From:	Vojin Janjic
To:	Water Permits
Subject:	FW: [EXTERNAL] - 03282024 National Pollutant Discharge Elimination System Discharge Monitoring Report for February for the Oak Ridge Y-12 National Security Complex (TN0002968)
Date:	Monday, April 1, 2024 7:57:36 AM

From: Hall, Eleanor Lynn [CONTR] <eleanor.hall@pxy12.doe.gov>
Sent: Monday, April 1, 2024 5:49 AM
To: Sarah Snyder <Sarah.Snyder@tn.gov>
Cc: Dana Casey <Dana.Casey@tn.gov>; kevin.crow@orcc.doe; Vojin Janjic <Vojin.Janjic@tn.gov>; Greg Mize <Greg.Mize@tn.gov>; Colby Morgan <Colby.Morgan@tn.gov>; Robert Ramsey <Robert.Ramsey@tn.gov>; EC_DMC <EC_DMC@pxy12.doe.gov>
Subject: [EXTERNAL] - 03282024 National Pollutant Discharge Elimination System Discharge Monitoring Report for February for the Oak Ridge Y-12 National Security Complex (TN0002968)

Attached is a letter from Diane R. McDaniel to Sarah Snyder dated March 28,2024, “National Pollutant Discharge Elimination System Discharge Monitoring Report for February for the Oak Ridge Y-12 National Security Complex (TN0002968).” The attached pdf file is an executed original.

Thank you,
Eleanor

consolidated nuclear security, llc
PANTEX PLANT | Y-12 NATIONAL SECURITY COMPLEX

301 Bear Creek Rd.
P.O. Box 2009
Oak Ridge, TN 37831-8111

Office: 865.576.9867
Fax: 865.241.4533

March 28, 2024

Ms. Sarah Snyder
Tennessee Department of Environment and Conservation
Knoxville Field Office
3711 Middlebrook Pike
Knoxville, Tennessee 37921-6538

Dear Ms. Snyder:

National Pollutant Discharge Elimination System Discharge Monitoring Report for February for the Oak Ridge Y-12 National Security Complex (TN0002968)

Enclosed are copies of the following documents required by the National Pollutant Discharge Elimination System (NPDES) permit effective October 1, 2022:

1. Y-12 National Security Complex Noncompliance Report for February 2024.

2. Outfall 200 Annual Biomonitoring Report for Permit Year 2023.

3. Biomonitoring report for the First quarter of CY2024.

Monitoring data collected for compliance with the NPDES permit is summarized and reported on Discharge Monitoring Report forms approved by your staff. This data is entered into NetDMR, and the forms are retained for our records.

If you have any questions or requests for additional information, please contact Kimberly Hanzelka at 865.574.1599.

Sincerely yours,

Diane McDaniel

Digitally signed by Diane McDaniel
Date: 2024.03.28 12:49:38 -04'00'

Diane R. McDaniel, Senior Director
Y-12 Environment, Safety and Health

DRM:kgh

Enclosure: As stated

C-2516

c/enc: Chloe L. Ashley, NPO
Dana Casey, TDEC
Kevin Crow, UCOR
Caitlin Hoch-Nussbaum
Vojin Janjic, TDEC
Kristopher K. Kinder
Alison K. Kring
Zachary P. Levasseur
Stacey E. Loveless
Greg Mize, TDEC
W. Colby Morgan, TDEC
Robert Ramsey, TDEC
Ashley Sexton, TDEC
Chuck Smolens, NPO
Steven M. Stone, NPO
Larissa W. Welch
Jan M. West
Laura Wilkerson, DOE
EC DMC – 1971352.5208 – RC

Enclosure 1
Letter, McDaniel to Snyder
Dated: March 28, 2024

Y-12 NATIONAL SECURITY COMPLEX
NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES)
EXCEEDANCES
FEBRUARY 2024

A. Noncompliances with Permit Effluent Limitations and Requirements

Analyses of water samples obtained during February 2024 revealed no exceedances of the NPDES permit limits at the Y-12 National Security Complex.

B. Other Events and Observations

None.

OAK RIDGE National Laboratory

PO Box 2008
Oak Ridge, TN 37831
(865) 341-0398| stevensonlm@ornl.gov

Date: December 2, 2022

To: K.G. Hanzelka (RC)

c: S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, A.M. Fortner

From: L.M. Stevenson, 1504, MS-6351 (865-341-0398).

RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted 2-9 Nov 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$_{25}$ (IC_{25}) for survival, growth, and/or reproduction were thus >100% (the highest concentration of effluent tested).

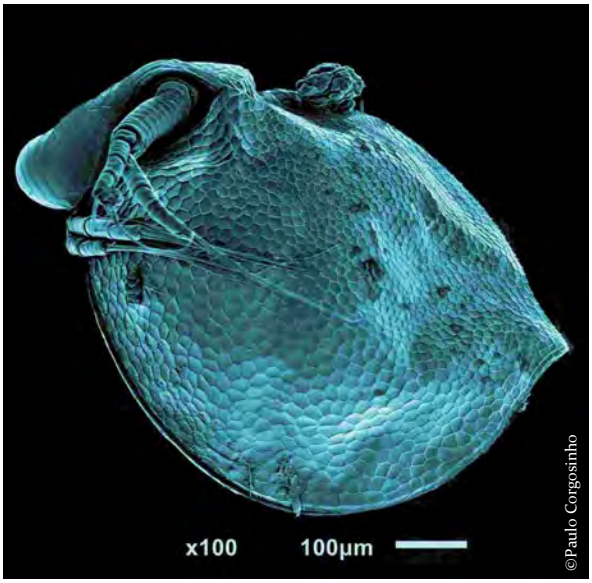
The NPDES permit states that toxicity will be demonstrated if the IC_{25} is less than or equal to the permit limit (50% effluent for Outfall 200). All of the results for all endpoints were within permit limits.

Outfall	Test Organism	Endpoint	IC_{25}
Outfall 200	Fathead minnow	Survival	>100%
		Growth	>100%
Outfall 200	*Ceriodaphnia dubia*	Survival	>100%
		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_{25}) 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 ± 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
pH (S.U.)	8.05	8.13	8.24
Conductivity (µS/cm)	418	398	415
Alkalinity (mg/L as $CaCO_3$)	114	128	141
Hardness (mg/L as $CaCO_3$)	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 ± 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 (4th 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; 4th 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_{25} for survival: 1.481 ± 0.756 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} for survival: 0.255 g NaCl/L
Central tendency of IC_{25} for reproduction: 1.027 ± 0.622 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} 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_{25} for reproduction was >100% effluent.

7.3 Summary of *Ceriodaphnia* toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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 $CaCO_3$)	82	105	105
Hardness (mg/L as $CaCO_3$)	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 (µS/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 Date: 10 November 2022

Report reviewed by: Louise Stevenson  Date: 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_{25}) 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 ± 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
pH (S.U.)	8.05	8.13	8.24
Conductivity (µS/cm)	418	398	415
Alkalinity (mg/L as $CaCO_3$)	114	128	141
Hardness (mg/L as $CaCO_3$)	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 ± 1 °C.

3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.

3.7 Mean dry weight at test initiation: 0.153 ± 0.006 mg (mean ± 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 (4th 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 ± 100 µL 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_{25} = 0.80 g KCl/L; 95% C.L. = 0.67-0.91 g KCl/L.

Growth IC_{25} = 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_{25} for survival: 0.837 ± 0.275 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} for survival: 0.164 g KCl/L

Central tendency of IC_{25} for growth: 0.92 ± 0.234 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} 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:

Survival

Concentration	Proportion surviving per replicate				Mean
	1	2	3	4	
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

Dry Weight

Concentration	Weight (mg) per replicate				Mean ± SD
	1	2	3	4	
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 ± 0.06
50%	0.64	0.6	0.68	0.59	0.63 ± 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_{25} for survival was >100% effluent.

7.2 Growth

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

7.3 Summary of fathead minnow toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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.)	8.11	8.20	8.08
Conductivity (µS/cm)	237	239	230
Alkalinity (mg/L as $CaCO_3$)	82	105	105
Hardness (mg/L as $CaCO_3$)	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 (µS/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.

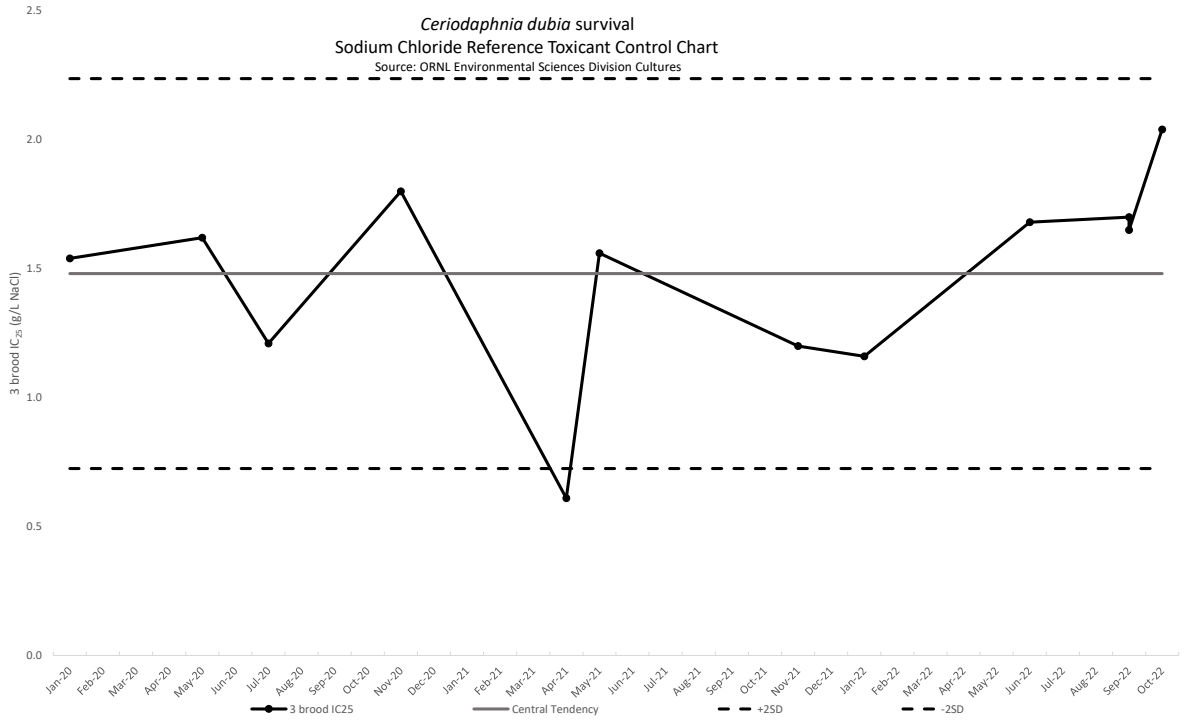
Instruments were calibrated and standardized according to manufacturer's instructions.

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 Date: 11 November 2022

Report reviewed by: Louise Stevenson Louise Stevenson Date: 2 December 2022

REFERENCE TOXICANT CONTROL CHARTS


Ceriodaphnia dubia survival
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC_{25} (g/L NaCl)
2.5
2.0
1.5
1.0
0.5
0.0
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
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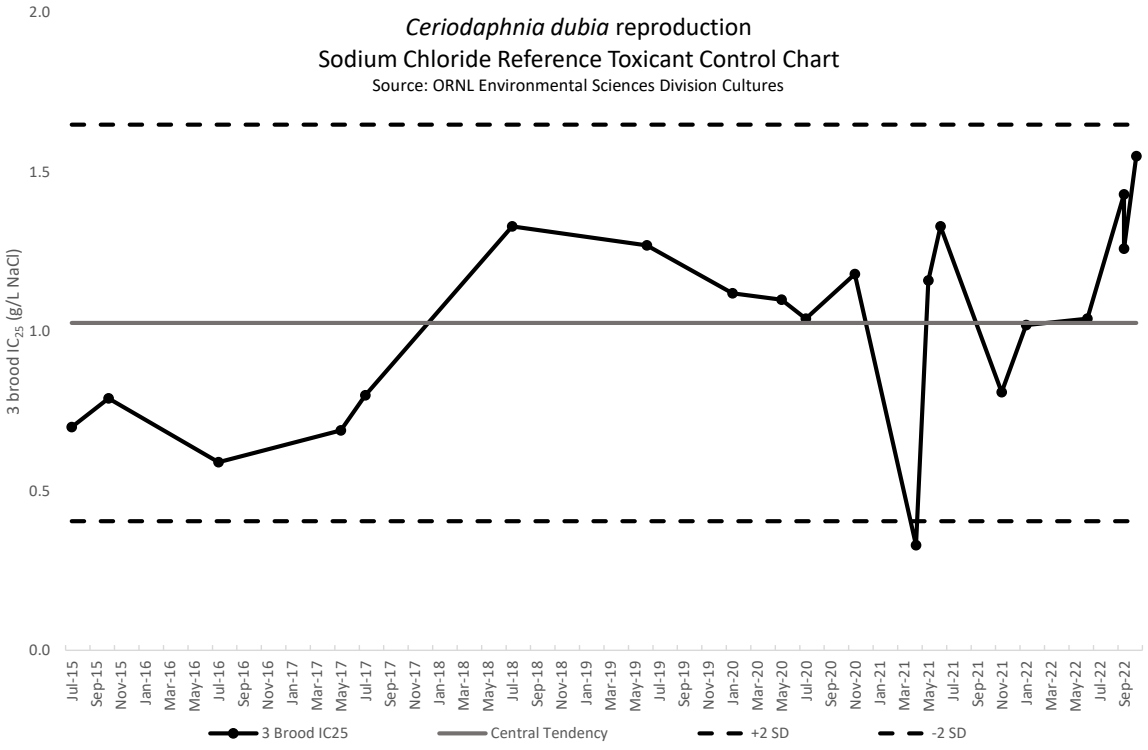
Nov-21
Dec-21
Jan-22
Feb-22
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
3 brood IC25
Central Tendency
+2SD
-2SD


Ceriodaphnia dubia reproduction
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.0
1.5
1.0
0.5
0.0
Jul-15
Sep-15
Nov-15
Jan-16
Mar-16
May-16
Jul-16
Sep-16
Nov-16
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Jul-17
Sep-17
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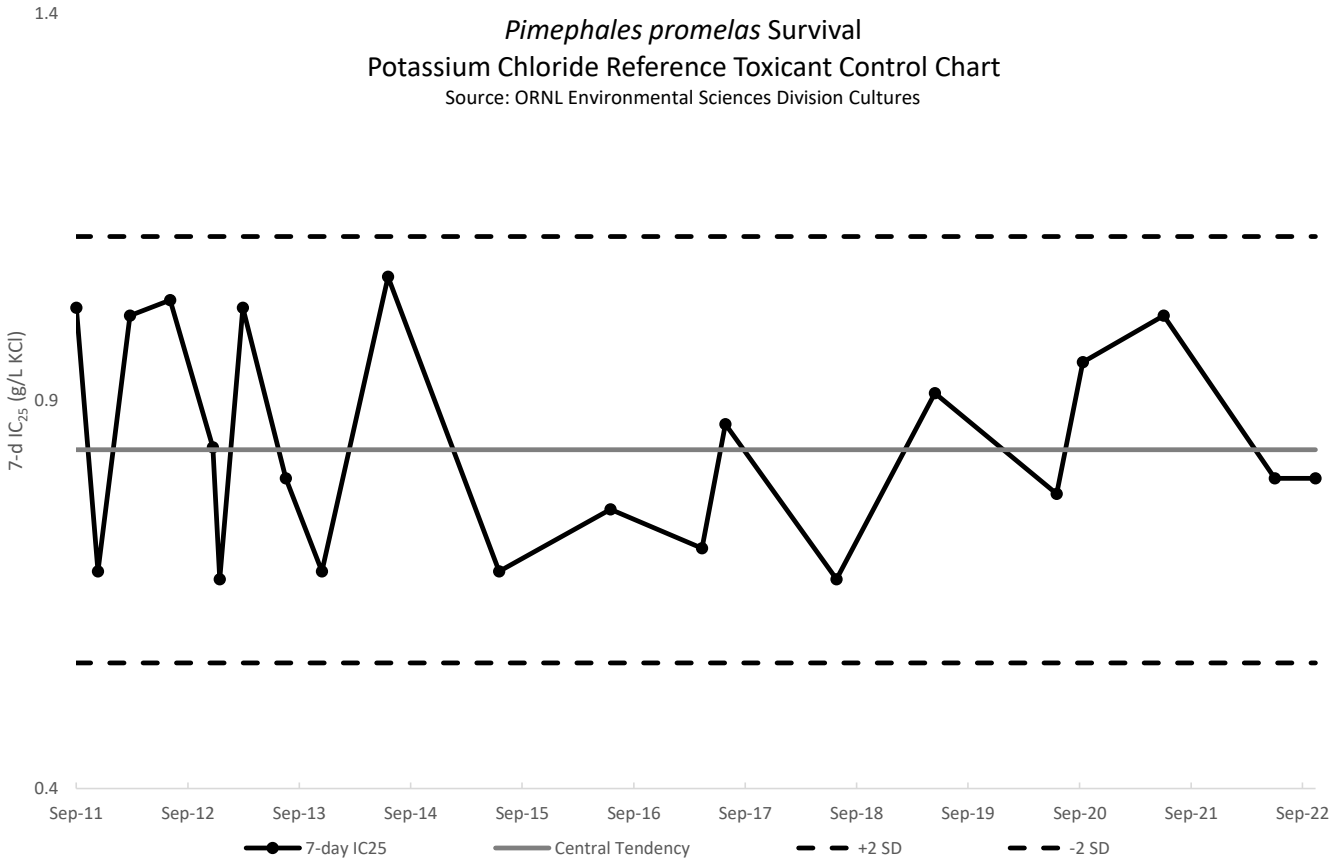
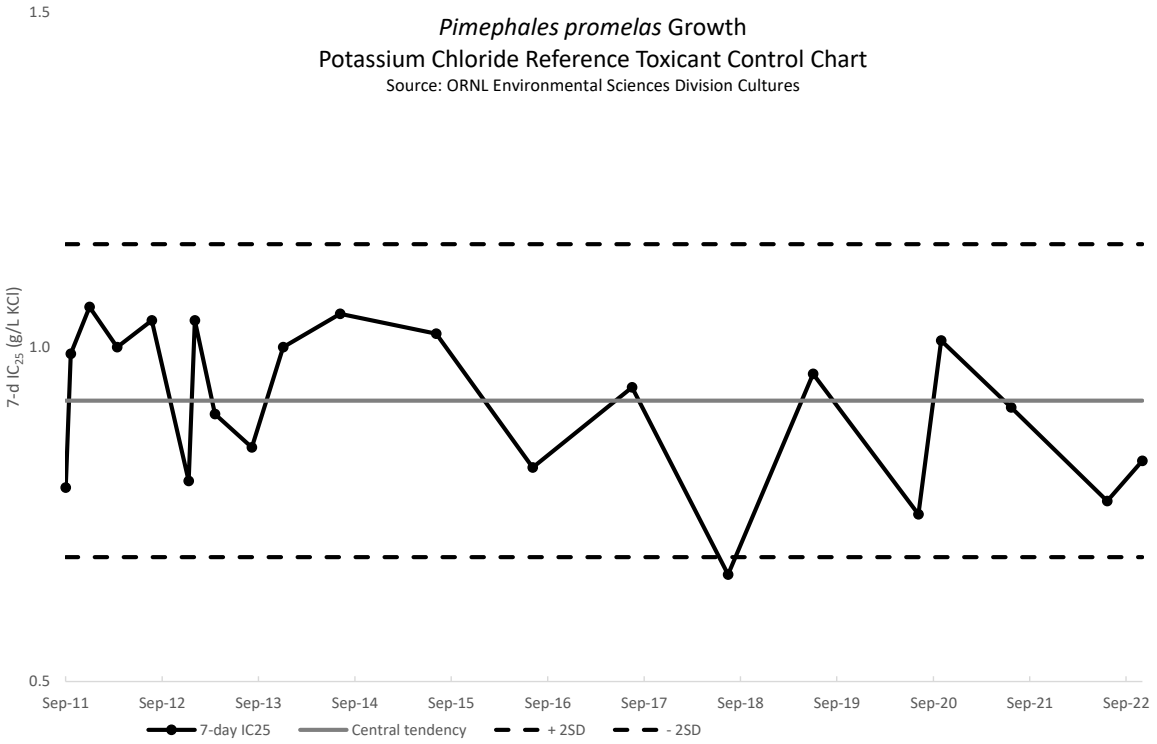
Sep-22
3 Brood IC25
Central Tendency
+2 SD
-2 SD

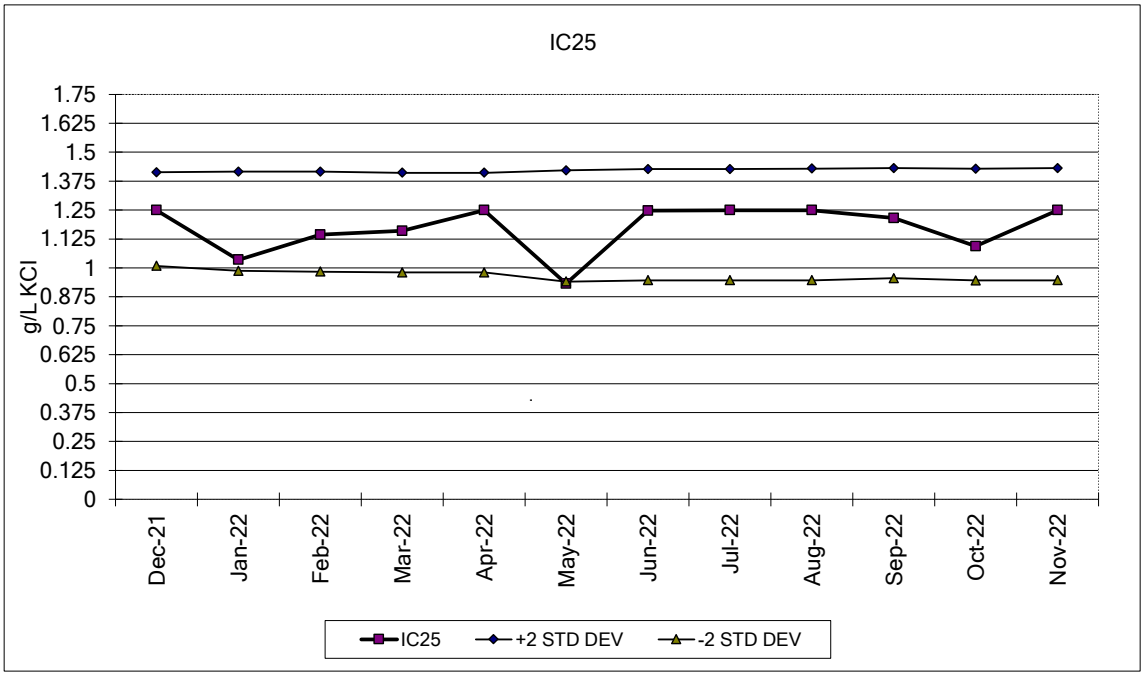

Pimephales promelas Survival
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
1.4
0.9
0.4
7-d IC25 (g/L KCl)
Sep-11
Sep-12
Sep-13
Sep-14
Sep-15
Sep-16
Sep-17
Sep-18
Sep-19
Sep-20
Sep-21
Sep-22
7-day IC25
Central Tendency
+2 SD
-2 SD


Pimephales promelas Growth
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
1.5
1.0
0.5
7-d IC_{25} (g/L KCl)
Sep-11
Sep-12
Sep-13
Sep-14
Sep-15
Sep-16
Sep-17
Sep-18
Sep-19
Sep-20
Sep-21
Sep-22
7-day IC25
Central tendency
+ 2SD
- 2SD

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

Date	NOEC (g/L KCl)	LOEC (g/L KCl)
Jun-22	0.50	1.0
Jul-22	0.50	1.0
Aug-22	0.50	1.0
Sep-22	0.50	1.0
Oct-22	0.50	1.0
Nov-22	0.50	1.0

IC 25 for Growth Test

Date	IC25 g/L KCl	95% Confidence (upper)	95% Confidence (lower)	Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
Jun-22	1.247	1.252	1.146	1.187	1.428	0.947
Jul-22	1.250	1.250	1.222	1.187	1.428	0.947
Aug-22	1.250	1.250	1.250	1.188	1.429	0.947
Sep-22	1.215	1.271	1.072	1.193	1.432	0.955
Oct-22	1.094	1.332	0.161	1.188	1.429	0.946
Nov-22	1.250	1.250	0.907	1.189	1.431	0.947

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

WATER CHEMISTRY DATA LOGSHEETS

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Daily Water Chemistry Log

Sponsor: Y12 Site/Treatment: OF100 Associated test numbers: CD-2978 FHM-1680

PK 11/8/22

Note: Not all parameters are required for all tests. All unused cells should be lined through or marked "NA."

