

RE:	Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted 2-9 Nov 2022
From:	L.M. Stevenson, 1504, MS-6351 (865-341-0398).
<b>c:</b>	S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, A.M. Fortner
To:	K.G. Hanzelka (RC)
Date:	December 2, 2022

Appended are the results of toxicity tests of effluent from Outfall 200 conducted 2-9 November 2022. The effluent was evaluated for toxicity with fathead minnows (*Pimephales promelas*) and water fleas (*Ceriodaphnia dubia*).

Effluent from Outfall 200 did not reduce fathead minnow survival or growth or *Ceriodaphnia* survival or reproduction by 25% or greater at any of the tested concentrations. For both species, the Inhibition Concentration<sub>25</sub> (IC<sub>25</sub>) for survival, growth, and/or reproduction were thus >100% (the highest concentration of effluent tested).

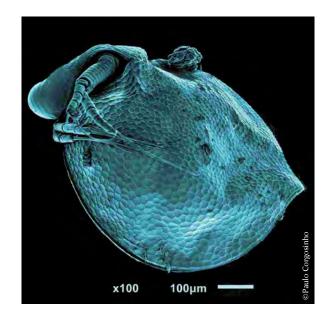
The NPDES permit states that toxicity will be demonstrated if the  $IC_{25}$  is less than or equal to the permit limit (50% effluent for Outfall 200). All of the results for all endpoints were within permit limits.

Outfall	Test Organism	Endpoint	IC <sub>25</sub>
Outfall	Fathead	Survival	>100%
200	minnow	Growth	>100%
Outfall	Ceriodaphnia	Survival	>100%
200	dubia	Reproduction	>100%

Please do not hesitate to call if you have any questions or comments.

Attachment

lms



# Ceriodaphnia dubia TOXICITY TEST REPORT

Test Number 2978 | Y-12 National Security Complex Outfall 200 | 10 November 2022

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

# STANDARD REPORT FORM CERIODAPHNIA 3-BROOD SURVIVAL AND REPRODUCTION TEST

Test Number 2978 Start Date: 2 November 2022 | End Date: 9 November 2022

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

The permit states that toxicity is demonstrated if the  $IC_{25}$  is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 1 November 2022 to 7 November 2022
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	11/1/2022	11/3/2022	11/6/2022
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	11/2/2022	11/42022	11/72022
Chain-of-Custody Form Number	031069	031070	031071
Sample Temperature (°C)	11.1	10.3	11.9
рН (S.U.)	8.05	8.13	8.24
Conductivity (µS/cm)	418	398	415
Alkalinity (mg/L as CaCO <sub>3</sub> )	114	128	141
Hardness (mg/L as CaCO <sub>3</sub> )	170	160	160
Chlorine (Free/Total) (mg/L)	0.01/0.00	0.01/0.00	0.01/0.00

#### 3. TEST ORGANISMS

- 3.1 Species: Ceriodaphnia dubia.
- 3.2 Life stage: Neonates ≤24 h old; all born within 8 h of each other.
- 3.3 Source: Environmental Sciences Division cultures.
- 3.4 Incubation water for cultures: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 3.5 Temperature of cultures:  $25 \pm 1$  °C.

### 4. TEST METHODS

- 4.1 Toxicity test method: Ceriodaphnia survival and reproduction test. Reference: EPA Test Method 1002.0, in P.A. Lewis et al., Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).
- 4.2 End points of test: Survival and reproduction.
- 4.3 Modifications or deviations to Method 1002.0: None.

- 4.4 Date and time test started: 11/2/2022, 11:28
- 4.5 Date and time test terminated: 11/9/2022, 11:00
- 4.6 Type and volume of test chambers: Polystyrene microbeakers, minimum 15mL each.
- 4.7 Number of Ceriodaphnia per test chamber: 1.
- 4.8 Number of replicates per treatment: 10.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Test temperature: Mean = 25.5 °C; range = 25.2-25.7 °C.
- 4.12 Treatment groups/concentrations: Control, 12.5%, 25%, 50%, 75% and 100% of full-strength effluent.
- 4.13 Feeding regime during test: 100 μL of yeast-Cerophyl-trout food (YCT) mixture and 100 μL of the green alga, *Selenastrum capricornutum*, per 15 mL of test solution every 24 h (EPA/821/R/02/013; 4<sup>th</sup> Ed., October 2002; or most recent version).

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Sodium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 12-19 Oct 2022.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival  $IC_{25}$  = 2.04 g NaCl/L; 95% C.L. = 1.14-2.17 g NaCl/L. Reproduction  $IC_{25}$  = 1.55 g NaCl/L; 95% C.L. = 1.30-1.63 g NaCl/L. The  $IC_{25}$ s were calculated by the EPA linear interpolation method.
- 5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $1.481 \pm 0.756$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for survival: 0.255 g NaCl/L

Central tendency of IC<sub>25</sub> for reproduction:  $1.027 \pm 0.622$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for reproduction: 0.303 g NaCl/L

A copy of the control chart is appended.

#### 6. CERIODAPHNIA TEST RESULTS

Copies of the toxicity test logsheets are appended.

6.1 Summary of results from the Ceriodaphnia toxicity test:

Effluent Concentration	Number of replicates	Number of animals surviving for 3 broods	Mean number of offspring per female (±SD)
Control	10	10	38.7 ± 3.1
12.5%	10	8	30.1 ± 13.9
25%	10	10	33.6 ± 12.3
50%	10	10	33.6 ± 8.4
75%	10	9	31.3 ± 11.9
100%	10	10	38.6 ± 5.5

#### 7. STATISTICAL ANALYSES

7.1 Survival

The calculated  $IC_{25}$  for survival was >100% effluent.

7.2 Reproduction

The calculated IC<sub>25</sub> for reproduction was >100% effluent.

7.3 Summary of Ceriodaphnia toxicity test results:

IC<sub>25</sub> for survival: >100%

IC<sub>25</sub> for reproduction: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
pH (S.U.)	8.11	8.20	8.08
Conductivity (µS/cm)	237	239	230
Alkalinity (mg/L as CaCO <sub>3</sub> )	82	105	105
Hardness (mg/L as CaCO₃)	108	114	114

#### 8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

Alkalinity, hardness, and chlorine were measured using a Hach SL1000 Portable Parallel Colorimeter.

Instruments were calibrated and standardized according to manufacturer's instructions.

All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Peijia KuDate: 10 November 2022Report reviewed by: Louise StevensonLouise StevensonDate: 2 December 2022



# Fathead Minnow TOXICITY TEST REPORT

Test Number 1686 | Y-12 National Security Complex Outfall 200 | 11 November 2022

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

# STANDARD REPORT FORM FATHEAD MINNOW SURVIVAL AND GROWTH TEST

Test Number 1686 Start Date: 2 November 2022 | End Date: 9 November 2022

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

Toxicity will be demonstrated if the  $IC_{25}$  is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.
- 1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 1 November 2022 to 7 November 2022
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process. Samples were used within sample holding time guidance outlined in EPA Test Method 1000.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	11/1/2022	11/3/2022	11/6/2022
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	11/2/2022	11/42022	11/72022
Chain-of-Custody Form Number	031069	031070	031071
Sample Temperature (°C)	11.1	10.3	11.9
рН (S.U.)	8.05	8.13	8.24
Conductivity (µS/cm)	418	398	415
Alkalinity (mg/L as CaCO₃)	114	128	141
Hardness (mg/L as CaCO <sub>3</sub> )	170	160	160
Chlorine (Free/Total) (mg/L)	0.01/0.00	0.01/0.00	0.01/0.00

#### 3. TEST ORGANISMS

- 3.1 Species: Fathead minnow (Pimephales promelas).
- 3.2 Hatch date: 31 October 2022 .
- 3.3 Life stage: Newly hatched larvae less than 48 h old.
- 3.4 Incubation water: Dechlorinated tap water.
- 3.5 Incubation temperature:  $25 \pm 1$  °C.
- 3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.
- 3.7 Mean dry weight at test initiation:  $0.153 \pm 0.006$  mg (mean  $\pm$  SD)
- 3.8 Diseases and treatment: None.

### 4. TEST METHODS

4.1 Toxicity test method: Fathead minnow larval survival and growth test. Reference: EPA Test Method 1000.0, in P.A. Lewis et al., *Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).

- 4.2 End points of test: Survival and growth.
- 4.3 Modifications or deviations to Method 1000.0: None.
- 4.4 Date and time test started: 11/2/2022, 10:19
- 4.5 Date and time test terminated: 11/9/2022, 9:39
- 4.6 Type and volume of test chambers: 600-mL borosilicate beakers, minimum 250 mL each.
- 4.7 Number of organisms per test chamber: 10.
- 4.8 Number of replicates per treatment: 4.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Acclimation of test organisms: Received larvae on 1 November 2022 at 15.0 °C.
- 4.12 Test temperature: Mean = 25.0 °C; range = 24.5-25.7 °C.
- 4.13 Treatment groups/concentrations: Control, 12.5%, 25%, 50%, 75% and 100% of full-strength effluent.
- 4.14 Feeding regime during test: Brine shrimp (*Artemia*) nauplii less than 24 h old; fed 600  $\pm$  100  $\mu$ L per beaker twice daily.

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Potassium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 2-9 Nov 2022.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival IC<sub>25</sub> = 0.80 g KCl/L; 95% C.L. = 0.67-0.91 g KCl/L. Growth IC<sub>25</sub> = 0.83 g KCl/L; 95% C.L. = 0.75-0.99 g KCl/L.

The  $IC_{25}s$  were calculated by the EPA linear interpolation method.

5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $0.837 \pm 0.275$  g KCl/L (mean  $\pm 2$  SD).

CV of IC\_{25} for survival: 0.164 g KCl/L

Central tendency of IC<sub>25</sub> for growth:  $0.92 \pm 0.234$  g KCl/L (mean  $\pm 2$  SD).

- CV of IC<sub>25</sub> for growth: 0.127 g KCl/L
- A copy of the control chart is appended.

## 6. FATHEAD MINNOW TEST RESULTS

Copies of the toxicity test logsheets are appended.

6.1 Summary of results from the fathead minnow toxicity test:

Concentration	1	2	3	4	Mean
Control	1	1	1	1	1
12.5%	1	1	1	1	1
25%	1	1	1	1	1
50%	1	1	1	1	1
75%	1	1	1	1	1
100%	1	1	1	1	1

Survival

#### Dry Weight

Concentration	1	2	3	4	Mean ± SD
Control	0.67	0.65	0.73	0.81	0.71 ± 0.07
12.5%	0.93	0.61	0.54	0.74	0.71 ± 0.17
25%	0.63	0.75	0.64	0.74	$0.69 \pm 0.06$
50%	0.64	0.6	0.68	0.59	$0.63 \pm 0.04$
75%	0.46	0.63	0.61	0.58	0.57 ± 0.08
100%	0.66	0.74	0.63	0.63	0.66 ± 0.05

#### 7. STATISTICAL ANALYSES

#### 7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

#### 7.2 Growth

The calculated IC<sub>25</sub> for growth was >100% effluent.

7.3 Summary of fathead minnow toxicity test results:

 $IC_{25}$  for survival: >100%

IC<sub>25</sub> for growth: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
рН (S.U.)	8.11	8.20	8.08
Conductivity (µS/cm)	237	239	230
Alkalinity (mg/L as CaCO₃)	82	105	105
Hardness (mg/L as CaCO <sub>3</sub> )	108	114	114

8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

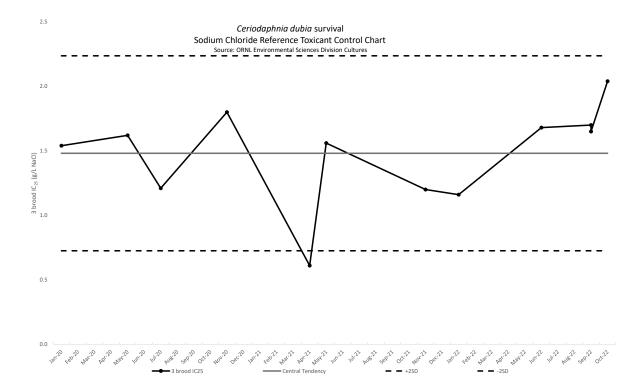
Alkalinity, hardness, and chlorine were measured using a Hach SL1000 Portable Parallel Colorimeter.

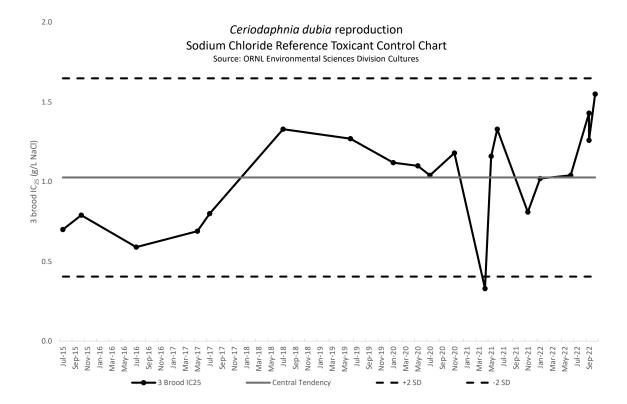
Instruments were calibrated and standardized according to manufacturer's instructions.

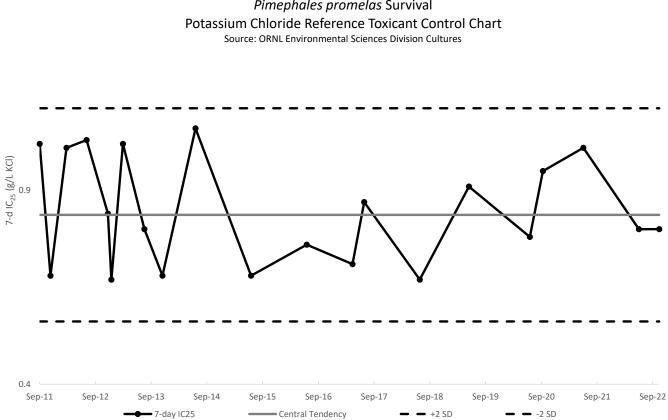
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by:Peijia KuDate: 11 November 2022Report reviewed by:Louise Stevenson Louise Hevenson Date: 2 December 2022

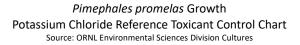
**REFERENCE TOXICANT CONTROL CHARTS** 

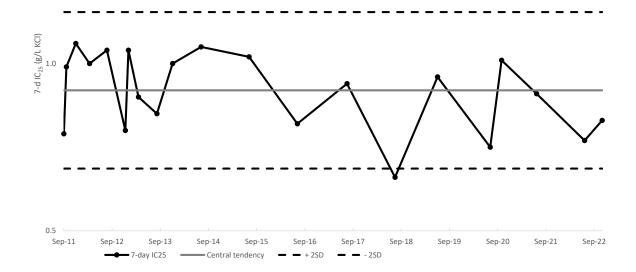






Pimephales promelas Survival

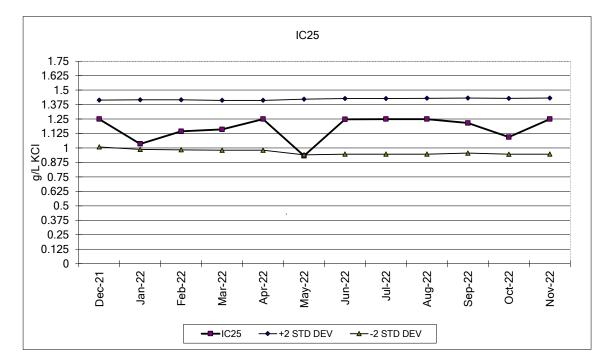






1300 Blue Spruce Drive, Suite C Fort Collins, Colorado 80524 Toll Free: 800/331-5916 Tel:970/484-5091 Fax:970/484-2514

Pimephales promelas



#### Chronic 7 Day Survival Test Data

#### IC 25 for Growth Test

Date	NOEC	LOEC	Date	IC25	95% Co	nfidence	Avg. IC25	+2 STD	-2 8
	(g/L KCI)	(g/L KCI)		g/L KCI	(upper)	(lower)	g/L KCI	DEV	D
Jun-22	0.50	1.0	Jun-22	1.247	1.252	1.146	1.187	1.428	0.5
Jul-22	0.50	1.0	Jul-22	1.250	1.250	1.222	1.187	1.428	0.9
Aug-22	0.50	1.0	Aug-22	1.250	1.250	1.250	1.188	1.429	0.
Sep-22	0.50	1.0	Sep-22	1.215	1.271	1.072	1.193	1.432	0.9
Oct-22	0.50	1.0	Oct-22	1.094	1.332	0.161	1.188	1.429	0.
Nov-22	0.50	1.0	Nov-22	1.250	1.250	0.907	1.189	1.431	0.

\*\*Current Test Dates: 11/2-9/2022

Aquatic BioSystems, Inc • Quality Research Organisms

WATER CHEMISTRY DATA LOGSHEETS

	1	36				hemistry l		CD-2978		
	PC	onsor: <u> </u>	Site/Treatm			Associated tes	i numbers:	A-168	-	
-	Not	e: Not all parameters	are required for	or all tests. Al	l unused cells			marked "NA		
_		Observation Day:	ONK	11/3/22 004	2 Mr	3 AME	4	5 NAV	6 MU	7 1/8
	-	Date/Initials:	11/2/2022	1322	14-22	11/5/22	1-4-22	1/2/22	11822	11-9-22 MUF
	Day	5-digit ORNL ID	SUCCC D	32924	32925	32925	32925	32926 Seecoc 12	32926	
-	Rec	c. temp. (°C) (New ✓) DMW Batch #	918	918	918	919	. 919		919	Thinn
_	3	Conductivity (µS/cm)		120	125	139	233	230	220	
	MA	Alkalinity (mg/L)	52	we	1000	105		1	100	VIIIIIII
0	292	Hardness (mg/L)	108	/	/	14	/	/	/	
v	N	pH (S.U.) Initia		8.14	8.13	8:20	8.6	8,08	8:00	
	ol:	Final CD/FHM	111111111111	8.48/9,01		8,30/7.79		8:39/1.93		8.45 17.85
	Control:	DO (mg/L) Initial	8.11	8.94	8.61	8.56	8102	8108	878	VIIIIIII
-	ő	Final CD/FHM	VITITITITI			8.44/757				8:70/145
		Conductivity (µS/cm)	and	150	2410	150	153	251	245	
		Alkalinity (mg/L)	/	/	1 /	/	/	/	1	
-		Hardness (mg/L)		/	/	/	/	/	/	
6	30	Chlorine (mg/L)	/	/	/	1		/	/	
e	N	pH (S.U.) Initia	1 8.06	8.10	8,14	8.18	801	8.13	813	
-	1	Final CD/FHM		8.55 /7.99	854/801	836/190	8.43 /8.01	8:45/-1.93	8.53 /198	8H8/195
-		DO (mg/L) Initial	8.88	8,95	875	842	8.83	8.85	8.89	
		Final CD/FHN		9.31 /1.83		8.53/7.41		895/7.16	8.96/7.22	8:76 /13
		Conductivity (µS/cm)	284	28	2108	23	274	26	270	
-		Alkalinity (mg/L)	/	/	/	-/	/	-/	/	
_	1.1	Hardness (mg/L)	/	/	1	/	/	/	/	
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	S	pH (S.U.) Initia	777777777777777777777777777777777777777	8.10	8.14	8.38/195	5.09	8.14	8:15	
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		DO (mg/L) Initial	9.00	916	8.98	8.48	4.01	9.00	9.14	
	-	Final CD/FHM	111111111111	9.20/7.71				898 118		885 133
-	:	Conductivity (µS/cm)	33	329	313	322	317	324	320 m	V/////////////////////////////////////
_		Alkalinity (mg/L) Hardness (mg/L)	-/		/	-/-	/			
0	2	Chlorine (mg/L)	/	/	/	MUSE	/	/	/	
Ð		pH (S.U.) Initia	8.07	8.06	8.11	813800	8,07	817	8.15	\/////////////////////////////////////
_	4.	Final CD/FHM		8.53/8.10	8.59/809	841/000	8.45 /801			8,49 8,12
		DO (mg/L) Initial	mannin	9.63	917	894	9.05	(9.41	9.68	11111111
		Final CD/FHN	11111111111111	9.13/272		8,60 /130		Nº III		8.80/1.61
-		Conductivity (µS/cm)	the fille fille	374	351	3103	351	311	368	V/////////////////////////////////////
		Alkalinity (mg/L)	1	1	/	/	/		/	
		Hardness (mg/L)	/	/	/		/	/	/	
2	5	Chlorine (mg/L)	/	1.	/	/	/	/	/	V/////////////////////////////////////
3	P	pH (S.U.) Initia	Sille	8105	8.12	8.08	8.07	\$.20	8.23	
		Final CD/FHM		8.55/8.10		8.42/1.04		8.49/804	8.54/8:12	8:53 8.17
		DO (mg/L) Initial	9.02	10.00	951	8.65	10:24	9.79	10.73	
-		Final CD/FHM		9.24/1.38		8.60 A.N	8,79 17.15			8.77 1.07
_		Conductivity (µS/cm)		424	398	402	397	415	416	
~		Alkalinity (mg/L)	114	-/	128	/	-/	141	/	<i>\////////////////////////////////////</i>
0	io	Hardness (mg/L)	170	/	160	/	1	1/10	/	<i>\////////////////////////////////////</i>
	1002	Chlorine (mg/L) F/T	0.01/0.00	am	0.01/0.00	CAN	211	201/000	0.00	
	5	pH (S.U.) Initia	VTTTTTTTTTTT	8.02	8.13	8.06	Sill	0.19	8.29	
		Final CD/FHM		8.55/8.15	8.61 /8.19	8,45/812	8,50 /8.07		8,55/8.17	8:33/02
-		DO (mg/L) Initial	10.01	10.40	9,50	SIL	10.01	10.10	10.13	
		Final CD/FHM	///////////////////////////////////////	11-11/135	19.19/7.43	04 4 K	8.29/12.93	8.88 /108	8,80 1.10	8 81/ 10

**CHAIN OF CUSTODY FORMS** 

# **ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY** CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 11/02/22 ESD TEST NAME TOX			NAME OF SAMPLER	A.L. GARLAN	5	CHAIN-OF-CUSTODY NO. 031069		
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG. TEMP (°C)	Темр # 7008 REMARKS	C12 # 4102 <0.05
OUTFALL 200	200	0730	C	1	~ IlLITERS	3°	11.1°C	<0.05
							-	
							-	
			-					
THERMOMETER NO.				10				
SAMPLES RELINQUISHED BY	8. Garland	ORNE SI	suple Ip:	= 32924	DA	11/2/22	TIME 0810	AM PM
SAMPLES RECEIVED BY	in a fler					11/2/22	TIME 0810	

# **ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY** CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 11/04/22	ESD TEST NAME	Tox	NAME OF SAMPLER	S J.T. WILLIAN	ns / D. CRAZ	ε	CHAIN-OF-CUSTODY NO. 031070
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG" <b>TEMP</b> (°C)	YSI TEMP, <b>REMARKS</b> LIZ #7008 #44102
OUTFALL 200	200	0730	С	1	~14 LITERS	30	10.3°c <0.05
	1	-	_		540	1	
	-			W.			
				211/4/2			
*							
	-						
	-						
-							
THERMOMETER NO.			OPul ca	1	C		
SAMPLES RELINQUISHED BY	T 1 7'00	)-	UHUSIM	ple ID=3292		ATE 11-4-22	
SAMPLES RECEIVED BY	T. Will				D	ATE	
UCN-18631 (3 3-92)	14-		0040/01 04/00	COMPOSITE (C) OR		11/4/22	0803 [PM

\* GRAB (G), 24 HR. COMPOSITE (C), OR OTHER (O; DESCRIBE)

# ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/Y) 11/07/22	ESD TEST NAME	OX	NAME OF SAMPLER	A.L. GARLAND	J.T. WILLIA	ms	CHAIN-OF-CUSTODY NO. 031071
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	TEMP REMARKS C12 # 7008 REMARKS # 4102 11.9 <0.05
OUTFALL 200	200	0725	C	1	~14 LITERS	3°	11.9 <0.05
		* *					
				.,			
				200			
				0.4. 11/2			
				~~	N.		
THERMOMETER NO.							
SAMPLES RELINQUISHED BY	<u> </u>	1			DA	TE	TIME DOTO RIAM
	R. Gaile	and			DA	1/7/22	TIME 0830 DAM
UCN-18631 (3 3-92)	mp			_		11/7/22	0830 DPM

GRAB (G), 24 HR. COMPOSITE (C), OR OTHER (O; DESCRIBE)

