WD		

EPA Test Method: EPA 821-R-02-012 (CHECK ONE)

Ceriodaphnia: 2002.0 X
Magna/pulex: 2021.0
Fathead: 2000.0
Trout: 2019.0
Americamysis: 2007.0
Cyprinodon: 2004.0
Menidia: 2006.0
OTHER:

ATS-T01
12/02/08

TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN-21-281

Date/Time/Initials	Comments/Activity

RANDOMIZATION CHART

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-261

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- 21-281

Day	Testing Location	Date	Time	Initials
0	25	5/12/21	1045	TP
1	25	5/13/21	0850	TP
2	25	5/14/21	1027	LAO
3				
4				
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- 21-281

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-21-282

TEST ORGANISM INFORMATION

Common Name: Fathead minnow

Scientific Name: *P. promelas*

Lot Number: FH1-518-9

Source: EA

Adults Isolated (Time, Date): _____

Neonates Pulled & Fed (Time, Date): _____ TP 5/12

Acclimation: <24 hrs Age: ~~6-7 days~~ 3-4 (5)

Culture Water (T/S): 25.0 °C 0 ppt

TEST SET-UP

TEST INITIATION

Date	Time	Initials	Activity
5/12/21	1021	TP	Dilutions Made
	1055		Test Vessels Filled
			Organisms Transferred
	1116	UAD	Head Counts

CONCENTRATION SERIES

Test Concentration	Volume Test Material	Final Volume
Control	0 ml	500 ml
6.25%	31.25 ml	
12.5%	62.5 ml	
25%	125 ml	
50%	250 ml	
100%	500 ml	

Comments:

INTERMEDIATE DILUTION PREPARATION AND FEEDING

DILUTION PREPARATION

Day	Date	Time	Initials	Sample / Diluent
0	5/12/21	1021	TP	ATI-280 / LDI-279
1				
2	5/14/21	0815	TP	ATI-280 / LDI-279
3				
4				
5				
6				

FEEDING

Food: *Artemia*

Day	Time, Initials, Amount	Time, Initials, Amount	Time, Initials, Amount
0			
1			
2		0835 MG 3 drops	
3			
4			
5			
6			

ACUTE TOXICITY TEST DATA SHEET

Project Number: 70019.TOX
Client: MWRD
QC Test Number: TN- 21-282
Test Material: Effluent
Accession Number: ATI-280
Dilution Water: Mod Hard
Accession Number: UDI-279

TEST ORGANISM
Common Name: Fathead minnow
Scientific Name: *P. promelas*

TARGET VALUES
Temp: 25±1 °C
pH: 6.0 - 9.0
Photoperiod: 16 l, 8 d
DO: >4.0 mg/L
Salinity: 0 ppt
Light Intensity: 50 - 100 fc

Beginning Date: 5/12/21 Time: 1055
Ending Date: 5/16/21 Time: 1014
TEST TYPE: (Static) / Flowthrough
(Renewal) / Non-renewal
Test Container: 1 L Beaker
Test Volume: 250 ml
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		24.0			7.8		8.1			8.0		8.5			324		332		
	B	10	9	9	9	8																				
6.25%	A	10	10	10	10	10	24.0		24.0			7.8		8.1			8.3		8.5			378		378		
	B	10	10	10	10	10																				
12.5%	A	10	10	10	10	10	24.1		24.0			7.8		8.0			8.4		8.6			438		431		
	B	10	10	10	10	10																				
25%	A	10	10	10	10	10	24.3		24.0			7.8		8.1			8.4		8.6			534		535		
	B	10	10	10	10	10																				
50%	A	10	10	10	10	9	24.6		24.0			7.8		8.1			8.4		8.6			750		751		
	B	10	10	9	9	8																				
100%	A	10	9	9	9	9	25.4		24.1			7.7		8.1			8.3		8.5			1176		1162		
	B	10	10	10	10	10																				
Meter Number							680		681			680	680 (b)	681			680		681			680		681		
Time		1116	1003	1032	1135	1014	1030		0822			1030		0822			1030		0822			1030		0822		
Initials		LAD	TP	LAD	TP	JAS	TP		TP			TP		TP			TP		TP			TP		TP		

(b) TP 5/14/21

EPA Test Method: EPA 821-R-02-012 (CHECK ONE)

Ceriodaphnia: 2002.0 Fathead: 2000.0 X
Magna/pulex: 2021.0 Trout: 2019.0
Americamysis: 2007.0 Menidia: 2006.0
Cyprinodon: 2004.0 OTHER:

ACUTE TOXICITY TEST DATA SHEET - OLD SOLUTIONS

Project Number: 70019.TOX
Client: MWRD
QC Test Number: TN-21-283
Test Material: Effluent
Accession Number: A11-280
Dilution Water: Mod Hard
Accession Number: L01-279