		Observation Day:	0 NJJ NJK	1 NJJ PK	2 MB	3 NJK AMF	4 NJJ AMF	5 NJW AMF	6 NJJ AMF	7 MB MAF
		Date/Initials:	11/2/2022	11/3/22	11.4.22	11/5/22	11-6-22	11/7/22	11/8/22	11-9-22
		5-digit ORNL ID	32924	32924	32925	32925	32925	32926	32926	
		Rec. temp. (°C) (New ✓)	SecCOC ☑	☐	SecCOC ☑	☐	☐	SecCOC ☑	☐	☐
①	Control: 25% DMW	DMW Batch #	918	918	918	919	919	919	919	
		Conductivity (µS/cm)	237	232	225	239	233	230	220	
		Alkalinity (mg/L)	92			105				
		Hardness (mg/L)	108			114				
		pH (S.U.) Initial	8.11	8.14	8.13	8.20	8.15	8.08	8.06	
		Final CD/FHM		8.48/8.07	8.52/8.01	8.30/7.79	8.38/7.91	8.39/7.93	8.47/7.98	8.45/7.86
		DO (mg/L) Initial	8.77	8.94	8.61	8.56	8.62	8.68	8.78	
		Final CD/FHM		9.36/7.93	9.19/7.59	8.44/7.51	8.54/7.10	8.82/7.40	8.81/7.47	8.70/7.45
②	12.5%	Conductivity (µS/cm)	260	256	246	259	253	251	245	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.06	8.10	8.14	8.18	8.07	8.13	8.13	
		Final CD/FHM		8.55/7.99	8.54/8.01	8.36/7.90	8.43/8.01	8.45/7.93	8.53/7.98	8.48/7.95
		DO (mg/L) Initial	8.88	8.85	8.75	8.42	8.83	8.85	8.89	
		Final CD/FHM		9.31/7.83	9.42/7.26	8.53/7.41	8.71/7.5	8.95/7.16	8.96/7.22	8.76/7.36
③	25%	Conductivity (µS/cm)	284	281	268	281	274	276	270	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.07	8.10	8.14	8.17	8.09	8.14	8.15	
		Final CD/FHM		8.53/8.03	8.60/8.02	8.38/7.95	8.44/8.00	8.46/7.97	8.52/8.00	8.50/7.97
		DO (mg/L) Initial	9.00	9.16	8.88	8.48	9.07	9.00	9.14	
		Final CD/FHM		9.20/7.71	9.40/7.26	8.58/7.31	8.72/7.11	8.98/7.18	8.93/7.16	8.85/7.33
④	50%	Conductivity (µS/cm)	331	329	313	322	317	324	320 (320)	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.07	8.06	8.11	8.13 8.00 MAF 11/5/22	8.07	8.17	8.18	
		Final CD/FHM		8.53/8.10	8.59/8.09	8.41/8.00	8.45/8.01	8.95/8.00	8.52/8.09	8.49/8.12
		DO (mg/L) Initial	9.25	9.63	9.17	8.54	9.05	9.41	9.68	
		Final CD/FHM		9.13/7.72	9.33/7.33	8.60/7.30	8.76/7.20	8.47/7.13	8.87/7.25	8.80/7.61
⑤	75%	Conductivity (µS/cm)	375	374	357	363	357	371	368	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.06	8.05	8.12	8.08	8.07	8.20	8.23	
		Final CD/FHM		8.55/8.10	8.60/8.14	8.42/8.04	8.48/8.06	8.49/8.04	8.54/8.12	8.53/8.17
		DO (mg/L) Initial	9.02	10.00	9.51	8.65	10.24	9.79	10.73	
		Final CD/FHM		9.24/7.38	9.21/7.40	8.60/7.22	8.79/7.15	8.88/7.14	8.80/7.21	8.77/7.07
⑥	100%	Conductivity (µS/cm)	418	424	398	402	397	415	416	
		Alkalinity (mg/L)	114		128			141		
		Hardness (mg/L)	170		160			160		
		Chlorine (mg/L) F/T	0.01/0.00		0.01/0.00			0.01/0.00		
		pH (S.U.) Initial	8.05	8.02	8.13	8.06	8.11	8.24	8.29	
		Final CD/FHM		8.55/8.15	8.61/8.19	8.45/8.12	8.50/8.07	8.48/8.07	8.55/8.17	8.53/8.21
		DO (mg/L) Initial	10.01	10.40	9.80	8.72	10.91	10.10	10.73	
		Final CD/FHM		9.21/7.36	9.19/7.43	8.62/7.28	8.79/6.93	8.88/7.08	8.80/7.16	8.81/7.61

CHAIN OF CUSTODY FORMS

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

DATE (MM/DD/YY)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
11/02/22	TOX	A.L. GARLAND / J.T. WILLIAMS	031069

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG. TEMP (°C)	TEMP #7008 REMARKS	Cl_2 #4102
OUTFALL 200	200	0730	C	1	~11 LITERS	3°	11.1°C	<0.05

THERMOMETER NO.

ORNL sample ID = 32924

SAMPLES RELINQUISHED BY: A. R. Garland	DATE: 11/2/22	TIME: 0810	☒ AM ☐ PM
SAMPLES RECEIVED BY: [signature]	DATE: 11/2/22	TIME: 0810	☒ AM ☐ 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)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
11/04/22	TOX	J.T. WILLIAMS / D. CRAZE	031070

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG. TEMP (°C)	YSI Temp. #7008	REMARKS	HACH Cl2 #4102
OUTFALL 200	200	0730	C	1	~14 LITERS	3°	10.3°C		<0.05

11/4/22

THERMOMETER NO.

ORM sample ID=32925

SAMPLES RELINQUISHED BY	DATE	TIME	
J.T. Williams	11-4-22	0803	☒ AM ☐ PM
SAMPLES RECEIVED BY	**DATE**	**TIME**	
	11/4/22	0803	☒ AM ☐ 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)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
11/07/22	TOX	A.L. GARLAND / J.T. WILLIAMS	031071

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C) FRIG	TEMP #7008	REMARKS	Cl_2 #4102
OUTFALL 200	200	0725	C	1	~14 LITERS	3°	11.9		<0.05

DSG 11/7/22

THERMOMETER NO.

SAMPLES RELINQUISHED BY: A. R. Garland	DATE: 11/7/22	TIME: 0830	☒ AM ☐ PM
SAMPLES RECEIVED BY:	DATE: 11/7/22	TIME: 0830	☑ AM ☐ PM

UCN-18631 (3 3-92)

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

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y12 **Site/Treatment:** OF200 **Test number:** CD 2978

Test begin date (Day 0)	Test end date	Test duration			Template number
11/02/2022	11/09/2022	7	☐ hours ☑ days	☐ NA ☑	6

Test Organism: ☑ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: Notes:

Isolated from:

Date:	11/01/2022	11/01/2022
Time:	10:00 am	5:50 pm

Hatch date: Delivery date:

PK 11/01/22

Test period: ☑ Chronic ☐ Acute

Test purpose: ☑ Regulatory ☐ Investigative

Test stage: ☐ Preliminary ☑ Analytical ☐ Re-test

Test type: ☑ Effluent ☐ Received waters ☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%	☑C ☐T	4 =	50%	☐C ☑T
2 =	12.5%	☐C ☑T	5 =	75%	☐C ☑T
3 =	25%	☐C ☑T	6 =	100%	☐C ☑T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe):

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 918

Source of Test Organisms:

☑ ESD cultures: Board numbers: ☐ NA ☑ 4727

☐ Vendor: ☐ Other (describe):

Water delivery dates:

☐ Not applicable

Sample ID:	32924	Date:	11-2-22	COC #:	031069
Sample ID:	32925	Date:	11/4/22	COC #:	031070
Sample ID:	32926	Date:	11/7/22	COC #:	031071

Record of Deviations from Method and/or Test Non-Conformities

Date	Description	Initial
11/7/22	Noticed late in the day (after running test) that YCT batch smelled like it had spoiled. Will switch to previous batch (9/13/22) for last day of test.	AMF
11/9/22	1 male in treatment 3 (cup 27)	AMF

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	[signature]	AMF	11/9/22
Data sheets QA:			
Data entered:	[signature]	AMF	11/9/22
Data entry QA:			

SIGNATURE _____ DATE ____ 20__
READ AND UNDERSTOOD _____ DATE ____ 20__

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FK 11/9/22

SIGNATURE
READ AND UNDERSTOOD
DATE
DATE
20
20

CHRONIC Daily Water/Feeding Log

Sponsor: Y12 Test site/treatment: OF200 Begin Date: 11/02/2022 End Date: 11/09/2022 Test Number: 2978

Daily Test Info		Temperature Information Therm. #: DD19		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10⁷/mL					Test Initiation, Water Change, or Test Termination				Sample Info
Test day	Date	Env. Chamber (°C)	Test Chamber (°C)	Food Type	Food Prep Date (YCT=10/18/22)	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 / pm	26.0 am / pm	YCT RASU	11/2/22 AMF 11/2/22	100 95	☑Yes	11:59 am / pm	11:28	12:27	32924	918	N/A
Day 1	11/03/22 PK	25.9 am / pm	25.2 am / pm	YCT RASU	11/2/22 AMF 11/2/22	100 91	☑Yes	10:59 am / pm	10:45	11:35	↓	918	
Day 2	11/04/22 AMF	25.8 am / pm	25.5 am / pm	YCT R	11/2/22 AMF 11/2/22	100 85 90 AMF 11/4/22	☑Yes	am / 1256 pm	1239	1328	32925	918	
Day 3	11/05/22 AMF	25.9 am / pm	25.4 am / pm	YCT R	10/18/22 11/2/22	100 83	☑Yes	1040 am / pm	1027	1137	↓	919	
Day 4	11/06/22 AMF	25.7 am / pm	25.6 am / pm	YCT R	10/18/22 11/6/22	100 88	☑Yes	1018 am / pm	1004	1116	↓	919	
Day 5	11/07/22 AMF	25.9 am / pm	25.6 am / pm	YCT R	10/18/22 11/6/22	100 91	☑Yes	1042 am / pm	1025	1128	32926	919	
Day 6	11/08/22 AMF	26.0 am / pm	25.7 am / pm	YCT R	10 9/13/22 11/6/22	100 89	☑Yes	1045 am / pm	1032	1149	↓	919	
Day 7	11/09/22 AMF	26.1 am / pm	25.5 am / pm				☐Yes	am / pm	1100	1221			↓

Notes:

Ceriodaphnia Chronic Daily Survival & Reproduction Log

Project: Y12 Test site/chemical: OF200 Test Number: 2978

Begin Date: 11/02/2022 End Date: 11/09/2022 Template Number: #6

PK 11/01/22

Codes: (-) Alive and No Reproduction; (*N*) Alive and Reproduction; (*xN*) Dead and Reproduction; (M) Male

Test Chamber	Treatment Number	Day: 1 PK Date: 11/03/22	2 AM PK 11/04/22	3 AM PK 11/05/22	4 AM PK 11/06/22	5 AM PK 11/07/22	6 AM PK 11/08/22	7 AM PK 11/09/22
1	5	-	-	7	6	-	16	21
2	2	-	-	8	-	14	21	22
3	6	-	-	6	-	15	20	21
4	3	-	-	4	-	11	19	20
5	3	-	-	7	11	-	17	21
6	6	-	-	8	-	-	21	26
7	5	-	-	6	-	-	22	22
8	4	-	-	5	11	-	20	21
9	5	-	-	-	13	-	21	23
10	4	-	-	6	12	-	17	24
11	2	-	-	6	14	-	20	24
12	3	-	-	6	-	14	20	18
13	4	-	-	7	-	14	21	19
14	1	-	-	8	11	-	22	24
15	1	-	-	4	10	-	21	24
16	5	-	-	6	13	-	20	22
17	1	-	-	6	11	-	23	26
18	2	-	-	7	X			
19	4	-	-	9	3	-	18	19
20	3	-	-	4	12	18	-	21
21	6	-	-	7	16	-	20	23
22	4	-	-	7	-	14	21	20
23	1	-	-	7	-	13	20	20
24	5	-	-	10	15	-	19	26
25	2	-	-	6	X			
26	3	-	-	2	13	-	19	24
27	3	-	-	-	-	-	-	- M
28	6	-	-	8	15	-	22	24
29	2	-	-	5	14	-	18	23
30	2	-	-	6	15	-	2	-
31	1	-	-	6	11	-	17	23
32	1	-	-	8	-	14	19	23
33	2	-	-	7	-	9	19	8
34	4	-	-	9	-	1	19	23
35	6	-	-	6	15	-	17	25
36	2	-	-	7	15	-	6	10
37	2	-	-	8	13	-	20	25
38	1	-	-	9	12	-	18	23
39	6	-	-	7	16	-	19	22
40	5	-	-	5	14	-	21	25
41	4	-	-	6	14	-	20	26
42	6	-	-	5	-	15	18	18
43	3	-	-	5	10	-	19	22
44	2	-	-	8	14	-	19	25
45	5	-	-	7	13	-	17	22
46	1	-	-	7	15	-	22	27
47	4	-	-	7	-	-	11	25
48	5	-	-	-	12	-	17	21
49	3	-	-	6	16	-	20	22
50	1	-	-	7	11	-	18	21
51	3	-	-	8	15	-	18	25
52	5	-	-	4	12	-	16	19
53	3	-	-	1	X			
54	6	-	-	8	13	-	21	18
55	4	-	-	7	14	-	20	25
56	4	-	-	7	16	-	-	2
57	6	-	-	5	3	-	21	25
58	3	-	-	7	13	-	22	27
59	1	-	-	6	10	-	23	3
60	6	-	-	7	14	18	-	19

Toxicity Test Information Sheet

Sponsor: 412 **Site/Treatment:** OF200 **Test number:** 1686

Test begin date (Day 0)	Test end date	Test duration	Template number
11-2-22	11-9-22	7 ☐ hours ☑ days	☑ NA ☐ _____

Test Organism: ☐ *Ceriodaphnia dubia* ☑ Fathead minnow ☐ Other: _____

Isolated from: Date: _____ Time: _____

Hatch date: 10-31-22 Delivery date: 11-1-22

Notes:

Test period	Test purpose	Test stage	Test type
☑ Chronic	☑ Regulatory	☐ Preliminary	☑ Effluent
☐ Acute	☐ Investigative	☑ Analytical	☐ Received waters
		☐ Re-test	☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%	☐ C ☐ T	4 =	50%	☐ C ☑ T
2 =	12.5%	☐ C ☑ T	5 =	75%	☐ C ☑ T
3 =	25%	☐ C ☑ T	6 =	100%	☐ C ☑ T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 918-919

11-2-22 MSS

Source of Test Organisms:

☐ ESD cultures: Board numbers: ☐ NA ☐ _____

☑ Vendor: ABS ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: 32924 Date: 11-2-22 COC #: 031069

Sample ID: 32925 Date: 11-4-22 COC #: 031070

Sample ID: 32926 Date: 11-7-22 COC #: 031071

Record of Deviations from Method and/or Test Non-Conformities

Date	*Description*	*Initial*
11-9-22	None.	MSS

Quality Assurance (QA) Record

Procedure	*Name*	*Initial*	*Date*
Test run by:	Nikki Jones	MSS	11-9-22
Data sheets QA:			
Data entered:	Nikki Jones	MSS	11-9-22
Data entry QA:			

CHRONIC Daily Water/Feeding Log

Sponsor: Y12 Test site/treatment: OF200 Begin Date: 11-2-22 End Date: 11-9-22 Test Number: 11686

Daily Test Info		Temperature Information Therm. #: DD19		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10^7/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	11-2-22 MJS	— am 25.5 pm	— am 25.0 pm	— B	— 11-1-22	— 111	☑Yes	— am 1532 pm	1019	1058	32924	918	N/A
Day 1	11-3-22 MJS	24.9 am 25.4 pm	24.5 am 25.1 pm	B B	11-2-22 11-2-22	96 111	☑Yes	0747 am 1344 pm	0949	1050	32924	918	
Day 2	11-4-22 MJS	25.2 am 26.1 pm	25.7 am 25.5 pm	B B	11-3-22 11-3-22	103 72	☑Yes	0805 am 1441 pm	1108	1208	32925	918	
Day 3	11-5-22 MJS	25.1 am 25.4 pm	24.7 am 24.9 pm	B B	11-4-22 11-4-22	95 83	☑Yes	0852 am 1313 pm	1053	1158	32925	919	
Day 4	11-6-22 MJS	25.1 am 25.7 pm	25.0 am 25.3 pm	B B	11-5-22 11-5-22	84 83	☑Yes	0753 am 1201 pm	0953	1039	32925	919	
Day 5	11-7-22 MJS	25.5 am 25.4 pm	25.0 am 24.9 pm	B B	11-6-22 11-6-22	102 72	☑Yes	0800 am 1508 pm	1016	1113	32926	919	
Day 6	11-8-22 MJS	25.3 am 25.3 pm	24.9 am 24.6 pm	B B	11-7-22 11-7-22	109 105	☑Yes	0800 am 1335 pm	1027	1110	32926	919	
Day 7	11-9-22 MJS	25.2 am ____ pm	24.6 am ____ pm				☐Yes	____ am ____ pm	0939	1143			

Notes:

11-2-22 MJS

Fathead Minnow Chronic Daily Survival Log

Sponsor: 412 **Test site/chemical:** OF200 **Test Number:** 1686

Begin Date: 11-2-22 **End Date:** 11-9-22

Comment Codes: C = Clear; D = Dead; Fg = Fungus; K = Killed by siphoning; M = Missing; Sk = Sick; SM = Small; SOR = Siphoned and returned; W = Wounded

Treatment Number and Desc.	Replicate Number	Position Number	Day 1 Date 11-3-22 MSF	Day 2 Date 11-4-22 MSF	Day 3 Date 11-5-22 MSF	Day 4 Date 11-6-22 MSF	Day 5 Date 11-7-22 MSF	Day 6 Date 11-8-22 MSF	Day 7 Date 11-9-22 MSF
1: DMW 25%	1	17	10	10	10	10	10	10	10
	2	24	10	10	10	10	10	10	10
	3	11	10	10	10	10	10	10	10
	4	4	10	10	10	10	10	10	10
2: 12.5%	1	2	10	10	10	10	10	10	10
	2	22	10	10	10	10	10	10	10
	3	13	10	10	10	10	10	10	10
	4	9	10	10	10	10	10	10 ISH	10
3: 25%	1	10	10	10	10	10	10	10	10
	2	5	10	10	10	10	10	10	10
	3	21	10	10	10	10	10	10	10
	4	1	10	10	10	10	10	10	10
4: 50%	1	16	10	10	10	10	10	10	10
	2	23	10	10	10	10	10	10	10
	3	14	10 ISK	10	10	10	10	10	10
	4	3	10	10	10	10	10	10	10
5: 75%	1	20	10	10	10	10	10	10	10
	2	8	10	10	10	10	10	10	10
	3	12	10	10 ISOR	10 ISOR	10	10	10	10
	4	19	10	10	10	10	10	10	10
6: 100%	1	15	10	10	10	10	10	10	10
	2	7	10	10	10	10	10	10	10
	3	18	10	10	10	10	10	10	10
	4	6	10	10	10	10	10	10	10

Random Assignment of Test Chambers

Project: VI2						
Test site/chemical: OF200						
Test number: 1686						
Starting position (on Table of Random Numbers): 16						
Assigned Numbers				Sample ID/Treatment	Replicate	Position
1	25	49	~~73~~	3-25%	4	1
2	26	50	~~74~~	2-12.5%	1	2
~~3~~	27	51	75	4-50%	1	3
4	28	52	~~76~~	1-DMW75%	4	4
5	29	53	~~77~~	3-25%	2	5
6	30	54	78	6-100%	4	6
7	~~31~~	55	79	6-100%	2	7
8	32	56	~~80~~	5-75%	2	8
~~9~~	33	57	81	2-12.5%	4	9
10	34	~~58~~	82	3-25%	1	10
~~11~~	35	59	83	1-DMW25%	3	11
~~12~~	36	60	84	5-75%	3	12
13	37	61	~~85~~	2-12.5%	3	13
14	38	62	~~86~~	4-50%	3	14
~~15~~	39	63	87	6-100%	1	15
16	~~40~~	64	88	4-50%	4	16
17	41	~~65~~	89	1-DMW25%	1	17
18	~~42~~	66	90	6-100%	3	18
19	~~43~~	67	91	5-~~50~~ 75%	4	19
20	~~44~~	68	92	5-75%	1	20
21	45	~~69~~	93	3-25%	3	21
22	~~46~~	70	94	2-12.5%	2	22
23	47	71	~~95~~	4-50%	2	23
24	~~48~~	72	96	1-DMW25%	2	24

11-2-22
NSB

Fathead Minnow Weight and Survival Data

Sponsor: YIZ	Test number: 16816
Test site/chemical: OF200	Balance ID: A009820
Test Start Date: 11-7-22	Test End Date: 11-9-22
Start Drying Date/Time: 11-9-22@ 1143am	End Drying Date/time: 11-10-22@ 0755

Treatment	Replicate	Pan Wt. (mg) Date: 11-8-22 Balance check: ☑	Pan + Larvae (mg) Date: 11-10-22 Balance check: ☑	Number Surviving
Initial	1	15.0695	16.6885	10
	2	15.0795	16.5980	10
	3	15.0915	16.6190	10
	4	15.0585	16.5215	10
1. DMW 25%	1	15.0225	21.7390	10
	2	15.0600	21.5085	10
	3	15.0310	22.3220	10
	4	15.0620	23.1220	10
2. 12.5%	1	15.0995	24.4125	10
	2	15.0240	21.1445	10
	3	15.1050	20.5430	10
	4	15.0360	22.4225	10
3. 25%	1	15.1660	21.4850	10
	2	15.0855	22.5550	10
	3	15.0290	21.4565	10
	4	15.0940	22.4880	10
4. 50%	1	15.0620	21.4360	10
	2	15.0670	21.0805	10
	3	15.0760	21.8270	10
	4	15.0745	20.9440	10
5. 75%	1	15.0340	19.6360 *	10
	2	15.1435	21.4260	10
	3	15.0830	21.1510	10
	4	15.0855	20.8780	10
6. 100%	1	15.0915	21.0465	10
	2	15.0665	22.4190	10
	3	15.0550	21.3810	10
	4	15.0670	21.3545	10

11-7-22 MS

11-7-22 MS

Environmental Sciences Division — Rev. 03 2020-10-28

* weight for 5-1 may be off due to fish sticking to KimWipe.