**TOXICITY TEST LOGSHEETS** 

Test begin date (Day 0)       Test end date       Test duration       Template num $(1/0^2/2^{opr})$ $11/0^2/2^{opr}$ $11/0^2/2^{op$	Test begin date (Day 0)       Test end date       Test duration       Template num         II/b9/2022       II/b9/2022       I hours & days       INA the 6         Test $0' Ceriodophnia dubia       Date: II/0/2022       II/0/2022       I hours & days       INA the 6         Test period       Test purpose       Test stage       Test type         Test period       Test purpose       Test type         Mumber       Treatment Description*       Type**         1       DNW 25%       \square C C DT       S       \exists Substance         Test ment Description*       Type**         1       DNW 25%       \square C C DT       S       \exists Substance         Test ment Description*       Type**         1       DNW 25%       \square C C DT       S       \exists Substance         Treatment Description*       Type**         1       DNW 25%       \square C C DT       S       \exists Substance         Test ment         Diste: Diversendestandestement$	Sponsor:	Y12		reatment:	t Informat OF Ze		Tost		
Image: Image	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	-								
Organism:       Isolated from:       Notes:       Notes:         Date:       11001/2002       15:50 pm       Delivery date:	Organism:       Isolated from:       Note:       Note:         Date: $1/(0)/2xxy$ $1/(0)/2xxy$ Hatch date:       Note:         Test period       Test purpose       Test stage       Test type         I Corronic       If Regulatory       If Analytical       If Received waters         I Acute       Investigative       If Analytical       If Buestide waters         Treatment descriptions:       If C IT       4 = $3^{-2}$ If C IT         1 =       DNW 25%       If C IT       5 = $77.7$ If C IT         2 = $1/2.5\%$ If C IT       5 = $77.7$ If C IT         3 = $a_5\%$ If C IT       5 = $77.7$ If C IT         3 = $a_5\%$ If C IT       5 = $77.7$ If C IT         3 = $a_5\%$ If C IT       5 = $77.7$ If C IT         3 = $a_5\%$ If C IT       5 = $77.7$ If C IT         3 = $a_5\%$ If C IT       5 = $77.7$ If C IT         3 = $a_5\%$ If C IT       5 = $77.7$ If C IT         4 =       Joo								Templa	te num
Test period       Test purpose       Test stage       Test type $\square$ Acute $\square$ Regulatory $\square$ Preliminary $\square$ Est function         Treatment descriptions:       Number       Treatment Description*       Type** $1 =$ $\underline{PMW} \ge 25\%$ $\square$ C $\square$ $4 =$ $59\%$ $\square$ C $\square$ $2 =$ $(2.5\%)$ $\square$ C $\square$ $4 =$ $59\%$ $\square$ C $\square$ $2 =$ $(2.5\%)$ $\square$ C $\square$ $4 =$ $59\%$ $\square$ C $\square$ $3 =$ $a_5\%$ $\square$ C $\square$ $f_{\square} =$ $\frac{1}{100\%}$ $\square$ C $\square$ * ItDMW, include Batch number       **C = Control, T = Treatment $D$ $I = \frac{1}{100\%}$ $\square$ C $\square$ $\square$ Not applicable $\square$ Other (describe): $\square$ $\square$ $\square$ $\square$ $\square$ Not applicable $\square$ Other (describe): $\square$ $\square$ $\square$ $\square$ $\square$ Not applicable       Sample D: $32924$ Date: $\square -2-21$ COC $\#$ $031069$ Source of Test Organisms: $\square$ $\square$ $\square$ $\square$ $\square$ $\square$ $\square$ $\square$ $\square$ Not applicable       Sample D:	Test period       Test purpose       Test stage       Test type $\square$ Acute $\square$ negulatory $\square$ Preliminary $\square$ Effluent $\square$ Acute $\square$ negulatory $\square$ Analytical $\square$ Received waters $\square$ meatment descriptions: $\square$ newstigative $\square$ Received waters $\square$ Substance $\square$ meatment descriptions: $\square$ preliminary $\square$ Received waters $\square$ as $\square$ $\square$ preliminary $\square$ Received waters $\square$ preliminary $\square$ meatment descriptions: $\square$ preliminary $\square$ Received waters $\square$ description: $\square$ preliminary $\square$ Received waters $\square$ as $\square$		Date: 11/01	$ z_{022} $ [1]	01/2022	Hatch date:				
$\Box$ Chronic $\Box$ Regulatory $\Box$ Preliminary $\Box$ Effinent $\Box$ Acute $\Box$ Investigative $\Box$ Analytical $\Box$ Received waters $\Box$ Retent Description* $\Box$ vpe** $\Box$ Retest $\Box$ Substance $T$ reatment Description* $\Box$ vpe** $\Box$ Retest $\Box$ Substance $T$ reatment Description* $\Box$ vpe** $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $2 = (2.3.\%)$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $3 = 3^{\circ} \beta_{\circ}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $3 = 3^{\circ} \beta_{\circ}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $3 = 3^{\circ} \beta_{\circ}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $f = \frac{1}{3^{\circ} \beta_{\circ}}$ $\Box$ C $\Box$ T $2^{\circ} 2^{\circ} \beta_{\circ}$ Didu t       Mineral Water (DMW) + Trace Metals       Batch number: $\underline{-918}$ $\Box$ C $\Box$ T         Source of Test Organisms: $\Box$ ESD cultures: Board numbers: $\Box$ NA ft $4^{-1}2^{-1}$ $\Box$ C $CC \#$ $\partial_{310^{\circ}6^{\circ}9}$ Sample ID: $\underline{3^{\circ}2^{\circ}2^{\circ}2^{\circ}$ Date:	$\Box$ Chronic $\Box$ Regulatory $\Box$ Preliminary $\Box$ Efficient $\Box$ Acute $\Box$ Investigative $\Box$ Analytical $\Box$ Received waters         Treatment descriptions: $\Box$ Rectest $\Box$ Substance $\Box$ Rectived waters $\Box$ Imperiment Description* $\Box$ reatment Description* $\Box$ repe** $\Box$ Rectest $\Box$ Substance $\Box$ Imperiment Description* $\Box$ repe** $\Box$ Rectest $\Box$ Substance $\Box$ C $\Box$ T $\Box$ = $D = \Delta$ restment Description* $\Box$ repe** $\Box$ Rectest $\Box$ C $\Box$ T $\exists$ = $D = \Delta$ restment Description* $\Box$ repe** $\Box$ restment Description* $\Box$ repe** $\exists$ = $D = \Delta$ restment Description* $\Box$ restment Description* $\Box$ repe** $\Box$ c $\Box$ T $\exists$ = $\Box$ restment Description* $\Box$ restment $\Box$ restment $\Box$ restment         Dilution Water Type: $\Box$ restment $\Box$ restment $\Box$ restment $\Box$ restment         Dilution Water Type: $\Box$ rest organisms: $\Box$ rest organisms: $\Box$ restment $\Box$ restment $\Box$ rest organisms: $\Box$ rest organisms: $\Box$ restment restment $\Box$ restment $\Box$ rest restment $\Box$ rest		Time: 10:00	am 5:	50 pm	Delivery date:		2002 Date of		
Number       Treatment Description*       Type**       Number       Treatment Description*       Type**         1 =       DMW 25% $\square C \square T$ 4 = $\Im \Im $ $\square C \square T$ 2 = $(2.5\%)$ $\square C \square T$ 5 = $\exists \neg \neg \land$ $\square C \square T$ 3 = $a_5\%$ $\square C \square T$ 5 = $\exists \neg \neg \land$ $\square C \square T$ 3 = $a_5\%$ $\square C \square T$ 5 = $\exists \neg \neg \land$ $\square C \square T$ 3 = $a_5\%$ $\square C \square T$ 5 = $\exists \neg \neg \land$ $\square C \square T$ 3 = $a_5\%$ $\square C \square T$ 6 = $I \square O \square \top$ $\square C \square T$ 3 = $a_5\%$ $\square C \square T$ 6 = $I \square O \square \top$ $\square C \square T$ 3 = $a_5\%$ $\square C \square T$ $= I \square O \square \top$ $\square C \square T$ $\square C \square T$ $\square Not applicable       \square O \square \square \square \square \square  \square O \square \square$	Number       Treatment Description*       Type**       Number       Treatment Description*       Type**         1 =       DMW 25% $D \in D$ 4 = $J \circ 2$ $C D T$ 5 = $J \times Z$ $C C T$ 2 = $(2.5\%)$ $\Box C D T$ 5 = $J \times Z$ $\Box C D T$ $T = Treatment Description*       Type**         3 =       a_2 \% \Box C D T       5 =       J \times Z \Box C D T         3 =       a_2 \% \Box C D T       6 =       I \circ C_Z \Box C D T         3 =       a_2 \% \Box C D T       6 =       I \circ C_Z \Box C D T         3 =       a_2 \% \Box C D T       6 =       I \circ C_Z \Box C D T         3 =       a_2 \% \Box C D T       7 =       I \subset D T       6 =       I \circ C_Z \Box C D T         3 =       a_2 \% \Box C D T       T = Treatment       I \cap C_Z \Box C D T         Dilution Water Type:         \Box D S = 0 \Box C D T I \cap C_Z \Box C D T I \cap C_Z         Date: I \cup I = 0 I \cap C_Z \Box C D H         Date: D = 2 O D C D T D = 2 O D T D = 2 $	Chronic Acute		Regulat	tory	□ Pre □ An	liminary alytical	J	☑ Effluent □ Received	waters
1 =       DMW 25% $D C \Box T$ 4 = $5 \circ h$ $\Box C \Box T$ 2 = $125\%$ $\Box C \Delta T$ 5 = $77.7$ $\Box C \Delta T$ 3 = $a 5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box C \Delta T$ 3 = $a 5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box C \Delta T$ 3 = $a 5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box C \Delta T$ 3 = $a 5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box C \Delta T$ 3 = $a 5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box \Delta T$ 3 = $a 5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box \Delta T$ 4 = $5 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box \Delta T$ 4 = $2 \%$ $\Box C \Delta T$ 6 = $1 \circ \delta h$ $\Box C \Delta T$ 4 = $2 5 \%$ $\Box T$ $\Box T$ $\Box T$ $\Box T$ $\Box T$ 4 = $2 5 \%$ $\Box T$ <td< td=""><td>1 =       DNW 25%       <math>D \subset DT</math>       4 =       <math>55\%</math> <math>C C DT</math>         2 =       <math>(2.5\%)</math> <math>C C DT</math>       5 =       <math>77\%</math> <math>C C DT</math>         3 =       <math>a5\%</math> <math>C C DT</math>       6 =       <math>700\%</math> <math>C C DT</math>         3 =       <math>a5\%</math> <math>C C DT</math>       6 =       <math>700\%</math> <math>C C DT</math>         3 =       <math>a5\%</math> <math>C C DT</math>       6 =       <math>700\%</math> <math>C C DT</math>         3 =       <math>a5\%</math> <math>C C DT</math>       6 =       <math>700\%</math> <math>C C DT</math>         3 =       <math>a5\%</math> <math>C C DT</math>       6 =       <math>700\%</math> <math>C DT</math>         3 =       <math>a5\%</math> <math>C C DT</math>       6 =       <math>700\%</math> <math>C DT</math>         4       <math>75\%</math> <math>C DT</math>       6 =       <math>70\%</math> <math>C DT</math>         10 tapplicable       <math>C Other (describe):</math> <math>Q10\%</math> <math>Q10\%</math>&lt;</td><td></td><td>~</td><td>intion*</td><td>Type**</td><td>Number</td><td>Treatment</td><td>Descriptio</td><td></td><td>The way</td></td<>	1 =       DNW 25% $D \subset DT$ 4 = $55\%$ $C C DT$ 2 = $(2.5\%)$ $C C DT$ 5 = $77\%$ $C C DT$ 3 = $a5\%$ $C C DT$ 6 = $700\%$ $C C DT$ 3 = $a5\%$ $C C DT$ 6 = $700\%$ $C C DT$ 3 = $a5\%$ $C C DT$ 6 = $700\%$ $C C DT$ 3 = $a5\%$ $C C DT$ 6 = $700\%$ $C C DT$ 3 = $a5\%$ $C C DT$ 6 = $700\%$ $C DT$ 3 = $a5\%$ $C C DT$ 6 = $700\%$ $C DT$ 4 $75\%$ $C DT$ 6 = $70\%$ $C DT$ 10 tapplicable $C Other (describe):$ $Q10\%$ <		~	intion*	Type**	Number	Treatment	Descriptio		The way
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $			-						
$3 =$ $3 \leq 2$ $\Box C \boxtimes T$ $6 =$ $I \otimes Z_{T}$ $\Box C \boxtimes T$ *If DMW, include Batch number       **C = Control, T = Treatment         Dilution Water Type: $\Box$ Not applicable $\Box$ Other (describe): $\Box$ $\blacksquare$ Not applicable $\Box$ Other (describe): $\Box$ $\blacksquare$ $\blacksquare$ Z5% Dilute Mineral Water (DMW) + Trace Metals       Batch number: $\P I \otimes$ Source of Test Organisms: $\blacksquare$ $\blacksquare$ $\blacksquare$ $\blacksquare$ ESD cultures: Board numbers: $\Box$ NA $\blacksquare$ $\blacksquare$ $\blacksquare$ $\blacksquare$ Vendor: $\Box$ $\Box$ Other (describe): $\blacksquare$ Water delivery dates: $\Box$ Other (describe): $\Box$ $\blacksquare$ $\blacksquare$ Not applicable       Sample ID: $32924$ Date: $11-220$ $COC \#$ : $031 \otimes 69$ Sample ID: $32925$ Date: $11/4/122$ $COC \#$ : $031 \otimes 69$ Sample ID: $32925$ Date: $11/4/122$ $COC \#$ : $031 \otimes 69$ Sample ID: $32925$ Date: $11/4/122$ $COC \#$ : $031 \otimes 71$ Date       Description       Initial       Initial       Initia       Initiai <t< td=""><td>3 = <math>3 =</math> <math>3 =</math> <math>2 =</math> <t< td=""><td></td><td></td><td></td><td></td><td></td><td>TrZ</td><td></td><td></td><td></td></t<></td></t<>	3 = $3 =$ $3 =$ $2 =$ <t< td=""><td></td><td></td><td></td><td></td><td></td><td>TrZ</td><td></td><td></td><td></td></t<>						TrZ			
Image: Second of the secon	Tripper       Tripper         Image: State of the state			_						
Dilution Water Type: $\square$ Not applicable $\square$ Other (describe): $\blacksquare$ 25% Dilute Mineral Water (DMW) + Trace Metals       Batch number: 918         Source of Test Organisms: $\blacksquare$ ESD cultures: Board numbers: $\square$ NA $\blacksquare$ 4727 $\square$ Vendor: $\square$ Other (describe): $\square$ Vendor: $\square$ Other (describe):         Water delivery dates: $\square$ Other (describe): $\square$ Not applicable       Sample ID: 32924       Date: 11-221 COC #: 031069         Sample ID:       32925       Date: 11/222 COC #: 031070         Sample ID:       329226       Date: 11/1/222 COC #: 031071         Network of Deviations from Method and/or Test Non-Conformities         Date       Description       Initia $\  1/2^{M}$ Noticed lake M the day (after munity fest) that YCF brack scelue #HY       HY $  U q ^{M}$ I near in theorem 3 (cup 2 1)       HY $  U q ^{M}$ I near in theorem 3 (cup 2 1)       HY $  U q ^{M}$ I near in theorem 3 (cup 2 1)       MY $  U q ^{M}$ I near in theorem 3 (cup 2 1)       MY $  U q ^{M}$ I near in theorem 3 (cup 2 1)       MY $  U q ^{M}$ I near in theorem 4 (que 2 1)       Initial $Date$	Dilution Water Type: $\square$ Not applicable $\square$ Other (describe): $\square$ 25% Dilute Mineral Water (DMW) + Trace Metals       Batch number: 918         Source of Test Organisms: $\square$ ESD cultures: Board numbers: $\square$ NA $\square$ $4727$ $\square$ Vendor: $\square$ Other (describe): $\square$ $\square$ Vendor: $\square$ Other (describe): $\square$ Water delivery dates: $\square$ Other (describe): $\square$ $\square$ Not applicable       Sample ID: $32924$ Date: $11-221$ $COC #$ : $031069$ Sample ID: $32925$ Date: $11/4/22$ $COC #$ : $031070$ Sample ID: $32924$ Date: $11/242$ $COC #$ : $031070$ Sample ID: $32925$ Date: $11/242$ $COC #$ : $031070$ Sample ID: $32924$ Date: $11/242$ $COC #$ : $031070$ Sample ID: $32925$ Date: $11/2422$ $COC #$ : $031070$ Sample ID: $32924$ Date: $11/2422$ $COC #$ : $031070$ Sample ID: $32925$ Date: $11/2422$ $COC #$ : $031070$ Date       Description       Initial $11/2121$ Noticed lack M flue day (after manuly fest) fluest VG batch scalus After procedure from whether of previou		10	C = Control			100%			СДІ
Record of Deviations from Method and/or Test Non-Conformities         Date       Description       Initia         IV1/2       Noticed lade M the day (after number test) that VCI back to swelled #HP       Initia         IV1/2       Noticed lade M the day (after number test) that VCI back to swelled #HP       Initia         Iver it had spoiled. Will switch to previous back (a(10/22) for       Initia         11/9/22       Inate in the the system of the sys	Record of Deviations from Method and/or Test Non-Conformities         Date       Description       Initial         W/1/22       Noticed lade M the day (after munning test) that YG batch smelled After like it had spoiled. will switch to previous batch (a[19(22) for liked day of test.       Initial         11/9/22       I male in treatment 3 (cup 27)       AMF         Initial         Ouality Assurance (QA) Record         Procedure         Name       Initial       Date         Test run by:       MMF       U/9/22         Data sheets QA:         Data entered:       MMA       Hruff       I/9/22         Data entry QA:       MMA       Hruff       I/9/22	Source of Tes	t Organisms:	l Water (D	MW) + Trace	e Metals		ber: <u>918</u>	3	
Date       Description       Initia         IV1/2       Noticed lade m the day (after number test) that V.T batch swelled Attributed in the day (after number test) that V.T batch swelled Attributed is that goiled. Will switch to previous batch (a(13/22) for       Initia         IV2       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         IV9/22       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         IV9/22       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         IV9/22       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         IV9/22       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         IV9/22       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         Ivest day of yest.       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         Ivest day of yest.       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         Ivest day of yest.       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         Ivest day of yest.       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         Ivest day of yest.       Ivest day of yest.       Ivest day of yest.       Ivest day of yest.         Ivest day of yest.	Date       Description       Initial         If 1/2       Noticed late M the day (after number test) that VG batch swelves Attribute is that goiled. Will writch to previous batch (al.a.22) for       Initial         Itest day of test.       Itest day of test.       Itest day of test.         II/9/22       Imale in the the day (after number to previous batch (al.a.22) for       Itest day of test.         II/9/22       Imale in the the test to previous batch (al.a.22) for       Itest day of test.         II/9/22       Imale in the test ment 3 (aup 27)       Itest         Quality Assurance (QA) Record       Initial       Date         Procedure       Name       Initial       Date         Test run by:       Image       Initial       Date         Data sheets QA:       Image       Image       Image         Data entered:       Image       Image       Image         Data entry QA:       Image       Image       Image	Source of Tes ESD Ven Water deliver	t Organisms: ) cultures: Board dor: y dates: applicable S S	l Water (D. d numbers: ample ID: ample ID:	$MW) + Trace$ $\Box NA U \_$ $\Box Other$ $32924$ $32925$	e Metals 	-727 1-2-21 C	20C #: 20C #:	031069	
III/9/22     I male in treatment 3 (aup 27)     AMF       Quality Assurance (QA) Record       Initial Date       Test run by:       Data sheets QA:     MMP     U/9/22       Data entered:     MMP     U/9/22	III/9/22     I male in treatment 3 (aup 27)     AMF       Quality Assurance (QA) Record       Procedure     Name     Initial     Date       Test run by:     OMP     AMF     II/9/22       Data sheets QA:     Initial     Date       Data entered:     OMA     Image: Non-     Image: Non-       Data entered:     Image: Non-     Image: Non-     Image: Non-	Source of Tes ESD Ven Water deliver	t Organisms: ) cultures: Board dor: ry dates: applicable S S S	l Water (D. d numbers: ample ID: ample ID: ample ID:	MW) + Trace DNA M Other 32924 32925 32926	e Metals 4 (describe): _ Date: _ / Date: _ / Date: _ /	1-2-22 0 14/22 0 12/22 0	COC #: COC #: COC #:	031069 031070 031071	
Procedure     Name     Initial     Date       Test run by:     Image: Comparent to the second s	Procedure     Name     Initial     Date       Test run by:     Image: Comparent to the second s	Source of Tes ESD Ven Water deliver Not	t Organisms: ) cultures: Board dor: y dates: applicable S S Record of Descriptic Noticed In Life if M	l Water (D) d numbers: ample ID: ample ID: ample ID: Deviation m adv M fw ad Spril	$MW) + Trace$ $\square NA II □ Other$ $32924$ $32925$ $32926$ ns from Me	e Metals 4 (describe): Date: _! _ Date: _! _ Date: _! _ thod and/c	-727 )-2-22 C 1/4/22 C 1/4/22 C 1/2/22 C pr Test Non-	СОС #: СОС #: СОС #: • Сопform	031069 031070 031071 ities ities	Initial
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	Daily	y Test Info	Tempe Inform Therm. #:_	nation		ood codes: M R= <i>Raphid</i> table algal cel	locelis, B= 1 density ra	st-cerophyl Brine shrin	ap)	Test I		Water Chang mination	ge, or Test	Sample Infe
S	Test day	Date	Eny. Chamber (C)	Test Chamber (C)	- Food Type	Food ( Prep Date	Volume (μL)	Confirm cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
	Day 0	11/02/22 PK	25.3 am	26.0 am	YCT RASU.	\$44722 ANFIES	n 100 95	VZY es	<u>11:59</u> am pm	11=28	12:27	32924	918	N/A
	Day 1	11/03/22pk	749 am	25.2 am pm	YCT PASIL	11/2/22	41 en	VEYes	<u>10:59</u> am pm	10:45	11:35	1	918	
	Day 2	1/04/22 MAF	25.8 am	25.5 am	YCT	11/2/22	100 mg	PYes m	am 1256 pm	1239	1328	32925	918	
	Day 3	11/05/22 AME	25.9 am	<u>25.4</u> am pm	YUT	10/18/22	100	EYes	1040 am pm	1027	1137	1	919	
	Day 4	11/06/22	<u>25.7</u> am pm	25.6 am	YOT	10/18/22	100 88	TYes	<u>1018</u> am pm	1004	1116	ł	919	
-	Day 5	11/07/22 AME	<u>25.9</u> am pm	25. 6 am	YOT	10/18/22	100	Yes	1042 am	1025	1128	32926	919	
	Day 6	11/08/22	26.0 am	<u>25:1</u> am pm	YCT	109/13/22	100	<b>⊉</b> Yes	<u>1045</u> am pm	1032	1149	V	919	
	Day 7	11/09/22	<u>24.1</u> am pm	25.5 am				□Yes	ana	1100	1221			¥

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11/12/27 Nd

CHRONIC Daily Water/Feeding Log

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		Project:	Y12	<i>iodaphnia</i> Chro Test site/cl	nemical:	0F200	Test Nu	mber: 2979	9	PK	1
105-2	·B	egin Date:C	11/02/2022 Codes: (-) Alive and I	No Reproduction; (	N) Alive and Repro	oduction; (xN) Dead	Templat and Reproduction;	te Number: <del></del> (M) Male	16	PK	19
11-14	Test Chamber	Treatment Number	Day: 1 PK Date: 11/03/20	2 000	3 1000	4 NJR 11/06/22	5 10 pk	6144 24	7 AHE		1
	1	5	-	-	1	6	-	16	21	-	1
2	2	2	1	-	8	1	14	20	22	1	
J.V.	3	6	-	-	4		15	19	21	-	
Sol	5	3.		-	7	-II	-		20 .	1	
-	6	6	-	-	8	-	-	17	20	-	
	7	5	-	-	6 5	Ī		22.	22		-
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10.10	16	5	-	-	4	10	-	20	22	-	-
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and the	27	36	-	-	-	-	-	-	- H		
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an in	30	2		-	6	14	-	22 1822	23		
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A A B	37	2	-	-	8	13	-	20	15	-	-
Sarris-	38	6		1	7	16		19	23		_
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		al Sciences Div	ision					R	ev. 02 2020-01-02	1	

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Sponsor:	112	Toxicity Test Site/Treatment:			Test nu	mbor 1	.686	37
Test begin d		Test end date		est duratio			plate number	
11-2-1	2 2	11-9-22	-		1	/		
Fest Organism:	Date:	Isolated from:	Fathead m Hatch date: <u>0</u> Delivery date: <u>1</u>		Not	es:		
Test period		Test purpose ☐ Regulatory ☐ Investigative	Test sta Preli Manal Re-tu	iminary lytical	Т	est type Effluent Received Substand	d waters	
Number	Treatment Deso	crintion* Type**	Number	Treatment	t Descripti	on*	Type**	
1.1.1.1	DMW25%		4 =		Descripti		C CT	
2=	1.4			50%				-
3= -	12.5%			75%				
	150	**C = Control, T= Treatment	0 -	100%				
ource of Tes	st Organisms D cultures: Boa	ard numbers: $\Box$ NA $\Box$	eribe):	Batch num	ber: <u>918</u>	919		11-2-72 1438
Source of Tes ESI Ver Water delive Date	% Dilute Miner st Organisms D cultures: Boa ndor:ABS ry dates: t applicable	s: ard numbers: $\Box$ NA $\Box$ $\Box$ Other Sample ID: $379711$ Sample ID: $379721$ Sample ID: $379720$ Sample ID: $379720$	e Metals (describe): Date: Date: Date:	1-12 ( -1-12 ( -1-12 (	coc #:_0 coc #:_0 coc #:_0	310109 31070 31071	Initiali	11-2-72 1285
E 259 Source of Tes □ ESI E Ver Water delive □ No	% Dilute Miner st Organisms D cultures: Boa ndor: <u>ABS</u> ry dates: t applicable <u>Record o</u>	s: ard numbers: $\Box$ NA $\Box$ $\Box$ Other Sample ID: 37974 Sample ID: 37975 Sample ID: 37976 Sample ID: 37976	e Metals (describe): Date: Date: Date:	1-12 ( -1-12 ( -1-12 (	coc #:_0 coc #:_0 coc #:_0	310109 31070 31071	Initial	11-2-72 1435
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Date Date Procedure	% Dilute Miner st Organisms D cultures: Boa ndor:ABS ry dates: t applicable ABS 	Sample ID: 37974 Sample	e Metals (describe): Date: Date: Date: ethod and/or	<u>272</u> <u>4-12</u> - <u>1-12</u> r Test Non-	COC #: COC #: COC #: -Conform	310109 31070 31071 nities		11-2-72 1435
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									and the second				
						CHRO	NIC Dai	ily Water/	Feeding	Log			
oonsor	y12 :	Fest site/tre	eatment: _(	)F200	)	Begin Dat	te: 11-7-	n End	d Date:	-9-22	_ Test Num	ber: 1686	0
	Test Info	Tempe	erature nation	(F	Feed ood codes: R= Raphi	docelis, B=	rmation ast-cerophyl Brine shrin nge = 3.0 - 3	np)	Test Ir		Vater Chang mination	ge, or Test	Sample Info
Test day	Date	Env. Chamber (C)	Test Chamber (C)	Food Type	Food Prep Date	Volume (µL)	Confirm cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	11-6-02 MF	am 25.5 pm	am <u>25.0</u> pm	B	1-1-1-12	-	⊠Yes	am 1532.pm	1019	1058	32924	918	NA
Day 1	11-3-22 MB	<u>74.9</u> am <u>15,4</u> pm	<u>24,5</u> am <u>25,1</u> pm	BB	11-2-22	96	₫Yes	0147 am 1344 pm	0949	1050	32924	918	
Day 2	11-4-72 Mt	<u>25.2</u> am <u>Del</u> pm	<u>15.7</u> am <u>15.5</u> pm	BB	11-3-22	103	⊠Yes	<u>0805</u> am 1441 pm	1108	1208	32925	918	
Day 3	11-5-22 148	<u>15.1</u> am 15.4 pm	24.9 pm	BB	11-4-72	55	⊠Yes	0852am 1313 pm	1053	1138	32925	919	
Day 4	11-6-22	<u>15:7</u> am <u>15:7</u> pm		BB	11-5-22 11-5-22	84	EYes	0153 am [20] pm	0953	1039	32975	919	
Day 5	11-7-72 Mr	and the second se	249 pm	BB	11-10-22	102 72	⊡ Yes	0800 am 608 pm	1016	1113	32926	919	
-	11-8-22	<u>15.3</u> am <u>15.3</u> pm	74.9 am	0402	11-7-22	109	⊠Yes	0800 am 1333 pm	1027	1110	32926	919	

Notes:

Environmental Sciences Division

Rev. 03 2020-06-05

	ues: c-cle	ai, D – Dead;	Day 1	Day 2	Day 3	Day 4	Day 5	oned and returned Day 6	, w = wounded Day 7
Treatment Number and Desc.	Replicate Number	Position Number	Date 185	Date MA 11-4-22	Date My	the second se	Date	Date 11-8-22 MS	Date
DAMAS	1	17	10	10	IQ.	10	10	10	10
DMW 25%	2	24	0	10	10	10	0	10	18
5%	3 4	- U		K	10	10	10	10	0
:		2	10	10	10	1.5	10	10	10
	1 2	n	10	10	10	10	10	10	10
12.5%	3	B	10	10	10	10	10	10	10
4- 14 14	4	à	10	10	18	10	lõ	NU OI	10
:	1	10	10	10	10	10	10	10	10
	2	5	10	10	ĬŎ	10	10	lŏ	10
25%	3	21	10	10	10	10	10	10	10
	4	1	10	10	10	10	10	10	10
1:	1	193	10	10	10	10	. 10	10	10
50%	2		10	10	0	10	10	10	10
N 60	3	14	10 14	0	10	10	10	10	10
	4	3	10	10	10	10	10	10	10
5:	1	20	10	10	10	10	10	10	10
75%	2	8	10	10 15012	0	10	10	10	10
120	4	12	10	10 100-	10.04-	10	10	10	10
5:	1	5	10	10	10	10	10	10	10
100	2	1	18	18	10	10	10	18	10
100%	3	18	10	10	10	10	6)	10	10
	4	6	10	10	D	10	ID	ĬŬ	ĬŎ
nvironmental	Sciences Divisi							Re	ev. 01 2019-05-

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**Random Assignment of Test Chambers** 

est num	ber:	086		*		
tarting p	position (	on Table	of Randor	n Numbers):		
	Assigned	Numbers	3	Sample ID/Treatment	Replicate	Position
1	25	49	73	3-25%	4	1
2	26	50	74	2-125%		2
3	27	51	75	4-50%		3
4	28	52	76	1-DMW25590	4	4
5	29	53	77	3-15%	2	5
6	30	54	78	4-100%	Ч	6.
7	31	55	79	12-100%	2	7
8	32	56	-80	5-75%	2	8
9	33	57	81	2-1252	4	9
10	34	58	82	3-2590	1	10
#	35	59	83	1-DMW2596	33	11
12	36	60	84	5-75%	3	. 12
13	37	61	85	2-12590	33	13
14	38	62	86	4-50%	3	14
15	39	63	87	6-10090		15
16	40	64	88	4-50%	4	16
17	41	65	89	1-DMW2596	1	17
18	42	66	90	6-100%	3	18
19	43	67	91	5-90% 75%	4	19
20	44	68	92	5-7590		20
21	45	69	: 93	3-25%	3	21
22	46	70	94	2-125%	22	22
23	47	71	95	4-50%	2	23
24	48	72	96	1-DMW2590	2	24

Sponsor: UI	2		Test nu	mber:	186		
Test site/chem	ical: 0F200	)	Balance	e ID: A(	09820		
Test Start Date	: 11-7-22		Test Er	nd Date:	11-9-22	2	
Start Drying D	Date/Time:   - 9			ying Date	e/time:   -	0-220	
		1143am				07	
Treatment	Replicate	Pan W Date:		Date: 11	arvae (mg) -10-11 heck:	Numb Surviv	
Initial	1	10	1695	16.6	895	10	
	2	5.0	795	105	980	10	
	3	5.0	915	16.6	190	10	
	4	15.	0585	165	25	10	
1. DIANA	1	150	NTS	217	390	10	
DMW ESC	2	15.0	000	25	1085	10	
5%	3	15	0370	23	12.0	10	
	4	15.0	01010	13.1	220	10	
2.	- 1	50	9995	241	115	10	4
12.5%	2	5	2240	211	145	10	
12.010	3	15.1	050	205	430	ĬŎ	
	4	15.0	1360	12.4	125	10	
3.	1	15.	1660	212	850	10	
15%	2	5.0	1855	29	50	10	
60 10	3	5.0	1290	21.4	565	10	
	4	150	X940	22.6	880	10	
4.	1	- 15.0	1020	11.4	360	10	
509	2	15.0	01010	21.0	805	10	
000	3	150	260	21.8	210	10	
	4	15.0	145	200	440	10	
5.	1	15.0	1340	19.6	360 *	10	
769	2	15,1	135	214	260	10	
1210	3	15.0	830	21.1	50	10	
	4	15.0	855	20.8	5780	0	
5.	1	15.0	915	210	465	10	
long	2	15.0	1005	ni	190	10	
100%	3	15,0	550	21.3	810	10	
	4	150	000	213	245	10	

\* weight for 5-1 may be off due to fish sticking to Kin Wipe.