TEST ORGANISM
Common Name: Fathead minnow
Scientific Name: *P. promelas*

TARGET VALUES
Temp: 25±1 °C
pH: 6.0 - 9.0
Photoperiod: 16 l, 8 d
DO: ≥4.0 mg/L
Salinity: 0 ppt
Light Intensity: 50 - 100 fc

Beginning Date: 5/12/21 Time: 1055
Ending Date: 5/16/21 Time: 1014
TEST TYPE: (Static) / Flowthrough
(Renewal) / Non-renewal
Test Container: 1 L Beaker
Test Volume: 250 ml
Test Duration: 96 hrs

Concentration	Rep	Number of Live Organisms 0	24	48	72	96	Temperature (°C) 0	24	48	72	96	pH 0	24	48	72	96	Dissolved Oxygen (mg/L) 0	24	48	72	96	Conductivity (µS/cm) 0	24	48	72	96
Control	A							24.8	24.3	24.1	24.3		7.9	8.2	8.2	8.7		8.4	8.5	8.3	8.6		329	329	340	337
	B																									
6.25%	A							25.2	24.9	25.1	24.9		7.9	8.2	8.2	8.5		8.2	8.3	8.0	8.3		379	379	384	382
	B																									
12.5%	A							25.3	25.0	25.3	25.1		7.9	8.2	8.2	8.4		8.0	8.3	7.8	8.0		437	439	439	437
	B																									
25%	A							25.4	25.0	25.4	25.3		7.9	8.1	8.2	8.2		8.0	8.2	7.6	8.0		531	534	538	535
	B																									
50%	A							25.4	25.0	25.5	25.4		7.9	8.1	8.1	8.1		7.9	8.1	7.6	7.9		736	743	751	747
	B																									
100%	A							25.5	25.0	25.5	25.5		8.0	8.1	8.0	8.1		7.7	8.1	7.6	7.7		1153	1155	1167	1172
	B																									
Meter Number								681	681	681	681		681	681	681	681		681	681	681	681		681	681	681	681
Time								0845	0830	0933	1009		0845	0830	0933	1009		0845	0830	0933	1009		0845	0830	0933	1009
Initials								TP	TP	TP	JR		TP	TP	TP	JR		TP	TP	TP	JR		TP	TP	TP	JR

TOXICOLOGY LABORATORY BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-283

Date/Time/Initials	Comments/Activity

RANDOMIZATION CHART

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-296

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 - 258

Day	Testing Location	Date	Time	Initials
0	26	5/12/21	1056	TP
1	26	5/13/21	0847	TP
2	26	5/14/21	1033	JAO
3	26	5/15/21	0940	TP
4	26	5/16/21	1009	JA
5				
6				
7				
8				
9				
10				
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TOXICOLOGY LABORATORY CORRECTION BENCH SHEET

Project Number: 70019.TOX

Client: MWRD

QC Test Number: TN- 21-282

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- 21-281, 282

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

		ON AIR				OFF AIR			
Date	Sample #	Initial DO (mg/L)	Time	Meter	Initials	Final DO (mg/L)	Time	Meter	Initials
5-12-21	ATI 280	10.3	1005	680	LAO	8.3	1015	680	LAO
5/14/21	ATI-280	9.7	0803	680	TP	8.3	0813	680	TP

ATTACHMENT II

Report Quality Assurance Record
(2 pages)

REPORT QUALITY ASSURANCE RECORD

Client: MWRD

Project Number: 70019.TOX

Author: Rachael Brooks

EA Report Number: 8559

REPORT CHECKLIST

QA/QC ITEM	REVIEWER	DATE
1. Samples collected, transported, and received according to study plan requirements.	[signature]	5/17/2021
2. Samples prepared and processed according to study plan requirements.	[signature]	5/17/2021
3. Data collected using calibrated instruments and equipment.	[signature]	5/17/2021
4. Calculations checked: - Hand calculations checked	[signature]	5/17/2021
- Documented and verified statistical procedure used.	[signature]	5/17/2021
5. Data input/statistical analyses complete and correct.	Jessa M Reinhart	5/27/2021
6. Reported results and facts checked against original sources.	Jessa M Reinhart	5/27/2021
7. Data presented in figures and tables correct and in agreement with text.	Jessa M Reinhart	5/27/2021
8. Results reviewed for compliance with study plan requirements.	[signature]	5/17/2021

	AUTHOR	DATE
9. Commentary reviewed and resolved.	[signature]	6/4/2021

10. All study plan and quality assurance/control requirements have been met and the report is approved:

PROJECT MANAGER	[signature]	6/1/21
QUALITY CONTROL OFFICER	Jessa M Reinhart	5/27/2021
SENIOR TECHNICAL OFFICER	[signature]	6/1/21