Random Assignment of Larvae to Test Chambers

Project: 412					Project: ESD				
Test site/chemical: OF200					Test site/chemical: KCl RefTox				
Test number: 1686					Test number: 1687				
Starting position (on Table of Random Numbers): 27					Starting position (on Table of Random Numbers): 6				
Assigned Numbers				Sample ID/Treatment	Replicate	Assigned Numbers				Sample ID/Treatment	Replicate
~~1~~	~~25~~	49	73	1. DMW 25%	~~16~~ 1 ~~25~~	~~1~~	25	49	~~73~~	1. DMW 25%	~~13~~ 1 ~~24~~
2	~~26~~	50	~~74~~		~~21~~ 2 ~~40~~	2	~~26~~	~~50~~	74		~~18~~ 2 ~~32~~
~~3~~	~~27~~	51	75		~~6~~ 3 ~~30~~	3	~~27~~	51	~~75~~		~~8~~ 3 ~~41~~
4	~~28~~	~~52~~	76		~~14~~ 4 ~~45~~	4	28	~~52~~	~~76~~		~~39~~ 4 ~~42~~
~~5~~	29	~~53~~	77	2. 12.5%	~~8~~ 1 ~~42~~	~~5~~	29	53	77	2. 0.25g/L	~~26~~ 1 ~~48~~
6	30	~~54~~	~~78~~		~~11~~ 2 ~~44~~	~~6~~	~~30~~	54	78		~~2~~ 2 ~~31~~
~~7~~	31	~~55~~	79		~~7~~ 3 ~~24~~	~~7~~	~~31~~	55	79		~~7~~ 3 ~~23~~
8	32	56	80		~~41~~ 4 ~~48~~	~~8~~	~~32~~	56	80		~~25~~ 4 ~~38~~
9	~~33~~	57	~~81~~	3. 25%	~~17~~ 1 ~~45~~	9	33	~~57~~	~~81~~	3. 0.50g/L	~~3~~ 1 ~~17~~
10	~~34~~	58	~~82~~		~~19~~ 2 ~~32~~	10	~~34~~	58	~~82~~		~~16~~ 2 ~~46~~
~~11~~	35	~~59~~	83		~~9~~ 3 ~~29~~	~~11~~	~~35~~	59	83		~~19~~ 3 ~~30~~
12	~~36~~	60	~~84~~		~~10~~ 4 ~~35~~	~~12~~	~~36~~	60	84		~~11~~ 4 ~~47~~
~~13~~	37	~~61~~	85	4. 50%	~~15~~ 1 ~~31~~	13	37	~~61~~	~~85~~	4. 1.00g/L	~~14~~ 1 ~~18~~
14	~~38~~	~~62~~	86		~~12~~ 2 ~~30~~	~~14~~	38	62	~~86~~		~~33~~ 2 ~~34~~
~~15~~	~~39~~	63	87		~~1~~ 3 ~~23~~	15	~~39~~	~~63~~	87		~~35~~ 3 ~~43~~
~~16~~	~~40~~	64	88		~~33~~ 4 ~~31~~	~~16~~	~~40~~	64	88		~~31~~ 4 ~~40~~
~~17~~	41	65	~~89~~	5. 75%	~~21~~ 1 ~~37~~	~~17~~	41	65	89	5. 1.25g/L	~~5~~ 1 ~~16~~
18	~~42~~	~~66~~	90		~~5~~ 2 ~~28~~	~~18~~	42	~~66~~	90		~~1~~ 2 ~~28~~
19	43	~~67~~	~~91~~		~~20~~ 3 ~~46~~	~~19~~	43	67	~~91~~		~~20~~ 3 ~~22~~
20	~~44~~	68	~~92~~		~~3~~ 4 ~~26~~	20	44	~~68~~	~~92~~		~~9~~ 4 ~~21~~
~~21~~	45	69	~~93~~	6. 100%	~~18~~ 1 ~~39~~	21	~~45~~	~~69~~	93	6. 1.50g/L	~~12~~ 1 ~~27~~
22	46	~~70~~	~~94~~		~~17~~ 2 ~~37~~	22	~~46~~	~~70~~	94		~~15~~ 2 ~~45~~
23	~~47~~	71	~~95~~		~~2~~ 3 ~~22~~	~~23~~	~~47~~	71	95		~~4~~ 3 ~~36~~
24	~~48~~	72	~~96~~		~~13~~ 4 ~~41~~	~~24~~	~~48~~	72	96		~~29~~ 4 ~~44~~

11-2-22 NM

Fathead Minnow Order & Shipment Log

Ordering Information:

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
10-31-22	16826 + 16827	ABS	800	1 day old on arrival	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	1706			
Thermometer ID	DD19	→	→	→	→			
Initials	MSS	→	→	→	→			

Comments (e.g. condition of larvae received):
Fed 2.18mL BS to each container @ 1510. MSS 110122
Fed 2.29mL BS to each container @ 0821. MSS 110222

MSS 11-9-22

OAK RIDGE National Laboratory

PO Box 2008
Oak Ridge, TN 37831
(865) 341-0398| stevensonlm@ornl.gov

Date: February 16, 2023

To: K.G. Hanzelka (RC)

c: S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, A.M. Fortner

From: L.M. Stevenson, 1504, MS-6351 (865-341-0398).

RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted January 25 to February 1, 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*).

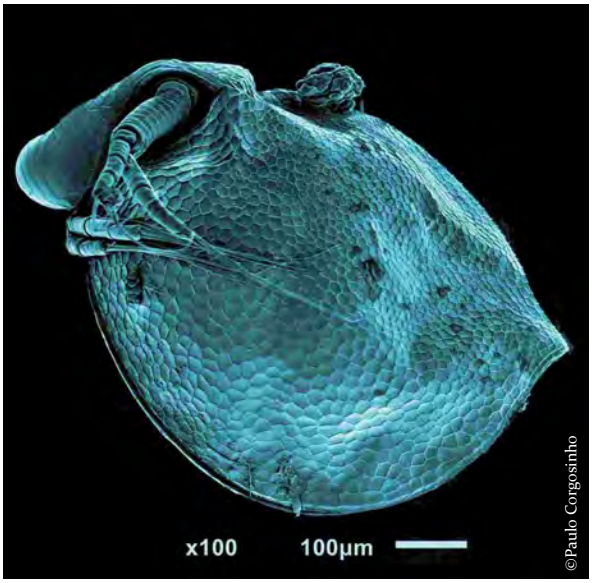
Effluent from Outfall 200 did not reduce fathead minnow survival or growth or *Ceriodaphnia* survival or reproduction by 25% or greater at any of the tested concentrations. For both species, the Inhibition Concentration$_{25}$ (IC_{25}) for survival, growth, and/or reproduction were thus >100% (the highest 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_{25}
Outfall 200	Fathead minnow	Survival	>100%
		Growth	>100%
Outfall 200	*Ceriodaphnia*	Survival	>100%
		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_{25}) 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: 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 ± 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
pH (S.U.)	8.12	8.04	8.01
Conductivity (µS/cm)	407	490	367
Alkalinity (mg/L as $CaCO_3$)	118	122	108
Hardness (mg/L as $CaCO_3$)	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 ± 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 (4th 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; 4th 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_{25} = 0.86 g NaCl/L; 95% C.I. = 0.40-1.53 g NaCl/L.
Reproduction IC_{25} = 0.84g NaCl/L; 95% C.I. = 0.46-1.47 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_{25} for survival: 1.433 ± 0.802 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} for survival: 0.280 g NaCl/L
Central tendency of IC_{25} for reproduction: 1.034 ± 0.610 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} 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_{25} for survival was >100% effluent.

7.2 Reproduction

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

7.3 Summary of *Ceriodaphnia* toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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 $CaCO_3$)	100	104	106
Hardness (mg/L as $CaCO_3$)	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. 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  Date: 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_{25}) 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 ± 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
pH (S.U.)	8.12	8.04	8.01
Conductivity (µS/cm)	407	490	367
Alkalinity (mg/L as $CaCO_3$)	118	122	108
Hardness (mg/L as $CaCO_3$)	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 ± 1 °C.

3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.

3.7 Mean dry weight at test initiation: 0.13 ± 0.006 mg (mean ± 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 (4th 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 ± 100 µL 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_{25} = 1.02 g KCl/L; 95% C.I. = 0.86-1.06 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_{25} for survival: 0.853 ± 0.274 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} for survival: 0.161 g KCl/L

Central tendency of IC_{25} for growth: 0.920 ± 0.236 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} for growth: 0.128 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

Concentration	Proportion surviving per replicate				Mean
	1	2	3	4	
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	Weight (mg) per replicate				Mean ± SD
	1	2	3	4	
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_{25} for survival was >100% effluent.

7.2 Growth

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

7.3 Summary of fathead minnow toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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 $CaCO_3$)	100	104	106
Hardness (mg/L as $CaCO_3$)	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.

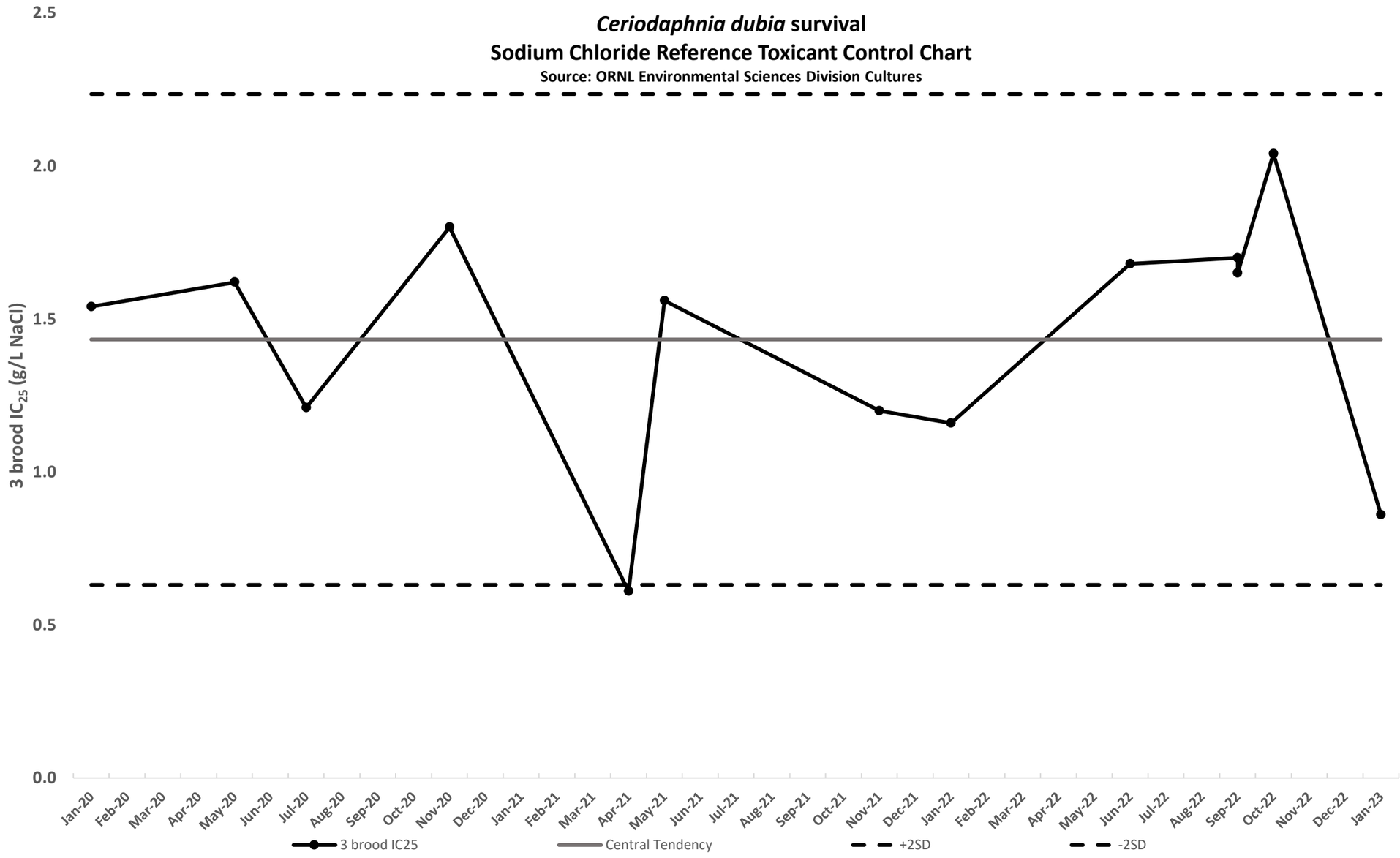
Instruments were calibrated and standardized according to manufacturer's instructions.

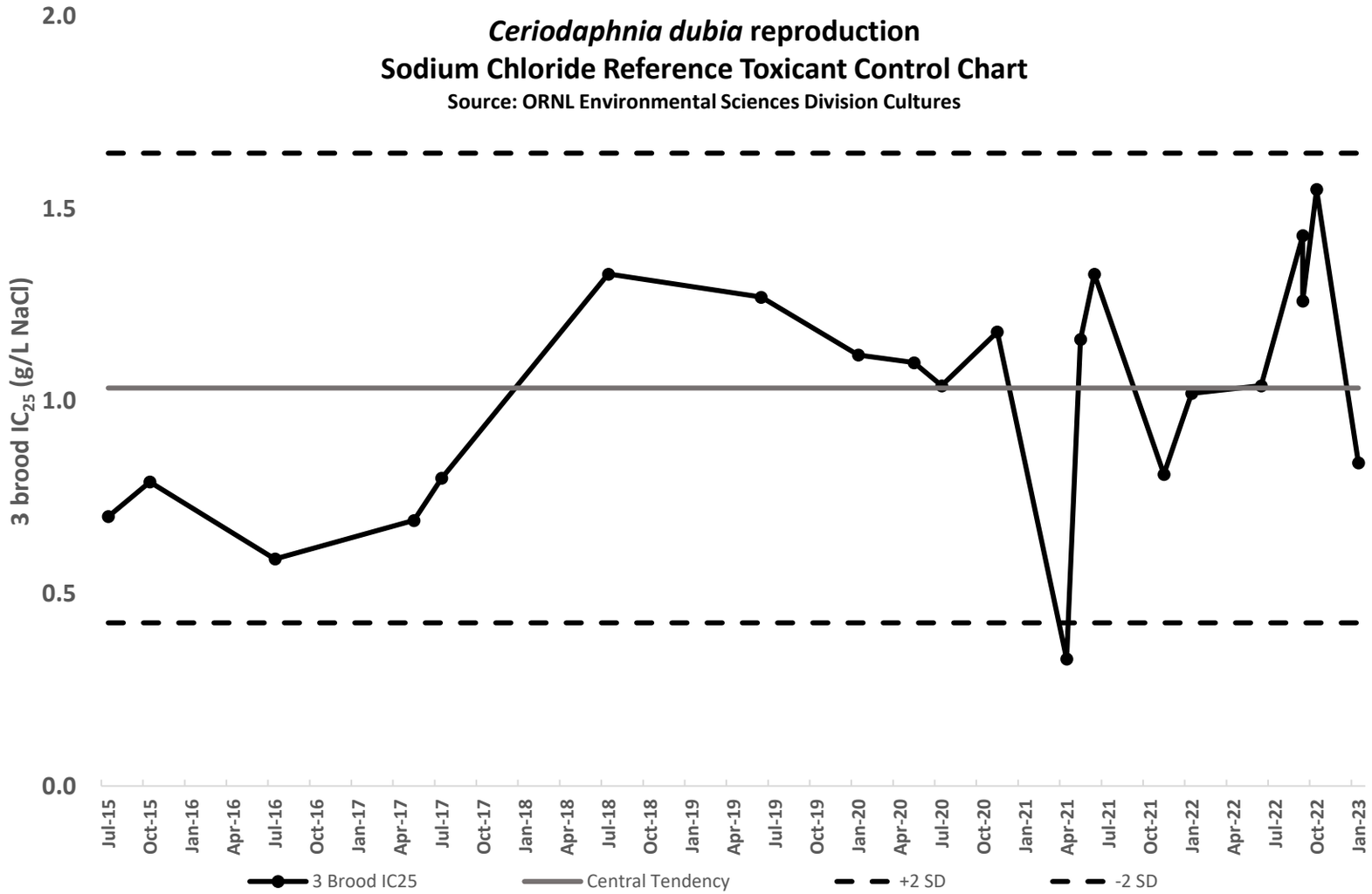
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

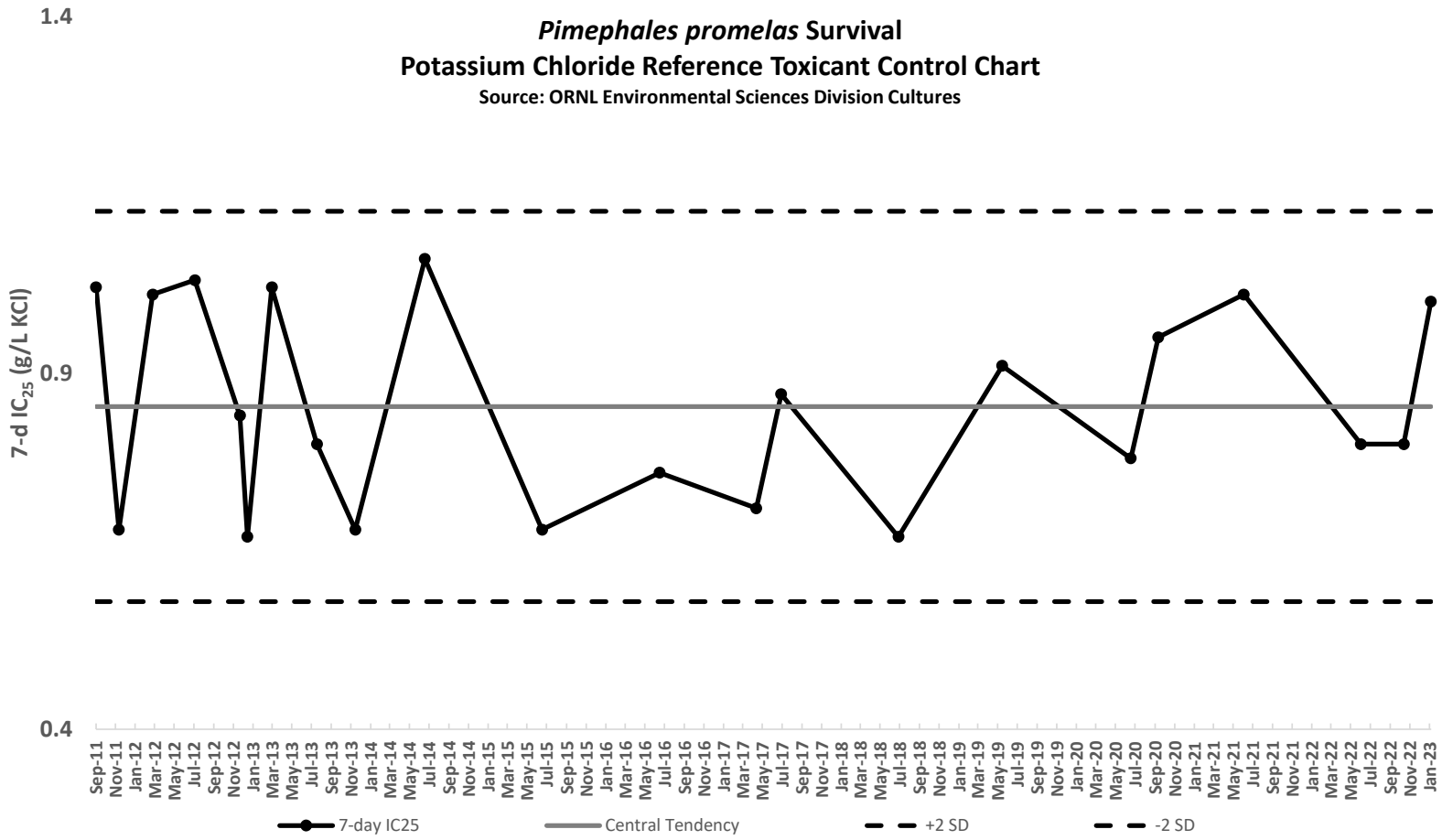
Report prepared by: Peijia Ku Date: 3 February 2023

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

REFERENCE TOXICANT CONTROL CHARTS


Ceriodaphnia dubia survival
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.5
2.0
1.5
1.0
0.5
0.0
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
3 brood IC25
Central Tendency
+2SD
-2SD


Ceriodaphnia dubia reproduction
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.0
1.5
1.0
0.5
0.0
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16
Jan-17
Apr-17
Jul-17
Oct-17
Jan-18
Apr-18
Jul-18
Oct-18
Jan-19
Apr-19
Jul-19
Oct-19
Jan-20
Apr-20
Jul-20
Oct-20
Jan-21
Apr-21
Jul-21
Oct-21
Jan-22
Apr-22
Jul-22
Oct-22
Jan-23
3 Brood IC25
Central Tendency
+2 SD
-2 SD


1.4
Pimephales promelas Survival
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC25 (g/L KCl)
0.9
0.4
Sep-11
Nov-11
Jan-12
Mar-12
May-12
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Sep-13
Nov-13
Jan-14
Mar-14
May-14
Jul-14
Sep-14
Nov-14
Jan-15
Mar-15
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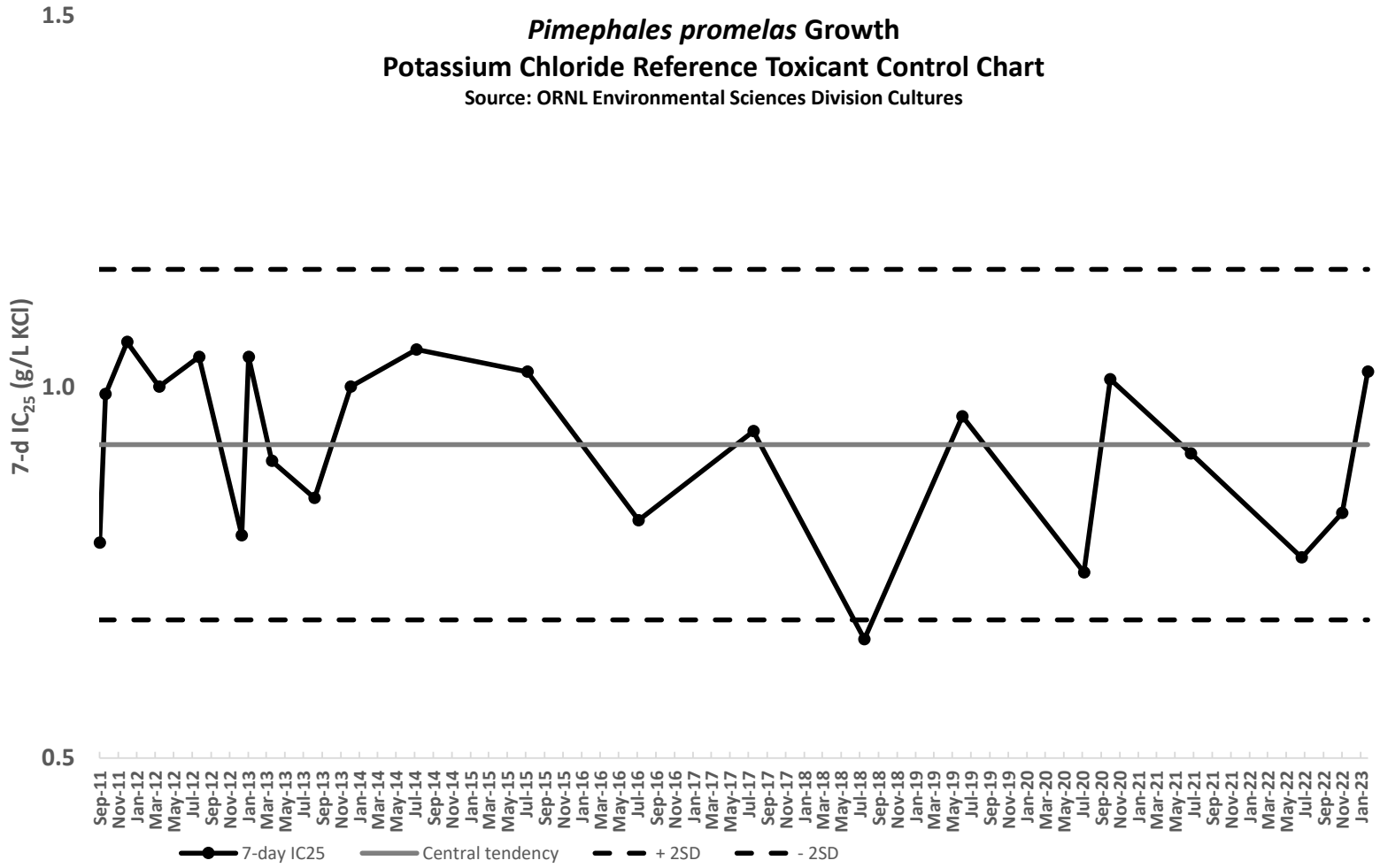
Nov-20
Jan-21
Mar-21
May-21
Jul-21
Sep-21
Nov-21
Jan-22
Mar-22
May-22
Jul-22
Sep-22
Nov-22
Jan-23
7-day IC25
Central Tendency
+2 SD
-2 SD