12-22 N88

roject: VI2	Project: 3
est site/chemical: 0F200	Test site/chemical: LCI Kef TOX
est number: 1686	Test number: 1087
tarting position (on Table of Random lumbers):	Starting position (on Table of Random Numbers):
Assigned Sample Numbers ID/Treatment Replicate	Assigned Sample Numbers ID/Treatment Replicate
25 49 73 1.	X 25 49 73 1. 13124
26 50 74 DMW 21 240	2 26 50 74 DMW 18 2 32
27 51 75 25% 16 330	3 27 51 75 7540 8 349
28 82 76 14 445	4 28 52 76 39 4 42
29 58 77 2. 8 142	8 29 53 77 2. Z6 1 48
30 54 78 125% X 2 14	6 20 54 78 D 25011 2 234
31 55 79 12.56 7 374	7 21 55 79 0.25g/L 73B
32 56 80 47 4 48	8 32 56 80 26438
33 57 81 3.	9 33 57 81 3. A 1 H
34 58 82 269 1 232	10 34 58 82 2 46
35 59 83 20 6 9 325	1 35 59 83 0,50g/L 19338
2 36 60 84	12 36 60 84
8 37 61 85 4. 18 1 31	13 37 61 85 4.
1 38 62 86 Cab #-230	14 38 62 86 300 1 33234
5 29 63 87 DOG X 325	15 39 63 87 1.00g/L 35 3 48
5 40 64 88 3B 4 3A	16 40 64 88 37 4 40
41 65 89 5. 2X 1375	17 41 65 89 5. 55 1 16
3 A2 66 90 764 \$ 2 B	18 42 66 90 1 15 11 X 2 28
43 61 91 Dlo 20 346	15 43 67 91 1.199/2 2032
) 44 68 92 B 4 26	20 44 58 92 94 24
45 69 93 6. 18 131	21 45 69 93 6. H 17
2 46 70 94 1124 1 2 27	
3 AT 71 95 100 to 2 3 72	22 46 70 94 $50g/L 15248$

Random Assignment of Larvae to Test Chambers

#### Fathead Minnow Order & Shipment Log

1

14 2 2 2 2 2 2 2 2 2

## Ordering Information:

1 1

5 1 3

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
10-31-22	1687 1687	ABS	800	I day old On annival	11-1-22	AMF	

Delivery Information:

Larva source	Approx. number received	Date/time received	Received by (Initials)	
ABS	880	11-1-22@ 0940	PEK	

Monitoring Interval	Hour									
	0	1	2	3	4	5	6	7		
Temperature (°C)	15.0 2:10 PM	20.9	21.8	22.6	23.4					
Time	2:10 pm	1441	1505	1540	106			1		
Thermometer ID	DDI9				~~>					
Initials	MA				>					
Comments (e.g. Fed 2.18mL Fed 2.29ml	BS to ear	larvae receir Ch Cohta Ch Contau	ved): inercis	10. MJ-1101 21 NJ-1102	22					
Fed 2.29ML BS to each container @ 0821. My 110222							Rev. 02	2020-10-23		

48

100

1 = 5



RE:	Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted January 25 to February 1, 2023
From:	L.M. Stevenson, 1504, MS-6351 (865-341-0398).
<b>c:</b>	S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, A.M. Fortner
То:	K.G. Hanzelka (RC)
Date:	February 16, 2023

Appended are the results of toxicity tests of effluent from Outfall 200 conducted from January 25 to February 1, 2023. The effluent was evaluated for toxicity with fathead minnows (*Pimephales promelas*) and water fleas (*Ceriodaphnia dubia*).

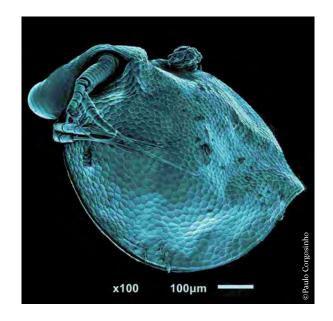
Effluent from Outfall 200 did not reduce fathead minnow survival or growth or *Ceriodaphnia* survival or reproduction by 25% or greater at any of the tested concentrations. For both species, the Inhibition Concentration<sub>25</sub> (IC<sub>25</sub>) for survival, growth, and/or reproduction were thus >100% (the highest concentration of effluent tested).

The NPDES permit states that toxicity will be demonstrated if the  $IC_{25}$  is less than or equal to the permit limit (50% effluent for Outfall 200). All of the results for all endpoints were within permit limits.

Outfall	Test Organism	Endpoint	IC <sub>25</sub>
Outfall	Fathead	Survival	>100%
200	minnow	Growth	>100%
Outfall	Ceriodaphnia	Survival	>100%
200		Reproduction	>100%

Please do not hesitate to call if you have any questions or comments.

Attachment lms



# Ceriodaphnia dubia TOXICITY TEST REPORT

Test Number 2979 | Y-12 National Security Complex Outfall 200 | 3 February 2023

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

### STANDARD REPORT FORM CERIODAPHNIA 3-BROOD SURVIVAL AND REPRODUCTION TEST

Test Number 2979 | Start Date: 25 January 2023 | End Date: 1 February 2023

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

The permit states that toxicity is demonstrated if the IC<sub>25</sub> is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 24 January 2023 to 30 January 2023
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	1/24/2023	1/26/2023	1/29/2023
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	1/25/2023	1/27/2023	1/30/2023
Chain-of-Custody Form Number	031072	031073	031074
Sample Temperature (°C)	10.1	10.8	7.2
рН (S.U.)	8.12	8.04	8.01
Conductivity (µS/cm)	407	490	367
Alkalinity (mg/L as CaCO₃)	118	122	108
Hardness (mg/L as CaCO₃)	190	200	150
Chlorine (Free/Total) (mg/L)	0.01/0.01	0.01/0.02	0.01/0.01

#### 3. TEST ORGANISMS

- 3.1 Species: Ceriodaphnia dubia.
- 3.2 Life stage: Neonates ≤24 h old; all born within 8 h of each other.
- 3.3 Source: Environmental Sciences Division cultures.
- 3.4 Incubation water for cultures: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 3.5 Temperature of cultures:  $25 \pm 1$  °C.

#### 4. TEST METHODS

- 4.1 Toxicity test method: Ceriodaphnia survival and reproduction test. Reference: EPA Test Method 1002.0, in P.A. Lewis et al., Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).
- 4.2 End points of test: Survival and reproduction.
- 4.3 Modifications or deviations to Method 1002.0: None.

- 4.4 Date and time test started: 1/25/2023, 11:55
- 4.5 Date and time test terminated: 2/1/2023, 13:10
- 4.6 Type and volume of test chambers: Polystyrene microbeakers, minimum 15mL each.
- 4.7 Number of Ceriodaphnia per test chamber: 1.
- 4.8 Number of replicates per treatment: 10.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Test temperature: Mean = 24.9 °C; range = 24.7-25.1 °C.
- 4.12 Treatment groups/concentrations: Control, 12.5%, 25%, 50%, 75% and 100% of full-strength effluent.
- 4.13 Feeding regime during test: 100 μL of yeast-Cerophyl-trout food (YCT) mixture and 100 μL of the green alga, *Selenastrum capricornutum*, per 15 mL of test solution every 24 h (EPA/821/R/02/013; 4<sup>th</sup> Ed., October 2002; or most recent version).

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Sodium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 01/25/2023 02/01/2023.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival IC<sub>25</sub> = 0.86 g NaCl/L; 95% C.I. = 0.40-1.53 g NaCl/L. Reproduction IC<sub>25</sub> = 0.84g NaCl/L; 95% C.I. = 0.46-1.47 g NaCl/L. The IC<sub>25</sub>s were calculated by the EPA linear interpolation method.
- 5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $1.433 \pm 0.802$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC25 for survival: 0.280 g NaCl/L

Central tendency of IC<sub>25</sub> for reproduction:  $1.034 \pm 0.610$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for reproduction: 0.295 g NaCl/L

A copy of the control chart is appended.

#### 6. CERIODAPHNIA TEST RESULTS

Copies of the toxicity test logsheets are appended.

6.1 Summary of results from the Ceriodaphnia toxicity test:

Effluent Concentration	Number of replicates	Number of animals surviving for 3 broods	Mean number of offspring per female (±SD)
Control	10	10	38.7 ± 4.5
12.5%	10	10	34.8 ± 4
25%	10	9	32.2 ± 5.4
50%	10	10	32.3 ± 2.7
75%	10	10	29.8 ± 7.2
100%	10	10	30.4 ± 3

#### 7. STATISTICAL ANALYSES

7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

7.2 Reproduction

The calculated IC<sub>25</sub> for reproduction was >100% effluent.

7.3 Summary of Ceriodaphnia toxicity test results:

IC<sub>25</sub> for survival: >100%

IC<sub>25</sub> for reproduction: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
pH (S.U.)	8.18	8.11	8.22
Conductivity (µS/cm)	239	239	240
Alkalinity (mg/L as CaCO₃)	100	104	106
Hardness (mg/L as CaCO₃)	130	120	120

#### 8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

Alkalinity, hardness, and chlorine were measured using a Hach SL1000 Portable Parallel Colorimeter.

Instruments were calibrated and standardized according to manufacturer's instructions.

All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Peijia KuDate: 3 February 2023Report reviewed by: Louise StevensonLouise StevensonDate: 15 February 2023



# Fathead Minnow TOXICITY TEST REPORT

Test Number 1688 | Y-12 National Security Complex Outfall 200 | 3 February 2023

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

### STANDARD REPORT FORM FATHEAD MINNOW SURVIVAL AND GROWTH TEST

Test Number 1688 | Start Date: 25 January 2023 | End Date: 1 February 2023

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

Toxicity will be demonstrated if the  $IC_{25}$  is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 24 January 2023 to 30 January 2023
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process. Samples were used within sample holding time guidance outlined in EPA Test Method 1000.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	1/24/2023	1/26/2023	1/29/2023
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	1/25/2023	1/27/2023	1/30/2023
Chain-of-Custody Form Number	031072	031073	031074
Sample Temperature (°C)	10.1	10.8	7.2
рН (S.U.)	8.12	8.04	8.01
Conductivity (µS/cm)	407	490	367
Alkalinity (mg/L as CaCO <sub>3</sub> )	118	122	108
Hardness (mg/L as CaCO <sub>3</sub> )	190	200	150
Chlorine (Free/Total) (mg/L)	0.01/0.01	0.01/0.02	0.01/0.01

#### 3. TEST ORGANISMS

- 3.1 Species: Fathead minnow (Pimephales promelas).
- 3.2 Hatch date: 23 January 2023 .
- 3.3 Life stage: Newly hatched larvae less than 48 h old.
- 3.4 Incubation water: Dechlorinated tap water.
- 3.5 Incubation temperature:  $25 \pm 1$  °C.
- 3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.
- 3.7 Mean dry weight at test initiation:  $0.13 \pm 0.006$  mg (mean  $\pm$  SD)
- 3.8 Diseases and treatment: None.

#### 4. TEST METHODS

4.1 Toxicity test method: Fathead minnow larval survival and growth test. Reference: EPA Test Method 1000.0, in P.A. Lewis et al., *Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).

- 4.2 End points of test: Survival and growth.
- 4.3 Modifications or deviations to Method 1000.0: Treatment 5, replicate 4 was lost overnight between Days 0 (test initiation) and 1 (the beaker for this replicate fell over). Test beaker temperature was outside of the allowable range (below 24°C at 23.8°C) on the morning Day 2 of the test (it was back within acceptable range by the afternoon and did not exceed the range for the rest of the test).
- 4.4 Date and time test started: 1/25/2023, 11:08
- 4.5 Date and time test terminated: 2/1/2023, 11:39
- 4.6 Type and volume of test chambers: 600-mL borosilicate beakers, minimum 250 mL each.
- 4.7 Number of organisms per test chamber: 10.
- 4.8 Number of replicates per treatment: 4.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Acclimation of test organisms: Received larvae on 24 January 2023 at 13.3 °C.
- 4.12 Test temperature: Mean = 25.2 °C; range = 23.8-25.9 °C.
- 4.13 Treatment groups/concentrations: Control, 12.5%, 25%, 50%, 75% and 100% of full-strength effluent.
- 4.14 Feeding regime during test: Brine shrimp (*Artemia*) nauplii less than 24 h old; fed 600  $\pm$  100  $\mu$ L per beaker twice daily.

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Potassium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 01/25/2023 02/01/2023.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival  $IC_{25}$  = 1.00 g KCl/L; 95% C.I. = 0.68-1.05 g KCl/L.

Growth IC<sub>25</sub> = 1.02 g KCI/L; 95% C.I. = 0.86-1.06 g KCI/L.

The  $IC_{25}s$  were calculated by the EPA linear interpolation method.

5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $0.853 \pm 0.274$  g KCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for survival: 0.161 g KCl/L

Central tendency of IC<sub>25</sub> for growth:  $0.920 \pm 0.236$  g KCI/L (mean  $\pm 2$  SD).

CV of  $IC_{25}$  for growth: 0.128 g KCI/L

A copy of the control chart is appended.

### 6. FATHEAD MINNOW TEST RESULTS

Copies of the toxicity test logsheets are appended.

#### 6.1 Summary of results from the fathead minnow toxicity test:

Survival	
----------	--

	Proportion surviving per replicate							
Concentration	1	2	3	4	Mean			
Control	1	1	1	1	1			
12.5%	1	1	0.9	1	0.98			
25%	1	1	1	1	1			
50%	1	1	1	1	1			
75%	1	1	1	LOST	1			
100%	1	1	1	1	1			

Dry Weight

Concentration	1	2	3	4	Mean ± SD
Control	0.51	0.46	0.51	0.53	0.5 ± 0.03
12.5%	0.5	0.57	0.48	0.56	0.53 ± 0.04
25%	0.54	0.51	0.48	0.57	0.52 ± 0.04
50%	0.56	0.66	0.58	0.54	0.58 ± 0.05
75%	0.6	0.51	0.62	LOST	0.58 ± 0.06
100%	0.55	0.5	0.59	0.57	0.55 ± 0.04

### 7. STATISTICAL ANALYSES

7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

#### 7.2 Growth

The calculated IC<sub>25</sub> for growth was >100% effluent.

7.3 Summary of fathead minnow toxicity test results:

IC<sub>25</sub> for survival: >100%

IC<sub>25</sub> for growth: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
pH (S.U.)	8.18	8.11	8.22
Conductivity (µS/cm)	239	239	240
Alkalinity (mg/L as CaCO <sub>3</sub> )	100	104	106
Hardness (mg/L as CaCO <sub>3</sub> )	130	120	120

8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity (µS/cm) was measured by EPA method 120.1 with a YSI 4310 meter. All values were corrected to 25°C. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

Alkalinity, hardness, and chlorine were measured using a Hach SL1000 Portable Parallel Colorimeter.

Instruments were calibrated and standardized according to manufacturer's instructions.

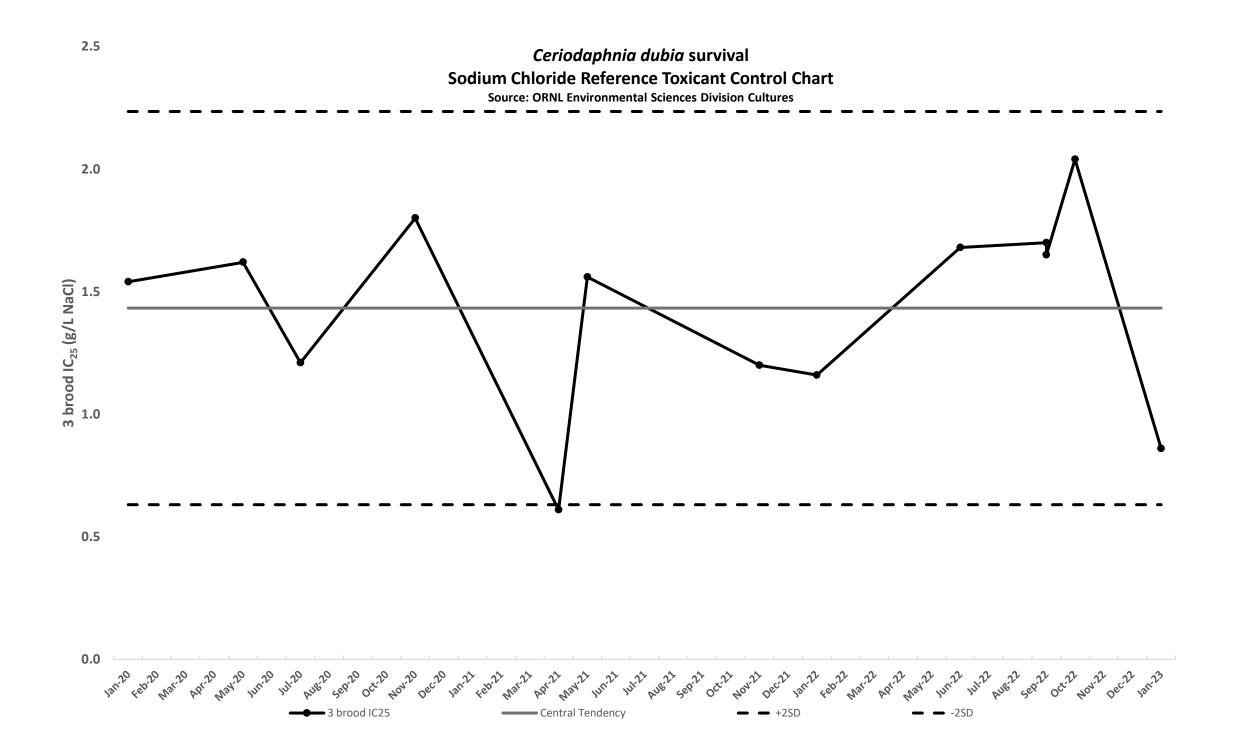
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

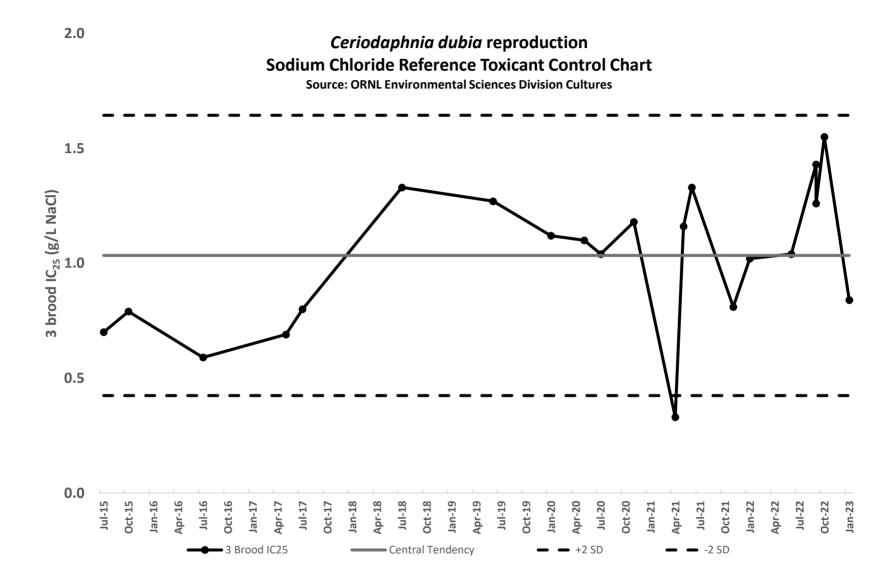
Report prepared by: Peijia Ku

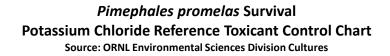
Date: 3 February 2023

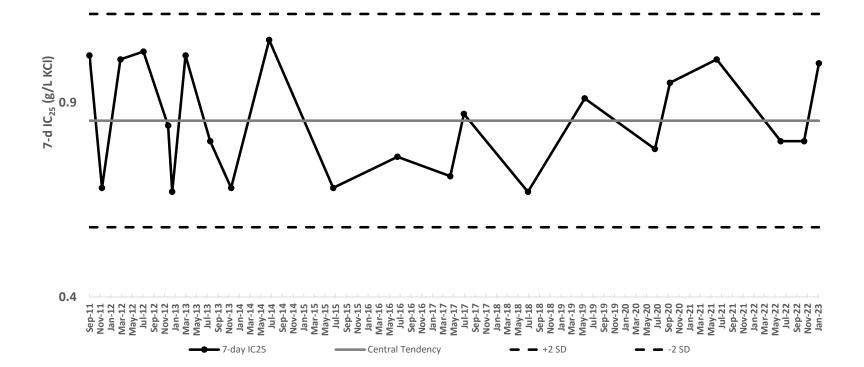
Report reviewed by: Louise Stevenson Louise Hevenson Date: 15 February 2023

**REFERENCE TOXICANT CONTROL CHARTS** 

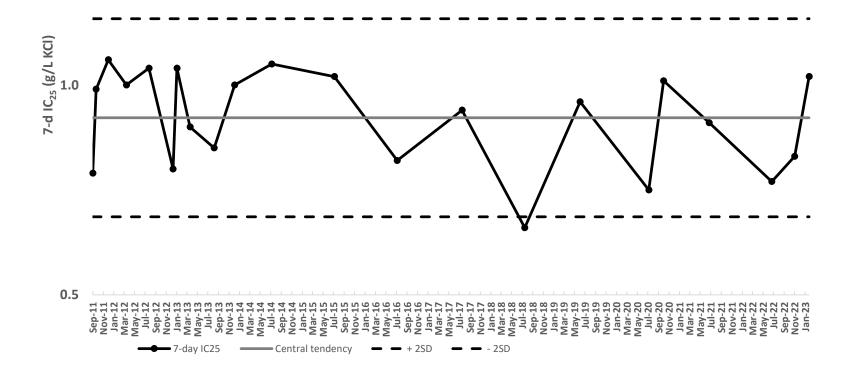








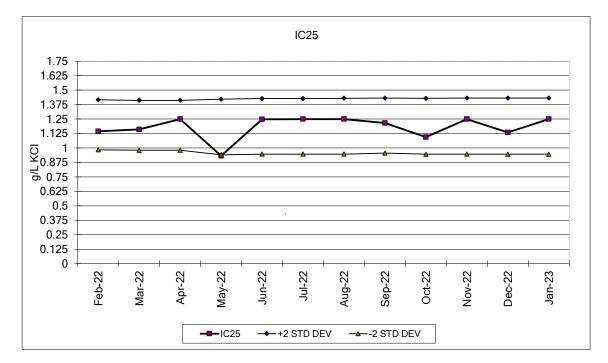
Pimephales promelas Growth Potassium Chloride Reference Toxicant Control Chart Source: ORNL Environmental Sciences Division Cultures





1300 Blue Spruce Drive, Suite C Fort Collins, Colorado 80524 Toll Free: 800/331-5916 Tel:970/484-5091 Fax:970/484-2514

Pimephales promelas



#### Chronic 7 Day Survival Test Data

#### IC 25 for Growth Test

Date	NOEC	LOEC	Date	IC25	95% Co	nfidence	Avg. IC25	+2 STD	-2 ST
	(g/L KCI)	(g/L KCI)		g/L KCI	(upper)	(lower)	g/L KCI	DEV	DEV
Aug-22	0.50	1.0	Aug-22	1.250	1.250	1.250	1.188	1.429	0.947
Sep-22	0.50	1.0	Sep-22	1.215	1.271	1.072	1.193	1.432	0.955
Oct-22	0.50	1.0	Oct-22	1.094	1.332	0.161	1.188	1.429	0.946
Nov-22	0.50	1.0	Nov-22	1.250	1.250	0.907	1.189	1.431	0.947
Dec-22	0.50	1.0	Dec-22	1.134	1.319	0.164	1.188	1.431	0.946
Jan-23	0.50	1.0	Jan-23	1.250	1.250	1.144	1.188	1.431	0.946

\*\*Current Test Dates: 1/4-11/23

Aquatic BioSystems, Inc • Quality Research Organisms

WATER CHEMISTRY DATA LOGSHEETS

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	Not	te Not all parameters a	re required for	or all tests Al.	unused cells	Should be lin	ed through of 4 PW/NJJ				NO NO
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		5-digit ORNL ID	33120	33120	33121	33121	パニー	331.22	1/31/23	(12/01/23	
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	and the second second	DMW Batch #	928	928	928	29 00 00	929 223	929	930		Re
	12	Conductivity (µS/cm)	239 100	235	227	239	233	233	240		
G	0	Alkalınıty (mg/L)	100			104_			100		The Party
J	12	Hardness (mg/L)	120			120			120		
		pH(SU) Initial	3131	8185	8.101	S.H. MX	8152	8.16	8.12		
	itrol	Final CD/FHM		8.59/8.05	8.49/802	8.44/802	8,72	8.71	5441:04	881 830	_   i
	Control	DO (mg/L) Initial Final CD/FHM	8.62 1111111111	908/136			8.83/152		832/33	857/776	
		Conductivity (µS/cm)	261	256	261	263	264	21+2	204 00 US6		
		Alkalınıty (mg/L)	1					6.0	000		
		Hardness (mg/L)									
	44	Chlorine (mg/L)	1	/					/		
$(\mathbf{\hat{o}})$	2		8042	8.178	3189	8.09	8120	8.172	8.24		al
-,	2:1-2	Final CD/FHM			8.52/8:04		8,49/8.09			8.812831	
	~	DO (mg/L) Initial	8.62	8.77	8.76	8,50	864	877	869		-21
	<b></b>	Final CD/FHM					8.91744		888/1.20	8.67/171	
	}	Conductivity (µS/cm)	283	278	295	301	297	20	-UL		
		Alkalinity (mg/L)									
	No	Hardness (mg/L) Chlorine (mg/L)	/		/				/		
$\odot$	126	pH(SU) Initial	8.110	3.11	8151	8.131	\$ 131	8.16	8.25		
O	6.1	Final CD/FHM		8.54/8.13		8.52/8.10	8 131 852 8,08	852/3.15	3.51/8.02	3.82/826	
		DO (mg/L) Initial	8.66	8,11	8.83	8.78	8 16	8.80	8.64		
		Final CD/FHM		8 90/1.33			896/131	8.72/743	8.51/1.21	3.70 /173	
		Conductivity (µS/cm)	325	373	360	365	363	296	301		
		Alkalınıty (mg/L)									
		Hardness (mg/L)									
Ð		Chlorine (mg/L)	8 11 0	Kaut	K	8.134	<u> </u>	00	001		-A)
Ŷ	N	pH(SU) Initial	8.112	8.14	3112	8.53/8/1	8112	8.12	Sill	874 833	
	l	Final CD/FHM DO (mg/L) Initial	8.60	8 rt 18.12 9.34	8.57/808 9.02	9,10	346/5.11 8.96	3.14/8.17 8.96	8.79		
		Final CD/FHM			893/163		9.01 1735			876-1.15	
	-	Conductivity (µS/cm)	367	307	424	425	424	331	333		
		Alkalınıty (mg/L)	001	7					300		
$\sim$		Hardness (mg/L)									
(5)	民	Chlorine (mg/L)	/				/				10
~	1rt	pH(SU) Initial	777777777777777	<u>k 8.11</u>	\$.099	8.10	8092	8.08	8:4		- 6
	1	Final CD/FHM		8.578.17	8.60/8.12	854/812		855 18.22	361 1200	9.879 5.31	
		DO (mg/L) Initial	8.68	9.95	9,13	903	9:3	945	8.108		
		Final CD/FHM	407	8.92/135	905 /165 490	892/153	936/133 488		SAN MUB	8.31 1.151	
,		Conductivity ( $\mu$ S/cm)	118	407	122	-+10	400	367	304		
		Alkalınıty (mg/L) Hardness (mg/L)	190	/	200	/	/	167			
G		Chlorine (mg/L)	0.01/001	/	0.01/0.02	/	/	2.01/001			10
Q	0	pH(SU) Initial		513	8.037	8.028	8.05	8.01	8.13		
	0	Final CD/FHM		8.56 /8.15		8,56/817	9.53/819	8 58 8.26	SEBREDS	8.84 (33)	
		DO (mg/L) Initial	7.66	9.60	7.89	10.72	7.53	10.00	9.19		
		Final CD/FHM		8.83/7.15		8.83/754		8.18 7.38	9.02/7.10	8 90 772	
^	En	vironmental Sciences D	Vivision ~1-2	5-23°0FW	water was	s very clo	udy. Mr		Rev 04 20	121-02-05	
						<u> </u>	$\sim$				

Alternative Real Real and Stanky and Alternative Real Alternative Re

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6. 4m

Spon Note

**CHAIN OF CUSTODY FORMS** 

# **ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY** CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 01/25/23	ESD TEST NAME	OX TEST	NAME OF SAMPLE	A. GARLAN	10 / J. WILLIN	IMS	CHAIN-OF-CUSTODY NO.	2
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	# 7008 TEMP REMARKS	# 410
OUTFALL#200	200	0815	C	1	~16 LITERS	3°	10.1	<0.05
						-		
-								
				of drigge				
THERMOMETER NO.		OPU SUM	ple ID=	33120		Ŧ		
SAMPLES RELINQUISHED BY	Garlan	0			D	ATE 1-25-2	23 TIME 0852	AM
SAMPLES RECEIVED BY Deijia	barlan Kn				D	ATE 1-25-23	TIME 0853	

PM

## **ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY** CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 01-27-23	ESD TEST NAME	DXTEST	A. GARL	AND /J. W	ILLIAMS	rnic	CHAIN-OF-CUSTODY NO. 031073
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	# 7008 REMARKS # 4102 TEMP: C/2
OUTFALI # 200	200	0725	C	1	~14Litters	3"	10.8
			Ap				
			- A	E AZZ			
					3		
	FON C	ample ID	-22171				
THERMOMETER NO.	OKIN D	unal 12	- 20141				
SAMPLES RECEIVED BY	Harlan	w			DATE	1/27/2	TIME OBOG PM

## ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/Y30/23	ESD TEST NAME	TEST	NAME OF SAMPLE		VILLIAMS	FRIG	CHAIN-OF-CUSTODY NO. 031074
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	ТЕМР (°С)	# 7009 REMARKS # 4/02 TEMP. C12 7.2 40.02
OUTFAIL# 200	200	0735	C.	ALG 1/36/13-	~ 151	3°	7.2 40.05
		5					
							- · · · ·
3							
	001 50						
THERMOMETER NO. SAMPLES RELINQUISHED BY		mple JD=	12512		DAT	re , ,	TIME AGOG DAM
ALLOU DO ODOCINED DV	Harlai	d			DAT	1/30/23	

**TOXICITY TEST LOGSHEETS** 

		Toxicity Test		on Sheet		20
Sponsor:	Y12	Site/Treatment:	OF21	00 Te	est numl	ber: 29
Test begin o	late (Day 0)	Test end date	T	est duration		Template nu
01/2	5/2023	02/01/2023	_7	. D hours	days	DNA 🗹
Test	Ceriodaph		□ Fathead m	innow	□ Oth	
Organism:	I	solated from:			Notes:	
8		and the second second	Hatch date:			+
	Time: 10.	20 1750	Delivery date:			
Test period	1	Test purpose	Test sta		Test	type
☑ Chronic □ Acute		Regulatory		iminary Intical	۲Ľ	Effluent Received waters
LI Acute		ы шvesugauve	Ana Re-t	est		Received waters Substance
Treatment d	lescriptions:					
Number	Treatment Desc		Number	Treatment De	scription*	
1 =	DMW 25%			50	%	
2 =	12.5%		5 =	- 75		
3 =	25%	□с µт	6 =	101		
*If DMW, includ		*C = Control, T= Treatment	1			
⊭ 25 Source of Te	est Organisms		Metals		928	- <u>9</u> 29,930
≠ 25 Source of Ta ⊭ ES	% Dilute Minera est Organisms 3D cultures: Boar	al Water (DMW) + Trace : rd numbers: □ NA □	Metals #4736.		928	<u>-</u> 929,930
Ø 25 Source of Te Ø ES □ Ve	% Dilute Minera est Organisms 3D cultures: Boar endor:	al Water (DMW) + Trace : rd numbers: □ NA □	Metals		928	- <u>9</u> 29,930
≠ 25 Source of Ta ⊭ ES	% Dilute Minera est Organisms: SD cultures: Boar endor:	al Water (DMW) + Trace : rd numbers: □ NA □ □ Other	• Metals • # 4736 . (describe): _		928	<u>-</u> 929,930
Ø 25 Source of Te Ø ES □ Ve Water delive	% Dilute Minera est Organisms D cultures: Boar endor: ery dates: ot applicable	al Water (DMW) + Trace rd numbers: □ NA □ □ Other 33120 Sample ID: <u>331872</u>	$\frac{\#4736}{(\text{describe})} = \frac{(018\%)}{\text{Date:} 0}$	4735 1/25123 COC	#:	31072
Ø 25 Source of Te Ø ES □ Ve Water delive	% Dilute Minera est Organisms: ED cultures: Boar endor: ery dates: ot applicable	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33120 Sample ID: $37127$ Sample ID: $37121$	Metals #4736. (describe): ( $(012^{S})$ Date: Date:	4735 11/25123 COC	#: #:	31072 31073
Ø 25 Source of Te Ø ES □ Ve Water delive	% Dilute Minera est Organisms: ED cultures: Boar endor: ery dates: ot applicable	al Water (DMW) + Trace rd numbers: □ NA □ □ Other 33120 Sample ID: <u>331872</u>	Metals #4736. (describe): ( $(012^{S})$ Date: Date:	4735 1/25123 COC	#: #:	31072
Ø 25 Source of Te Ø ES □ Ve Water delive	% Dilute Minera est Organisms SD cultures: Boar endor: ery dates: ot applicable	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33120 Sample ID: $37127$ Sample ID: $37121$	$\frac{\#4736}{(\text{describe})}$ $\frac{(0)}{2}$ $\frac{(0)}{2}$ $Date: 0$ $Date: 0$ $Date: 0$	4735 1/25125 COC 1/27123 COC 1/30/23 COC	#: #: #:	71072 31073 31074
Ø 25 Source of Te Ø ES □ Ve Water delive □ No Date	% Dilute Minera est Organisms: D cultures: Boar endor: ery dates: ot applicable Record of Descripti	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33 20 Sample ID: $33 27$ Sample ID: $33 27$ Sample ID: $33 27$ f Deviations from Meton	$# 4736.$ $(describe):(o) \neq )$ $Date:Date:Date:Date:Date:$	4735 1/25125 COC 1/27123 COC 1/80/23 COC	#: #: #: nformiti	3107-2 3107-2 31073 31074 ies
Ø 25 Source of Te Ø ES □ Ve Water delive □ No	% Dilute Minera est Organisms: D cultures: Boar endor: ery dates: ot applicable Record of Descripti	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33120 Sample ID: $33127$ Sample ID: $33127$ Sample ID: $33127$	$# 4736.$ $(describe):(o) \neq )$ $Date:Date:Date:Date:Date:$	4735 1/25125 COC 1/27123 COC 1/80/23 COC	#: #: #: nformiti	3107-2 3107-2 31073 31074 ies
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Ø 25 Source of Te Ø ES □ Ve Water delive □ No Date	% Dilute Minera est Organisms: D cultures: Boar endor: ery dates: ot applicable Record of Descripti	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33 20 Sample ID: $33 27$ Sample ID: $33 27$ Sample ID: $33 27$ f Deviations from Meton	$# 4736.$ $(describe):(o) \neq )$ $Date:Date:Date:Date:Date:$	4735 1/25125 COC 1/27123 COC 1/80/23 COC	#: #: #: nformiti	3107-2 3107-2 31073 31074 ies
Ø 25 Source of Te Ø ES □ Ve Water delive □ No Date	% Dilute Minera est Organisms: D cultures: Boar endor: ery dates: ot applicable Record of Descripti	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33 20 Sample ID: $33 27$ Sample ID: $33 27$ Sample ID: $33 27$ f Deviations from Meton	$# 4736.$ $(describe):(o) \neq )$ $Date:Date:Date:Date:Date:$	4735 1/25125 COC 1/27123 COC 1/80/23 COC	#: #: #: nformiti	3107-2 3107-2 31073 31074 ies
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Ø 25 Source of Te Ø ES □ Ve Water delive □ No Date V2]01/907	% Dilute Minera         est Organisms:         SD cultures: Boar         endor:         ery dates:         ot applicable         Record of         Descripti         Small bubble         Name	al Water (DMW) + Trace rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33 20 Sample ID: $33 27$ Sample ID: $33 27$ f Deviations from Me on S (i.e., po) in treatmost Quality Assur- ne	Metals #4736. (describe):	4735 1/25/23 COC 1/27/23 COC 1/20/23 COC r Test Non-Co	#: 0 #: 0 #: 0 nformiti ervation	31072 31072 31074 ies 1,cDattoured P
Ø 25 Source of Te Ø ES Ø Ve Water delive Ø No Date Ø 2 10 1 / 9007 Procedure Test run by:	% Dilute Minera         est Organisms:         SD cultures: Boar         endor:            ery dates:         ot applicable         Record of         Descripti         Smit bubble         Name	al Water (DMW) + Trace ind numbers: $\Box$ NA $\Box$ rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33120 Sample ID: $37121$ Sample ID: $37121$ Sample ID: $37127$ <b>f Deviations from Me</b> on S (i.e., $D0$ ) in treatment <b>Quality Assur</b> ne Pei)The Ken	Metals #4736. (describe):	4735 1/25/23 COC 1/27/23 COC 1/20/23 COC Test Non-Co 1/20/23 COC Test Non-Co 1/20/23 COC Record Initia	#: 0 #: 0 #: 0 nformiti e+vation	3107-2 31073 31074 ies 1, cD attached P
	% Dilute Minera         est Organisms:         SD cultures: Boar         endor:            ery dates:         ot applicable         Record of         Descripti         Smit bubble         Name         OA:	al Water (DMW) + Trace ind numbers: $\Box$ NA $\Box$ rd numbers: $\Box$ NA $\Box$ and other 33120 (Construction) Sample ID: $33127$ Sample ID: $33127$ f Deviations from Me fon s (i.e., $00$ ) in treatment Quality Assur- ne Peijin ku	Metals #4736. (describe):	4735 1/25/23 COC 1/27/23 COC 1/30/23 COC r Test Non-Co ups (just obs Record Initi P	#: 0 #: 0 #: 0 mformiti ervation, al D K	3107-2 3107-2 31074 ies 1, CD attached P 02/01/20 2/2/202
	% Dilute Minera         est Organisms:         SD cultures: Boar         endor:         ery dates:         ot applicable         Record of         Descripti         > Smal bubble         Name         QA:         (Name)	al Water (DMW) + Trace ind numbers: $\Box$ NA $\Box$ rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33120 Sample ID: $37121$ Sample ID: $37121$ Sample ID: $37121$ Sample ID: $37127$ f Deviations from Me fon S (i.e., $DO$ ) in treatment S (i.e., $DO$ ) in treatment Pei)The Ken May Learne Pei)The Ken	Metals #4736. (describe):	4735 1/25125 coc $1/27123 coc 1/30/23 cocr Test Non-Coups (just obsRecordInitiPM$	#: 0 #: 0 #: 0 mformiti ervation, al D K JF	31072 31073 31074 ies 1, cD attached P 2/01/200 2/2/202 52/01/200
Ø 25 Source of Te Ø ES Ø Ve Water delive Ø No Ø No Ø Date Ø Procedure Test run by: Data sheets Q Data entered: Data entry Qa	% Dilute Minera         est Organisms:         SD cultures: Boar         endor:         ery dates:         ot applicable         Record of         Descripti         > Smal bubble         Name         OA:         A:	al Water (DMW) + Trace ind numbers: $\Box$ NA $\Box$ rd numbers: $\Box$ NA $\Box$ and other 33120 Sample ID: $33127$ Sample ID: $33127$ f Deviations from Me fon s (i.e., $\forall 0$ ) in treatment Quality Assur- ne Peijita ka	Metals #4736. (describe):	4735 1/25/23 COC 1/27/23 COC 1/30/23 COC r Test Non-Co ups (just obs Record Initi P	#: 0 #: 0 #: 0 mformiti ervation, al D K JF	2/07-2 3107-2 3107-2 31074 ies Init I, CD attacked P 02/01/20 2/20 2/2/20 2/20
Date Procedure Test run by: Data entered: Data entry Q. invironmental Sei	% Dilute Minera         est Organisms:         SD cultures: Boar         endor:         ery dates:         ot applicable         Record of         Descripti         > Smal bubble         Name         OA:         A:	al Water (DMW) + Trace ind numbers: $\Box$ NA $\Box$ rd numbers: $\Box$ NA $\Box$ $\Box$ Other 33120 ( Sample ID: $37121$ Sample ID: $37121$ Sample ID: $37121$ Sample ID: $37127$ f Deviations from Me on S (i.e., $DO$ ) in treatment D of the sum S (i.e., $DO$ ) in treatment D of the sum D of the sum $D$	Metals #4736. (describe):	4735 1/25125 coc $1/27123 coc 1/30/23 cocr Test Non-Coups (just obsRecordInitiPM$	#: 0 #: 0 #: 0 mformiti e+vation, e+vation, al D K JF	31072 31073 31074 ies 1, cD attached P 2/01/200 2/2/202 52/01/200

CHRONIC Daily Water/Feeding Log

arg       zs $\mathcal{E}_{1, \text{fines}}$ (C)       Type       Date       (III)       density       III       III       Number         Day 0       01/25/23       H32 am       24.8 am       yeT       12/06/22       100       Myes       am       11.35       13.15       33/120       100         Day 0       01/25/23       Pm       Pm       pm       yeT       12/06/22       100       123/2       am       11.35       13.15       33/120       100       928         Day 1       01/26/23       Pm       Pm       Pm       RASU       01/24/22       100       123/2       am       11.50       11.55       13.15       33/120       128         Day 2       01/24/23       Pm       Pm       Pm       RASU       01/24/3       95       Pm       11:50       12.24       33/12.1       928         Day 2       01/28/32       Pk       Pm       Pm       RASU       01/24/3       95       Pm       11:51       12.24       33/12.1       928         Day 3       01/28/32       Pk       Pm       Pm       RASU       01/24/3       95       1002       112.6       929       929         Day 3	Analyte
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Day 7 02/01/23 pxpmpmpmpmpmpm1140 1310	V
Notes:	NIA
Environmental Sciences Division	

19	roject:	V12	Test site/c	conic Daily Surv hemical: 0F2	00 .	Test Nu	umber: 297	19 Pl
·B	egin Date:	01/25/202)	End Date:	(N) Alive and Renn	oduction: (~N)	Templa ead and Reproduction	te Number:	1 01/2
					and the second sec	strend of some life the support of the local of the support	A REAL PROPERTY AND ADDRESS OF TAXABLE PARTY.	
Test Chamber	Number	Day: 1 PK Date: 01/26/22	01/27/23	01/28/23	1/29/23	< 5 pk 01/30/23	01/31/23	7 PK
and the second se	5	Date. 01/2012	01/21/-5	6	8		16	
1 2	1		-	1	12	-	23	24
3	5	-	-	6	-	8	19	2.0
4	ī	-	-	7	-		22	26
5	6.	1 -	-	7	-	12	18	22 .
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12	5			6		12	16	19
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26	6.	-		6		X	15	2)
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28	3			1	8	-	17	28
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33	6	-		6	-	8	10	20
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35	2	-	-	8	9	-	18	25
36	2	~	-	. 7	-	11	17	. 21
37	5	-	-	1	11		18	23
38	6	-		1	1	-	17	24
39	3	-	-	8	10			20
40		-		6		9	14	20
41.	4		1		-	1.1	19	20
42	4	-	-	75	9	-	20	24
43	3		-	5	-	6	17	23
45	. 1	-	-	1	11	-	21	. 11
46	5 .	-	-	1	-	10	17	22
47	6	-	-	1	8		18	20
48	1		)	7.	12		25	26
49	5	-	-	6	-	10		T -
50		-	-		6		17	72
51	3	-	-	1	9		17	20
52	6	-		1	8	5	15	.20
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60	6	1.	-	6	-	il	16	17

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STILLSUL: VI	12	Site/Tre	atment:	07200		Test num	ber:	588
Sponsor: _ Y Fest begin da 	ate (Day 0)	Test en 02-01		т 1	<b>Γest duratio</b> _ □ hours	. (		e number
Fest Organism:		ohnia dubia Isolated from:	_	Fathead n Hatch date: 0 Delivery date: 1	1-23-23	Ot Notes:	her:	
Test period Chronic Acute		Test purpos	У	Test st: □ Prel □ Ana □ Re-	liminary alytical		t type Effluent Received wa Substance	aters
Number	Treatment De	scription*	Type**	Number	Treatment	Description	1* Tv	/pe**
1 TUILIE	MW 25%		C DT	4 =	609	courption		C DT
2 = 1	1620		DCOT		769			c th
3= . 7	169		DCDT		1009			CIEN
	Batch number	**C = Control, T=		-	100 10			
ource of Tes	st Organism	eral Water (DM	W) + Trace	ribe): e Metals	Batch num	ber: <u>928-</u>	930	
ource of Tes DESI Ver Vater deliver	st Organism D cultures: Bo ndor: <u>AP55</u>	eral Water (DM 1 <b>s:</b> pard numbers: E	W) + Trace ] NA □ _ □ Other	e Metals (describe): _			-	(S4 =
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CHRONIC Daily Water/Feeding Log

Dail	y Test Info		erature nation DD19	Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= <i>Raphidocelis</i> , B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10 <sup>7</sup> /mL						Test Initiation, Water Change, or Test Termination					
Test day	Date	Env. Chamber (C)	Test Chamber (C)	Food Type	Food Prep Date	Volume (µL)	Confirm cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte		
Day 0	01-25-23 14	am 260,4 pm	am 	B	01-24-23	- 59	₫Yes	<u>am</u> 1516 pm	1108	1129	33120	928	NA		
Day 1	01-21e-23 Mot	<u>24.2</u> am <u>24.2</u> pm	<u>25,5</u> am <u>15,6</u> pm	BB	01-25-23	72 94	DYes	0830 am	1031	1136	33120	928			
Day 2	0-71-73 MJ	<u>24.1</u> am <u>25.3</u> pm	<u>23.8</u> am <u>24.8</u> pm	BB	01-26-23	67	⊠Yes	<u>0837</u> am <u>613</u> pm	1114	1212	33121	928			
Day 3	0178-73 MS	<u>15.10</u> am <u>15.1</u> pm	<u>15:2</u> am <u>25.4</u> pm	BB	01-27-23	18	ØYes	<u>0144</u> am 1202 pm	0994	1038	33121	929			
Day 4	0-29-23 M35	<u>168</u> am 15.8 pm	<u>8.4</u> am <u>25.9</u> pm	BB	01-28-23	083 89	₫Yes	0 <u>130</u> am 1130 pm	0930	1009	33121	929			
Day 5	01-30-23	26.0 am 262.0 pm	15.0 am 15.5 Mm	BB	01-29-23	100	<b>⊡</b> Yes	0816 am 1310 pm	1047	1138	33122	929			
Day 6	01-31-23 Mt	<u>24.8</u> am 24.8 pm	<u>25.1</u> am <u>24.9</u> pm	B	01-30-23	72 96	⊡Yes	0821_am 1441_pm	1031	1116	33/22	930			
Day 7	02-01-13 M88	<u>24.1</u> am pm	<u>13.9</u> am pm				QYes	am pm	105	1139			V		

Notes:

			01-1570 MAX
	111111		La state de la segura de la segur
[]	1/17	Fathead Minnow Chronic Daily Survival L	Log Test Number: 1488

			Fa	thead Minnov	w Chronic Dai	ly Survival Log	g		
Sponsor:	412		_ Test site/ch	emical: OFU	00		Test Numbe	r: 1688	
<b>Begin Date:</b>			End Date:	02-01-23					
Comment Co			Fg = Fungus; K =	Killed by siphoni	ng; M = Missing;	Sk = Sick; SM = S	mall; SOR = Siph	oned and returned	; W = Wounded
Treatment Number	Replicate	Position	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
and Desc.	Number	Number	Date 01-26-73,145	Date 01-27-73 MA	Date MY	Date 145	Date 195	01-31-23	Date 141
1:	1	7	10	10	10	10	10	10	10
DMW	2	6	10	10	10	10	10	10	10
DMW 25%	4	8	8	0	10	10	. 10	10	10
2:	1	5	10	10	10	10	10	10	10
NCG	. 2	21	1ð	13	10	10	10	10	10
12.5%	3	9	g 10	ġ	q	q	q	9	9
	4	18	10	10	10	10	10	10	lÒ
3:	1	24	10	10	- 10	10	10	10	10
25%	2 3	10	10	10	W	0	10	0	10
60 40	4	2	10	10	10	10	10	10	10
4:	1	14	10	10	10	- W	10	10	10
Gall	2	5	10	10	10	10	lõ	10	10
50%	3	23	10	10	10	10	10	10	lõ
	4	16	10	10	Ő	10	10	16	10
5:	1	13	10	10	0	10	10	01	10
75%	2 3	4	10	10	10	10	[0]	10	10
100	4	20 22		10	10	10	10	10	10
6:	1	3	10	10	10	10 ISK	10	10	10
Ima	2	12	iŏ	10	10	10	0	10	10
100%	3	19	10	10	0	10	lÕ	10	10
	4	17	10 15014	10	10 .	0	D	10	10
Environmental S	sciences Divisio	on						Re	ev. 01 2019-05-28
Environmental S	1. J								

11-15-19 19/1

Random Assignment of Test Chambers

St HUIL	ber: 10	XX				
	100	00 n Table c	fRandor	n Numbers): 7		
	Assigned	A DECEMBER OF A	1 Randoi	Sample ID/Treatment	Replicate	Position
1	25	49	73	1-DMW2590	3	1
2	26	50	74	3-15%	4	2
3	27	51	75	6-100%		3
4	28	52	76	5-69	2	4
5	29	53	77	2-125%	T	5
6	30	54	78	1-DMW2522	2	6
7	31	55	79	1- DAW 2520	1	7
8	32	56	80	1-DMW 2520	4	8
9	33	57	81	2-12-576	3	9
10	34	58	82	3-15%	3	10
11	35	59	83	3-25%	2	11
12	36	60	84	10-10090	2	12
13	37	61	85	5-752	1	13
14	38	62	86	4-50%	1	14
15	39	63	87	4-50%	2	15
16	40	64	88	4-50%	4	16
17	41	65	89	6-100%	4	17
18	42	66	90	2-12.5%	4	18
19	43	67	91	10-100%	C.	19
20	44	68	92	5-75%	3	20
21	45	69	93	2-12.52	2	21
22	46	70	94	5-75%	4	22
23	47	71	95	4-50%	3	23
24	48	72	96	3-1520		24