Pimephales promelas Growth
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC25 (g/L KCl)
1.5
1.0
0.5
Sep-11
Nov-11
Jan-12
Mar-12
May-12
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Sep-13
Nov-13
Jan-14
Mar-14
May-14
Jul-14
Sep-14
Nov-14
Jan-15
Mar-15
May-15
Jul-15
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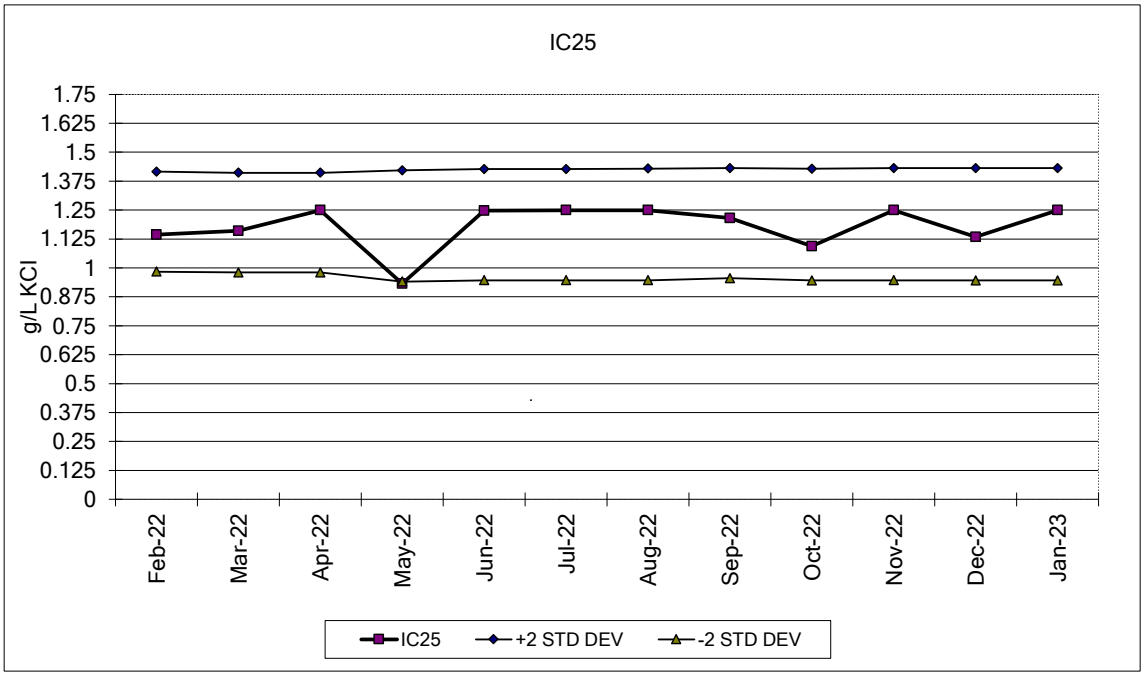
Jul-19
Sep-19
Nov-19
Jan-20
Mar-20
May-20
Jul-20
Sep-20
Nov-20
Jan-21
Mar-21
May-21
Jul-21
Sep-21
Nov-21
Jan-22
Mar-22
May-22
Jul-22
Sep-22
Nov-22
Jan-23
7-day IC25
Central tendency
+ 2SD
- 2SD

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

Date	NOEC (g/L KCl)	LOEC (g/L KCl)
Aug-22	0.50	1.0
Sep-22	0.50	1.0
Oct-22	0.50	1.0
Nov-22	0.50	1.0
Dec-22	0.50	1.0
Jan-23	0.50	1.0

IC 25 for Growth Test

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

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

WATER CHEMISTRY DATA LOGSHEETS

Daily Water Chemistry Log

CD - 2979

Sponsor Y12 Site/Treatment 07-200 Associated test numbers FHM-1688

PK 01/24/23

Note Not all parameters are required for all tests All unused cells should be lined through or marked "NA"

	Observation Day	0 PK/NJJ	1 PK/NJJ	2 PK/NJJ	3 PK/NJJ	4 PK/NJJ	5 PK/NJJ	6 PK/NJJ	7 PK/NJJ
	Date/Initials	01/25/2023	01/26/23	1/27/23	1/28/23	1/29/23	1/30/23	1/31/23	02/01/23
	5-digit ORNL ID	33120	33120	33121	33121	33121	33122	33122	
	Rec temp (°C) (New ✓)	service ☒	service ☐	service ☐	service ☐	see note ☐	See Cal ☐	service ☐	☐
① 25% DMW Control	DMW Batch #	928	928	928	929	929	929	930	
	Conductivity (µS/cm)	239	235	227	239	233	233	240	
	Alkalinity (mg/L)	100			104			100	
	Hardness (mg/L)	130			120			120	
	pH (S U) Initial	8.181	8.185	8.101	8.11 MK	8.152	8.16	8.22	
	Final CD/FHM		8.53/8.08	8.49/8.02	8.49/8.02	8.45/8.02	8.47/8.12	8.42/8.04	8.81/8.36
	DO (mg/L) Initial	8.62	8.73	8.75	8.31	8.72	8.71	8.71	
	Final CD/FHM		9.08/7.36	8.87/7.60	8.97/7.78	8.83/7.52	8.72/7.51	8.32/7.33	8.57/7.76
② 12.5% 0.5 gal	Conductivity (µS/cm)	261	256	261	268	264	242	256	
	Alkalinity (mg/L)								
	Hardness (mg/L)								
	Chlorine (mg/L)								
	pH (S U) Initial	8.042	8.178	8.184	8.09	8.120	8.172	8.24	
	Final CD/FHM		8.53/8.10	8.52/8.04	8.51/8.01	8.49/8.05	8.41/8.14	8.50/8.01	8.81/8.37
	DO (mg/L) Initial	8.62	8.77	8.76	8.80	8.64	8.77	8.64	
	Final CD/FHM		8.69/7.42	8.86/7.52	8.95/7.67	8.91/7.44	8.71/7.52	8.88/7.20	8.67/7.71
③ 25% 1.0 gal	Conductivity (µS/cm)	283	278	295	301	297	260	272	
	Alkalinity (mg/L)								
	Hardness (mg/L)								
	Chlorine (mg/L)								
	pH (S U) Initial	8.110	8.17	8.151	8.131	8.131	8.16	8.25	
	Final CD/FHM		8.54/8.13	8.55/8.07	8.52/8.10	8.52/8.08	8.52/8.15	8.51/8.02	8.82/8.35
	DO (mg/L) Initial	8.66	8.77	8.83	8.78	8.76	8.86	8.64	
	Final CD/FHM		8.90/7.33	8.86/7.56	8.98/7.53	8.96/7.37	8.72/7.43	8.81/7.21	8.70/7.73
④ 50% 1.5 gal	Conductivity (µS/cm)	325	323	360	365	363	296	304	
	Alkalinity (mg/L)								
	Hardness (mg/L)								
	Chlorine (mg/L)								
	pH (S U) Initial	8.112	8.14	8.112	8.134	8.112	8.12	8.21	
	Final CD/FHM		8.56/8.12	8.57/8.08	8.53/8.11	8.56/8.11	8.54/8.17	8.52/8.02	8.84/8.33
	DO (mg/L) Initial	8.60	8.84	9.02	9.10	8.96	8.96	8.79	
	Final CD/FHM		8.92/7.28	8.93/7.53	8.93/7.51	9.01/7.35	8.77/7.37	8.84/7.8	8.76/7.65
⑤ 75% 2.0 gal	Conductivity (µS/cm)	367	361	424	425	424	331	333	
	Alkalinity (mg/L)								
	Hardness (mg/L)								
	Chlorine (mg/L)								
	pH (S U) Initial	8.102 MK	8.11	8.099	8.10	8.092	8.08	8.24	
	Final CD/FHM		8.57/8.17	8.60/8.12	8.54/8.12	8.57/8.14	8.55/8.22	8.51/8.00	8.83/8.31
	DO (mg/L) Initial	8.68	9.55	9.13	9.03	9.03	9.45	8.68	
	Final CD/FHM		8.92/7.35	9.05/7.65	8.92/7.53	9.06/7.33	8.77/7.41	8.96/7.16	8.81/7.67
⑥ 100% 2.5 gal	Conductivity (µS/cm)	407	407	490	490	488	367	366	
	Alkalinity (mg/L)	118		122			108		
	Hardness (mg/L)	190		200			150		
	Chlorine (mg/L) PT	0.01/0.01		0.01/0.02			0.01/0.01		
	pH (S U) Initial	8.115	8.13	8.037	8.028	8.051	8.01	8.13	
	Final CD/FHM		8.56/8.15	8.59/8.11	8.56/8.17	8.53/8.19	8.58/8.26	8.53/8.05	8.84/8.33
	DO (mg/L) Initial	9.66	9.60	9.89	10.72	7.53	10.00	9.79	
	Final CD/FHM		8.83/7.15	9.03/7.42	8.83/7.54	9.07/7.30	8.75/7.38	9.02/7.16	8.90/7.72

Environmental Sciences Division 1-25-23 0F20 water was very cloudy. MK

Rev 04 2021-02-05

CHAIN OF CUSTODY FORMS

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

DATE (MM/DD/YY)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
01/25/23	TOXTEST	A. GARLAND / J. WILLIAMS	031072

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C) FRIG	REMARKS #7008 TEMP	REMARKS #4102 Cl_2
OUTFALL #200	200	0815	C	1	~16 LITERS	3°	10.1	<0.05
		ORN SAMPLE ID=	33120					

MR 01-25-23

THERMOMETER NO.

SAMPLES RELINQUISHED BY	DATE	TIME	
A. K. Garland	1-25-23	0852	☒ AM ☐ PM
SAMPLES RECEIVED BY	**DATE**	**TIME**	
Peijia Ku	1-25-23	0853	☒ AM ☐ 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)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
01-27-23	TOXTEST	A. GARLAND / J. WILLIAMS FRIG	031073

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS
OUTFALL # 200	200	0725	C	1	~14 LITERS	3°	#7008 TEMP: 10.8 #4102 C/2
	ORNL Sample ID = 33121						

MDLs 01/27/23

THERMOMETER NO.

SAMPLES RELINQUISHED BY: A. Garland	DATE: 1/27/23	TIME: 0806	☒ AM ☐ PM
SAMPLES RECEIVED BY: [signature]	DATE: 01-27-23	TIME: 0806	☐ AM ☒ 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)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
01/30/23	TOX TEST	A.GARLAND / J. WILLIAMS ERIG	031074

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS	
							#7009 Temp.	# 4102 Cl_2
OUTFALL #200	200	0735	C	ALG 1/30/23 1	~15L	3°	7.2	<0.05
	ORAU Sample ID = 33122							

THERMOMETER NO.

SAMPLES RELINQUISHED BY: R. Garland	DATE: 1/30/23	TIME: 0828	☒ AM ☐ PM
SAMPLES RECEIVED BY: Reijia Kan	DATE: 1/30/23	TIME: 0828	☒ AM ☐ PM

UCN-18631 (3 3-92)

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

TOXICITY TEST LOGSHEETS

196 | PROJECT NAME _____ NOTEBOOK NO.

Toxicity Test Information Sheet

Sponsor: Y12 **Site/Treatment:** OF200 **Test number:** 2979

Test begin date (Day 0)	Test end date	Test duration	Template number
01/25/2023	02/01/2023	7 ☐ hours ☑ days ☐ NA	☑ 1

Test Organism: ☑ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: _____

Isolated from:

Date: 01/24/23 | 01/24/23

Time: 1020 | 1750

Hatch date: _____

Delivery date: _____

Notes: PK 01/24/23

Test period: ☑ Chronic ☐ Acute

Test purpose: ☑ Regulatory ☐ Investigative

Test stage: ☐ Preliminary ☑ Analytical ☐ Re-test

Test type: ☑ Effluent ☐ Received waters ☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%	☑C ☐T	4 =	50%	☐C ☑T
2 =	12.5%	☐C ☑T	5 =	75%	☐C ☑T
3 =	25%	☐C ☑T	6 =	100%	☐C ☑T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 928, 929, 930

Source of Test Organisms:

☑ ESD cultures: Board numbers: ☐ NA ☐ #4736, 4735

☐ Vendor: _____ ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: ~~031072~~ 33120 PK(01/25) Date: 01/25/23 COC #: 031072

Sample ID: 33121 Date: 01/27/23 COC #: 031073

Sample ID: 33122 Date: 01/30/23 COC #: 031074

Record of Deviations from Method and/or Test Non-Conformities

Date	Description	Initial
02/01/2023	Small bubbles (i.e. DO) in treatment 5 & 6 cups (just observation), CD attached	PK

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	Peijia Ku	PK	02/01/2023
Data sheets QA:	[signature]	AMF	2/2/2023
Data entered:	Peijia Ku	PK	02/01/2023
Data entry QA:	[signature]	AMF	2/2/2023

SIGNATURE _____ DATE _____ 20

READ AND UNDERSTOOD _____ DATE _____ 20

CHRONIC Daily Water/Feeding Log

Sponsor: Y12 Test site/treatment: OF200 Begin Date: 01/25/2023 End Date: 02/01/2023 Test Number: 2979

Daily Test Info		Temperature Information Therm. #: DD20		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10⁷/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	01/25/23 PK	~~1435~~ 25.6 (time) am / pm	~~24.8~~ PK am / pm (cutting) 25.1 PK	YCT / RASU	12/06/22 / 01/24/23	100 / 100	☑Yes	am / 1235 pm	11:55	1315	33120 (1125 PK) ~~031072~~ (typo)	928	N/A
Day 1	01/26/23 PK	25.7 am / pm	~~24.8~~ am / pm	YCT / RASU	12/06/22 / 01/24/23	100 / 96	☑Yes	1120 am / pm	11:02	11:~~50~~	↓	928	
Day 2	01/27/23 PK	25.5 am / pm	25.0 am / pm	YCT / RASU	01/10/23 / 01/24/23	100 / 95	☑Yes	~~1220~~ am / pm	11:51	12~~40~~	33121	928	
Day 3	01/28/23 PK	25.5 am / pm	24.9 am / pm	YCT / RASU	01/10/23 / 01/24/23	100 / 89	☑Yes	1004 am / pm	0935	1120	↓	929	
Day 4	01/29/23 PK	~~26.0~~ am / pm	24.7 am / pm	YCT / RASU	01/10/23 / 01/24/23	100 / 88	☑Yes	1002 am / pm	0931	1056	↓	929	
Day 5	01/30/23 PK	25.6 am / pm	25.0 am / pm	YCT / RASU	01/10/23 / 01/24/23	100 / 99	☑Yes	1050 am / pm	1025	1138	33122	929	
Day 6	01/31/23 PK	25.5 am / pm	25.0 am / pm	YCT / RASU	01/10/23 / ~~01/24/23~~	100 / ~~89~~ PK	☑Yes	1055 am / pm	1030	1210	↓	930	
Day 7	02/01/23 PK	25.7 am / pm	25.0 am / pm	~~YCT~~ / ~~RASU~~ PK			☐Yes	am / pm	1140	1310			N/A

Notes:

01/24/23 PK

198

Ceriodaphnia Chronic Daily Survival & Reproduction Log

Project: Y12 Test site/chemical: OF200 Test Number: 2979

Begin Date: 01/25/2023 End Date: 02/01/2023 Template Number: 1

PK 01/24/23

Codes: (-) Alive and No Reproduction; (*N*) Alive and Reproduction; (*xN*) Dead and Reproduction; (M) Male

Test Chamber	Treatment Number	Day: 1 PK Date: 01/26/23	2 PK 01/27/23	3 PK 01/28/23	4 PK 01/29/23	5 PK 01/30/23	6 PK 01/31/23	7 PK 02/01/23
1	5	-	-	6	8	-	16	24
2	1	-	-	7	12	-	23	24
3	5	-	-	6	-	8	19	20
4	1	-	-	7	-	12	22	26
5	6	-	-	7	-	10	18	22
6	1	-	-	7	-	12	22	27
7	4	-	-	7	-	8	16	18
8	4	-	-	7	10	-	21	23
9	6	-	-	6	-	10	14	22
10	2	-	-	8	12	-	15	19
11	1	-	-	7	9	-	21	29
12	5	-	-	6	-	12	16	19
13	1	-	-	6	-	10	20	22
14	6	-	-	6	-	10	14	21
15	5	-	-	5	-	9	18	18
16	4	-	-	6	8	1	16	19
17	1	-	-	7	14	-	22	27
18	2	-	-	7	9	-	18	26
19	2	-	-	8	9	-	18	25
20	4	-	-	8	10	-	10	23
21	2	-	-	7	14	-	20	21
22	3	-	-	7	-	11	19	18
23	2	-	-	6	-	13	17	20
24	2	-	-	7	-	12	16	21
25	3	-	-	6	-	9	20	22
26	6	-	-	6	-	8	15	25
27	3	-	-	7	14	X		
28	3	-	-	5	6	-	16	20
29	4	-	-	7	8	-	17	28
30	3	-	-	7	10	-	15	25
31	6	-	-	4	-	8	17	17
32	4	-	-	7	-	9	17	20
33	6	-	-	6	-	8	10	20
34	4	-	-	7	9	-	16	20
35	2	-	-	8	9	-	18	25
36	2	-	-	7	-	11	17	21
37	5	-	-	7	11	-	18	23
38	6	-	-	7	7	-	17	24
39	3	-	-	9	10	-	19	25
40	5	-	-	8	10	-	14	9
41	4	-	-	6	-	9	15	20
42	2	-	-	7	-	11	19	22
43	4	-	-	5	9	-	20	24
44	3	-	-	5	-	6	17	23
45	1	-	-	7	11	-	21	11
46	5	-	-	7	-	10	17	22
47	6	-	-	7	8	-	18	20
48	1	-	-	7	12	-	25	26
49	5	-	-	6	-	10	-	-
50	1	-	-	6	6	-	17	22
51	3	-	-	7	9	-	17	20
52	6	-	-	7	8	-	15	20
53	3	-	-	6	11	-	17	24
54	5	-	-	7	8	-	2	15
55	4	-	-	8	-	10	16	21
56	3	-	-	7	-	9	21	23
57	2	-	-	4	-	5	16	16
58	5	-	-	7	10	-	17	26
59	1	-	-	7	-	9	19	23
60	6	-	-	6	-	11	16	17

20 20

Toxicity Test Information Sheet

Sponsor: 412 Site/Treatment: OF200 Test number: 1688

Test begin date (Day 0)	Test end date	Test duration	Template number
01-25-23	02-01-23	7 ☐ hours ☑ days	☑ NA ☐

Test Organism: ☐ *Ceriodaphnia dubia* ☑ Fathead minnow ☐ Other: _____

Isolated from: Date: _____ Time: _____

Hatch date: 01-23-23 Delivery date: 01-24-23

Notes:

Test period	Test purpose	Test stage	Test type
☑ Chronic	☑ Regulatory	☐ Preliminary	☑ Effluent
☐ Acute	☐ Investigative	☑ Analytical	☐ Received waters
		☐ Re-test	☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%	☑ C ☐ T	4 =	50%	☐ C ☑ T
2 =	12.5%	☐ C ☑ T	5 =	75%	☐ C ☑ T
3 =	25%	☐ C ☑ T	6 =	100%	☐ C ☑ T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 928-930

Source of Test Organisms:

☐ ESD cultures: Board numbers: ☐ NA ☐ _____

☑ Vendor: ABS ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: 33120 Date: 01-25-23 COC #: 031072
Sample ID: 33124 Date: 01-27-23 COC #: 031073
Sample ID: 33122 Date: 01-30-23 COC #: 031074

NSS 01-25-23

Record of Deviations from Method and/or Test Non-Conformities

Date	Description	Initial
01-27-23	Test beaker temperature was below 24°C.	NSS
01-26-23	Trt 5, replicate 4 beaker overturned + was lost.	NSS

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	Nikki Jones	NSS	02-01-23
Data sheets QA:	[signature]	AMF	2/3/23
Data entered:	Nikki Jones	NSS	02-01-23
Data entry QA:	[signature]	AMF	2/3/23

CHRONIC Daily Water/Feeding Log

Sponsor: UI2 Test site/treatment: OF200 Begin Date: 01-25-23 End Date: 02-01-23 Test Number: 1688

Daily Test Info		Temperature Information Therm. #: 0019		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10⁷/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	01-25-23 MS	— am 26.4 pm	— am 25.9 pm	— B	— 01-24-23	— 59	☑Yes	— am 1516 pm	1108	1129	33120	928	N/A
Day 1	01-26-23 MS	26.2 am 26.2 pm	25.5 am 25.6 pm	B B	01-25-23 01-25-23	72 94	☐Yes	0830 am 1607 pm	1031	1136	33120	928	
Day 2	01-27-23 MS	24.1 am 25.3 pm	23.8 am 24.8 pm	B B	01-26-23 01-26-23	67 86	☑Yes	0837 am 1513 pm	1114	1212	33121	928	
Day 3	01-28-23 MS	25.10 am 25.7 pm	25.2 am 25.4 pm	B B	01-27-23 01-27-23	98 143	☑Yes	0744 am 1222 pm	0954	1038	33121	929	
Day 4	01-29-23 MS	25.8 am 25.8 pm	25.4 am 25.4 pm	B B	01-28-23 01-28-23	083 89	☑Yes	0730 am 1130 pm	0930	1009	33121	929	
Day 5	01-30-23 MS	26.0 am ~~26.2~~ MS pm 25.9	25.6 am ~~25.8~~ MS pm 25.4	B B	01-29-23 01-29-23	100 130	☑Yes	0816 am 1310 pm	1047	1138	33122	929	
Day 6	01-31-23 MS	26.1 am 24.8 pm	25.7 am 24.9 pm	B B	01-30-23 01-30-23	72 96	☑Yes	0827 am 1441 pm	1031	1116	33122	930	
Day 7	02-01-23 MS	24.1 am — pm	23.9 am — pm				☐Yes	— am — pm	1015	1139			↓