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Fathead Minnow	Weight and	<b>Survival Data</b>
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Sponsor: U12			Test number: USS					
Test site/chem			Balance	ID: 170099	520			
Test Start Date		3		d Date: 02-0	-	3		
Start Drying D	ate/Time: 02-0	-230/129	End Dr	ying Date/time	:021	N-BC		
						105		
Treatment	Replicate	Pan Wt. Date: <u>02</u> Balance c	-01-23,	Pan + Larvae Date: 01-02- Balance check:	US_	Number Surviving		
Initial	1	15.11	10	104270	)	10		
	2	15.10	10	10.3515	)	10		
	3	15:10	05	10.4209	5	10		
	4	15.0	105	10.4419	5	10		
1.	1	1511	40	20.24005	5	10		
DMW	2	1500	10	19.644	0	10		
25%	3	1511	20	10.7580	)	10		
1210	4	1510	30	20.3615	5	10		
2.	1	149	695	20.0099	5	10		
NEG	2	150	040	20782	0	10		
12.5%	3	150	315	197890	5	9		
	4	15.0	970	20.6500	1	10		
3.	1	1500	180	20.4715	5	10		
NG	2	14.90	120	20.0025		10		
25%	3	ISE	325	19.9810	)	10		
	4	50	510	20.7055	5	10		
4.	1	5.14	15	20.7540	)	10		
629	2	617	195	21.0865	-	10		
2010	3	15.0	295	20.8555		10		
	4	15.19	510	20,51055	5	10		
5.	1	15.17	170	21755		10		
va	2	150	45	20.1395	)	10		
Db	3	15:06	80	21:2615		10		
	4	5.15	15	-		-		
6.	1	611	25	20.624	0	10		
	2	617	30	20/1090	)	10		
100%	3	1570	95	211204	5	10		
	4	1512	370	20.70.20		10		

01-25-23 MS T

Project: 412						BI			-2	
Test site/chemical	CA. CO. CO. CO. CO. CO. CO. CO. CO. CO. CO			Test	site/	/chem	nical:	140 Ref To	X	1
Test number: 1685			Test number: 1089					1		
Starting position ( Numbers):	on Table of Ran	dom		Starting position (on Table of Random Numbers):						
Assigned Numbers	Sample ID/Treatment	Replicate			Assi Num	gned bers		Sample ID/Treatment	Replicate	
1 25 49 73	1.	BA 1		1	25	49	73	1.	BYB1	
2 26 50 74	DMW ZSB	24362		×	26	50	74	DMW 25%	28.4/2	
8 27 51 75	75%	1.263		3	27	51	75	25%	1343	
4 28 52 76		1/4		4	28	52	76	~ ~	13354	
\$ 29 53 77	2.	KA1		5	29	53	77	2.	32.421	
\$ 30 54 78	9.3%	242		6	30	54	78	-0.25	3k, v 12	
7 31 55 79	1, 540	42443		1	31	55	79	all	10453	
8 32 56 80		13:54		8	32	56	80	510	4/4/4	
8 33 57 81	3.	2561		9	33	57	81	3.	31,401	
10 34 58 82	1846	1,102	7	10	34	58	82	0.50 g/L	1332	
1 35 59 83	10.00	8.453		X	35	59	83	all.	1/3	
12 36 60 84	4	39.404		12	36	60	84	510	1244	
13 37 61 85	4.	133		13	37	61	85	4.	B1B1	
14 38 62 86	272	9432		14	38	62	86	1.00	X12	
15 39 63 87	51.50	1303		15	39	\$3	81	all	8:3/3	-
16 40 64 88		1/2/14		16	40	64	88	0.	1.104	
41 65 89	5.	1671		V	41	65	89	5.	24361	
18 42 66 90	749	B722		18	42	66	90	1.25	1,762	
19 43 67 91	1740	X123.		19	43	61	91	all	PHY 3	
20 44 68 92		25.474		20	44	68	92	-)10	16:264	
21 AS 69 93	6.	103/1		21	45	69	93	6.	1711	
22 46 70 94	1009	46.482		22	46	70	94	150	122	
23 AT 71 95	(VU vo	W7B3		23	47	71	95	all	12383	
24 48 72 96		14.384		24	48	72	96	91-	1364	8 012

Random Assignment of Larvae to Test Chambers

## Fathead Minnow Order & Shipment Log

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1.1.1

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Ordering Information:

1. S.

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
01-23-23	1488	ABS	600	I day old on annual	01-24-23	AMF	

Delivery Information:

Larva source	Approx. number received	Date/time received	Received by (Initials)
ABS	1040	01-24-23 C 1210	MSF

Monitoring	Hour							
Interval	0	1	2	3	4	5	6	7
Temperature (°C)	13:3	20.4	22.4					
Time	1350	1415	1436					
Thermometer ID	DD19			X				
Initials	LAS						1	· · · · · ·
Comments (e.g. Fed Anliy-Sh Fed 2mL BS	redded B 830.01-1	f larvae recei Sflalus C 5-23 Mt	ved): 1430.01-24	-73 MB				
Environmental Science							Rev. 02	2020-10-28



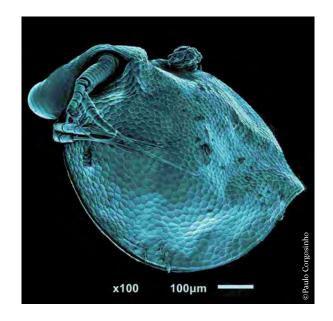
RE:	Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted May 3-10, 2023
From:	L.M. Stevenson, 1504, MS-6351 (865-341-0398).
c:	S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, A.M. Fortner
To:	K.G. Hanzelka (RC)
Date:	May 23, 2023

Appended are the results of toxicity tests of effluent from Outfall 200 conducted from May 3 to May 10, 2023. The effluent was evaluated for toxicity with fathead minnows (*Pimephales promelas*) and water fleas (*Ceriodaphnia dubia*). For both species, the Inhibition Concentration<sub>25</sub> (IC<sub>25</sub>) for survival, growth, and/or reproduction for organisms exposed to effluent from Outfall 200 was >100% (the highest concentration of effluent tested). The NPDES permit states that toxicity will be demonstrated if the IC<sub>25</sub> is less than or equal to the permit limit (50% effluent for Outfall 200). All of the results for all endpoints were within permit limits.

Outfall	Test Organism	Endpoint	IC <sub>25</sub>
Outfall	Fathead	Survival	>100%
200	minnow	Growth	>100%
Outfall	Ceriodaphnia	Survival	>100%
200		Reproduction	>100%

Please do not hesitate to call if you have any questions or comments.

Attachment lms



# Ceriodaphnia dubia TOXICITY TEST REPORT

Test Number 2985 | Y-12 National Security Complex Outfall 200 | 17 May 2023

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

## STANDARD REPORT FORM CERIODAPHNIA 3-BROOD SURVIVAL AND REPRODUCTION TEST

Test Number 2985 | Start Date: 3 May 2023 | End Date: 10 May 2023

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

The permit states that toxicity is demonstrated if the IC<sub>25</sub> is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 2 May 2023 to 8 May 2023
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	5/2/2023	5/4/2023	5/7/2023
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	5/3/2023	5/5/2023	5/8/2023
Chain-of-Custody Form Number	031101	031102	031103
Sample Temperature (°C)	14.3	11.6	14.7
рН (S.U.)	8.16	8.13	8.24
Conductivity (µS/cm)	544	525	418
Alkalinity (mg/L as CaCO <sub>3</sub> )	124	140	132
Hardness (mg/L as CaCO <sub>3</sub> )	290	250	200
Chlorine (Free/Total) (mg/L)	0.02/0.01	0.01/0.01	0.01/0.02

#### 3. TEST ORGANISMS

- 3.1 Species: Ceriodaphnia dubia.
- 3.2 Life stage: Neonates ≤24 h old; all born within 8 h of each other.
- 3.3 Source: Environmental Sciences Division cultures.
- 3.4 Incubation water for cultures: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 3.5 Temperature of cultures:  $25 \pm 1$  °C.

#### 4. TEST METHODS

- 4.1 Toxicity test method: Ceriodaphnia survival and reproduction test. Reference: EPA Test Method 1002.0, in P.A. Lewis et al., Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).
- 4.2 End points of test: Survival and reproduction.
- 4.3 Modifications or deviations to Method 1002.0: None.

- 4.4 Date and time test started: 5/3/2023, 18:03
- 4.5 Date and time test terminated: 5/10/2023, 11:50
- 4.6 Type and volume of test chambers: Polystyrene microbeakers, minimum 15mL each.
- 4.7 Number of Ceriodaphnia per test chamber: 1.
- 4.8 Number of replicates per treatment: 10.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Test temperature: Mean = 25.2 °C; range = 25.0-25.3 °C.
- 4.12 Treatment groups/concentrations: Control, 12.5%, 25%, 50%, 75% and 100% of full-strength effluent.
- 4.13 Feeding regime during test: 100 μL of yeast-Cerophyl-trout food (YCT) mixture and 3 x 10<sup>6</sup> cells of the green alga *Raphidocelis subcapitata* per 15 mL of test solution every 24 h from an algal stock with a concentration 3.0 3.5 x 10<sup>7</sup> cells/mL (EPA/821/R/02/013; 4<sup>th</sup> Ed., October 2002; or most recent version).

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Sodium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 04/19/2023 04/26/2023.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival IC<sub>25</sub> = 2.24 g NaCl/L; 95% C.I. = 1.74-2.32 g NaCl/L. Reproduction IC<sub>25</sub> = 1.38 g NaCl/L; 95% C.I. = 1.18-1.57 g NaCl/L. The IC<sub>25</sub>s were calculated by the EPA linear interpolation method.
- 5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $1.49 \pm 0.851$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for survival: 0.285 g NaCl/L

Central tendency of IC<sub>25</sub> for reproduction:  $1.063 \pm 0.619$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for reproduction: 0.291 g NaCl/L

A copy of the control chart is appended.

#### 6. CERIODAPHNIA TEST RESULTS

Copies of the toxicity test logsheets are appended.

6.1 Summary of results from the Ceriodaphnia toxicity test:

Effluent Concentration	Number of replicates	Number of animals surviving for 3 broods	Mean number of offspring per female (±SD)
Control	10	10	30.7 ± 7.3
12.5%	10	10	20.7 ± 10.7
25%	10	10	24.7 ± 10.5
50%	10	10	26.9 ± 11.3
75%	10	10	30 ± 7.5
100%	10	10	28.9 ± 10.2

#### 7. STATISTICAL ANALYSES

7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

7.2 Reproduction

The calculated IC<sub>25</sub> for reproduction was >100% effluent.

7.3 Summary of Ceriodaphnia toxicity test results:

IC<sub>25</sub> for survival: >100%

IC<sub>25</sub> for reproduction: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
pH (S.U.)	8.21	8.23	8.04
Conductivity (µS/cm)	241	235	246
Alkalinity (mg/L as CaCO₃)	80	80	120
Hardness (mg/L as CaCO₃)	130	130	130

#### 8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

Alkalinity, hardness, and chlorine were measured using a Hach SL1000 Portable Parallel Colorimeter.

Instruments were calibrated and standardized according to manufacturer's instructions.

All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Peijia Ku Report reviewed by: Louise Stevenson Date: 17 May 2023

Date: 22 May 2023 Louise Hevenson



# Fathead Minnow TOXICITY TEST REPORT

Test Number 1690 | Y-12 National Security Complex Outfall 200 | 17 May 2023

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

## STANDARD REPORT FORM FATHEAD MINNOW SURVIVAL AND GROWTH TEST

Test Number 1692 | Start Date: 3 May 2023 | End Date: 10 May 2023

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

Toxicity will be demonstrated if the  $IC_{25}$  is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 2 May 2023 to 8 May 2023
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process. Samples were used within sample holding time guidance outlined in EPA Test Method 1000.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	5/2/2023	5/4/2023	5/7/2023
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	5/3/2023	5/5/2023	5/8/2023
Chain-of-Custody Form Number	031101	031102	031103
Sample Temperature (°C)	14.3	11.6	14.7
рН (S.U.)	8.16	8.13	8.24
Conductivity (µS/cm)	544	525	418
Alkalinity (mg/L as CaCO <sub>3</sub> )	124	140	132
Hardness (mg/L as CaCO <sub>3</sub> )	290	250	200
Chlorine (Free/Total) (mg/L)	0.02/0.01	0.01/0.01	0.01/0.02

#### 3. TEST ORGANISMS

- 3.1 Species: Fathead minnow (Pimephales promelas).
- 3.2 Hatch date: 1 May 2023 .
- 3.3 Life stage: Newly hatched larvae less than 48 h old.
- 3.4 Incubation water: Dechlorinated tap water.
- 3.5 Incubation temperature:  $25 \pm 1$  °C.
- 3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.
- 3.7 Mean dry weight at test initiation: 0.129 mg
- 3.8 Diseases and treatment: None.

### 4. TEST METHODS

4.1 Toxicity test method: Fathead minnow larval survival and growth test. Reference: EPA Test Method 1000.0, in P.A. Lewis et al., *Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).

- 4.2 End points of test: Survival and growth.
- 4.3 Modifications or deviations to Method 1000.0: None.
- 4.4 Date and time test started: 5/3/2023, 16:30
- 4.5 Date and time test terminated: 5/10/2023, 16:30
- 4.6 Type and volume of test chambers: 600-mL borosilicate beakers, minimum 250 mL each.
- 4.7 Number of organisms per test chamber: 10.
- 4.8 Number of replicates per treatment: 4.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Acclimation of test organisms: Received larvae on 2 May 2023 at 13.5 °C.
- 4.12 Test temperature: Mean = 25.6 °C; range = 24.5-25.9 °C.
- 4.13 Treatment groups/concentrations: Control, 12.5%, 25%, 50%, 75% and 100% of full-strength effluent.
- 4.14 Feeding regime during test: Brine shrimp (*Artemia*) nauplii less than 24 h old; fed 1500 ± 100 shrimp per beaker twice daily.

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Potassium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 05/03/2023 05/10/2023.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival  $IC_{25} = 1.01$  g KCl/L; 95% C.I. = 0.68-1.04 g KCl/L.

Growth IC<sub>25</sub> = 1.01 g KCI/L; 95% C.I. = 0.72-1.04 g KCI/L.

The IC<sub>25</sub>s were calculated by the EPA linear interpolation method.

5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival: 0.868  $\pm$  0.27 g KCI/L (mean  $\pm$  2 SD).

CV of IC25 for survival: 0.156 g KCI/L

Central tendency of IC<sub>25</sub> for growth:  $0.920 \pm 0.228$  g KCI/L (mean  $\pm 2$  SD).

- CV of IC<sub>25</sub> for growth: 0.125 g KCl/L
- A copy of the control chart is appended.

## 6. FATHEAD MINNOW TEST RESULTS

Copies of the toxicity test logsheets are appended.

6.1 Summary of results from the fathead minnow toxicity test:

	Proportion surviving per replicate				
Concentration	1	2	3	4	Mean
Control	1	1	1	1	1
12.5%	1	1	0.9	1	0.98
25%	1	1	0.8	1	0.95
50%	0.9	1	1	1	0.98
75%	1	0.9	1	1	0.98
100%	1	1	1	1	1

Survival

### Dry Weight

		Weight (mg) per replicate				
Concentration	1	2	3	4	Mean ± SD	
Control	0.82	0.78	0.75	0.75	0.78 ± 0.03	
12.5%	0.86	0.67	0.69	0.79	$0.75 \pm 0.09$	
25%	0.74	0.87	0.71	0.77	$0.77 \pm 0.07$	
50%	0.73	0.85	0.91	0.7	0.8 ± 0.1	
75%	0.75	0.7	0.72	0.82	$0.75 \pm 0.05$	
100%	0.71	0.69	0.82	0.78	0.75 ± 0.06	

#### 7. STATISTICAL ANALYSES

#### 7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

#### 7.2 Growth

The calculated IC<sub>25</sub> for growth was >100% effluent.

7.3 Summary of fathead minnow toxicity test results:

 $IC_{25}$  for survival: >100%

IC<sub>25</sub> for growth: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
рН (S.U.)	8.21	8.23	8.04
Conductivity (µS/cm)	241	235	246
Alkalinity (mg/L as CaCO₃)	80	80	120
Hardness (mg/L as CaCO₃)	130	130	130

8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. All values were corrected to 25°C. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

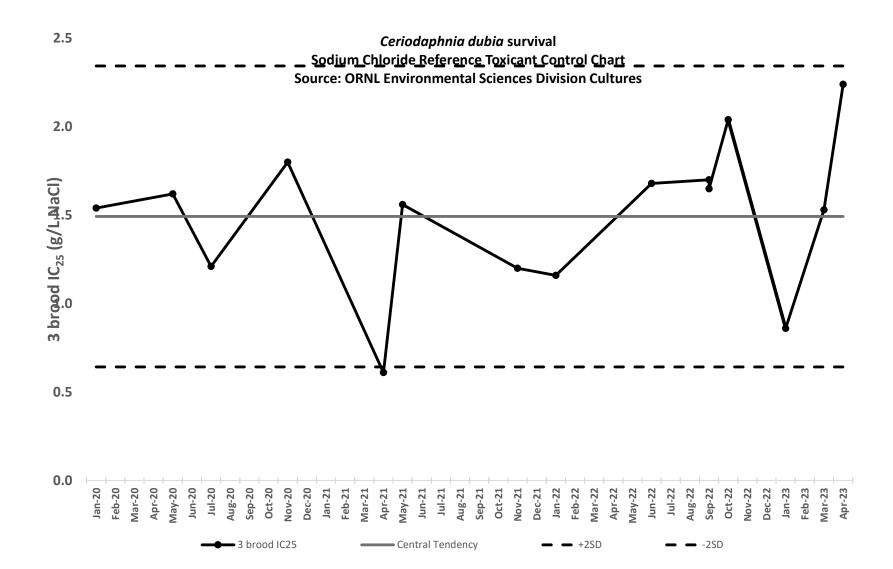
Alkalinity, hardness, and chlorine were measured using a Hach SL1000 Portable Parallel Colorimeter.

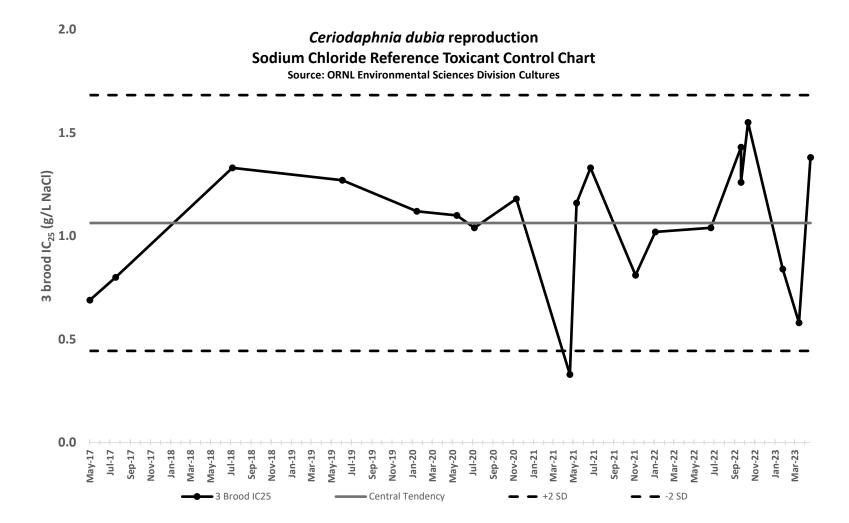
Instruments were calibrated and standardized according to manufacturer's instructions.

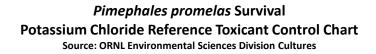
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

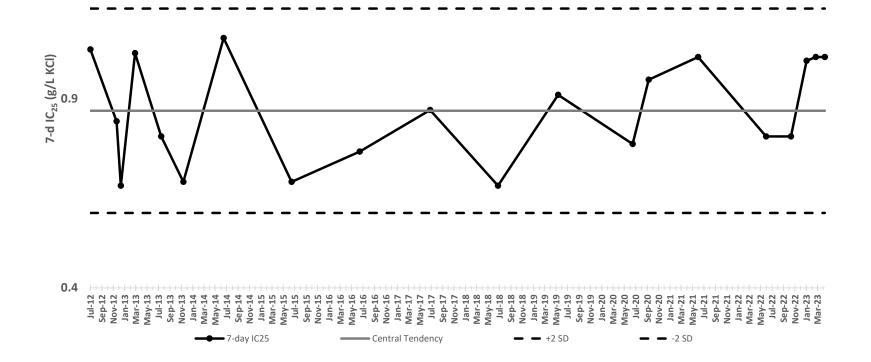
Report prepared by: Peijia Ku	Date: 18 May 2023		
Report reviewed by: Louise Stevenson	Date: 22 May 2023	louise	Hevensor

**REFERENCE TOXICANT CONTROL CHARTS** 

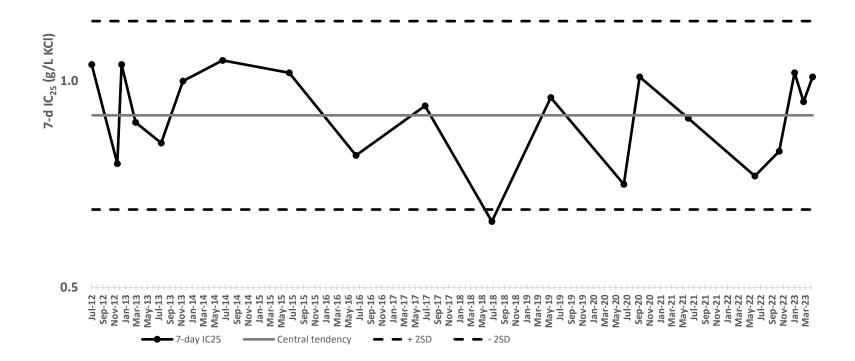








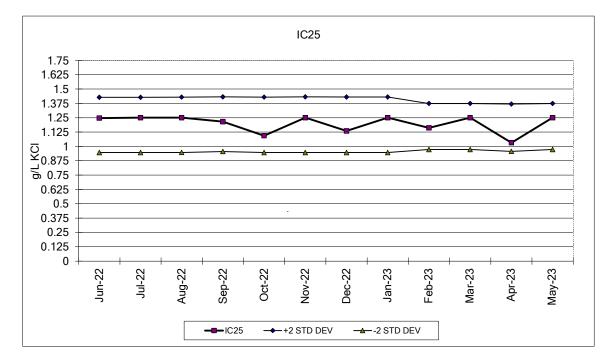
Pimephales promelas Growth Potassium Chloride Reference Toxicant Control Chart Source: ORNL Environmental Sciences Division Cultures





1300 Blue Spruce Drive, Suite C Fort Collins, Colorado 80524 Toll Free: 800/331-5916 Tel:970/484-5091 Fax:970/484-2514

Pimephales promelas



#### Chronic 7 Day Survival Test Data

#### IC 25 for Growth Test

Date	NOEC	LOEC	Date	IC25	95% Co	onfidence	Avg. IC25	+2 STD	-2 STD
	(g/L KCI)	(g/L KCI)		g/L KCI	(upper)	(lower)	g/L KCI	DEV	DEV
D 00	0 50	1.0	D 00	1 104	1 0 1 0	0.104	1 100	1 401	0.040
Dec-22	0.50	1.0	Dec-22	1.134	1.319	0.164	1.188	1.431	0.946
Jan-23	0.50	1.0	Jan-23	1.250	1.250	1.144	1.188	1.431	0.946
Feb-23	0.50	1.0	Feb-23	1.162	1.303	-0.506	1.173	1.374	0.972
Mar-23	0.50	1.0	Mar-23	1.250	1.250	1.210	1.173	1.374	0.972
Apr-23	0.50	1.0	Apr-23	1.032	1.272	0.023	1.163	1.369	0.957
May-23	0.50	1.0	May-23	1.250	1.250	1.141	1.173	1.374	0.973

\*\*Current Test Dates: 5/3-10/2023

Aquatic BioSystems, Inc • Quality Research Organisms

WATER CHEMISTRY DATA LOGSHEETS

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146									
			Daily	Water Cl	nemistry l	00			
_	Sponsor: YI2	Site/Treatme	ent: 07-24	Water CI	Associated tes	t numbers:(	D 2985	FHM I	692
	Note: Not all parameters a		r all tests. All	unused cells	should be lin	ed through or	marked "NA	**	-
PK	Observation Day: Date/Initials:	0 5333 PK	1 514/2384	2 PK	3 pk 5/6/23	4 1K 517123	5 1K 518/23		7
05/02/2	5-digit ORNL ID	33286-		33287	2/6/+3		33288-	519123	5/10/23
	Rec. temp. (°C) (New ✓)	securit-		secont.			secol -		X
	DMW Batch #	947	947	947	947	950	950 239	950	111
Ø	Conductivity (μS/cm) Alkalinity (mg/L)	241	239	235	227	246		228	
4	Hardness (mg/L)	130	/	/		130	/		
	pH (S.U.) Initial	8.207	8.19	P-23	8.20	8.04	8.20	8.05	
	Final CD/FHM DO (mg/L) Initial	<u>MANNAN</u>	8.59/8.05		8.50 / 8.04		8.55 7.90	8.57 / 7.91	8.28 8.10
	DO (mg/L) Initial Final CD/FHM	8.63	8.63	8.69	8.60	8.60 8.79 [7.10	X LO	X 4 G	CONTRACTOR OF THE OWNER OF
	Conductivity (µS/cm)	277	277	271	261	280	261	251	8.41/7.43
0	S Alkalinity (mg/L)	/	/	/	-0.	/	/	1	111
3	Hardness (mg/L)	/	/	/	/	/	/		Children -
	Chlorine (mg/L)	2.0	0.19	~	0.22	Car	8.18	9.45	
	PH (S.U.) Initial	8.19	8.19 8.62/8.09	8.11	8.23	8.25		8.05	82×100
	2 DO (mg/L) Initial	8.63	8.69	8.70	8.70	8.45	8.56	8.58	07) [ 8.0]
	Final CD/FHM	<u>MAANA</u>	8.68/7.39	9.16/ 6.97		8.90 7.05	8.86 6.36	8,92 6.81	851/7.44
	Conductivity (µS/cm)	317	318	308	299	316	284	275	
6	Alkalinity (mg/L)		-/	-/-	-/		-/-	-/	
	Hardness (mg/L)	/	/	/	/	/	/	/	7000
	O HI(CII) Initial	8.18	8:20	8.14	8.20	8.24	8.27	8.17	
	Final CD/FHM		8.51 8.08	8.60/8.12		8.5918.09	8.61 7.96	8.63 7.98	8.36 8.04
	DO (mg/L) Initial	8.77	8.76	8.72	8:76	8.54	8.36	8.74	057/75
	Final CD/FHM Conductivity (µS/cm)	399	8.70/7.45 396	381	8,83 /7.50	382	327	9.03/701	R 8.95 130
0	Alkalinity (mg/L)	311	210	281	11-	100	101	/	
$(\mathfrak{P})$	Hardness (mg/L)	/	/	/	/	/		/	
	Chlorine (mg/L)	/		/	/	/	/		
	pH (S.U.) Initial	8.16	8.19	8.14	8.18	8.22	8.25	8.17 8.67   8.01	839 8.07
	Final CD/FHM DO (mg/L) Initial	8.76	8.55/8.14 8.77	8.87	8.78	8.58	8-74	8.95	00110-1
	Final CD/FHM	anna ann			8.96 7.18		8.99/6.62	9.14 6.81	8.5417.49
0	Conductivity (µS/cm)	470	472	451	450	453	371	368	<u>11/60</u>
A	Alkalinity (mg/L)	/	-/-	/	-/-	-/-	-/-		
	Hardness (mg/L)	/	/	1	/	/	/	/	
	pH (S.U.) Initial	8.13	8.19	8.14	8.18	8.22	8.24	8:15	- 19ak
	Final CD/FHM		8.59/8.20	8.66 / 8.19	8.63 8.22		8.66 8.08		842/8.05
	DO (mg/L) Initial	9.02	8.91	8.99	8.90	8.61	8.60	9.02	053/736
	Final CD/FHM		8-71/7.47	9.11/7.11	9.09/ 7.19		9.04 0.65	9.18/6.42 415	0.75
	Conductivity (µS/cm) 8 Alkalinity (mg/L)	544	549	525	520	525	418	TI	
P	Hardness (mg/L)	290	/	250	/		200	/	
	Chlorine (mg/L) F/T	0,02/0.01	/	0.01/0.01		1	50.01/0.02	0.11	
	pH(S.U.) Initial	8.16	8.19	8.13	8.18	8.21	8.24	8.14	8.44 8.05
	C Final CD/FHM DO (mg/L) Initial	9.12	8.64 /8.24	9.11	8.67/821 8.83	8.64	9.58	9.20	
	Final CD/FHM		8.78/7.31	9.17 / 6.87		9.25 7.00	9.09 6.20	92016.69	8.58 7.3
	Environmental Sciences D			,,				Rev. 04 20	21-02-05