Notes:

01-25-23 MS

Fathead Minnow Chronic Daily Survival Log

UI2 Test site/chemical: OF200 Test Number: 1688

Fathead Minnow Chronic Daily Survival Log

Sponsor: 412 **Test site/chemical:** OF200 **Test Number:** 1688

Begin Date: 01-25-23 **End Date:** 02-01-23

Comment Codes: C = Clear; D = Dead; Fg = Fungus; K = Killed by siphoning; M = Missing; Sk = Sick; SM = Small; SOR = Siphoned and returned; W = Wounded

Treatment Number and Desc.	Replicate Number	Position Number	Day 1 Date 01-26-23 MSS	Day 2 Date 01-27-23 MSS	Day 3 Date 01-28-23 MSS	Day 4 Date 01-29-23 MSS	Day 5 Date 01-30-23 MSS	Day 6 Date 01-31-23 MSS	Day 7 Date 02-01-23 MSS
1: DMW 25%	1	7	10	10	10	10	10	10	10
	2	6	10	10	10	10	10	10	10
	3	1	10	10	10	10	10	10	10
	4	8	10	10	10	10	10	10	10
2: 12.5%	1	5	10	10	10	10	10	10	10
	2	21	10	10	10	10	10	10	10
	3	9	9 ID	9	9	9	9	9	9
	4	18	10	10	10	10	10	10	10
3: 25%	1	24	10	10	10	10	10	10	10
	2	11	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10	10
	4	2	10	10	10	10	10	10	10
4: 50%	1	14	10	10	10	10	10	10	10
	2	15	10	10	10	10	10	10	10
	3	23	10	10	10	10	10	10	10
	4	16	10	10	10	10	10	10	10
5: 75%	1	13	10	10	10	10	10	10	10
	2	4	10	10	10	10	10	10	10
	3	20	10	10	10	10	10	10	10
	4	22	—	—	—	—	—	—	—
6: 100%	1	3	10	10	10	10 Sk	10	10	10
	2	12	10	10	10	10	10	10	10
	3	19	10	10	10	10	10	10	10
	4	17	10 SOR	10	10	10	10	10	10

Random Assignment of Test Chambers

Project: U12						
Test site/chemical: OF400						
Test number: 11088						
Starting position (on Table of Random Numbers): 7						
Assigned Numbers				Sample ID/Treatment	Replicate	Position
~~1~~	25	49	73	1-DMW 25%	3	1
2	~~26~~	50	74	3-25%	4	2
3	27	51	~~75~~	6-100%	1	3
4	28	52	~~76~~	5-75%	2	4
~~5~~	29	53	77	2-12.5%	1	5
~~6~~	30	54	78	1-DMW 25%	2	6
7	~~31~~	55	79	1-DMW 25%	1	7
~~8~~	32	56	80	1-DMW 25%	4	8
9	33	~~57~~	81	2-12.5%	3	9
10	~~34~~	58	82	3-25%	3	10
11	~~35~~	59	83	3-25%	2	11
~~12~~	36	60	84	6-100%	2	12
13	37	61	~~85~~	5-75%	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	6-100%	3	19
20	44	68	~~92~~	5-75%	3	20
21	~~45~~	69	93	2-12.5%	2	21
22	46	~~70~~	94	5-75%	4	22
~~23~~	47	71	95	4-50%	3	23
~~24~~	48	72	96	3-25%	1	24

01-25-23 NM

Environmental Sciences Division Rev. 03 2020-10-28

Fathead Minnow Weight and Survival Data

Sponsor: YI2	Test number: 1688
Test site/chemical: OF200	Balance ID: A009820
Test Start Date: 01-25-23	Test End Date: 02-01-23
Start Drying Date/Time: 02-01-23@1839	End Drying Date/time: 02-02-23@ 1017

Treatment	Replicate	Pan Wt. (mg) Date: 02-01-23 Balance check: ☑	Pan + Larvae (mg) Date: 02-02-23 Balance check: ☑	Number Surviving
Initial	1	15.1170	16.4270	10
	2	15.1070	16.3575	10
	3	15.1005	16.4205	10
	4	15.0705	16.4415	10
1. DMW 25%	1	15.1140	20.2605	10
	2	15.0020	19.6440	10
	3	15.1120	20.2580	10
	4	15.1030	20.3605	10
2. 12.5%	1	14.9695	20.0095	10
	2	15.0040	20.7820	10
	3	15.0375	19.7895	9
	4	15.0970	20.6500	10
3. 25%	1	15.0980	20.4715	10
	2	14.9920	20.0025	10
	3	15.1535	19.9810	10
	4	15.0510	20.7055	10
4. 50%	1	15.1405	20.7540	10
	2	15.1295	21.0865	10
	3	15.0595	20.8555	10
	4	15.1510	20.5655	10
5. 75%	1	15.1270	21.1755	10
	2	15.0745	20.1385	10
	3	15.0680	21.2615	10
	4	15.1515	—	—
6. 100%	1	15.1135	20.6240	10
	2	15.1230	20.1690	10
	3	15.2095	21.1395	10
	4	15.1370	20.7935	10

01-25-23 MS

01-25-23 MS

Random Assignment of Larvae to Test Chambers

Project: 412								Project: ESD							
Test site/chemical: OF100								Test site/chemical: KCl Ref Tox							
Test number: 1688								Test number: 1689							
Starting position (on Table of Random Numbers): 1								Starting position (on Table of Random Numbers): 29							
Assigned Numbers				Sample ID/Treatment	Replicate			Assigned Numbers				Sample ID/Treatment	Replicate		
1	25	49	73	1. DMW 25%	1			1	25	49	73	1. DMW 25%	1		
2	26	50	74		2			2	26	50	74		2		
3	27	51	75		3			3	27	51	75		3		
4	28	52	76		4			4	28	52	76		4		
5	29	53	77	2. 9.3%	1			5	29	53	77	2. 0.25 g/L	1		
6	30	54	78		2			6	30	54	78		2		
7	31	55	79		3			7	31	55	79		3		
8	32	56	80		4			8	32	56	80		4		
9	33	57	81	3. 18%	1			9	33	57	81	3. 0.50 g/L	1		
10	34	58	82		2			10	34	58	82		2		
11	35	59	83		3			11	35	59	83		3		
12	36	60	84		4			12	36	60	84		4		
13	37	61	85	4. 37%	1			13	37	61	85	4. 1.00 g/L	1		
14	38	62	86		2			14	38	62	86		2		
15	39	63	87		3			15	39	63	87		3		
16	40	64	88		4			16	40	64	88		4		
17	41	65	89	5. 74%	1			17	41	65	89	5. 1.25 g/L	1		
18	42	66	90		2			18	42	66	90		2		
19	43	67	91		3			19	43	67	91		3		
20	44	68	92		4			20	44	68	92		4		
21	45	69	93	6. 100%	1			21	45	69	93	6. 1.50 g/L	1		
22	46	70	94		2			22	46	70	94		2		
23	47	71	95		3			23	47	71	95		3		
24	48	72	96		4			24	48	72	96		4		

NBT 01-25-23

Fathead Minnow Order & Shipment Log

Ordering Information:

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
01-23-23	1688 + 1689	ABS	600	1 day old on arrival	01-24-23	AMF	

Delivery Information:

Larva source	Approx. number received	Date/time received	Received by (Initials)
ABS	600	01-24-23 @ 1210	MS

Monitoring Interval	Hour							
	0	1	2	3	4	5	6	7
Temperature (°C)	13.3	20.4	22.4					
Time	1350	1415	1436					
Thermometer ID	DD19							
Initials	MS							

Comments (e.g. condition of larvae received):
Fed finely shredded ~~BS~~ flakes @ 1430. 01-24-23 MS
Fed 2mL BS @ 830. 01-25-23 MS

OAK RIDGE National Laboratory

PO Box 2008
Oak Ridge, TN 37831
(865) 341-0398| stevensonlm@ornl.gov

Date: May 23, 2023

To: K.G. Hanzelka (RC)

c: S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, A.M. Fortner

From: L.M. Stevenson, 1504, MS-6351 (865-341-0398).

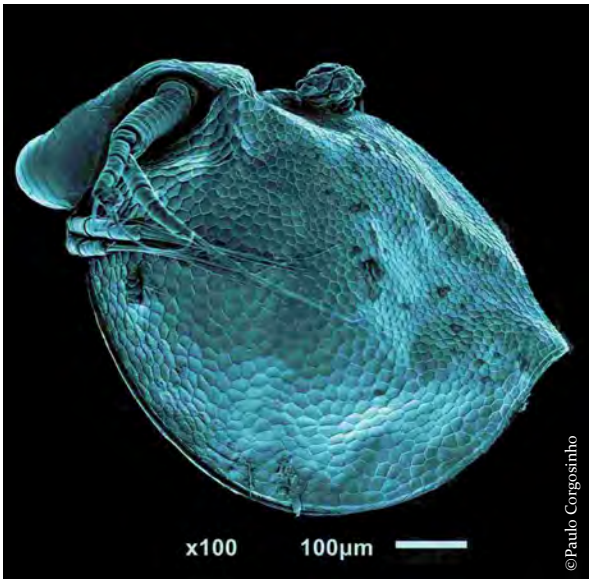
RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted May 3-10, 2023**

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$_{25}$ (IC_{25}) 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_{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_{25}
Outfall 200	Fathead minnow	Survival	>100%
		Growth	>100%
Outfall 200	*Ceriodaphnia*	Survival	>100%
		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_{25}) 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: 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 ± 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
pH (S.U.)	8.16	8.13	8.24
Conductivity (µS/cm)	544	525	418
Alkalinity (mg/L as $CaCO_3$)	124	140	132
Hardness (mg/L as $CaCO_3$)	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 ± 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 (4th 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 10^6 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 10^7 cells/mL (EPA/821/R/02/013; 4th 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_{25} = 2.24 g NaCl/L; 95% C.I. = 1.74-2.32 g NaCl/L.
Reproduction IC_{25} = 1.38 g NaCl/L; 95% C.I. = 1.18-1.57 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_{25} for survival: 1.49 ± 0.851 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} for survival: 0.285 g NaCl/L
Central tendency of IC_{25} for reproduction: 1.063 ± 0.619 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} 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_{25} for survival was >100% effluent.

7.2 Reproduction

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

7.3 Summary of *Ceriodaphnia* toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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 $CaCO_3$)	80	80	120
Hardness (mg/L as $CaCO_3$)	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 (µS/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 Date: 17 May 2023

Report reviewed by: Louise Stevenson Date: 22 May 2023 Louise Stevenson

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_{25}) 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 ± 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
pH (S.U.)	8.16	8.13	8.24
Conductivity (µS/cm)	544	525	418
Alkalinity (mg/L as $CaCO_3$)	124	140	132
Hardness (mg/L as $CaCO_3$)	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 ± 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 (4th 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_{25} = 1.01 g KCl/L; 95% C.I. = 0.72-1.04 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_{25} for survival: 0.868 ± 0.27 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} for survival: 0.156 g KCl/L

Central tendency of IC_{25} for growth: 0.920 ± 0.228 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} 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:

Survival

Concentration	Proportion surviving per replicate				Mean
	1	2	3	4	
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

Dry Weight

Concentration	Weight (mg) per replicate				Mean ± SD
	1	2	3	4	
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 ± 0.09
25%	0.74	0.87	0.71	0.77	0.77 ± 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 ± 0.05
100%	0.71	0.69	0.82	0.78	0.75 ± 0.06

7. STATISTICAL ANALYSES

7.1 Survival

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

7.2 Growth

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

7.3 Summary of fathead minnow toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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.)	8.21	8.23	8.04
Conductivity (µS/cm)	241	235	246
Alkalinity (mg/L as $CaCO_3$)	80	80	120
Hardness (mg/L as $CaCO_3$)	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 (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.

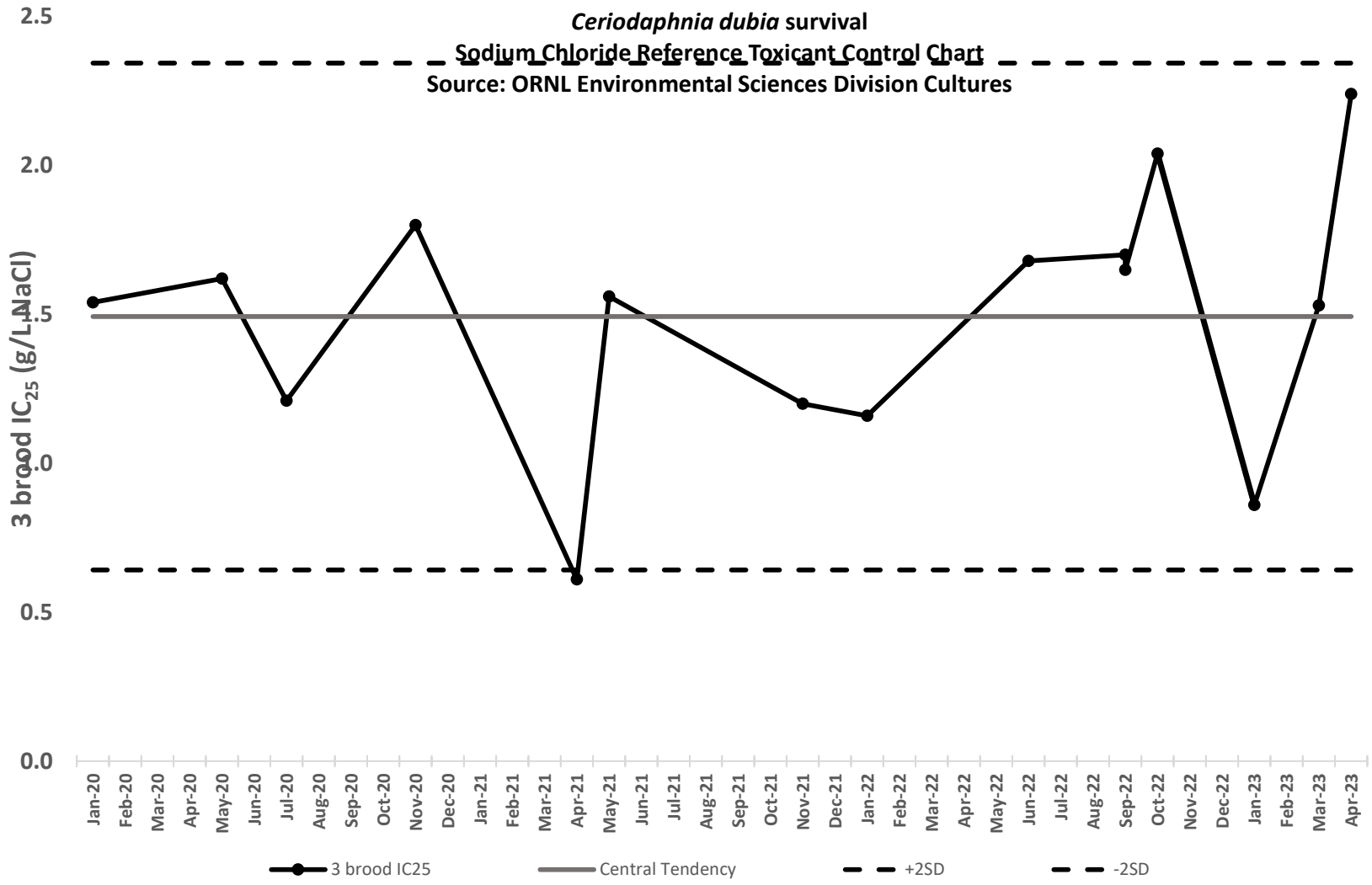
Instruments were calibrated and standardized according to manufacturer's instructions.

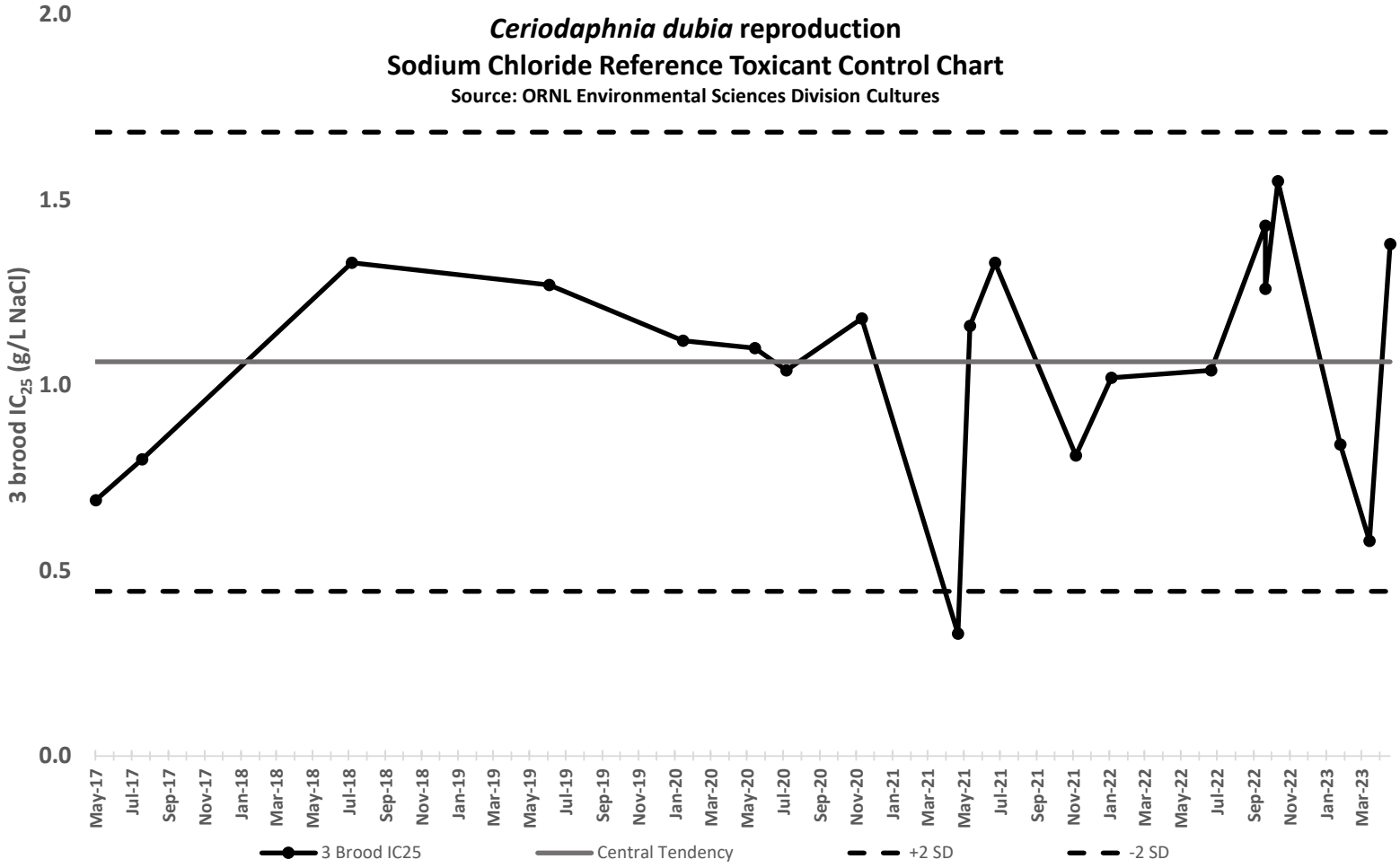
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Peijia Ku Date: 18 May 2023

Report reviewed by: Louise Stevenson Date: 22 May 2023 Louise Stevenson

REFERENCE TOXICANT CONTROL CHARTS


Ceriodaphnia dubia survival
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.5
2.0
1.5
1.0
0.5
0.0
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Mar-23
Apr-23
3 brood IC25
Central Tendency
+2SD
-2SD


Ceriodaphnia dubia reproduction
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.0
1.5
1.0
0.5
0.0
May-17
Jul-17
Sep-17
Nov-17
Jan-18
Mar-18
May-18
Jul-18
Sep-18
Nov-18
Jan-19
Mar-19
May-19
Jul-19
Sep-19
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Sep-21
Nov-21
Jan-22
Mar-22
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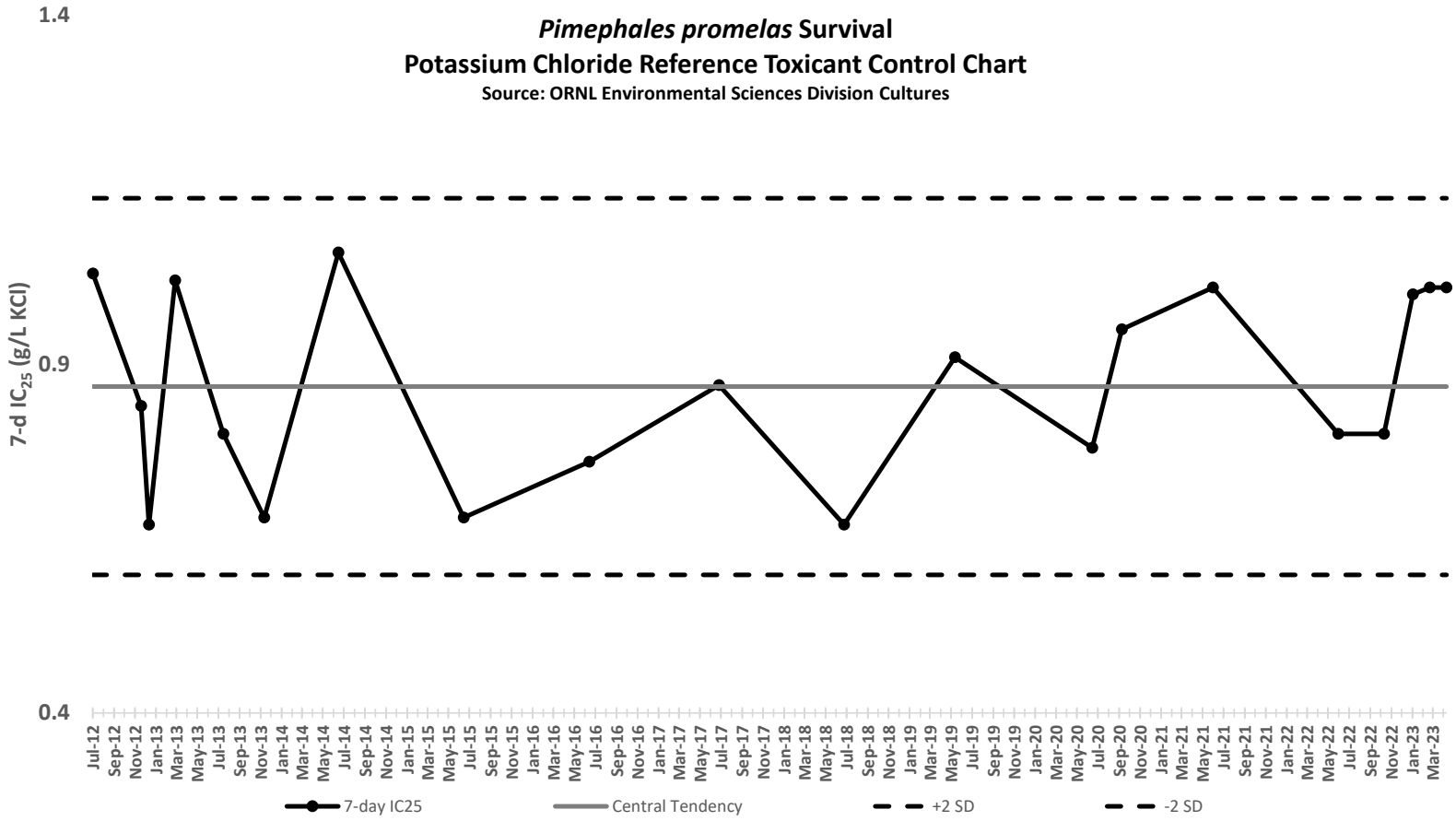
Sep-22
Nov-22
Jan-23
Mar-23
3 Brood IC25
Central Tendency
+2 SD
-2 SD