**CHAIN OF CUSTODY FORMS** 

# ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 05/03/23	ESD TEST NAME	OX TEST	NAME OF SAMPLERS	GARLAND /	KIILIAMS	FRIG	CHAIN-OF-CUSTODY NO.	101
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	# 7009 REM	ARKS # 4102 C/2
TOX TEST	200	1240	C	/	~181	40	14.3°	<0.05
		_						
		_						
<u></u>								
					10			
			1					
							-	
THERMOMETER NO.								
	D. D. I.	0 0			DA	TE	TIME	AM
SAMPLES RECEIVED BY	S. Sac	land			DA		TIME	
UCN-18631 (3 3-92)	eijia Ku		RAB (G). 24 HR.	COMPOSITE (C). OR	OTHER (O; DESCRIBE)	5/3/23	13	DE XPM

# ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 05/05/23	ESD TEST NAME	XTEST	NAME OF SAMPLER	GARLAND	/WILLIAMS	EDIC		31102
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	#7009 TEMP	REMARKS # 4102
TOX TEST	200	07/0	C	1	~ 17Liters	4°	11.6°	<0.05
							-	
			A	8 4. 5 S				-
				1.5				
					23			
		_						
THERMOMETER NO.								
SAMPLES RELINQUISHED BY	2.1 4.1	0			DAT	E	TIME	802 AM
SAMPLES RECEIVED BY	& Sull	ua			DAT	5/5/23	TIME	802 [2 AM 0802 [2 AM 0 PM

UCN-18631 (3 3-92

\* GRAB (G), 24 HR. COMPOSITE (C), OR OTHER (O; DESCRIBE)

# ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/YY), 05/08/23	ESD TEST NAME	XTEST	NAME OF SAMPLER	GARLANDI	WILLIAM	5 FAIR	CHAIN-OF-CUSTODY NO	1103
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	40000	MARKS_ #4102
TOXTEST	200	0715	C	/		40	14.7	<0.05
			_					
			-					
				ha .				
				2 de				
					CF B			
					X			
			_					
THERMOMETER NO.								
SAMPLES RELINQUISHED BY	Saila	nd		-		DATE 5/8/2	3 TIME OE	323 (AM) DPM
SAMPLES BECEIVED BY	jia ku			t	OTHER (O: DESCRIBE)	DATE 05/08/		23 DPM

**TOXICITY TEST LOGSHEETS** 

				T. C			NOTEB	OOK V
		То	xicity Test	Informati	ion Sheet		2	-
Sponsor:	412	Site/I	reatment:	0F2	00	Test n	umber: <mark>2</mark>	98
Test begin dat	te (Day 0)							ate num b
		105						t vo
						uays		- <u>-</u>
Fest V	Cerioda			□ Fathead n	ninnow		Other:	
Organism:		Isolated from:	11.1.22			N	otes:	
	Date: 5	13/23 5	14/00	Hatch date:			*	
	Time:	10:00pm 5.	<u></u>	Delivery date: _		-		
Test period		Test purp	pose	Test st	age		Test type	
VE Chronic		Regula		□ Pre.	liminary		Effluent Effluent	
□ Acute		□ Investi	gative	↓ Ana □ Re-	alytical		□ Received □ Substance	
Freatment des	criptions	:					- Substance	
		escription*	Type**	Number	Treatmen	nt Descrip	tion*	Type**
	DWW S		X C DT			% OF-2		COT
		07-200						CUT
		0F200				20 DF2		CUT
*If DMW, include B					. 70	40 01-0		
				and the second				
1 .	applicable	 				-	Jata	
□ Not a	applicable Dilute Mir	neral Water (D				-	<del>-1 ,95</del> 0	
□ Not a 25% 1 Source of Test	applicable Dilute Min <b>Organis</b>	neral Water (D	OMW) + Trace	Metals		-	-1 ,950	
□ Not a 25% I Source of Test	applicable Dilute Min <b>Organis</b> cultures: E	neral Water (D ms:	0MW) + Trace s: □ NA □	e Metals 4762		-	-1 ,950	
□ Not a 25% I Source of Test V□ ESD □ Vende	applicable Dilute Min Organist cultures: E lor:	neral Water (D <b>ms:</b> 30ard numbers	0MW) + Trace s: □ NA □	e Metals 4762		-	<u>-1 ,9</u> 50	
□ Not a 25% 1 Source of Test V□ ESD □ Vende Water delivery	applicable Dilute Min Organish cultures: E lor: 7 dates:	neral Water (D ms: 30ard numbers	0MW) + Trace s: □ NA □ □ Other	e Metals 4762 (describe): _	Batch nun	nber: <u>94</u>		
□ Not a 25% 1 Source of Test V□ ESD □ Vende Water delivery	applicable Dilute Min Organist cultures: E lor:	neral Water (D ms: Board numbers Sample ID	0MW) + Trace :: □ NA □ □ Other : 33286	Metals <u>4762</u> (describe): _ Date:	Batch nun	nber: <u>94</u>	031101	
□ Not a 25% 1 Source of Test V□ ESD □ Vende Water delivery	applicable Dilute Min Organish cultures: E lor: 7 dates:	neral Water (D ms: Board numbers Sample ID Sample ID	DMW) + Trace $DMW) + Trace$ $DMW = 0$ $DMW$	<u>4162</u> (describe): _ _ Date: _ Date:	Batch nun	nber: <u>94</u> COC #: COC #:	03110	
□ Not a 25% 1 Source of Test V□ ESD □ Vende Water delivery	applicable Dilute Min Organish cultures: E lor: 7 dates:	neral Water (D ms: Board numbers Sample ID Sample ID	0MW) + Trace :: □ NA □ □ Other : 33286	<u>4162</u> (describe): _ _ Date: _ Date:	Batch nun	nber: <u>94</u> COC #: COC #:	031101	
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Test day	Date	Eny. Chamber (C)	Test Chamber (C)	Food Type	Food Prep Date	Volume (µL)	Confirm cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	05/03/23 PZ	am 26.0 pm		YUT R	3121123 513123	001 100	EYes 3.5E7	am pm	1803	1900	33286	947	NA
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Day 2	05/05/23 PIC	am 25.7 pm	am 25:3 pm	YLT	3/21/23 5/3/23	100	12 Yes 3.33E+07	am pm	1510	1600	33287	947	
Day 3	05/06/23 PK	<u>25.7</u> am pm	<u>25.3</u> am pm	YUT R	3/21/23	100	VEIYes 3.28 Eto]	<u>1625</u> am pm	1610	1700		9.4-7	
Day 4	05(07/23 pic	<u>25.7</u> am pm	<u>25.5</u> am pm	YCT R	3/21/23	(00)	2Yes	<u>1705</u> am pm	1450	1500		950	
Day 5		25.8 am	25.3 am pm	YCT R	3 21/23 513  23	(00)	VYes 3.25E+07	<u>1535</u> am pm	1515	1635	33288	950	-
Day 6	at also	<u>27.6</u> am pm		YUT R	3/21/23	100	12 Yes 2.34 E+0	<u>1518</u> am	15.04	1630	1	950	
Day 7	1. John "	>5.3 am					- TYes	am	1125	1150			

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mental Sciences Division

		Toxicity Test					
ponsor:	412	Site/Treatment:	07200	Test num	iber:	<b>69</b> 2	2
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## CHRONIC Daily Water/Feeding Log

Sponsor:	412	Test site/treatment:	0F200	Begin Date:	05/03/2013 End Date:	05/10/2023 Test Number: _	1692

Daily	y Test Info	Tempe Inforn Therm. #:_	nation		ood codes: S= Selene	strum, B=	rmation ast-cerophyl Brine shrim e = 3.0 to 3.	p)	Test Initiation, Water Change, or Test Termination				Sample Info
Test day	Date	Env. Chamber (C)	Test Chamber (C)	Food Type	Food Prep Date	Volume (µL)	Confirm aigae B count	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	05/03/23 PEL/NUN	am Zle Y pm	am 25,3 pm	В	05/02/22	195	□NA ØYes	am 1152pm	1630	1742	33,286	94-7	NA
Day 1	05104/23 plc	26.2 am 26.3 pm	25.7 am 25.9 pm	B	05/03/20		□NA ØYes	1030 am 1625 pm	1325	1420	J	947	
Day 2	05/05/23	26.2 am 26.4 pm	25.7 am 25.9 pm	BB	05/04/23	139	□NA IØYes	0830 am 1455 pm	1200	1255	33287	947	
Day 3	05/06/23 DE	26.6 am	26.° am	B	05/05/23		□NA ØYes	0910 am 1604 pm	1450	1750		947	
Day 4	25/07/23 PK	26.1 am 27.9 pm	25.5 am 25.5 pm	BB	05/06/23		□NA ØYes	0950 am 1621 pm	1240	1340	1	950	
Day 5	07/08/23 PK	26.0 am 26,/ pm	25.5 am 25.7 pm	6 B	05/07/23		□NA ØYes	0845 am 1505 pm	1250	1335	37288	950	
Day 6	05/09/23 PK	26.0 am 26.0 pm	25.5 am 25.5 pm	BB	05/08/23	91 59	□NA ØYes	0935 am 1700pm	1210	1310		950	
Day 7	05/10/23 PK	26:0 am pm	25.5 am pm		/				1500	1630-			inX

Notes:

Environmental Sciences Division

Rev. 01 2019-05-28

74

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			Fa	thead Minnov	v Chronic Dai	ly Survival Lo	g		P. 05/1
Sponsor:	¥12		Test site/ch	emical:	OFDO	Test Number: 1692			
Begin Date:			End Date:				11 00D 01 1		
	des: C = Cle	ar; D = Dead;	Fg = Fungus; K = 1				1		
Treatment Number and Desc.	Replicate Number	Position Number	Day 1 Date 05/04/23 PK	Day 2 Date 05/05/23 PK	Day 3 Date	Day 4 Date D5/07/23 /K	Day 5 Date PK 03/08/23	Day 6 . Date PK 05/09/23	Day 7 Date PK 05/10/23
1:	1	18	10	10	10	10	10	10	10
25% pmw	- 2	24	10	10	10	. 10	10	10	10
	3	11	,0	10	10	10	10	10	10
	4	14	10	10	10	10	10	10	10
2: 12.5% 07-200	1	13	10	10	10	10	10	10	AID BP
	2	10	(0	10	10	10	10	10	10
	3	. 16	9 D	9	9	9	PK 09	9	9
	4 -	15.	10	10	iÐ	10	.10 .	10	10
3: 25%	1	21	10	10	10	PIC \$ 100	10	10	10
	2	12 .	10	10	pt 10 @	10	10	10	10
0F200	3	19	9 1D	9	8.10	8	00	8	8
1.1	4	3	10	ID	10	10	10	10	10
4: 50% 0F200	1	7	10	10	10	9' ID	4	9	9
	2	20	10	10	10 ISK	10	10	10	10
	3	1	10	10	10	10	10 0	10	10
	4	22	100 15 15 15 P	10	10	10	10	10	10
5: 75% 07-200	1	23	10	10	10	10	10	10	10
	2	4	1.0	10	PK 609 1D	9	9	9	9
	• 3	17	10	10	10	10	10	10	10
	4	2	10	10	10	10	10	10	10
6: 1007	1	5	10	10 ZEM	10 24M	10 25M	10 25M	10 25M	10 25m
	2	9	(0	40	10	10	10	10	10
07-200	3	6	10	10	10	10	12	. 10	10
	4	8	10	10	10	10	10	10	10

Environmental Sciences Division

75

#### **Random Assignment of Test Chambers** V12 DF200 Project: Test site/chemical: Test Number: Starting Position Number (on Table of Random Numbers): \_\_\_\_ PK-Sample ID/Treatment Replicate Assigned Numbers Position 05/02/23 50% OF200 - 3 75% 07-200 21% 0F200 3. 75% OF-200 -2 6. 100% UF200 -1 100% UF200 -3 10% 0F200 4--1 100% DF200 6-100% 0F200 -2 6. 12.5% 07-200 -2 N 25% DMW 1. -3 25% OF200 -2 125% 07-200 -1 25% PMW 1. 12.5% 05-200 2. -4 12.5% 07-200 2--3 -3 75% 01-200 5. 25% DMW 1--1 25% 0F200 3--3 56% 0F-200 4 --2 21% 0F200 3-70-50% JF200 4--4 75% OF200 5. 25% DMW

Environmental Sciences Division

Rev. 02 2020-05-28

Sponsor:	YIZ		Test number: 1692					
Test site/chemi		50	Balance ID: Aco 9820					
Test Start Date:			Test End Date: 05/10/2023					
				ying Date/time: 05				
10		1.0,		- *				
Treatment	Replicate	Date:	t. (mg) 05/09/23 check:	Pan + Larvae (mg) Date: $05/11/23$ Balance check: $12$	Number Surviving			
Initial	1	15	.1175	16.4000	10			
	2		10915	/	,			
er .	3	15.0130			/			
	4	15.	0090		/			
1.	1	15.0785		23.2285	10			
	2	15	.0645		10			
25% DMW	3	15	.2950	22.8305	(0			
	4	15	2245	22.7680	10			
2.	1	15	1305	23.7075	10			
10/	2	14	1785	21.8920	10			
12.5%	3		0895	PK 20 21.9525	9			
07-200	4		2905	23.1545	10			
3.	1		2900]	22.7785	10			
25%	2 ·		2875	23.9815	10			
05-200	3	17	3600	22,3025	8			
	4		3370	23.0570	10			
4	1	1.	33307	22.5860	9			
50%	2		855	23.7705	10			
0F200	3		1690	24.4465	(0			
	4		3905	22.3495	10			
5.	1		3705	22.8755	10			
75%	2		3250	22.3335	9			
07-200	3	15.	1295	22.3390	10			
	4		3605	23.5665	01			
6.	1	-	3685	22.4470	10			
100%	2		210	22.2235	10			
100% 0F200	3		3780	23,6150	10			
	4	15.3780		23.1690	10			

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	1.		101.	ref Test site/che	emical: D	-250	; KL	1	Test Number:	16/2;
ting P	ositio	n Nu	mber	(on Table of Rai	ndom Numb	ers): _3	5,11.	. 1		
-	Assigned			Sample		Assigned Numbers		Sample	Replica	
	Numbers		ID/Treatment	Replicate	ID/Treatment					
X	25	49	73	1.	14,221 V	X	25 49	73	1.	2,451
2	26	30	74	off DHAL)	3,82 V	2 2	26 30	\$4	25% PMW	18,352
X	22	51	75	25% DINW	10,403 V	3 2	27 31	75	25/5 1000	29,43
4	28	52	76		39; 3/4 J	4	38 32	76		1,324
5	29	33	.77	2.	5,231 V	5/2	29 53	X7	2.	3,131
8	30	54	78	12.5% JF200	21,442 V	6 3	30 34	78	0.750/6	22,282 .
X	31	55	79	121210 1	27,293 1	X	N 55	79	az5g1L Kcj	9,233
8	32	56	80		1,374 J	8 3	32 36	80		7,214
6	33	57	81	3.	18,241 J	8	33 57	81	3.	16,171
10	34	58	82	25% 0F-200	41,482 V	NO 3	34 38	82	0.53/2	11,142
N	35	59	83	0F200	19,313 J	N 3	35 59	83	3. 0.5д/L КС	20,303 1
12	36	60	84		15,204 1	12 3	36 80	84		15,424 1
R	32	61	85	4. ~~	25,301 1	13 3	37 61	85	4.	34,471
14	38	82	86	4. 50% OF200	28,352 V	1	38 62	86	1.09/1	5,82
No	38	63	87	CF2CV	16,45 V	15	63	88	KU	19,463
16	40	64	38		7,334 √	16 2	NQ 64	88	1.1.1.1	26,404 V
17	41	05		5.	4,321 V		1 65	82	5.	33, BAB9
18	42	66	30	5. 75% 0F200	2,132 1		12 66	30	1.25g/L KC1	6,272 8
19	43	75	91	. 0F200	6,263 V		13 62	1	Kri	4,443 .
20	44	68	92		12,394 1		N 88	92	1.01	37.484
21	45	80		6.	9,421 1		NS 69	93	6.	31,431
22	46	70	94	6. 100%	38,472 V	>	16 70	94	1.50g/L	10,122
23	745	71	26	07-2010	11,173 V	-	XX	95	1.50g/L KCI	36,383
24	48	72	96		43,464 V	-	18 72	96	rul	24,254

Environmental Sciences Division

Rev. 01 2019-04-25

#### Fathead Minnow Order & Shipment Log

Ordering Information:

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
05/01/23	1692	ABS	600	iday old on arrival	05/02/23	AMF	

Delivery Information:

Larva source	Approx. number received	Date/time received	Received by (Initials)
ABS	660	05/02/23 1300	PK

Monitoring	Hour							
Interval	0	1	2	3	4	5	6	7
Temperature (°C)	13.5	22.5		23.4				
Time	1305	14:25		1625				
Thermometer ID	0019	DD19		1019				
Initials	PK	PK		PK				

Comments (e.g. condition of larvae received):

Environmental Sciences Division

Rev. 02 2020-10-28

05/02/23

84



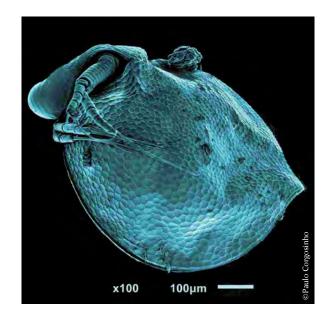
RE:	Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted August 9-16, 2023
From:	L.M. Stevenson, 1504, MS-6351 (865-341-0398).
<b>c:</b>	S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, T. Bordeau
То:	K.G. Hanzelka (RC)
Date:	August 28, 2023

Appended are the results of toxicity tests of effluent from Outfall 200 conducted from August 9 to August 16, 2023. The effluent was evaluated for toxicity with fathead minnows (*Pimephales promelas*) and water fleas (*Ceriodaphnia dubia*). Effluent from Outfall 200 did not reduce fathead minnow survival or growth or *Ceriodaphnia* survival or reproduction by 25% or greater at any of the tested concentrations compared to the control. For both species, the Inhibition Concentration<sub>25</sub> (IC<sub>25</sub>) for survival, growth, and/or reproduction for organisms exposed to effluent from Outfall 200 was >100% (the highest concentration of effluent tested). The NPDES permit states that toxicity will be demonstrated if the IC<sub>25</sub> is less than or equal to the permit limit (50% effluent for Outfall 200). All of the results for all endpoints were within permit limits.

Outfall	Test Organism	Endpoint	IC <sub>25</sub>
Outfall	Fathead	Survival	>100%
200	minnow	Growth	>100%
Outfall	Ceriodaphnia	Survival	>100%
200		Reproduction	>100%

Please do not hesitate to call if you have any questions or comments.

Attachment lms



# Ceriodaphnia dubia TOXICITY TEST REPORT

Test Number 2992 | Y-12 National Security Complex Outfall 200 | 17 August 2023

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

### STANDARD REPORT FORM CERIODAPHNIA 3-BROOD SURVIVAL AND REPRODUCTION TEST

Test Number 2992 | Start Date: 9 August 2023 | End Date: 16 August 2023

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

The permit states that toxicity is demonstrated if the  $IC_{25}$  is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 8 August 2023 to 14 August 2023
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	8/8/2023	8/10/2023	8/13/2023
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	8/9/2023	8/11/2023	8/14/2023
Chain-of-Custody Form Number	031138	031139	031140
Sample Temperature (°C)	11.6	12.9	10.8
рН (S.U.)	8.43	8.07	8.14
Conductivity (µS/cm)	601	463	498
Alkalinity (mg/L as CaCO <sub>3</sub> )	168	138	162
Hardness (mg/L as CaCO <sub>3</sub> )	260	230	200
Chlorine (Free/Total) (mg/L)	0.01/0.01	0.02/0.03	0.01/0.01

#### 3. TEST ORGANISMS

- 3.1 Species: Ceriodaphnia dubia.
- 3.2 Life stage: Neonates ≤24 h old; all born within 8 h of each other.
- 3.3 Source: Environmental Sciences Division cultures.
- 3.4 Incubation water for cultures: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 3.5 Temperature of cultures: 25  $\pm$  1 °C.

### 4. TEST METHODS

- 4.1 Toxicity test method: Ceriodaphnia survival and reproduction test. Reference: EPA Test Method 1002.0, in P.A. Lewis et al., Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).
- 4.2 End points of test: Survival and reproduction.

- 4.3 Modifications or deviations to Method 1002.0: Used the dilution series suggest by the EPA WET method manuals: Control, 6.25%, 12.50%, 25%, 50%, and 100% of full-strength effluent. Although different from the dilutions listed in the permit, these dilutions still facilitate calculation of an IC25 concentration (the measured endpoint for toxicity) and include 100% effluent, the permit limit (PL), 0.5 \* PL, 0.25 \* PL, and control.
- 4.4 Date and time test started: 8/9/2023, 10:30
- 4.5 Date and time test terminated: 8/16/2023, 9:40
- 4.6 Type and volume of test chambers: Polystyrene microbeakers, minimum 15mL each.
- 4.7 Number of Ceriodaphnia per test chamber: 1.
- 4.8 Number of replicates per treatment: 10.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Test temperature: Mean = 25.1 °C; range = 24.6 25.3 °C.
- 4.12 Treatment groups/concentrations: Control, 6.25%, 12.5%, 25%, 50% and 100% of full-strength effluent.
- 4.13 Feeding regime during test: 100 μL of yeast-Cerophyl-trout food (YCT) mixture and 3 x 10<sup>6</sup> cells of the green alga *Raphidocelis subcapitata* per 15 mL of test solution every 24 h from an algal stock with a concentration 3.0 3.5 x 10<sup>7</sup> cells/mL (EPA/821/R/02/013; 4<sup>th</sup> Ed., October 2002; or most recent version).

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Sodium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 07/19/2023 07/26/2023.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].

5.4 Survival IC<sub>25</sub> = 2.34 g NaCl/L; 95% C.L. = 1.98-2.47 g NaCl/L.

Reproduction  $IC_{25}$  = 1.49 g NaCl/L; 95% C.L. = 1.34-1.63 g NaCl/L.

The  $IC_{25}s$  were calculated by the EPA linear interpolation method.

5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $1.55 \pm 0.925$  g NaCl/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for survival: 0.299 g NaCl/L Central tendency of IC<sub>25</sub> for reproduction: 1.10  $\pm$  0.621 g NaCl/L (mean  $\pm$  2 *SD*). CV of IC<sub>25</sub> for reproduction: 0.281 g NaCl/L A copy of the control chart is appended.

#### 6. CERIODAPHNIA TEST RESULTS

Copies of the toxicity test logsheets are appended.

6.1 Summary of results from the Ceriodaphnia toxicity test:

Effluent Concentration	Number of replicates	Number of animals surviving for 3 broods	Mean number of offspring per female (±SD)
Control	10	8	30.2 ± 13.5
6.25%	10	8	27.2 ± 14.8
12.5%	10	8	31.2 ± 13.9
25%	10	8	28 ± 13.6
50%	10	9	36.8 ± 13.5
100%	10	8	25.3 ± 12.9

#### 7. STATISTICAL ANALYSES

#### 7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

#### 7.2 Reproduction

The calculated IC<sub>25</sub> for reproduction was >100% effluent.

7.3 Summary of Ceriodaphnia toxicity test results:

 $IC_{25}$  for survival: >100%

IC<sub>25</sub> for reproduction: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
рН (S.U.)	8.14	8.03	8.15
Conductivity (µS/cm)	242	234	225
Alkalinity (mg/L as CaCO₃)	104	102	102
Hardness (mg/L as CaCO <sub>3</sub> )	120	100	100

#### 8.2 Physical and chemical methods

pH, conductivity, and dissolved oxygen were measured using a YSI MultiLab 4010-3W.

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. All values were corrected to 25°C. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

Alkalinity was measured by titrating 50-mL samples with 0.01 N HCl to an endpoint pH of 4.5 (EPA method 310.1).

Hardness was determined by titrating 50-mL samples with EDTA to a colorimetric endpoint using Eriochrome Black T (EPA method 130.2).

Chlorine was measured using a Hach SL1000 Portable Parallel Colorimeter.

Instruments were calibrated and standardized according to manufacturer's instructions.

All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Trystan A. Bordeau	l	Date: 17 August 2023
Report reviewed by: Louise Stevenson	Louise Hevensoz	Date: 28 August 2023



# Fathead Minnow TOXICITY TEST REPORT

Test Number 1699 | Y-12 National Security Complex Outfall 200 | 17 August 2023

Toxicology Laboratory Principal Investigator: Dr. Louise Stevenson Environmental Sciences Division Oak Ridge National Laboratory Building 1504 P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 341-0398

### STANDARD REPORT FORM FATHEAD MINNOW SURVIVAL AND GROWTH TEST

Test Number 1699 | Start Date: 9 August 2023 | End Date: 16 August 2023

#### 1. INTRODUCTION

- 1.1 Permit Number: TN0002968
- 1.2 Toxicity testing requirements of permit: A 3-brood *Ceriodaphnia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test shall be conducted annually. All tests will be conducted using a minimum of three 24-hour composite samples of final effluent. The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction (IC<sub>25</sub>) in survival, reproduction, or growth of the test organisms as compared to the controls.

Toxicity will be demonstrated if the  $IC_{25}$  is less than or equal to the permit limit. The permit limit for Outfall 200 is 50% whole effluent.

- 1.3 Plant location: Y-12 National Security Complex.
- 1.4 Name of receiving water body: East Fork Poplar Creek.

1.5 Contractor: Toxicology Laboratory Environmental Sciences Division Oak Ridge National Laboratory P.O. Box 2008, MS 6351 Oak Ridge, TN 37831-6351 (865) 576-3459

#### 2. SAMPLE

- 2.1 Sample description: Effluent from Outfall 200.
- 2.2 Sampling point: NPDES Outfall 200.
- 2.3 Sampling period: 8 August 2023 to 14 August 2023
- 2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.
- 2.5 Samples were used immediately then stored at  $4 \pm 2$  °C to be used for two or three days during the daily effluent renewal process. Samples were used within sample holding time guidance outlined in EPA Test Method 1000.

- 2.6 Sample pre-treatment: Sample temperature was raised to 25 ± 1 °C in a warm water bath prior to test initiation and daily test renewal.
- 2.7 Sample information:

Parameter	Sample 1	Sample 2	Sample 3
Collection Start Date	8/8/2023	8/10/2023	8/13/2023
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	8/9/2023	8/11/2023	8/14/2023
Chain-of-Custody Form Number	031138	031139	031140
Sample Temperature (°C)	11.6	12.9	10.8
рН (S.U.)	8.43	8.07	8.14
Conductivity (µS/cm)	601	463	498
Alkalinity (mg/L as CaCO <sub>3</sub> )	168	138	162
Hardness (mg/L as CaCO <sub>3</sub> )	260	230	200
Chlorine (Free/Total) (mg/L)	0.01/0.01	0.02/0.03	0.01/0.01

#### 3. TEST ORGANISMS

- 3.1 Species: Fathead minnow (Pimephales promelas).
- 3.2 Hatch date: 7 August 2023 .
- 3.3 Life stage: Newly hatched larvae less than 48 h old.
- 3.4 Incubation water: Dechlorinated tap water.
- 3.5 Incubation temperature: 25  $\pm$  1 °C.
- 3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.
- 3.7 Mean dry weight at test initiation: 0.158 mg ± 0.012
- 3.8 Diseases and treatment: None.

### 4. TEST METHODS

4.1 Toxicity test method: Fathead minnow larval survival and growth test. Reference: EPA Test Method 1000.0, in P.A. Lewis et al., *Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA/821/R/02/013 (4<sup>th</sup> Ed., October 2002; or most recent version).

- 4.2 End points of test: Survival and growth.
- 4.3 Modifications or deviations to Method 1000.0: Used the dilution series suggest by the EPA WET method manuals: Control, 6.25%, 12.50%, 25%, 50%, and 100% of full-strength effluent. Although different from the dilutions listed in the permit, these dilutions still facilitate calculation of an IC25 concentration (the measured endpoint for toxicity) and include 100% effluent, the permit limit (PL), 0.5 \* PL, 0.25 \* PL, and control.
- 4.4 Date and time test started: 8/9/2023, 12:41
- 4.5 Date and time test terminated: 8/16/2023, 12:08
- 4.6 Type and volume of test chambers: 600-mL borosilicate beakers, minimum 250 mL each.
- 4.7 Number of organisms per test chamber: 10.
- 4.8 Number of replicates per treatment: 4.
- 4.9 Dilution/control water: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 4.10 Renewal period: 24 h
- 4.11 Acclimation of test organisms: Received larvae on 7 August 2023 at 12.9 °C.
- 4.12 Test temperature: Mean =  $25.5 \degree$ C; range =  $24.5 25.8 \degree$ C.
- 4.13 Treatment groups/concentrations: Control, 6.25%, 12.5%, 25%, 50%, and 100% of fullstrength effluent.
- 4.14 Feeding regime during test: Brine shrimp (*Artemia*) nauplii less than 24 h old; fed 1500 ± 100 shrimp per beaker twice daily.

#### 5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Potassium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 07/19/2023 07/26/2023.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival IC<sub>25</sub> = 1.04 g KCl/L; 95% C.I. = 0.80 1.09 g KCl/L.

Growth  $IC_{25} = 1.03$  g KCl/L; 95% C.I. = 0.65 - 1.08 g KCl/L.

The  $IC_{25}s$  were calculated by the EPA linear interpolation method.

5.5 We report the most recent 20 tests, as recommended by EPA Chronic test guidelines (EPA, 2002).

Central tendency of IC<sub>25</sub> for survival:  $0.868 \pm 0.275$  g KCl/L (mean  $\pm 2$  SD).

CV of IC25 for survival: 0.158 g KCI/L

Central tendency of IC<sub>25</sub> for growth:  $0.916 \pm 0.227$  g KCI/L (mean  $\pm 2$  SD).

CV of IC<sub>25</sub> for growth: 0.124 g KCl/L

A copy of the control chart is appended.

### 6. FATHEAD MINNOW TEST RESULTS

Copies of the toxicity test logsheets are appended.

#### 6.1 Summary of results from the fathead minnow toxicity test:

#### Survival

	J				
Concentration	1	2	3	4	Mean
Control	0.9	1	1	1	0.98
6.25%	1	0.9	1	0.9	0.95
12.5%	1	1	1	1	1
25%	1	1	1	1	1
50%	1	1	0.9	1	0.98
100%	1	1	1	1	1

Dry Weight

		Weight (mg)	) per replicate		
Concentration	1	2	3	4	Mean ± SD
Control	1.06	0.94	1.10	0.93	1.01 ± 0.08
6.25%	1.00	0.94	1.00	1.01	$0.99 \pm 0.03$
12.5%	1.06	1.01	1.05	1.02	1.04 ± 0.02
25%	1.05	1.06	1.01	0.99	1.03 ± 0.03
50%	0.98	1.01	1.13	1.19	1.08 ± 0.1
100%	1.18	1.06	1.10	1.20	1.13 ± 0.07

### 7. STATISTICAL ANALYSES

7.1 Survival

The calculated IC<sub>25</sub> for survival was >100% effluent.

#### 7.2 Growth

The calculated IC<sub>25</sub> for growth was >100% effluent.

7.3 Summary of fathead minnow toxicity test results:

IC<sub>25</sub> for survival: >100%

IC<sub>25</sub> for growth: >100%

#### 8. SUMMARY OF CHEMICAL ANALYSES

8.1 Water quality of control water:

Parameter	Sample 1	Sample 2	Sample 3
pH (S.U.)	8.14	8.03	8.15
Conductivity (µS/cm)	242	234	225
Alkalinity (mg/L as CaCO₃)	104	102	102
Hardness (mg/L as CaCO <sub>3</sub> )	120	100	100

8.2 Physical and chemical methods

The pH was measured by EPA method 150.1 with a YSI 4130 pH meter. The meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Conductivity ( $\mu$ S/cm) was measured by EPA method 120.1 with a YSI 4310 meter. All values were corrected to 25°C. The meters were verified using certified reference standards.

Dissolved oxygen (mg/L) was measured by EPA method 360.1 with a YSI 4410W dissolved oxygen meter. The meter was calibrated in accordance with the manufacturer's instructions.

Alkalinity was measured by titrating 50-mL samples with 0.01 N HCl to an endpoint pH of 4.5 (EPA method 310.1).

Hardness was determined by titrating 50-mL samples with EDTA to a colorimetric endpoint using Eriochrome Black T (EPA method 130.2).

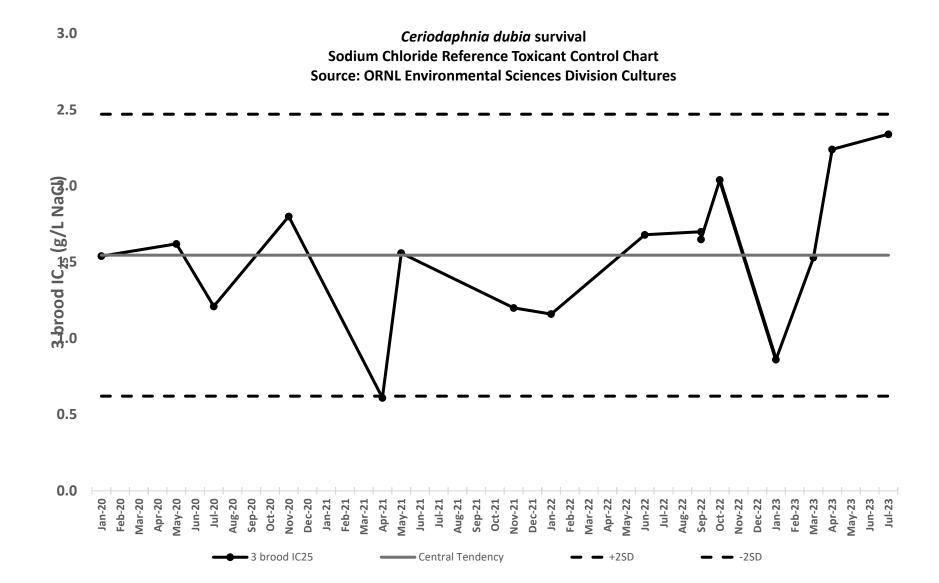
Chlorine was measured using a Hach SL1000 Portable Parallel Colorimeter.

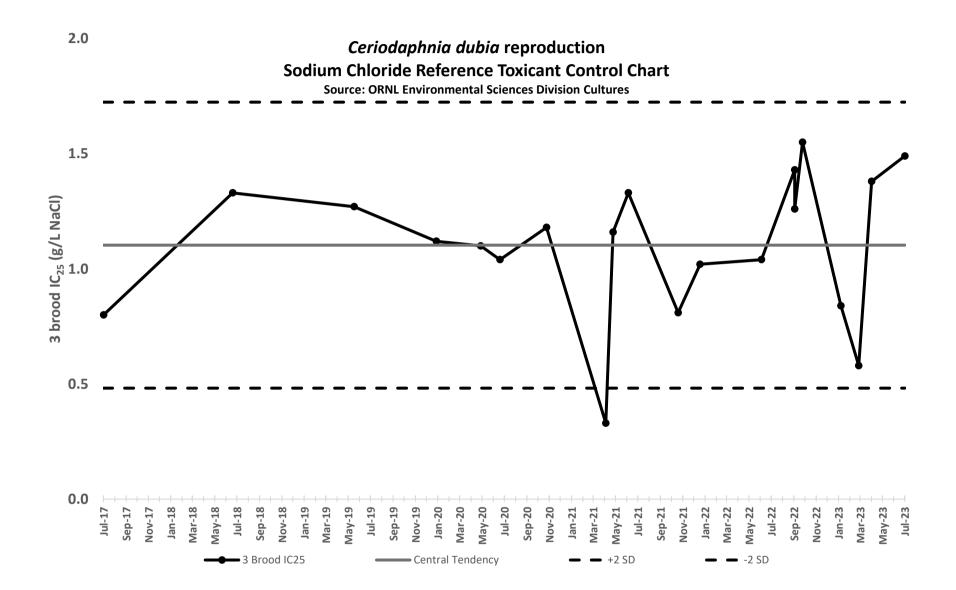
Instruments were calibrated and standardized according to manufacturer's instructions.

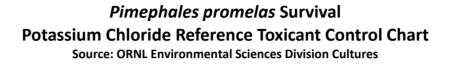
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

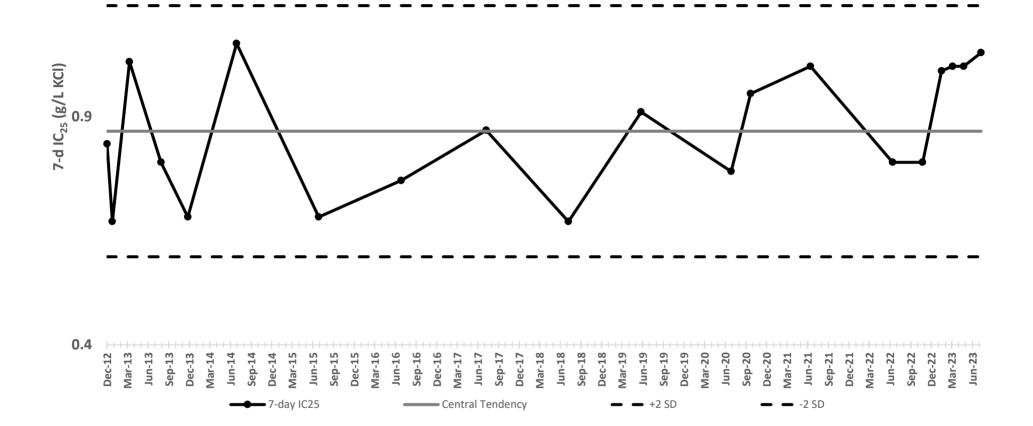
Report prepared by: Trystan A. BordeauDate: 17 August 2023Report reviewed by: Louise Stevenson Louise StevensonDate: 28 August 2023

**REFERENCE TOXICANT CONTROL CHARTS** 

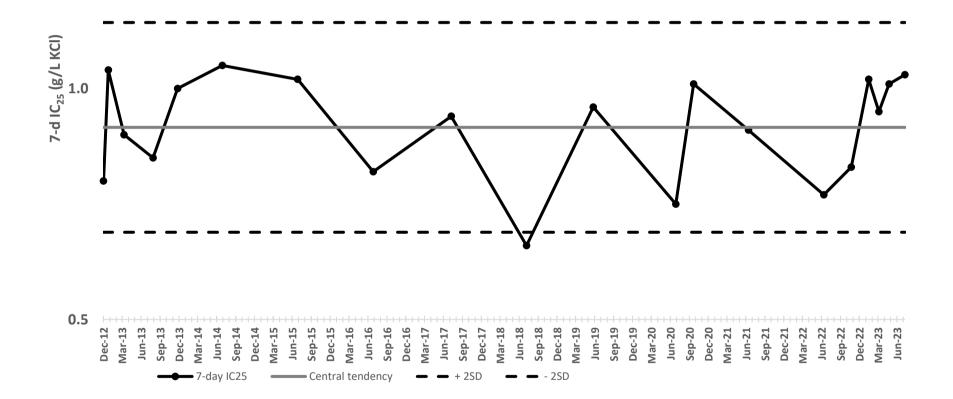








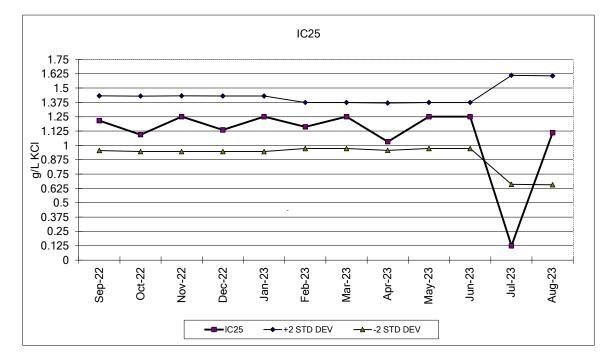
Pimephales promelas Growth Potassium Chloride Reference Toxicant Control Chart Source: ORNL Environmental Sciences Division Cultures





1300 Blue Spruce Drive, Suite C Fort Collins, Colorado 80524 Toll Free: 800/331-5916 Tel:970/484-5091 Fax:970/484-2514

Pimephales promelas



#### Chronic 7 Day Survival Test Data

#### IC 25 for Growth Test

Date	NOEC	LOEC	Date	IC25	95% Co	onfidence	Avg. IC25	+2 STD	-2 STD
	(g/L KCI)	(g/L KCI)		g/L KCI	(upper)	(lower)	g/L KCI	DEV	DEV
Mar-23	0.50	1.0	Mar-23	1.250	1.250	1.210	1.173	1.374	0.972
Apr-23	0.50	1.0	Apr-23	1.032	1.272	0.023	1.163	1.369	0.957
May-23	0.50	1.0	May-23	1.250	1.250	1.141	1.173	1.374	0.973
Jun-23	0.50	1.0	Jun-23	1.250	1.250	1.250	1.173	1.374	0.97
Jul-23	0.50	1.0	Jul-23	0.125	1.705	0.074	1.136	1.611	0.660
Aug-23	0.50	1.0	Aug-23	1.110	1.316	0.320	1.131	1.606	0.65

\*\*Current Test Dates: 8/3-10/2023

Aquatic BioSystems, Inc • Quality Research Organisms

WATER CHEMISTRY DATA LOGSHEETS

05	05/1	bnsor: 1-12	Site /T		Water Cl	hemistry l	Log	ELLINA	11-80 10	
1	Spo	onsor: <u><u>y</u>-12</u>	Site/Treatm							-
	INOT	te: Not all parameters a Observation Day:	0 PA/THE							-
		Date/Initials:	108/09/23	08/10/23	08/11/23	-	8113/23	114/23	8/15/23	08/18
-	-	5-digit ORNL ID	33462 -	>	33463-	811-1-5		33464 -	During	- Holton
	Rec	c. temp. (°C) (New ✓)	secure \$		seccor .			See or the		2
		DMW Batch #	9.70	970	970	970	970	DE 470/11		8
-	3	Conductivity (µS/cm)	242	231	230	211	201	7192/274	22524300	27
~	A	Alkalinity (mg/L)	104	/			/	1020	102	
9	S	Hardness (mg/L)	120	/		/	/	100	100	2
-	0	pH(S.U.) Initial	8.14	811	8.01	8.24	8.16	27.95/8.1	8.15	1
_	:lo	Final CD/FHM		8.21/7.59	832/7.98	8.37/7.94		8.43/7.8	8.38/7.88	235
	Control:	DO (mg/L) Initial	8.43	8.42	8.40	8.34	8.30	******		
-	Ŭ	Final CD/FHM	an a	8.34/6.67	8.50 7.37	84417.35		8.65/65	8.49/7.32	841
_		Conductivity (µS/cm)	266	255	PL= 523	234	232	PK2237252		1
		Alkalinity (mg/L)	/	/	/	/	/	1	1	
		Hardness (mg/L)	/	/	/	/	/	/	/	
M		Chlorine (mg/L)	/		/		/	/	/	1
	N	pH (S.U.) Initial	8.20	8.10	8.03	7.99	8.03	15.098.19	8.31	
	é	Final CD/FHM	200000000000000000000000000000000000000	8.36/7.94		8.277.44	8.44/7.91	8.19/78	839/783	837
_		DO (mg/L) Initial	8.45	8.34	8.37	8.413	8.25	8461825	5.34	1
		Final CD/FHM				850/7.40		8.28/7.0	8.28/7.17	64.6
		Conductivity (µS/cm)	289	279	PK28033	244	252	202	200	£
		Alkalinity (mg/L)	/	/	/	/	/	/	1	
		Hardness (mg/L)	/	/	/	/	/	/	/	
3	22	Chlorine (mg/L)	2	0.114	805			1	/	2
)	2.5	pH (S.U.) Initial	8.22	8.14	8.05	7.497	8.07	8.09	8.22	-
	-	Final CD/FHM		841/7.92	8.37/7.97	8.27/7.94	8.46/7.91	8.43/7.84		8.96
		DO (mg/L) Initial	8.44	8.35	8.44	8.52	8.31	8.23	8.40	10000
_		Final CD/FHM		8.64/6.45		8.53/7.50	8.71/7.05	8.64 1.05		BILL.
		Conductivity (µS/cm)	339	330	P13== 290	277	268	302	295	
-		Alkalinity (mg/L)	/	/	-/-	/	/	/	1	
6		Hardness (mg/L) Chlorine (mg/L)	/	/	/	/	/	/	/	-
9		pH (S.U.) Initial	8.23	8.19	8.02	7.998	8.09	010	421	-
-	M	Final CD/FHM			8.40/7.99		8.71/7.93	8.43 7.87	5.21 841/7.83	24)
_		DO (mg/L) Initial	8.43	8.71	143.938.5	91.25	8.61	8.26	541/7 85	= 1/
		Final CD/FHM	1		8-62/6.77	8.52.1711	8.71/7.12	8.57/7.04		Rut
	-	Conductivity (µS/cm)	434	431	341	340	337	399	363	0-11
_		Alkalinity (mg/L)	171	1	11/	5.0	11/	-17	203	
		Hardness (mg/L)	/	/	/	/	/	/	/	
>	Ne	CONTRACTOR - CONTRACTOR - CONTRACTOR	/	/	/	/	/	/	/	
-	29	Chlorine (mg/L) pH (S.U.) Initial Einal CD/EHM	8.23	8.20	8.02	7.478	8.08	8.20	3.19	
		Final CD/FHM			8.42/801	8.39/8.01	8.45/7.89	8.50/7.90		841
		DO (mg/L) Initial	8.69	8.94	8.53	8.781	9.07	8.30	5.64	
_		Final CD/FHM		8.60/6.07				the second se		840
		Conductivity (µS/cm)	601	624	463	465	466	498	497	
		Alkalinity (mg/L)	168	1	138	/	/	162	/	1
_		Hardness (mg/L)	260	/	230	/	/	200 1	1	
$\langle$	8	Chlorine (mg/L)F/T	10.01/0.01	/	0.02/0.03	/	/	20.01/0.0	1	1
-	100%	pH (S.U.) Initial		8.24	8.07	7.93	7.99	8.14	516	-
_		Final CD/FHM		8.54/816	8.44/8.12	8:34/810	8.41/7.98			8.48
		DO (mg/L) Initial	8.91	9.45	9.72	8.98	10.12	9.77	8.70	1
-		Final CD/FHM		8.42/0.51			8.40/0.72			CT LAR

E

**CHAIN OF CUSTODY FORMS** 

### ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/Y) OB/09/23	ESD TEST NAME	UX TEST	NAME OF SAMPLER	SARLAND 3	WILLIAMS	FRIG	CHAIN-OF-CUSTODY NO 031138
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	<u>#7009</u> REMARKS <u>#5102</u> <u>ТЕМР</u> <u>С/2</u> 11.6 <0.05
TOX TEST	200	0730	С	1	13L	4°	11.6 <0.05
	-			m/ -			
			081	0 4/23 1/2			
	/					- F	
THERMOMETER NO.							
	thong Id	rland	-		DA	TE 8/9/2	3 TIME O810 AM
SAMPLES RECEIVED BY	Reijia Kur	CULT -CA			DA	TE 8/9/23	

## ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 08/11/23	ESD TEST NAME	DX TEST	NAME OF SAMPLER	ARLAND /D.	CRAZE	FRIG	CHAIN-OF-CUSTODY NO.	39
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	ТЕМР (°С)	# 7009 TEMP	# 5102
TOX TEST	200	0735	C	1	~ 16L	3°	12.9	<0.05
			4.0	Pil				
				×. E	1/20			
				1	23			
						-		
THERMOMETER NO.								
SAMPLES RELINQUISHED BY	S. Salla	nd				ATE 8/11/23	TIME OBIC	
UCN-18631 (3 3-92)	NIN VI	* G	PAR (G) 24 HR	COMPOSITE (C) OR	OTHER (O: DESCRIBE)	- Miles		

## ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY CHAIN-OF-CUSTODY

DATE (MM/DD/YY)	ESD TEST.NAME.	TEST	NAME OF SAMPLERS	RUAND / 3	T. WILLIAMS	FRIG	CHAIN-OF-CUSTODY NO.	40
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	H 7009 REMARK	EINI
TOX TEST	200	0730	C	1	~17 L	3°	10.8	<0.05
				E.C.	\$			
					14/23	5		
		2 0			DA		TIME	12/m
ll.	R. Yall	and			Da:	8/14/23	3 11ME 0827	