1.4
Pimephales promelas Survival
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC25 (g/L KCl)
0.9
0.4
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Sep-13
Nov-13
Jan-14
Mar-14
May-14
Jul-14
Sep-14
Nov-14
Jan-15
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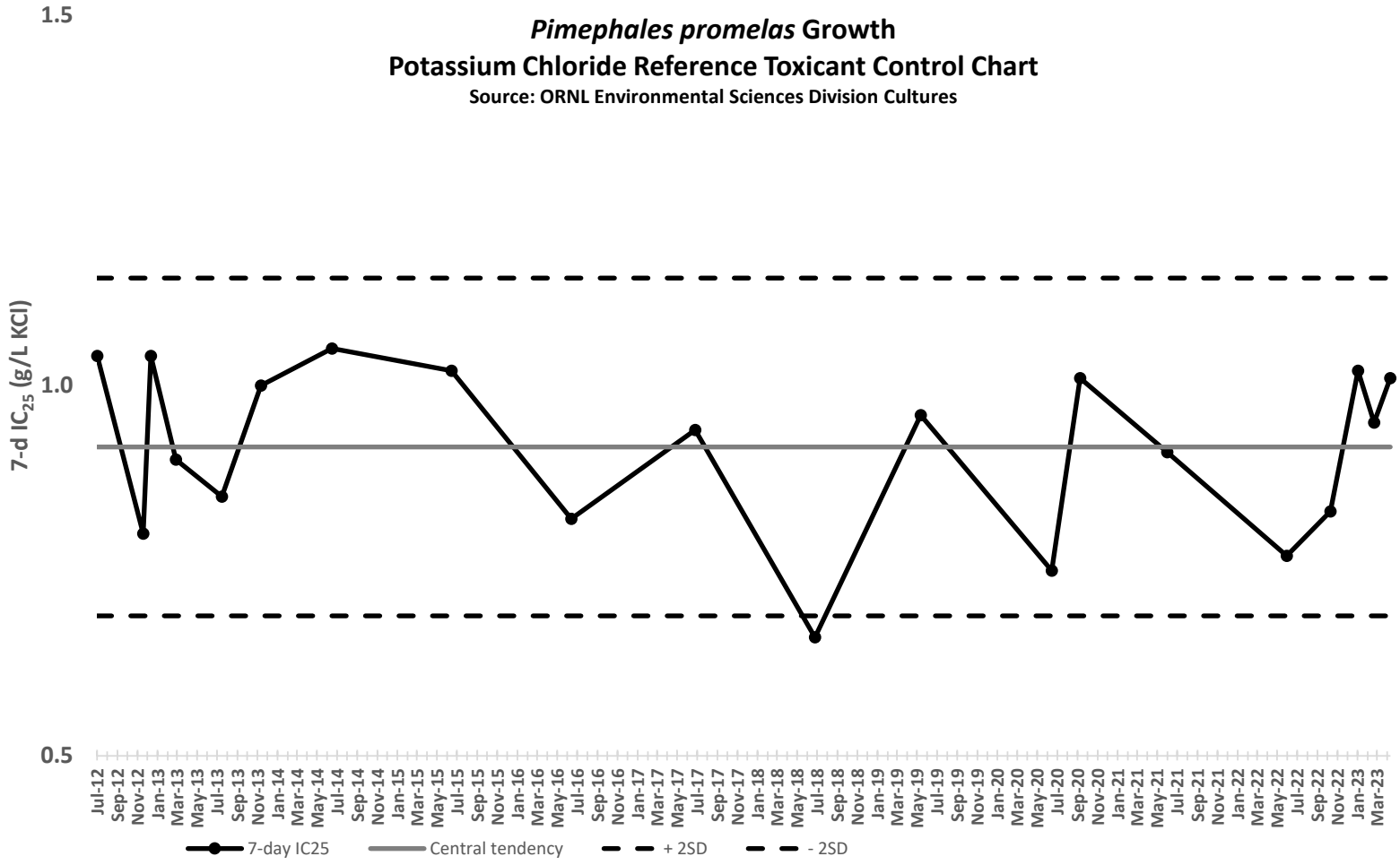
Jul-22
Sep-22
Nov-22
Jan-23
Mar-23
7-day IC25
Central Tendency
+2 SD
-2 SD


1.5
Pimephales promelas Growth
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC25 (g/L KCl)
1.0
0.5
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Sep-13
Nov-13
Jan-14
Mar-14
May-14
Jul-14
Sep-14
Nov-14
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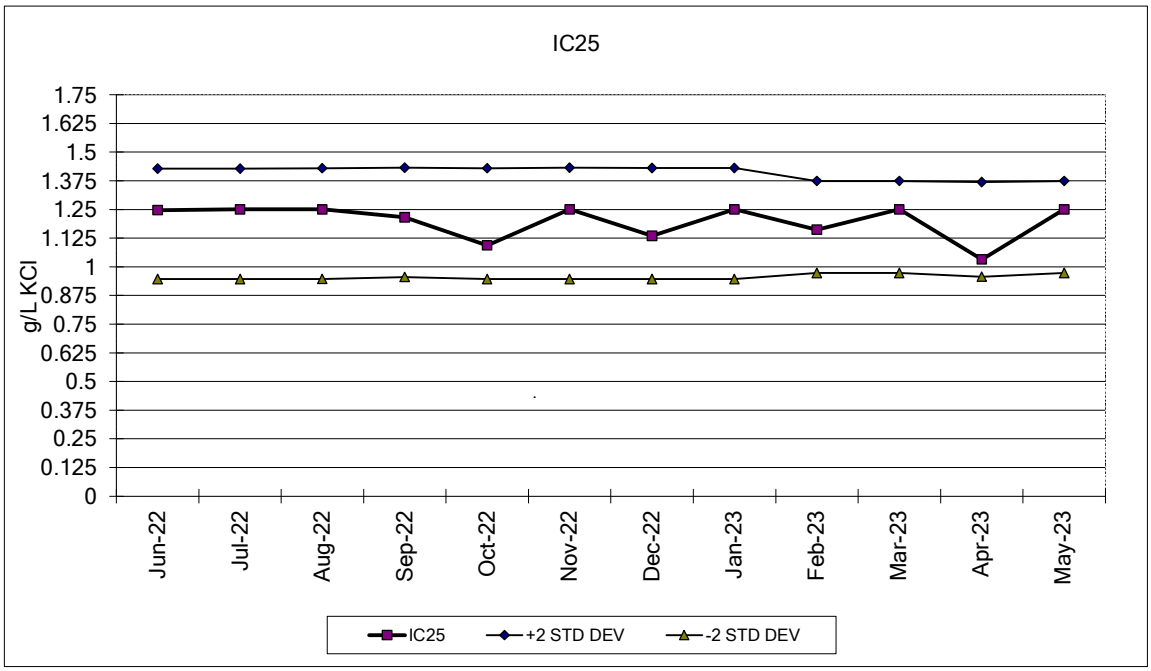
Mar-21
May-21
Jul-21
Sep-21
Nov-21
Jan-22
Mar-22
May-22
Jul-22
Sep-22
Nov-22
Jan-23
Mar-23
7-day IC25
Central tendency
+ 2SD
- 2SD

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

Date	NOEC (g/L KCl)	LOEC (g/L KCl)
Dec-22	0.50	1.0
Jan-23	0.50	1.0
Feb-23	0.50	1.0
Mar-23	0.50	1.0
Apr-23	0.50	1.0
May-23	0.50	1.0

IC 25 for Growth Test

Date	IC25 g/L KCl	95% Confidence (upper)	95% Confidence (lower)	Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
Dec-22	1.134	1.319	0.164	1.188	1.431	0.946
Jan-23	1.250	1.250	1.144	1.188	1.431	0.946
Feb-23	1.162	1.303	-0.506	1.173	1.374	0.972
Mar-23	1.250	1.250	1.210	1.173	1.374	0.972
Apr-23	1.032	1.272	0.023	1.163	1.369	0.957
May-23	1.250	1.250	1.141	1.173	1.374	0.973

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

WATER CHEMISTRY DATA LOGSHEETS

Daily Water Chemistry Log

Sponsor: Y12 Site/Treatment: OF-200 Associated test numbers: CD-7985 ; FHM1692

Note: Not all parameters are required for all tests. All unused cells should be lined through or marked "NA."

PK 05/02/23

		Observation Day:	0	1	2 PK	3 PK	4 PK	5 PK	6 PK	7
		Date/Initials:	5/3/23 PK	5/4/23 PK	5/5/23	5/6/23	5/7/23	5/8/23	5/9/23	5/10/23
		5-digit ORNL ID	33286	→	33287	→	→	33288	→	→
		Rec. temp. (°C) (New ✓)	secoc ☑	☐	secoc ☑	☐	☐	secoc ☑	☐	☐
①	Control: DI 5% PM4	DMW Batch #	947	947	947	947	950	950	950	
		Conductivity (µS/cm)	241	239	235	224	246	239	228	
		Alkalinity (mg/L)	80				120			
		Hardness (mg/L)	130				130			
		pH (S.U.) Initial	8.207	8.19	8.23	8.20	8.04	8.20	8.05	
		Final CD/FHM		8.59/8.05	8.50/7.99	8.50/8.04	8.54/7.93	8.55/7.90	8.5/7.91	8.28/8.10
		DO (mg/L) Initial	8.63	8.63	8.69	8.60	8.60	8.60	8.59	
		Final CD/FHM		8.71/7.76	9.09/6.97	8.78/7.15	8.79/7.10	8.50/5.99	8.79/6.48	8.47/7.43
②	12.5% OF200	Conductivity (µS/cm)	277	277	271	261	280	261	251	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.19	8.19	8.11	8.23	8.25	8.18	8.05	
		Final CD/FHM		8.62/8.09	8.57/8.08	8.54/8.06	8.59/8.02	8.61/7.93	8.63/7.98	8.35/8.01
		DO (mg/L) Initial	8.63	8.69	8.70	8.70	8.45	8.56	8.58	
		Final CD/FHM		8.68/7.39	9.16/6.97	8.81/7.14	8.90/7.05	8.86/6.36	8.92/6.81	8.51/7.49
③	25% OF200	Conductivity (µS/cm)	317	318	308	299	316	284	275	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) 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	8.61/8.10	8.59/8.09	8.61/7.96	8.65/7.98	8.36/8.04
		DO (mg/L) Initial	8.77	8.76	8.72	8.76	8.54	8.36	8.74	
		Final CD/FHM		8.70/7.45	8.96/7.30	8.83/7.30	8.98/7.41	8.89/6.55	9.03/[illegible]	8.52/7.50
④	50% OF200	Conductivity (µS/cm)	399	396	381	375	382	327	322 7.17	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.16	8.19	8.14	8.18	8.22	8.25	8.17	
		Final CD/FHM		8.55/8.14	8.60/8.15	8.59/8.17	8.68/8.23	8.65/8.05	8.67/8.01	8.39/8.07
		DO (mg/L) Initial	8.76	8.77	8.87	8.78	8.58	8.74	8.95	
		Final CD/FHM		8.69/7.53	9.02/7.14	8.96/7.18	9.17/7.27	8.99/6.62	9.14/6.81	8.54/7.49
⑤	75% OF200	Conductivity (µS/cm)	470	472	451	450	453	371	368	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.13	8.19	8.14	8.18	8.22	8.24	8.15	
		Final CD/FHM		8.59/8.20	8.66/8.19	8.63/8.22	8.67/8.13	8.66/8.08	8.68/8.04	8.42/8.05
		DO (mg/L) Initial	9.02	8.91	8.99	8.90	8.61	8.60	9.02	
		Final CD/FHM		8.71/7.47	9.11/7.11	9.09/7.19	9.21/7.18	9.02/6.63	9.18/6.42	8.53/7.36
⑥	100% OF200	Conductivity (µS/cm)	544	549	525	520	525	418	415	
		Alkalinity (mg/L)	124		140			132		
		Hardness (mg/L)	290		250			200		
		Chlorine (mg/L) F/T	0.02/0.01		0.01/0.01			0.01/0.02		
		pH (S.U.) Initial	8.16	8.19	8.13	8.18	8.21	8.24	8.14	
		Final CD/FHM		8.64/8.24	8.67/8.25	8.67/8.21	8.70/8.22	8.68/8.18	8.68/8.15	8.44/8.05
		DO (mg/L) Initial	9.12	8.91	9.11	8.83	8.64	9.58	9.20	
		Final CD/FHM		8.78/7.31	9.17/6.87	9.15/7.26	9.25/7.00	9.09/6.20	8.20/6.69	8.58/7.37

CHAIN OF CUSTODY FORMS

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

DATE (MM/DD/YY): 05/03/23 | ESD TEST NAME: TOX TEST | NAME OF SAMPLERS: GARLAND/WILLIAMS FRIG | CHAIN-OF-CUSTODY NO.: 031101

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS: #7009 TEMP. / # 4102 Cl2
TOX TEST	200	1240	C	1	~18L	4°	14.3° / <0.05

THERMOMETER NO.

SAMPLES RELINQUISHED BY: A. L. Garland	DATE: 5/3/23	TIME: 1308	☐ AM ☒ PM
SAMPLES RECEIVED BY: Peijia Ku	DATE: 5/3/23	TIME: 1308	☐ AM ☒ 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): 05/05/23 | ESD TEST NAME: TOXTEST | NAME OF SAMPLERS: GARLAND/WILLIAMS | CHAIN-OF-CUSTODY NO.: 031102

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	#7009 TEMP / REMARKS #4102 Cl2
TOX TEST	200	0710	C	1	~17 LITERS	4°	11.6° / <0.05

C.L.G. 5/5/23

THERMOMETER NO.

SAMPLES RELINQUISHED BY: C.L. Garland	DATE: 5/5/23	TIME: 0802	☒ AM ☐ PM
SAMPLES RECEIVED BY: Prijia Ku	DATE: 5/5/23	TIME: 0802	☒ AM ☐ 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: TOXTEST | NAME OF SAMPLERS: GARLAND/WILLIAMS | CHAIN-OF-CUSTODY NO.: 031103

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS
						FRIG	#4102
						#7009 TEMP	Cl2
TOXTEST	200	0715	C	1	~16.5L	4°	14.7 / <0.05

A. L. Garland 5/8/23

THERMOMETER NO.

SAMPLES RELINQUISHED BY: A. L. Garland	DATE: 5/8/23	TIME: 0823	☒ AM ☐ PM
SAMPLES RECEIVED BY: Peijia Ku	DATE: 05/08/23	TIME: 0823	☒ AM ☐ PM

UCN-18631 (3 3-92)

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

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y12 **Site/Treatment:** OF200 **Test number:** 2985

PK 05/04/23

Test begin date (Day 0)	Test end date	Test duration				Template number
05/03/2023	05/10/2023	7	☐ hours	☑ days	☐ NA	☑ 3 PK

Test Organism: ☑ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: _____ Notes:

Isolated from:

Date:	5/3/23	5/4/23
Time:	10:00 pm	5:55 am

Hatch date: _____ Delivery date: _____

Test period: ☑ Chronic ☐ Acute

Test purpose: ☑ Regulatory ☐ Investigative

Test stage: ☐ Preliminary ☑ Analytical ☐ Re-test

Test type: ☑ Effluent ☐ Received waters ☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%	☒ C ☐ T	4 =	50% OF200	☐ C ☑ T
2 =	12.5% OF200	☐ C ☒ T	5 =	75% OF200	☐ C ☑ T
3 =	25% OF200	☐ C ☒ T	6 =	100% OF200	☐ C ☑ T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 947, 950

Source of Test Organisms:

☑ ESD cultures: Board numbers: ☐ NA ☐ 4762

☐ Vendor: _____ ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: 33286 Date: 05/03/23 COC #: 031101
Sample ID: 33287 Date: 05/05/23 COC #: 031102
Sample ID: 33288 Date: 05/08/23 COC #: 031103

Record of Deviations from Method and/or Test Non-Conformities

Date	*Description*	*Initial*
05/10/23	None	PK

Quality Assurance (QA) Record

Procedure	*Name*	*Initial*	*Date*
Test run by:	Peijia Ku	PK	05/10/23
Data sheets QA:	[illegible]	[illegible]	05-11-23
Data entered:	Peijia Ku	PK	05/10/23
Data entry QA:	[illegible]	[illegible]	05-11-23

CHRONIC Daily Water/Feeding Log

Sponsor: Y12 Test site/treatment: DF200 Begin Date: 05/03/2023 End Date: 05/10/2023 Test Number: 2985

Daily Test Info		Temperature Information Therm. #: PD20		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10⁷/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	05/03/23 PK	___ am 26.0 pm	___ am 25.0 pm	YCT R	3/21/23 5/3/23	100 86	☑Yes 3.50E7	___ am 1830 pm	1803	1900	33286	947	NA
Day 1	05/04/23 PK	___ am 26.1 pm	___ am 25.0 pm	YCT R	3/21/23 5/3/23	100 91	☑Yes 3.30E+07	___ am 1650 pm	1630	1725	↓	947	
Day 2	05/05/23 PK	___ am 25.7 pm	___ am 25.3 pm	YCT R	3/21/23 5/3/23	100 90	☑Yes 3.33E+07	___ am 1525 pm	1510	1600	33287	947	
Day 3	05/06/23 PK	25.7 am ___ pm	25.3 am ___ pm	YCT R	3/21/23 5/3/23	100 91	☑Yes 3.28E+07	1625 am ___ pm	1610	1700	↓	947	
Day 4	05/07/23 PK	25.7 am ___ pm	25.5 am ___ pm	YCT R	3/21/23 5/3/23	100 92	☑Yes 3.25E+07	1505 am ___ pm	1450	1600	↓	950	
Day 5	05/08/23 PK	25.8 am ___ pm	~~25.8~~ 25.3 am PK ___ pm	YCT R	3/21/23 5/3/23	100 92	☑Yes 3.25E+07	1535 am ___ pm	1515	1635	33288	950	
Day 6	05/09/23 PK	25.6 am ___ pm	25.3 am ___ pm	YCT R	3/21/23 5/3/23	100 90	☑Yes 3.34E+07	1518 am ___ pm	1504	1630	↓	950	
Day 7	05/10/23 PK	25.3 am ___ pm	25.0 am ___ pm				☐Yes	___ am ___ pm	1125	1150			↓

Notes:

Ceriodaphnia Chronic Daily Survival & Reproduction Log

Project: Y12 Test site/chemical: 0.5-200 Test Number: 2985

Begin Date: 05/03/2023 End Date: 05/10/2023 Template Number: 3 PK

Codes: (-) Alive and No Reproduction; (*N*) Alive and Reproduction; (*x*N) Dead and Reproduction; (M) Male

Test Chamber	Treatment Number	Day: 1 PK Date: 05/04/23	2 PK 05/05/23	3 PK 05/06/23	4 PK 05/07/23	5 PK 05/08/23	6 PK 05/09/23	7 PK 05/10/23
1	2	-	-	-	7	11	-	
2	6	-	-	-	5	13	18 PK	16
3	5	-	-	-	6	13	13	18
4	3	-	-	-	7	1	-	-
5	5	-	-	-	5	17	17	2
6	6	-	-	-	6	14	16	-
7	4	-	-	-	5	12	-	-
8	4	-	-	5	-	13	17	-
9	1	-	-	-	6	5	13	-
10	1	-	-	-	6	12	17	-
11	5	-	-	-	6	10	-	15
12	3	-	-	-	5	12	-	14
13	6	-	-	-	6	15	17	-
14	6	-	-	-	1	14	15	-
15	4	-	-	-	6	11	11	-
16	1	-	-	-	6	14	16	-
17	1	-	-	-	-	13	13	-
18	2	-	-	-	4	9	19	-
19	6	-	-	5	-	9	17	-
20	2	-	-	-	5	-	10	-
21	4	-	-	-	6	12	20	-
22	2	-	-	-	7	2	4	-
23	1	-	-	-	6	13	15	-
24	4	-	-	-	7	12	17	-
25	6	-	-	-	-	1	17	-
26	4	-	-	-	5	2	11	-
27	6	-	-	8	-	16	17	-
28	6	-	-	5	-	10	18	-
29	2	-	-	PK 8 -	-	8	-	-
30	3	-	-	-	4	4	11	-
31	3	-	-	-	5	12	16	-
32	5	-	-	-	6	12	18	-
33	4	-	-	-	8	12	16	-
34	2	-	-	-	6	12	16	-
35	2	-	-	-	5	12	16	-
36	5	-	-	-	7	13	16	-
37	2	-	-	-	6	3	2	-
38	1	-	-	-	6	10	15	-
39	3	-	-	-	-	15	-	-
40	5	-	-	-	6	6	7	
41	6	-	-	-	6	14	14	-
42	4	-	-	-	-	2	1	-
43	2	-	-	-	7	4	-	-
44	5	-	-	-	6	12	14	
45	3	-	-	-	7	13	15	-
46	3	-	-	-	6	11	18	-
47	3	-	-	-	4	13	-	-
48	5	-	-	5	-	14	14	-
49	4	-	-	-	7	12	14	-
50	4	-	-	-	5	6	14	-
51	1	-	-	-	6	8	-	18
52	1	-	-	-	5	13	16	-
53	3	-	-	-	2	17	18	-
54	1	-	-	-	6	14	15	-
55	1	-	-	-	6	14	18	-
56	2	-	-	-	6	12	14	-
57	5	-	-	-	5	12	14	-
58	3	-	-	-	6	11	14	-
59	5	-	-	-	5	5	16	-
60	6	-	-	-	4	5	-	16

Toxicity Test Information Sheet

Sponsor: 4/12 Site/Treatment: OF-200 Test number: 1692

Test begin date (Day 0)	Test end date	Test duration	Template number
05/03/2023	05/10/2023	7 ☐ hours ☑ days	☑ NA ☐ _____

PK 05/02/23

Test Organism: ☐ *Ceriodaphnia dubia* ☑ Fathead minnow ☐ Other: _____

Isolated from: Date: _____ Time: _____

PK Hatch date: 05/04/2023 Delivery date: 05/02/2023

Notes:

Test period: ☑ Chronic ☐ Acute

Test purpose: ☑ Regulatory ☐ Investigative

Test stage: ☐ Preliminary ☑ Analytical ☐ Re-test

Test type: ☑ Effluent ☐ Received waters ☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%	☑C ☐T	4 =	50%	☐C ☑T
2 =	12.5%	☐C ☑T	5 =	75%	☐C ☑T
3 =	25%	☐C ☑T	6 =	100%	☐C ☑T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 947, 950

Source of Test Organisms:

☐ ESD cultures: Board numbers: ☐ NA ☐ _____

☑ Vendor: ABS ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: 33286 Date: 05/03/23 COC #: 031101
Sample ID: 33287 Date: 05/03/23 COC #: 031102
Sample ID: 33288 Date: 05/08/23 COC #: 031103

Record of Deviations from Method and/or Test Non-Conformities

Date	*Description*	*Initial*
05/10/23	NA	PK

Quality Assurance (QA) Record

Procedure	*Name*	*Initial*	*Date*
Test run by:	Peijia Hu	PK	05/10/23
Data sheets QA:	[illegible]	[illegible]	05-12-23
Data entered:	Peijia Hu	PK	05/10/23
Data entry QA:	[illegible]	[illegible]	05-12-23

PF 05/02/23

CHRONIC Daily Water/Feeding Log

Sponsor: YI2 Test site/treatment: OF200 Begin Date: 05/03/2023 End Date: 05/10/2023 Test Number: 1692