**TOXICITY TEST LOGSHEETS** 

	PROJEC		and the second				_1101	EBOOKING	
			Toxicity Test					000	
Sponsor:	4-12	Sit	e/Treatment:_	OFZ	00	Test nu	mber 🖌	2992	
Test begi	in date (Day	0) Te	est end date	T	est durati	ion	Tem	plate number	
081	69/23	08	116/23	7	· 🗆 hours	s 🖬 days	D NA	₫_1	
Fest Organisn	n: Date:	Isolated fr	ia om: 08/08/23 1620	□ Fathead m Hatch date: Delivery date:		Not	Other: tes:		
Test per Chron	nic e		<b>µrpose</b> gulatory vestigative	Test sta Preli Ana Re-t	iminary lytical		est type Effluer Receiv Substa	nt ed waters	
	nt descriptio		* Trma**	Mumber	Treatmo	mt Descript	ion*	Trucks	
Number		t Description	* <u>Type**</u>			ent Descript	1011.	Type**	
1=	0.011					25%			
2 =		70		1		50%			
3 =	12.5			f 6=	4	10%		DCBT	
IT DIVIW, ID	ICITICE DRICH HUIT		ntrol, T= Treatment						
Dilution `	Water Type	:: ble	□ Other (des r (DMW) + Trac		Batch nu	umber: <u>9</u> -	10, 911		
Dilution ` C Source of	Water Type Not applicat 25% Dilute 1 f Test Organ	: Mineral Wate nisms: s: Board num	□ Other (des er (DMW) + Trac bers: □ NA ☑_	ce Metals		umber: <u>9</u>	<u>10, 9</u> 71		
Dilution C Source of	Water Type Not applicat 25% Dilute 1 f Test Organ	: Mineral Wate nisms: s: Board num	□ Other (des r (DMW) + Trac	ce Metals			<u>10, 9</u> 71		
Dilution ` Source of	Water Type Not applicat 25% Dilute 1 f Test Organ	: ole Mineral Wate nisms: s: Board num	□ Other (des er (DMW) + Trac bers: □ NA ☑_	ce Metals		umber: <u>9</u> -	10, 911		
Dilution Source of Water de	Water Type Not applicat 25% Dilute f Test Organ ESD culture Vendor:	ble Mineral Wate nisms: s: Board num s: ble Sample Sample	□ Other (des er (DMW) + Trac bers: □ NA ☑_	ce Metals <b>4790 - 6</b> er (describe): Date:0	4791 3/091>3	_COC #: COC #:	03113 03113	38	
Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: elivery dates Not applicat	i ole Mineral Wate nisms: s: Board num s: ble Sample Sample Sample	□ Other (des er (DMW) + Trac bers: □ NA ☑ □ Othe = ID: <u>33462</u>	ce Metals 	4791 3/091>3 8/11/23 8/11/23	_ COC #: _ COC #: _ COC #:	03113 03113 03114	38 39 40	
Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: livery dates Not applicat	Mineral Wate Mineral Wate nisms: s: Board num s: Board	□ Other (des or (DMW) + Trac bers: □ NA $\boxed{2}$ □ Othe □ D: <u>33462</u> ations from M	ce Metals          u?90 - 1         er (describe):         Date:         Date:	4791 3/091>3 8/11/23 98/1423 or Test No	_COC #: COC #: COC #: <b>DN-Confor</b>	03113 03114 03114 mities	38 39 40 Initial	
Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: livery dates Not applicat	Mineral Wate Mineral Wate nisms: s: Board num s: Board	$\Box \text{ Other (deserved)}$ or (DMW) + Trade bers: $\Box \text{ NA } \Box 4$ bers: $\Box \text{ Other (deserved)}$ bers: $\Box \text{ NA } \Box 4$ bers: $\Box \text{ NA } \Box 4$ bers: $\Box \text{ SA } 462$ bers: $\underline{33464}$	ce Metals          u?90 - 1         er (describe):         Date:         Date:	4791 3/091>3 8/11/23 98/1423 or Test No	_COC #: COC #: COC #: <b>DN-Confor</b>	03113 03114 03114 mities	38 39 40	
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Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: livery dates Not applicat	Mineral Wate Mineral Wate nisms: s: Board num s: Board	□ Other (des or (DMW) + Trac bers: □ NA $^{-}$ □ Othe □ D: <u>33462</u> □ D: <u>33464</u> ations from M <u>&gt; Wenz 0, 6.25</u>	ce Metals <b>4790</b> - 1 er (describe): Date:0 Date:0 Iethod and/o ,12.5, 25, 5	4791 3/091>3 8/11/23 98/14/23 or Test No 0, 10% (3	_COC #: COC #: COC #: <b>DN-Confor</b>	03113 03114 03114 mities	38 39 40 Initial	
Dilution	Water Type Not applicat 25% Dilute 1 125% Di	ele Mineral Wate nisms: s: Board num s: ble Sample Sample Sample cord of Devi escription	□ Other (des or (DMW) + Trac bers: □ NA $\boxed{2}$ □ Othe □ D: <u>33462</u> ations from M	ce Metals <b>4790</b> - 1 er (describe): Date:0 Date:0 Iethod and/o ,12.5, 25, 5	4791 3/091>3 8/11/23 98/14/23 or Test No 0, 10% (3	COC #: COC #: COC #: COC #: Dn-Confor	03113 03113 03114 mities	38 39 40 Initial	
Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: elivery dates Not applicat Not applicat	initian service Name	□ Other (des or (DMW) + Trac bers: □ NA $\Box$ □ Othe □ Othe □ D: <u><math>33462</math></u> □ D: <u><math>33464</math></u> ations from M <u>b</u> Were 0, <u>6.25</u>	ce Metals <b>4790</b> - 1 er (describe): Date:0 Date:0 Iethod and/o ,12.5, 25, 5	4791 3/091>3 8/11/23 98/14/23 or Test No 0, 10% (3	COC #: COC #: COC #: Dn-Confor tanded dib	03113 03113 03114 mities -tiay, Date	38 39 40 Initial	
Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: elivery dates Not applicat 25 Dr 25 Dr 25 Dr 26	initial Water Mineral Water nisms: s: Board num s: ble Sample Sample Sample cord of Devi Sample scription Intion serie	□ Other (des or (DMW) + Trac bers: □ NA $\Box$ □ Othe • D: <u>33462</u> • D: <u>33464</u> ations from M <u>&gt; Were 0, 6.25</u> Quality Assu- a. km	ce Metals <b>4790</b> - 4 er (describe): Date:0 Date:0 Iethod and/o ,125, 25, 5 urance (QA)	4791 3/091>3 8/11/23 98/14/23 or Test No 0, 10% (3	COC #: COC #: COC #: Dn-Confor tanded dib	03113 03114 03114 mities +tisy, Date 08/	38 39 40 Initial Ple 16/2023	
Dilution	Water Type Not applicat 25% Dilute 1 f Test Organ ESD culture Vendor: elivery dates Not applicat 23 Dr 23 Dr 23 Dr 23 Dr 23 Dr 23 Cr 23 Cr 24 Cr 25 Cr 25 Cr 26 Cr 26 Cr 27 Cr	Mineral Wate Mineral Wate nisms: s: Board num s: ble Samp	□ Other (des or (DMW) + Trac bers: □ NA $\Box$ □ Othe □ Othe □ D: <u><math>33462</math></u> □ D: <u><math>33464</math></u> ations from M <u>b</u> Were 0, <u>6.25</u>	ce Metals  4790 - 4  r (describe): Date:0 Date:0 Date:0 Date:0 Iethod and/0 ,12,5, 25, 5  urance (QA)	4791 3/091>3 8/11/23 98/14/23 or Test No 0, 10% (3	COC #: COC #: COC #: Dn-Confor tanded dib	03113 03113 03114 mities =tio), Date 08/ 08/ 08/ 08/	38 39 40 Initial	

Dail	ly Test Info		erature nation DD200		ood codes:	docelis, B=	st-cerophy Brine shrin	ap)	Test Ir		Water Chang mination	ge, or Test	Samj	ole Info
Test day	Date	Env. Chamber (C)	Test Chamber (C)	Food Type	Food Prep Date	Volume (µL)	Confirm cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	An	alyte
Day 0	08109/23 PK	<u>25.5</u> am pm	<u>25,0</u> am pm	YCT RASU.	07/25/23	100	VZ Yes	<u>1050</u> am pm	1030	1120	33462	970	N	A · ·
Day 1	08/10/23 PK	<u>.25.8</u> am pm	<u>25.0</u> am pm	YET RASU	07/25/23	100	√ZYes	<u>1118</u> am pm	1100	1149		970		•
Day 2	08/11/23 pk	25.0 am	23.2 am	RASIN	07/25/23 08/08/23	96	VZ Yes	<u>1130</u> am pm	1110	1205	33463	970		
Day 3	08/12/23 PK	<u>25-7</u> am pm	24.6 am		07/25/23	100	VZ Yes	1620 am	1000	1110		970		
Day 4	08/13/23 PIL	<u>26.0</u> am pm	<u>25.2.</u> am pm	YCT RASU	07/25/23	94	YZYes	1020 am pm	1007	(130	$\downarrow$	970		
Day 5	08/14/23 PK	23-8 am	25.3 am	YUT RASU	07/25/23		(ZYes	1(20 am pm	1100	1240	33464	971	•	
Day 6	08/15/23 PK	<u>27.9</u> am pm	<u>27.3</u> am pm	yct Rasu	07/25/2) 08/08/23	(00 92	<b>V</b> ZYes	1000 am	0940	1145	33464	971		
Day 7	08/16 (73	am	am	pk			□Yes	am . pm	0831	0940 PK				V

22

CHRONIC Daily Water/Feeding Log

Environmental Sciences Division 200

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08/08/23

NOTEBOOK NO. Rev. 03 2020-06-05

249

	250		VI2 Cer			ival & Reprodu	iction Log	1 204	2 44
14	P	Project:	08/09/23	_ Test site/cl End Date:			Test Nt Templa	te Number: 294	7 7
1		C	odes: (-) Alive and 1	No Reproduction; (	N) Alive and Repro	oduction; (xN) Dead			-
	Test	Treatment	Day: 1 PK	2 PK	3 PK	4 .94	5 PK	6 PK	TP
	Chamber	Number	Date: 08 10 23	08/11/23		08/13/23	08/14/23	08/15/23	08/16/23
-	1	6	-	~	8	-	10	26	-
-	2	3	-		5	-	13	-23 15	18
	4	6	-	-	9	9		14	23 -
	5	4.		-	5	10	X		
sh	6	1	-	-	7	1	13	23	*
	7 8	2 3	-	-	5	10	-	15.	13
	- 9	5	-	-	55	14	1 5	14	22
1	10	2	-	-	.X2 -				-
~	11	1	-	-	7	-	15.	16	6 -
-	12	5	-	-	7		11	21	-44
-	- 13	1	-	.1	1.	-	8	20	16
	14	4	-	-	56	12	-	16	-
	16	3	-	-	7		17	20	16
2-	17	5		-	5	. 10	-	22	. 4
2	18	.6	-	-	5	13	4-	16	14
5	<u>19</u> 20.	2	-	1	4 X2 -	15	-	-	R.A.
-	20.	4	-		4	-	9	12	-
	22	4		-	6	-	17	19	18
	23	4	-	-	65	12	-	25	20 _
	- 24	2	-	-	X4 -				-
Na_	25	1		-	6	-	14	22	
2	20	4.	-		X4 -		-	+6	27
2	28	1	-	-	X				
1	- 29	4	-	-	-	11	-	24	22
	30	1		-	6	7	6	防水家-	
-	31	2	-		58	-	13	25	23
Y	32 .	3			6	7	.14	10 23	19 -
	34	1	-		6	-	10	21	13 -
	- 35	3	-	-	6	5	-	20	24
	36	5	-	-	. 9	12	-	19	19
<u></u>	37	3	-	-	2	12 13	-	22	14
5	39	4	-	-	34	6	-	29 8	25
-	40	5	-	-	4	12		24	23
1	41.	3	-	-	5	-	9	20	2.0
-	42	6		-	5	-	13	22	21
~ <u> </u>	43	5		-	X2 -	13		20	
	44		-		6	10	15	20 24	123
	- 46	2.	-		7	-	-	18	27
4	47	1	-	-	8	. 14		15	22
1	48	5			8		16	26	21 -
	49 50	3	-	-	4.	14	. 10 X	17	25
	51	6	-	-	Q	-	13	26	16
	52	2	-	-	86	-	13	19	20 -
	53	2	-	-	6	-	. 13	14	12
	- 54	3			6	. 4	. 1	X	
	55 56	26	-	-	6 9		15 X	22	21
	57	6	-		- 1.	14	X	17	
-	58	2	-		6	-	10	19	
-	59	1	-		G	10	X3 -		-
	60	4			4	8	BK malili	millin	here -
	Environmen	tal Sciences Div	/1S1011				PK 03/14		Rev. 02 2000-01

		Transister (Trans	Information Class		1.45 - 10-10
			Information Sheet		169
ponsor: <u>\</u> -	-12	Site/Treatment:	0F200	Test nu	mber
Fest begin date	e (Day 0)	Test end date	end date Test durati		Template numb
08-09-2	3	08-16-23	□ hours	Adays	XNA D
fest l	□ Ceriodaphnia	dubia	Fathead minnow		Other:
Organism:	Isola	ted from:		Not	
	Date:		Hatch date: 08107/23		4
	Time:		Delivery date: 08108123	-	
Test period		st purpose	Test stage	Т	'est type
Chronic		Regulatory	Preliminary		Effluent
□ Acute	. L	] Investigative	Analytical		□ Received waters □ Substance
Creatment des	criptions:				
	eatment Descrip			nt Descripti	ion* Type**
1 =	DAW 251.	EC DŢ	4=	25.1.	DC M
2=	6.25.1.			50 1.	
3 =	12.50%			00'1.	
TEDIAW include B		= Control, T= Treatment			
		□ Other (desc Vater (DMW) + Trac	rribe): e Metals Batch nur	nber: <b>9</b> 7	<u>0-9</u> 11
⊠25% Source of Test	Dilute Mineral V Organisms:	Vater (DMW) + Trace		nber: <u>9</u> 1	<u>0-9</u> 71
⊠25% Source of Test	Dilute Mineral V Organisms:	and the second		nber: <b>97</b>	<u>0-9</u> 71
⊠25% Source of Test	Dilute Mineral V Organisms: cultures: Board :	Vater (DMW) + Trace	e Metals Batch nur		<u>0-9</u> 71
⊠25% Source of Test □ ESD	Dilute Mineral V Organisms: cultures: Board : or:ASS	Vater (DMW) + Trace numbers: □ NA □	e Metals Batch nur	nber: <b>٩٦</b>	<u>0-9</u> 11
⊠25% Source of Test □ESD ⊠Vend Water delivery	Dilute Mineral V Organisms: cultures: Board r or: <u>ABS</u> v dates:	Vater (DMW) + Trace numbers: □ NA □ □ Other	e Metals Batch nur (describe):		
⊠25% Source of Test □ESD ⊠Vend Water delivery	Dilute Mineral V Organisms: cultures: Board : or: <u>ABS</u> v dates: pplicable San San	Vater (DMW) + Trace numbers: □ NA □ □ Other nple ID: <u>33462</u> nple ID: <u>33463</u>	e Metals Batch nur (describe): Date: Date:	COC #: COC #:	0-971
⊠25% Source of Test □ESD ⊠Vend Water delivery	Dilute Mineral V Organisms: cultures: Board : or: <u>ABS</u> v dates: pplicable San San	Vater (DMW) + Trace numbers: □ NA □ □ Other nple ID: <b>_33u</b> હ <b>2</b>	e Metals Batch nur (describe): Date: Date:	COC #:	031138
⊠25% Source of Test □ESD ⊠Vend Water delivery	Dilute Mineral V Organisms: cultures: Board : or: <b>ASS</b> v dates: upplicable San San San	Vater (DMW) + Trace numbers: □ NA □ □ Other nple ID: <u>33462</u> nple ID: <u>33463</u> nple ID: <u>33464</u>	e Metals Batch nur (describe): Date: Date: Date: Date: Date: Date: Date: Date: Date: Date: Date:	COC #: COC #: COC #:	031138 031138 031139 031140
⊠ 25% Source of Test □ ESD ⊠ Vend Water delivery □ Not a	Dilute Mineral V Organisms: cultures: Board : or: <u>A8S</u> v dates: upplicable San San San	Water (DMW) + Trace numbers:  NA  Other Dother NDIE ID: <u>33462</u> NDIE ID: <u>33463</u> NDIE ID: <u>33464</u> Deviations from Mo	e Metals Batch nur (describe): Date: Date:	COC #: COC #: COC #:	031138 031138 031139 031140 mities
⊠25% Source of Test □ESD ⊠Vend Water delivery	Dilute Mineral V Organisms: cultures: Board r or: <u>A8S</u> v dates: upplicable San San <u>Record of I</u> Description	Water (DMW) + Trace numbers:  NA  Other Deviations from Mo	e Metals Batch nur (describe): Date: Date: Date: Date: ethod and/or Test Nor	COC #: COC #: COC #: <b>n-Conform</b>	031138 031138 031139 031140 mities Initial
⊠ 25% Source of Test □ ESD ⊠ Vend Water delivery □ Not a	Dilute Mineral V Organisms: cultures: Board r or: <u>A8S</u> v dates: upplicable San San <u>Record of I</u> Description	Water (DMW) + Trace numbers:  NA  Other Deviations from Mo	e Metals Batch nur (describe): Date: Date: Date: Date: Date: Date: Date: Date: Date: Date: Date:	COC #: COC #: COC #: <b>n-Conform</b>	031138 031138 031139 031140 mities Initial
⊠ 25% Source of Test □ ESD ☑ Vend Vater delivery □ Not a	Dilute Mineral V Organisms: cultures: Board r or: <u>A8S</u> v dates: upplicable San San <u>Record of I</u> Description	Water (DMW) + Trace numbers:  NA  Other Deviations from Mo	e Metals Batch nur (describe): Date: Date: Date: Date: ethod and/or Test Nor	COC #: COC #: COC #: <b>n-Conform</b>	031138 031138 031139 031140 mities Initial
¥25% Source of Test ☐ ESD ¥ Vend Vater delivery ☐ Not a	Dilute Mineral V Organisms: cultures: Board r or: <u>A8S</u> v dates: upplicable San San <u>Record of I</u> Description	Water (DMW) + Trace numbers:  NA  Other Deviations from Mo	e Metals Batch nur (describe): Date: Date: Date: Date: ethod and/or Test Nor	COC #: COC #: COC #: <b>n-Conform</b>	031138 031138 031139 031140 mities Initial
¥25% Source of Test ☐ ESD ¥ Vend Vater delivery ☐ Not a	Dilute Mineral V Organisms: cultures: Board r or: <u>A8S</u> v dates: upplicable San San <u>Record of I</u> Description	Vater (DMW) + Trace numbers: D NA D Other Deviations from Ma Deviations from Ma	e Metals Batch nur (describe): Date: Date: Date: Date: ethod and/or Test Nor \$,12.5,25,50, cost	COC #: COC #: COC #: <b>n-Conform</b>	031138 031138 031139 031140 mities Initial
Z25%: ource of Test □ ESD ✓ Vend Vater delivery □ Not a Date OSII:6/2>	Dilute Mineral V Organisms: cultures: Board : or:ASS or:ASS or dates: pplicable San San San Record of I Description	Vater (DMW) + Trace numbers: D NA D Other Deviations from Ma Deviations from Ma	e Metals Batch nur (describe): Date: Date: Date: Date: ethod and/or Test Nor	COC #: COC #: COC #: n-Conform	031138 031138 031139 031140 mities Initial tendoral allothing TAB
25% Source of Test □ ESD ✓ Vend Vend Vater delivery □ Not a Date OSIISIZS Procedure	Dilute Mineral V Organisms: cultures: Board : or:ASS or:ASS or dates: pplicable San San Record of I Description Diudion Series Name	Vater (DMW) + Trace numbers:  NA  Other  nple ID: <u>33462</u> nple ID: <u>33463</u> nple ID: <u>33464</u> Deviations from Mages were: 0,6.2	e Metals Batch nur (describe): Date: osloel23 Date: oslul23 Date: oslul23 ethod and/or Test Nor 5,12.5,25,50, ene rance (QA) Record	COC #: COC #: COC #: n-Conform	031138 031138 031139 031140 mities Initial tendoral officities TAB
25% :     Source of Test     □ ESD     ✓ Vend     Vend     Vater delivery     □ Not a     Date     OSlis(23	Dilute Mineral V Organisms: cultures: Board r or:ABS v dates: upplicable San San Record of I Description Diution Series Name Name	Vater (DMW) + Trace numbers: DNA D Dother mple ID: 33462 nple ID: 33463 nple ID: 33463 Deviations from Ma es were: 0,6.2 Quality Assu	e Metals Batch nur (describe): Date: osloel23 Date: oslul23 Date: oslul23 ethod and/or Test Nor 5,12.5,25,50, ene rance (QA) Record	COC #: COC #: COC #: n-Conform a 1001. (s	031138 031138 031139 031140 mities Hendord Weth Tris
Z 25%. ource of Test     □ ESD     X Vend Vater delivery     □ Not a  Date OSIN(2) Procedure Test run by: Data sheets QA	Dilute Mineral V Organisms: cultures: Board : or: ASS dates: applicable Sau Sau Record of I Description Diution Sector Name Trys : Pei	Vater (DMW) + Trace numbers:  NA  Other nple ID: <u>33462</u> nple ID: <u>33463</u> nple ID: <u>33464</u> Deviations from Ma es were: 0, 6.2 Quality Assu Fan A. Dorotcor	e Metals Batch nur (describe): Date: osloel23 Date: oslul23 Date: oslul23 ethod and/or Test Nor 5,12.5,25,50, ene rance (QA) Record	COC #: COC #: n-Conform a. 1001. (s Initial Initial PK	031138 031138 031139 031140 mitties Hendord allating THB Date Date 03109 [23 08/18/25
¥25% ource of Test ☐ ESD ¥ Vend Vater delivery ☐ Not a Date ©\$114(23) Procedure	Dilute Mineral V Organisms: cultures: Board : or: ASS dates: applicable Sau Sau Perior Second of I Description Divence Second Name Tryst	Vater (DMW) + Trace numbers: DNA D Dother mple ID: 33462 nple ID: 33463 nple ID: 33463 Deviations from Ma es were: 0,6.2 Quality Assu	e Metals Batch nur (describe): Date: osloel23 Date: oslul23 Date: oslul23 ethod and/or Test Nor 5,12.5,25,50, ene rance (QA) Record	COC #: COC #: COC #: n-Conform a 1001. (s	031138 031138 031139 031140 mities Hendord Weth Tris

CHRONIC Daily Water/Feeding Log

#### CHRONIC Daily Water/Feeding Log

Sponsor: Y-12 Test site/treatment:

ient: 0F200

Begin Date: 08 109 23 End Date: 08 16 123 Test Number: 1699

Dail	y Test Info		erature nation DD 19.		ood codes:	docelis, B=	ast-cerophy Brine shrin	np)	Test .		Water Chang rmination	ge, or Test	Sample Info
Test day	Date	Eny. Chamber (C)	Test Chamber (C)	- Food Type	Food Prep Date	Volume (µL)	Confirm cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	08/09/23 PK/TH	<u>25.6</u> am pm	<u>24.5</u> am pm	В	t3/03/23	62	VZ Yes	<u>1315</u> atm pm	1241	1338	33462	970	NA
Day 1	08/10/23	26.0 am		B B	08104123	68	₩Yes	0901 am 1418 pm	1141	1311	33462	970	NIA
Day 2	05/11/25	<u>26.0</u> am <u>26.0</u> pm	25.8 am	BB	8110123	68	∎Yes	0839 am 1413 pm	1158	1303	33463	970	N/A
Day 3	08112/23	26.1 am 26.0 pm		BB	05/11/23	68	₩Yes	0841 am 1428 pm	1053	1156	33463	970	NIA
Day 4	08/13/23	26.1 am 26.0 pm	25.6 am	8	08112123	69	₽Yes	0832 am 1402 .pm	1058	1231	33463	970	NIA
Day 5	08/14/23	26.1 am 26.0 pm	25.6 am 25.7 pm	B	08/13/23	125	<b>₽</b> Yes	0530 am 1520 pm	1142	1232	33464	970	N/A
Day 6	08/15/23	26.2 am 26.1 pm	25.8 am 25.6 pm	ß	0514/23	७५ ५५	PYes .	0830 am 1445 pm	1032	1128	33464	971	NIA
Day 7	08116123	<u>25:7</u> am pm	25.5 am			~	DYes	am pm	1052	1208			NIA

08/09/23

Notes:

Environmental Sciences Division

Rev. 03 2020-06-05

1

119

Sponsor: Begin Date:		09/22	End Date:	emical: 0	F 600		1 est ivuilib	er: 1699	
				Killed by siphonin	ng; M = Missing;	Sk = Sick; SM = 3	Small; SOR = Siph	noned and returned	l; W = Wounde
Treatment	Replicate	Position	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Number and Desc.	Number	Number	Date wis	Date mo	Date me oslizizs	Date ms 08/13/23	Datems 08/14/23	Date me	Date m
1:	1	22	10	16	10	10	9 ID	9	٩
25.1.	2	1	10	10	10	10	10	10	10
DAL	3	23	10	10	10	10	16	10	10
	4	24	10	10	10	10	10	10	10
2:	. 1	HE IN	. 10	10	10	10	10	16	10
1 151	2	5	10	10 ISM	10	10	9 ID	9	4
6.25%	3	10	10	10	10	10	10	10	10
	4 .	21	. 10	10	10	10	9 ID .	9	9
3:	1	ч	10	10	10	16	10	10	10
12 Chil	2	13	10	10	10	10	10	10	10
12.50%	3	15	10	10	10	10	10	10	10
	4	19	10	LD	10	10	10	LD	10
4:	1	16	10	io	10	10	10	10	10
	2	רו	10	10	10	16	10	10	10
251.	3	3	10	10	10	10	10 4	10	10
	4	20	10	16	10	10	10	10	10
5:	1	2	10	10	10	10	10	16	10
50%	2	8	10	10	10	10	LO	10	10
501.	• 3	9	. 10	10	10	LD	10	9 ID	9
n	4	12	10	10	10	10	10	10	10
6:	1	6	10	10	10	10	10	10	10
	2	18	10	10	ID	10	10	10	10
100%	3	٦	10	10	10	10	10		10
	4	11	10	10	10	10	10	10	10
Environmental	Sciences Divisi	ion			- 14 P	1 1 1 1	·	Re	ev. 01 2019-05
-							-		

Treatment	Replicate	Тез Сир 1	Cup 2	-
Treatment #1 25		Cupi	Cup 2	7
		21	41	_
1	2	21 /	24	
1		9 1	37 /	
1	3	34	19	_
1	4	34	19	
	257.			
2	1	39 🖌	26	
2	2	23 🖌	42 /	_
2	3	46 🖌	4	
2	4	17	5 /	_
Treatment #3 12.	50.1.			
3	1	6 1	33	
3	2	43	14	
3	3	32 🖌	3 🖌	
3	4	31 🖌	27 🖌	
Treatment #4 23	(.			
4	1	16 🖌	2	
4	2	35 🖌	8 1	
4	3	7 🖌	15	
4	4	13 /	11 /	
Treatment #5 50 ·	1.			0009/23 TAB
5	1	22 /	18 🖌	1000
5	2	25 🖌	44 🗸	
5	3	29 /	47 /	
5	4	36 /	40 1	
Treatment #6 100	»1.	-		
6	1	20 🖌	48	
6	2	38 -	10 1	
6	3	45 1	30	
6	4	1	12	

22			signment of test chambers	
Project:	4-12	_ Test site/c	chemical: orzoo Test r	number: <u>١७٩٩</u>
Position	Treatment #	Replicate	Sample ID	
1	1	2	251. DMW	
2	5	1	so ·1.	
3	4	3	25.1.	
4	3	1	12.51.	
5	2	2	6.25.1.	
6	6	1	100%.	
7	6	3	100%.	
8	5	2	50%	
9	5	3	251.	
10	2	3	6.25.1.	-
11	6	4	100%.	
12	5	4	20.1	
13	3	2	12.5%	55.12 776
14	2	1	6.25%	
15	3	3	12.5.1.	-
16	4	1	25.1.	
17	4	2	25.1.	
18	6	2	100%	
19	3	4	(2.5.1.	-
20	4	4	25%.	
21	2	4	6.25%.	-
22	1	1 2	251. Dam	
23	1	3 <b>z</b> .	.51. Dmu	-
24	1	4 2	LS.1. DAW	-

1	Fathead Min	now Wei	ight and	d Survival Data	* .	
Sponsor: y-	-12		Test nu	mber: 1699		
Test site/chem	ical: 0F20	D	Balance ID: A 009820			
Test Start Date	08104123		Test Er	nd Date: osliol2"	3	
	Date/Time: SII		End Dr	ying Date/time: Sw		
Treatment	Replicate	Date: 8/16/23		Pan + Larvae (mg) Date: <b>g</b> 117/23 Balance check: <b>G</b>	Number Surviving	
Initial	1	15.3320		10. 9070	10	
	2	15.40	076	17.2095	10	
	3	15.4		16. 4080	10	
	4	15.40		16. 9900	10	
1.	1	15.3		25. 9065	++ 9 %	
251.	2	15.4		24.8680	18	
DMW	3	15.5055		26.5025	10	
	4	15.4665		24.7730	10	
2.	1			25.2715	10	
6.25-1.	2	15.5940		25.0230	+0.951	
6.00 1.	3	15.3540		25.3725	10	
	4		650	25.3600	+29%	
3.	1	15.40	55	26.0365	10	
	2 .	15,4355		25.5590	10	
12.50%	3	15.4605		25. 9330	10	
	4	15.3055		25.5100	10	
4.	1	15.31		25.8730	10	
251	2	15.27		25.8920	10	
251.	3	15.43		25.5745	10	
	4 .	15.4170		25.2915	10	
5.	1	15.38		25.1820	10	
50%	2	15.15		25.2615	10	
501.	3	15.1445				
	4	15.33		27.2440	9	
i.	1	15.25		27.0455	10	
1001.	2	15.14		25.6980	10	
	3	15.21		26.2150	10	
	4	15.2270		27.2485	10	

Environmental Sciences Division

5/01/13 TAB

Rev. 03 2020-10-28

123

# 124

### Fathead Minnow Order & Shipment Log

Ordering Information:

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
of loth?	,uga	ABS	400	ciday pm Hatch	05105123	148	N/A

Delivery Information:

Larva source	Approx. number received	Date/time received	Received by (Initials)	
ABS	440	08108123 12:39	THE	

Monitoring	Hour								
Interval	0	1	2	. 3	4	5	6	7	
Temperature (°C)	12.9	24.2							
Time	12:48	13:59							
Thermometer ID	DDIQ	PIGE						-	
Initials	THO	mo	1						

Comments (e.g. condition of larvae received):

Environmental Sciences Division

Rev. 02 2020-10-28