Daily Test Info		Temperature Information Therm. #: DD19		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, S= *Selenestrum*, B=Brine shrimp) Acceptable cell density range = 3.0 to 3.5 x10^7/mL					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 algae/B count	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	05/03/23 PEK/NJJ	am 26.4 pm	am 25.3 pm	B	05/02/23	195	☐NA ☑Yes	am 1752 pm	1630	1742	33286	947	NA
Day 1	05/04/23 PK	26.2 am 26.3 pm	25.7 am 25.9 pm	B B	05/03/23 05/03/23	79 94	☐NA ☑Yes	1030 am 1625 pm	1325	1420	↓	947	
Day 2	05/05/23 PK	26.2 am 26.4 pm	25.7 am 25.9 pm	B B	05/04/23 05/04/23	109 100	☐NA ☑Yes	0830 am 1455 pm	1200	1255	33287	947	
Day 3	05/06/23 PK	26.6 am 25.3 pm	26.0 am 24.8 pm	B B	05/05/23 05/05/23	78 85	☐NA ☑Yes	0910 am 1604 pm	1450	1550	↓	947	
Day 4	05/07/23 PK	26.1 am 25.9 pm	25.5 am 25.5 pm	B B	05/06/23 05/06/23	91 101	☐NA ☑Yes	0950 am 1621 pm	1240	1340	↓	950	
Day 5	05/08/23 PK	26.0 am 26.1 pm	25.5 am 25.7 pm	B B	05/07/23 05/07/23	80 77	☐NA ☑Yes	0845 am 1505 pm	1250	1335	33288	950	
Day 6	05/09/23 PK	26.0 am 26.0 pm	25.5 am 25.5 pm	B B	05/08/23 05/08/23	91 59	☐NA ☑Yes	0935 am 1700 pm	1210	1310	↓	950	
Day 7	05/10/23 PK	26.0 am pm	25.5 am pm						1500	1630			NA

Notes:

Fathead Minnow Chronic Daily Survival Log

PK 05/02/23

Sponsor: Y12 Test site/chemical: OF200 Test Number: 1692

Begin Date: 05/03/2023 End Date: 05/10/2023

Comment Codes: C = Clear; D = Dead; Fg = Fungus; K = Killed by siphoning; M = Missing; Sk = Sick; SM = Small; SOR = Siphoned and returned; W = Wounded

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 05/06/23 PK	Day 4 Date 05/07/23 PK	Day 5 Date 05/08/23 PK	Day 6 Date 05/09/23 PK	Day 7 Date 05/10/23 PK
1: 25% PMW	1	18	10	10	10	10	10	10	10
	2	24	10	10	10	10	10	10	10
	3	11	10	10	10	10	10	10	10
	4	14	10	10	10	10	10	10	10
2: 12.5% OF200	1	13	10	10	10	10	10	10	10 PK
	2	10	10	10	10	10	10	10	10
	3	16	9 ID	9	9	9	PK 9	9	9
	4	15	10	10	10	10	10	10	10
3: 25% OF200	1	21	10	10	10	PK 10	10	10	10
	2	12	10	10	PK 10	10	10	10	10
	3	19	9 ID	9	8 ID	8	8	8	8
	4	3	10	10	10	10	10	10	10
4: 50% OF200	1	7	10	10	10	9 ID	9	9	9
	2	20	10	10	10 ISK	10	10	10	10
	3	1	10	10	10	10	10	10	10
	4	22	10 PK ISK PK	10	10	10	10	10	10
5: 75% OF200	1	23	10	10	10	10	10	10	10
	2	4	10	10	PK 9 ID	9	9	9	9
	3	17	10	10	10	10	10	10	10
	4	2	10	10	10	10	10	10	10
6: 100% OF200	1	5	10	10 2SM	10 2SM	10 2SM	10 2SM	10 2SM	10 2SM
	2	9	10	10	10	10	10	10	10
	3	6	10	10	10	10	10	10	10
	4	8	10	10	10	10	10	10	10

Random Assignment of Test Chambers

Project: Y12 **Test site/chemical:** OF200 **Test Number:** 1692

Starting Position Number (on Table of Random Numbers): 29

PK
05/02/23

Assigned Numbers				Sample ID/Treatment		Replicate	Position
1	~~25~~	49	73	50% OF200	4	3	1
2	~~26~~	50	74	75% OF200	5	4	2
~~3~~	27	51	75	25% OF200	3	4	3
4	28	~~52~~	76	75% OF200	5	2	4
~~5~~	29	53	77	100% OF200	6	1	5
6	30	~~54~~	78	100% OF200	6	3	6
~~7~~	31	55	79	50% OF200	4	1	7
8	32	56	~~80~~	100% OF200	6	4	8
9	~~33~~	57	81	100% OF200	6	2	9
10	~~34~~	58	82	12.5% OF200	2	2	10
~~11~~	35	59	83	25% DMW	1	3	11
12	36	60	~~84~~	25% OF200	3	2	12
~~13~~	37	61	85	12.5% OF200	2	1	13
14	38	~~62~~	86	25% DMW	1	4	14
15	39	63	~~87~~	12.5% OF200	2	4	15
16	~~40~~	64	88	12.5% OF200	2	3	16
~~17~~	41	65	89	75% OF200	5	3	17
18	~~42~~	66	90	25% DMW	1	1	18
19	43	~~67~~	91	25% OF200	3	3	19
~~20~~	44	68	92	50% OF200	4	2	20
~~21~~	45	69	93	25% OF200	3	1	21
22	46	~~70~~	94	50% OF200	4	4	22
~~23~~	47	71	95	75% OF200	5	1	23
24	~~48~~	72	96	25% DMW	1	2	24

PK 05/02/23

Fathead Minnow Weight and Survival Data

Sponsor: Y12	Test number: 1692
Test site/chemical: UF-200	Balance ID: A009820
Test Start Date: 05/03/2023	Test End Date: 05/10/2023
Start Drying Date/Time: 05/10/23, 1620	End Drying Date/time: 05/11/23, 1200

Treatment	Replicate	Pan Wt. (mg) Date: 05/09/23 Balance check: ☑	Pan + Larvae (mg) Date: 05/11/23 Balance check: ☑	Number Surviving
Initial	1	15.1175	16.4080	10
	2	15.0915		
	3	15.0130		
	4	15.0090		
1. 25% DMW	1	15.0785	23.2285	10
	2	15.0645	22.8365	10
	3	15.2950	23.8305	10
	4	15.2295	22.7680	10
2. 12.5% UF-200	1	15.1305	23.7075	10
	2	15.1785	21.8920	10
	3	15.0895	PK 21.9525	9
	4	15.2905	23.1545	10
3. 25% UF-200	1	15.2900	22.7785	10
	2	15.2875	23.9815	10
	3	15.3600	22.3025	8
	4	15.3370	23.0570	10
4. 50% UF-200	1	15.3330	22.5860	9
	2	15.2855	23.7705	10
	3	15.1690	24.4465	10
	4	15.3905	22.3495	10
5. 75% UF-200	1	15.3705	22.8755	10
	2	15.3250	22.3345	9
	3	15.1295	22.3390	10
	4	15.3605	23.5665	10
6. 100% UF-200	1	15.3685	22.4470	10
	2	15.3290	22.2235	10
	3	15.3780	23.6150	10
	4	15.3780	23.1690	10

Random Assignment of Larvae to Test Chambers

Project: Y12. KCl. Ref **Test site/chemical:** OF-200 ; KCl **Test Number:** 1692 ; 1693

Starting Position Number (on Table of Random Numbers): 3, 11

PK 05/02/23

Assigned Numbers				Sample ID/Treatment	Replicate	Assigned Numbers				Sample ID/Treatment	Replicate
1	25	49	73	1. 25% DMW	14,221 ✓	1	25	49	73	1. 25% DMW	2,451 ✓
2	26	50	74		3,822 ✓	2	26	50	74		18,352 ✓
3	27	51	75		10,403 ✓	3	27	51	75		29,483 ✓
4	28	52	76		34,364 ✓	4	28	52	76		1,324 ✓
5	29	53	77	2. 12.5% OF200	5,231 ✓	5	29	53	77	2. 0.25g/L KCl	3,131 ✓
6	30	54	78		21,442 ✓	6	30	54	78		22,282 ✓
7	31	55	79		27,293 ✓	7	31	55	79		9,233 ✓
8	32	56	80		1,374 ✓	8	32	56	80		7,214 ✓
9	33	57	81	3. 25% OF200	18,241 ✓	9	33	57	81	3. 0.5g/L KCl	16,171 ✓
10	34	58	82		41,482 ✓	10	34	58	82		11,142 ✓
11	35	59	83		19,313 ✓	11	35	59	83		20,303 ✓
12	36	60	84		15,204 ✓	12	36	60	84		15,424 ✓
13	37	61	85	4. 50% OF200	25,301 ✓	13	37	61	85	4. 1.0g/L KCl	34,471 ✓
14	38	62	86		28,352 ✓	14	38	62	86		5,852 ✓
15	39	63	87		16,443 ✓	15	39	63	87		19,463 ✓
16	40	64	88		7,334 ✓	16	40	64	88		26,404 ✓
17	41	65	89	5. 75% OF200	4,321 ✓	17	41	65	89	5. 1.25g/L KCl	33,[illegible]89 ✓
18	42	66	90		2,132 ✓	18	42	66	90		6,272 ✓
19	43	67	91		6,263 ✓	19	43	67	91		4,443 ✓
20	44	68	92		12,394 ✓	20	44	68	92		37,484 ✓
21	45	69	93	6. 100% OF200	9,421 ✓	21	45	69	93	6. 1.50g/L KCl	31,431 ✓
22	46	70	94		38,472 ✓	22	46	70	94		10,122 ✓
23	47	71	95		11,173 ✓	23	47	71	95		36,383 ✓
24	48	72	96		43,464 ✓	24	48	72	96		24,254 ✓

PK 05/02/23

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 + 1693	ABS	600	1 day 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 Interval	Hour							
	0	1	2	3	4	5	6	7
Temperature (°C)	13.5	22.5		23.4				
Time	1305	1425		1625				
Thermometer ID	DD19	DD19		DD19				
Initials	PK	PK		PK				
Comments (e.g. condition of larvae received):								

OAK RIDGE National Laboratory

PO Box 2008
Oak Ridge, TN 37831
(865) 341-0398| stevensonlm@ornl.gov

Date: August 28, 2023

To: K.G. Hanzelka (RC)

c: S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, T. Bordeau

From: L.M. Stevenson, 1504, MS-6351 (865-341-0398).

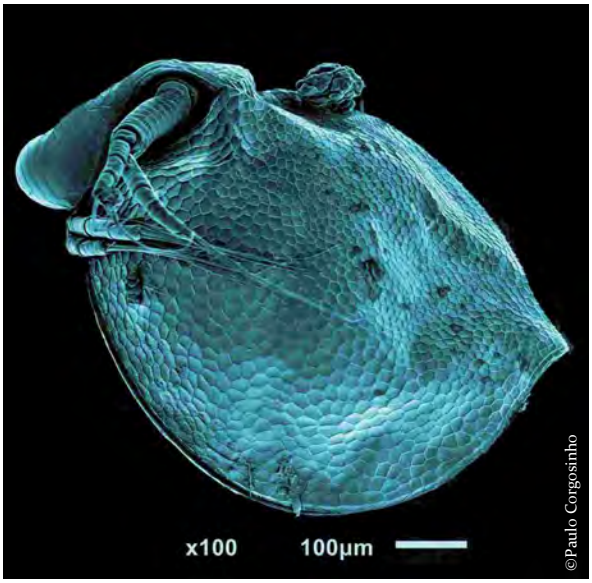
RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted August 9-16, 2023**

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$_{25}$ (IC_{25}) 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_{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_{25}
Outfall 200	Fathead minnow	Survival	>100%
		Growth	>100%
Outfall 200	*Ceriodaphnia*	Survival	>100%
		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_{25}) 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 ± 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
pH (S.U.)	8.43	8.07	8.14
Conductivity (µS/cm)	601	463	498
Alkalinity (mg/L as $CaCO_3$)	168	138	162
Hardness (mg/L as $CaCO_3$)	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 ± 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 (4th 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 10^6 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 10^7 cells/mL (EPA/821/R/02/013; 4th 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_{25} = 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_{25} for survival: 1.55 ± 0.925 g NaCl/L (mean ± 2 *SD*).

CV of IC_{25} for survival: 0.299 g NaCl/L

Central tendency of IC_{25} for reproduction: 1.10 ± 0.621 g NaCl/L (mean ± 2 *SD*).

CV of IC_{25} 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_{25} for survival was >100% effluent.

7.2 Reproduction

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

7.3 Summary of *Ceriodaphnia* toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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.)	8.14	8.03	8.15
Conductivity (µS/cm)	242	234	225
Alkalinity (mg/L as $CaCO_3$)	104	102	102
Hardness (mg/L as $CaCO_3$)	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 (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 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 Date: 17 August 2023

Report reviewed by: Louise Stevenson Louise Stevenson 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_{25}) 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 ± 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
pH (S.U.)	8.43	8.07	8.14
Conductivity (µS/cm)	601	463	498
Alkalinity (mg/L as $CaCO_3$)	168	138	162
Hardness (mg/L as $CaCO_3$)	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 ± 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 (4th 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 °C; range = 24.5 – 25.8 °C.

4.13 Treatment groups/concentrations: Control, 6.25%, 12.5%, 25%, 50%, 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: 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_{25} = 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_{25} for survival: 0.868 ± 0.275 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} for survival: 0.158 g KCl/L

Central tendency of IC_{25} for growth: 0.916 ± 0.227 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} 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

Concentration	Proportion surviving per replicate				Mean
	1	2	3	4	
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

Concentration	Weight (mg) per replicate				Mean ± SD
	1	2	3	4	
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 ± 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_{25} for survival was >100% effluent.

7.2 Growth

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

7.3 Summary of fathead minnow toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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 $CaCO_3$)	104	102	102
Hardness (mg/L as $CaCO_3$)	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 (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 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.

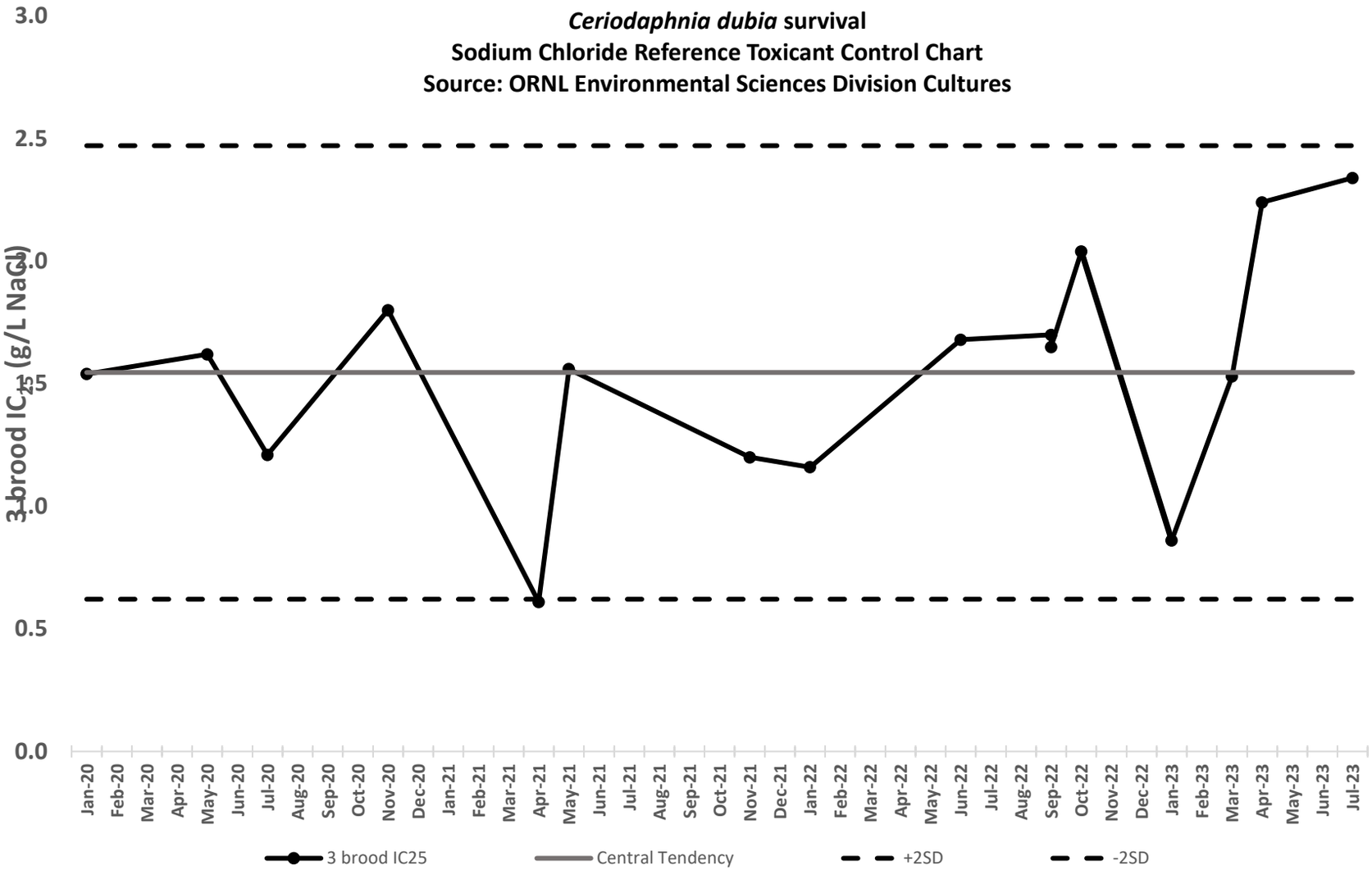
Instruments were calibrated and standardized according to manufacturer's instructions.

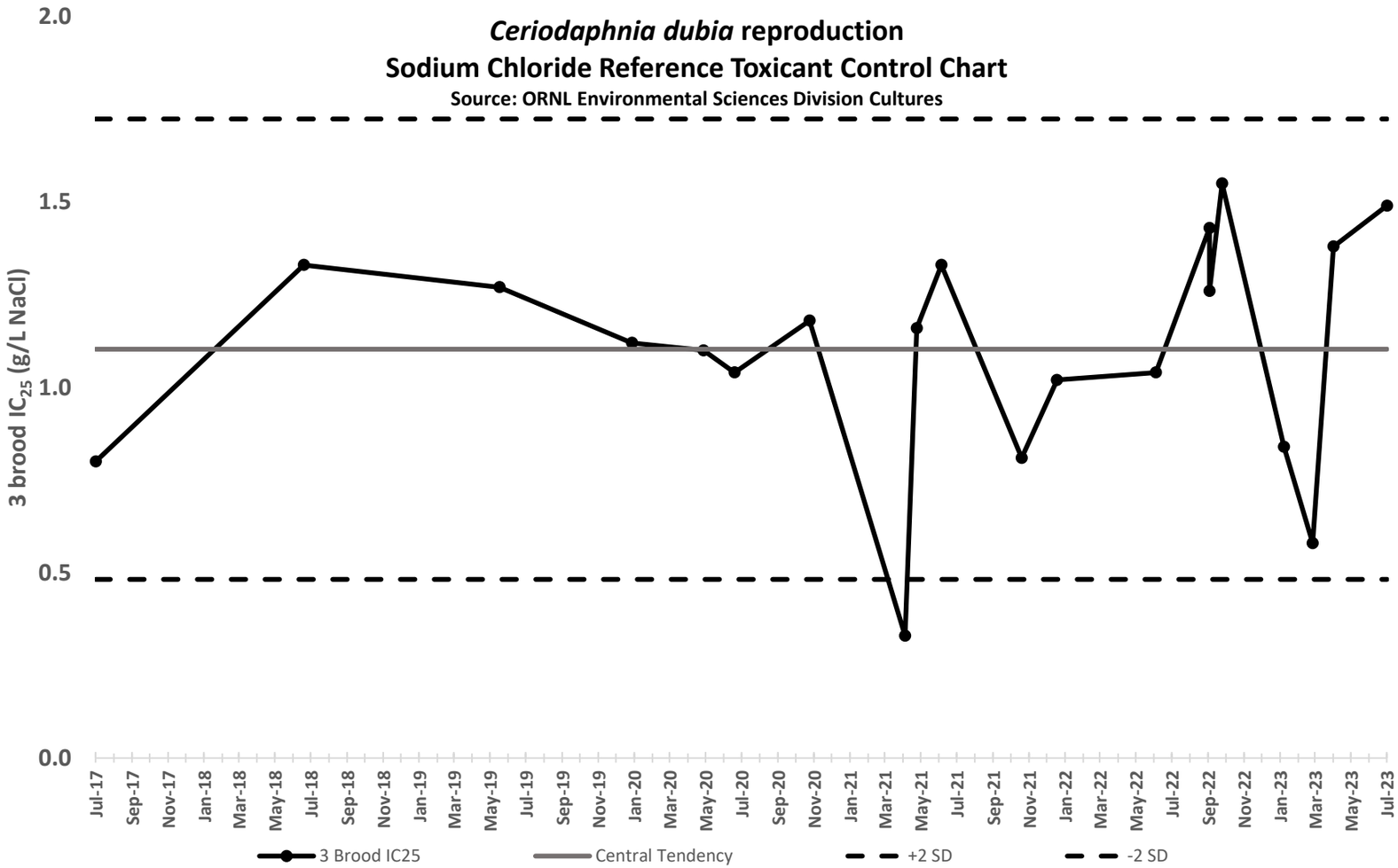
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Trystan A. Bordeau Date: 17 August 2023

Report reviewed by: Louise Stevenson Louise Stevenson Date: 28 August 2023

REFERENCE TOXICANT CONTROL CHARTS


Ceriodaphnia dubia survival
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Mar-23
Apr-23
May-23
Jun-23
Jul-23
3 brood IC25
Central Tendency
+2SD
-2SD


Ceriodaphnia dubia reproduction
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.0
1.5
1.0
0.5
0.0
Jul-17
Sep-17
Nov-17
Jan-18
Mar-18
May-18
Jul-18
Sep-18
Nov-18
Jan-19
Mar-19
May-19
Jul-19
Sep-19
Nov-19
Jan-20
Mar-20
May-20
Jul-20
Sep-20
Nov-20
Jan-21
Mar-21
May-21
Jul-21
Sep-21
Nov-21
Jan-22
Mar-22
May-22
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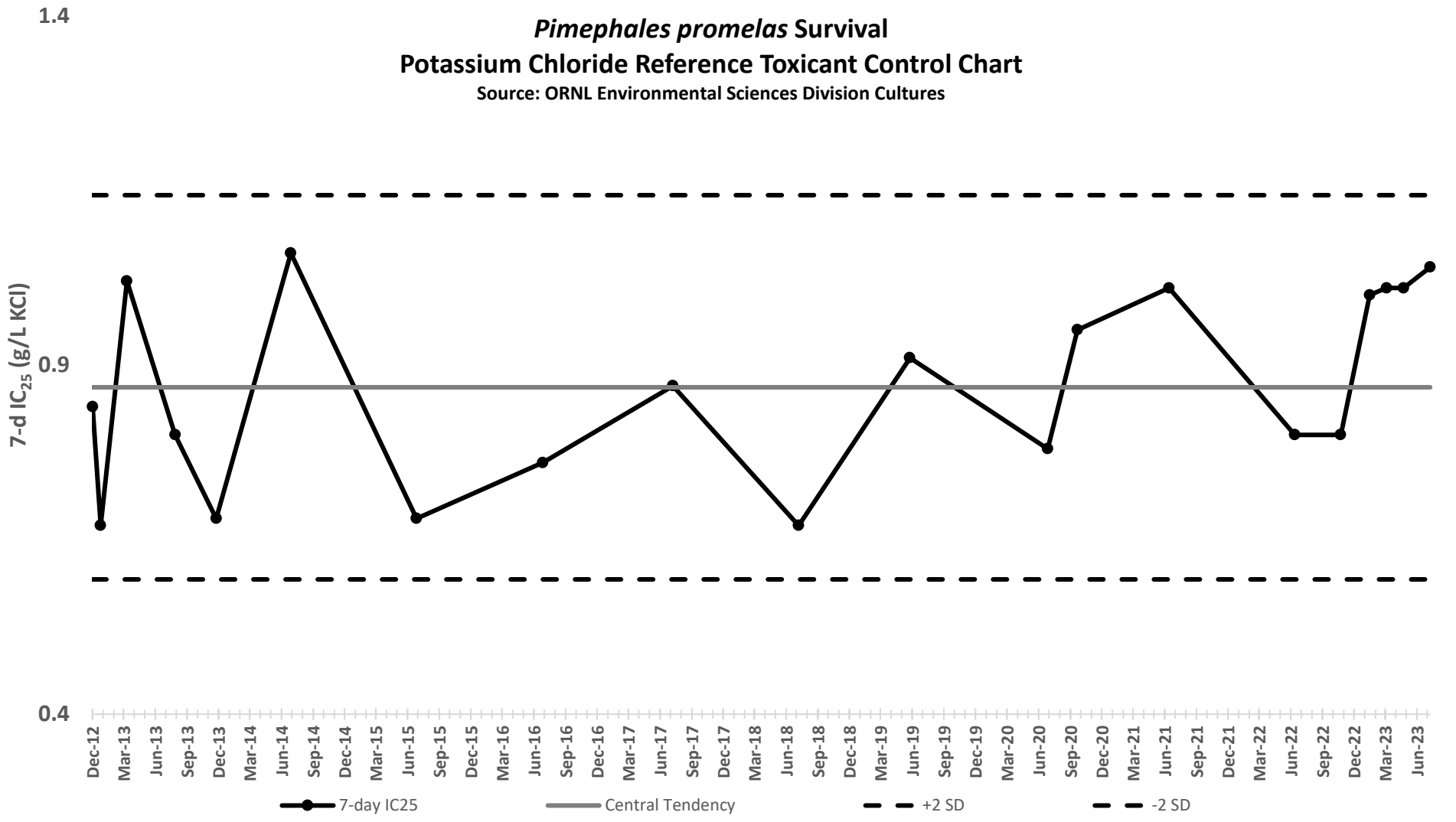
Mar-23
May-23
Jul-23
3 Brood IC25
Central Tendency
+2 SD
-2 SD


Pimephales promelas Survival
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC25 (g/L KCl)
1.4
0.9
0.4
Dec-12
Mar-13
Jun-13
Sep-13
Dec-13
Mar-14
Jun-14
Sep-14
Dec-14
Mar-15
Jun-15
Sep-15
Dec-15
Mar-16
Jun-16
Sep-16
Dec-16
Mar-17
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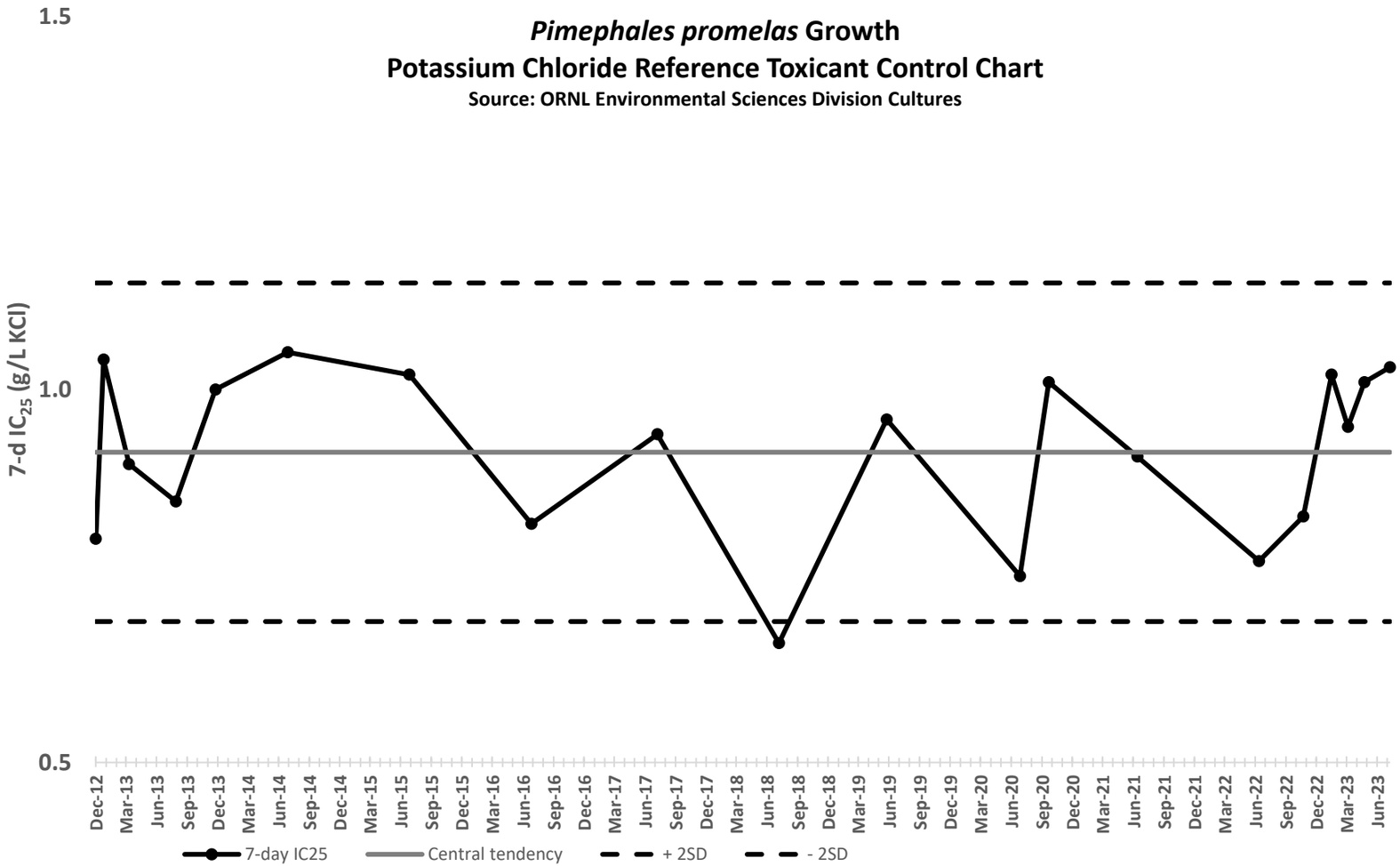
Mar-21
Jun-21
Sep-21
Dec-21
Mar-22
Jun-22
Sep-22
Dec-22
Mar-23
Jun-23
7-day IC25
Central Tendency
+2 SD
-2 SD


Pimephales promelas Growth
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC_{25} (g/L KCl)
1.5
1.0
0.5
Dec-12
Mar-13
Jun-13
Sep-13
Dec-13
Mar-14
Jun-14
Sep-14
Dec-14
Mar-15
Jun-15
Sep-15
Dec-15
Mar-16
Jun-16
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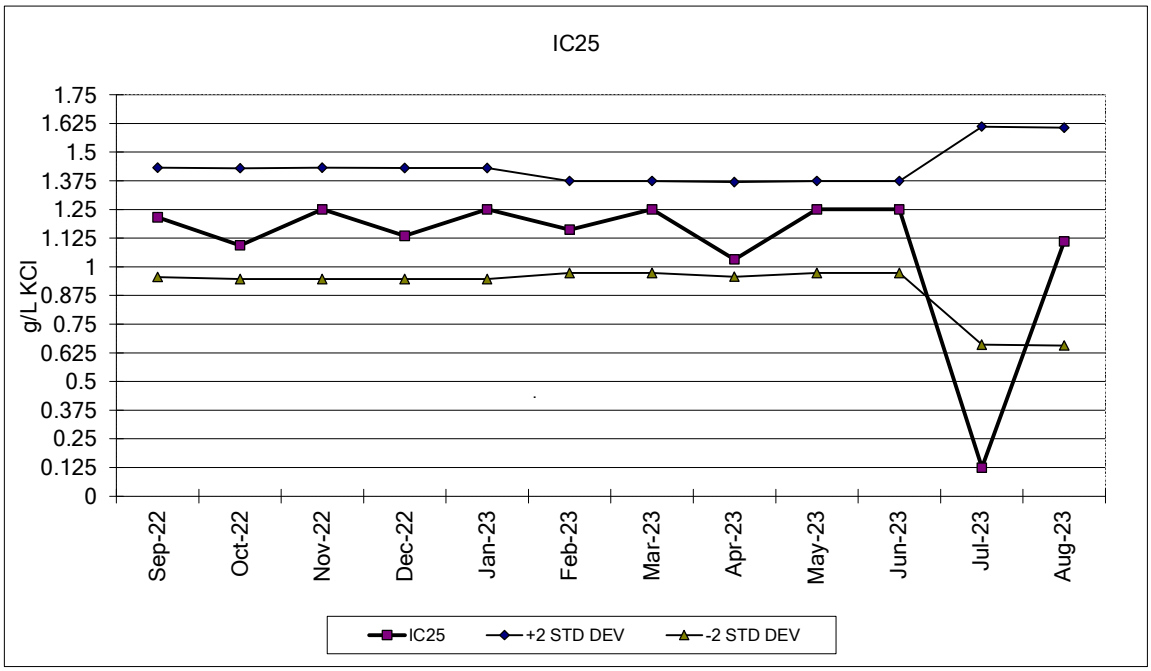
Jun-20
Sep-20
Dec-20
Mar-21
Jun-21
Sep-21
Dec-21
Mar-22
Jun-22
Sep-22
Dec-22
Mar-23
Jun-23
7-day IC25
Central tendency
+ 2SD
- 2SD

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

Date	NOEC (g/L KCl)	LOEC (g/L KCl)
Mar-23	0.50	1.0
Apr-23	0.50	1.0
May-23	0.50	1.0
Jun-23	0.50	1.0
Jul-23	0.50	1.0
Aug-23	0.50	1.0

IC 25 for Growth Test

Date	IC25 g/L KCl	95% Confidence (upper)	95% Confidence (lower)	Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
Mar-23	1.250	1.250	1.210	1.173	1.374	0.972
Apr-23	1.032	1.272	0.023	1.163	1.369	0.957
May-23	1.250	1.250	1.141	1.173	1.374	0.973
Jun-23	1.250	1.250	1.250	1.173	1.374	0.973
Jul-23	0.125	1.705	0.074	1.136	1.611	0.660
Aug-23	1.110	1.316	0.320	1.131	1.606	0.657

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

WATER CHEMISTRY DATA LOGSHEETS

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05/08/23 TRO

Daily Water Chemistry Log

Sponsor: Y-12 Site/Treatment: 0F200 Associated test numbers: FHM 1699 / CD 2992

Note: Not all parameters are required for all tests. All unused cells should be lined through or marked "NA."

		Observation Day:	0 PK/TAB	1 PK/TAB	2 PK/TAB	3 PK/TAB	4 PK/TAB	5 PK/TAB	PK/TAB	7 mPK
		Date/Initials:	08/09/23	08/10/23	08/11/23	8/12/23	8/13/23	8/14/23	8/15/23	08/16/23
		5-digit ORNL ID	33462	→	33463	→	→	33464	→	→
		Rec. temp. (°C) (New ✓)	ice ☑	☐	ice ☑	☐	☐	see ☑	☐	☐
①	Control: 25% pmw	DMW Batch #	970	970	970	970	970	~~970~~ 971	971	
		Conductivity (µS/cm)	242	231	230	211	201	PK ~~242~~ 244	~~225~~ 248 PK	
		Alkalinity (mg/L)	104					102	102	
		Hardness (mg/L)	120					100	100	
		pH (S.U.) Initial	8.14	8.11	8.01	8.24	8.16	PK ~~7.95~~ 8.13	8.15	
		Final CD/FHM		8.21/7.89	8.32/7.98	8.37/7.94	8.42/7.88	8.43/7.88	8.38/7.88	8.35/7.90
		DO (mg/L) Initial	8.43	8.42	8.40	8.34	8.30	PK ~~8.39~~ 8.44	8.23	
		Final CD/FHM		8.34/6.67	8.50/7.37	8.44/7.35	8.64/7.08	8.65/6.57	8.49/7.32	8.47/7.35
②	6.25%	Conductivity (µS/cm)	266	255	PK ~~235~~ 233	234	232	PK ~~223~~ 232	245	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.20	8.10	8.03	7.99	8.03	PK ~~8.09~~ 8.19	8.31	
		Final CD/FHM		8.36/7.94	8.34/7.90	8.27/7.84	8.44/7.91	8.19/7.86	8.39/7.83	8.37/7.92
		DO (mg/L) Initial	8.45	8.34	8.37	8.41	8.25	PK ~~8.46~~ 8.25	8.34	
		Final CD/FHM		8.53/6.78	8.53/7.03	8.50/7.40	8.65/6.94	8.28/7.03	8.28/7.27	8.48/7.11
③	12.5%	Conductivity (µS/cm)	289	279	PK ~~280~~ 283	244	252	282	260	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.22	8.14	8.05	7.99	8.07	8.09	8.22	
		Final CD/FHM		8.41/7.92	8.37/7.97	8.27/7.94	8.46/7.91	8.43/7.84	8.38/7.83	8.36/7.85
		DO (mg/L) Initial	8.44	8.35	8.44	8.52	8.31	8.23	8.40	
		Final CD/FHM		8.64/6.45	8.61/7.17	8.53/7.50	8.71/7.05	8.64/7.05	8.31/6.75	8.49/6.91
④	25%	Conductivity (µS/cm)	339	330	PK ~~334~~ 290	277	268	302	295	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.23	8.19	8.09	7.99	8.09	8.20	8.21	
		Final CD/FHM		8.44/7.97	8.40/7.99	8.31/8.00	8.71/7.93	8.43/7.87	8.41/7.83	8.41/7.90
		DO (mg/L) Initial	8.43	8.71	PK ~~8.43~~ 8.59	8.63	8.61	8.26	8.49	
		Final CD/FHM		8.70/6.38	8.62/6.77	8.52/7.14	8.71/7.02	8.57/7.04	8.31/6.72	8.47/6.93
⑤	50%	Conductivity (µS/cm)	434	431	341	340	337	369	363	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.23	8.20	8.02	7.97	8.08	8.20	8.19	
		Final CD/FHM		8.49/7.97	8.42/8.01	8.39/8.01	8.45/7.89	8.50/7.90	8.41/7.90	8.43/7.95
		DO (mg/L) Initial	8.69	8.94	8.53	8.78	9.07	8.30	8.64	
		Final CD/FHM		8.60/6.07	8.56/6.77	8.41/7.34	8.58/6.60	8.53/7.01	8.36/6.58	8.46/6.91
⑥	100%	Conductivity (µS/cm)	601	624	463	465	466	498	497	
		Alkalinity (mg/L)	168		138			162		
		Hardness (mg/L)	260		230			200		
		Chlorine (mg/L) FT	0.01/0.01		0.02/0.03			0.01/0.01		
		pH (S.U.) Initial	8.43	8.24	8.07	7.93	7.99	8.14	8.16	
		Final CD/FHM		8.54/8.16	8.44/8.12	8.34/8.10	8.41/7.98	8.40/8.02	8.42/8.04	8.48/8.13
		DO (mg/L) Initial	8.91	9.45	9.72	8.98	10.12	9.77	8.70	
		Final CD/FHM		8.42/6.51	8.47/7.35	8.23/6.97	8.40/6.72	8.34/7.00	7.95/6.52	8.41/6.85

CHAIN OF CUSTODY FORMS

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

DATE (MM/DD/YY)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
08/09/23	TOX TEST	A. GARLAND / J. WILLIAMS FRIG	031138

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS #7009 TEMP	REMARKS #5102 Cl2
TOX TEST	200	0730	C	1	13L	4°	11.6	<0.05

08/09/23 PIC

THERMOMETER NO.

SAMPLES RELINQUISHED BY: Anthony Garland	DATE: 8/9/23	TIME: 0810	☒ AM ☐ PM
SAMPLES RECEIVED BY: Peijia Ku	DATE: 8/9/23	TIME: 0811	☒ AM ☐ 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): 08/11/23 | ESD TEST NAME: TOX TEST | NAME OF SAMPLERS: A. GARLAND / D. CRAZE | FRIG | CHAIN-OF-CUSTODY NO. 031139

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS #7009 TEMP	REMARKS #5102 Cl_2
TOX TEST	200	0735	C	1	~ 16L	3°	12.9	<0.05

A. G. 8/11/23

THERMOMETER NO.

SAMPLES RELINQUISHED BY: A. G. Garland — DATE: 8/11/23 — TIME: 0810 ☒ AM ☐ PM

SAMPLES RECEIVED BY: [illegible] — DATE: 8/11/23 — TIME: 0810 ☒ AM ☐ 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): 08/14/23 | ESD TEST NAME: TOX TEST | NAME OF SAMPLERS: A. GARLAND / J. WILLIAMS FRIG | CHAIN-OF-CUSTODY NO.: 031140

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS: #7009 TEMP / 5102 Cl2
TOX TEST	200	0730	C	1	~17 L	3°	10.8 / <0.05

A.R.G. 8/14/23

THERMOMETER NO.

SAMPLES RELINQUISHED BY	A. R. Garland	DATE 8/14/23	TIME 0827	☒ AM ☐ PM
SAMPLES RECEIVED BY	Peijia Ku	DATE 08/14/23	TIME 0827	☒ AM ☐ PM

UCN-18631 (3 3-92)

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

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

2992

Sponsor: Y-12 Site/Treatment: OF 200 Test number

Test begin date (Day 0)	Test end date	Test duration	Template number
08/09/23	08/16/23	7 ☐ hours ☑ days ☐ NA	☑ 1

Test Organism: ☑ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: _____ Notes:

Isolated from:		
Date:	08/08/23	08/08/23
Time:	0838	1620

Hatch date: _____ Delivery date: _____

Test period	Test purpose	Test stage	Test type
☑ Chronic	☑ Regulatory	☐ Preliminary	☑ Effluent
☐ Acute	☐ Investigative	☑ Analytical	☐ Received waters
		☐ Re-test	☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	25% DMW	☑C ☐T	4 =	25%	☐C ☑T
2 =	6.25%	☐C ☑T	5 =	50%	☐C ☑T
3 =	12.5%	☐C ☑T	6 =	100%	☐C ☑T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☑ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 970, 971

Source of Test Organisms:

☑ ESD cultures: Board numbers: ☐ NA ☑ 4790 - 4791

☐ Vendor: _____ ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: 33462 Date: 08/09/23 COC #: 031138
Sample ID: 33463 Date: 08/11/23 COC #: 031139
Sample ID: 33464 Date: 08/14/23 COC #: 031140

Record of Deviations from Method and/or Test Non-Conformities

Date	Description	Initial
08/16/23	Dilution series were 0, 6.25, 12.5, 25, 50, 100% (standard dilution)	PK

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	Peijia Ku	PK	08/16/2023
Data sheets QA:	Trystan A. Bordeau	TAB	08/17/2023
Data entered:	Peijia Ku	PK	08/16/2023
Data entry QA:	Trystan A. Bordeau	TAB	08/17/2023

READ AND UNDERSTOOD _____ DATE _____ 20

CHRONIC Daily Water/Feeding Log

CHRONIC Daily Water/Feeding Log

Sponsor: Y12 Test site/treatment: OF200 Begin Date: 08/09/23 End Date: 08/16/23 Test Number: 2992

Daily Test Info		Temperature Information Therm. #: DD20		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10⁷/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	08/09/23 PK	25.5 am / pm	25.0 am / pm	YCT RASU	07/25/23 08/08/23	100 95	☑Yes	1050 am / pm	1030	1120	33462	970	NA
Day 1	08/10/23 PK	25.8 am / pm	25.0 am / pm	YCT RASU	07/25/23 08/08/23	100 95	☑Yes	1118 am / pm	1100	1149	↓	970	
Day 2	08/11/23 PK	25.6 am / pm	25.2 am / pm	YCT RASU	07/25/23 08/08/23	100 96	☑Yes	1130 am / pm	1110	1205	33463	970	
Day 3	08/12/23 PK	25.7 am / pm	24.6 am / pm	YCT RASU	07/25/23 08/08/23	100 94	☑Yes	1020 am / pm	1000	1110	↓	970	
Day 4	08/13/23 PK	26.0 am / pm	25.2 am / pm	YCT RASU	07/25/23 08/08/23	100 94	☑Yes	1020 am / pm	1007	1130	↓	970	
Day 5	08/14/23 PK	25.8 am / pm	25.3 am / pm	YCT RASU	07/25/23 08/08/23	100 93	☑Yes	1120 am / pm	1100	1240	33464	971	
Day 6	08/15/23 PK	25.9 am / pm	25.3 am / pm	YCT RASU	07/25/23 08/08/23	100 92	☑Yes	1000 am / pm	0940	1145	33469	971	
Day 7	08/16/23	am / pm	am / pm	PK			☐Yes	am / pm	0831	0940 PK			↓

Notes:

08/08/23 TPD

Ceriodaphnia Chronic Daily Survival & Reproduction Log

Project: Y12 Test site/chemical: JF-200 Test Number: 2992

Begin Date: 08/09/23 End Date: 08/16/23 Template Number: 7

Codes: (-) Alive and No Reproduction; (*N*) Alive and Reproduction; (*xN*) Dead and Reproduction; (M) Male

Test Chamber	Treatment Number	Day: 1 PK Date: 08/10/23	2 PK 08/11/23	3 PK 08/12/23	4 PK 08/13/23	5 PK 08/14/23	6 PK 08/15/23	7 PK 08/16/23
1	6	-	-	8	-	10	26	-
2	3	-	-	5	-	13	23	18
3	6	-	-	1	-	2	15	16
4	6	-	-	9	9	-	14	23
5	4	-	-	5	10	X-		
6	1	-	-	7	-	13	23	*
7	2	-	-	5	10	-	15	13
8	3	-	-	5	14	-	22	22
9	5	-	-	5	10	-	14	-
10	2	-	-	X2				
11	1	-	-	7	-	15	16	6
12	5	-	-	7	-	11	21	24
13	1	-	-	7	-	8	20	16
14	4	-	-	5	12	-	16	-
15	6	-	-	6	-	-	-	-
16	3	-	-	7	-	17	20	16
17	5	-	-	5	10	-	22	4
18	6	-	-	5	13	1-	16	14
19	2	-	-	4	15	-	-	M-
20	3	-	-	X2				
21	4	-	-	4	-	9	12	-
22	4	-	-	6	-	17	19	18
23	4	-	-	5	12	-	25	24
24	2	-	-	X4				
25	1	-	-	6	-	14	22	-
26	4	-	-	6	-	-	22	27
27	4	-	-	X4				
28	1	-	-	X-				
29	4	-	-	-	11	-	24	22
30	1	-	-	6	7	6	PK 08/14 X-	-
31	2	-	-	5	-	13	25	23
32	1	-	-	8	-	14	10	-
33	3	-	-	6	7	-	23	19
34	1	-	-	6	-	10	21	13
35	3	-	-	6	5	-	20	24
36	5	-	-	9	12	-	19	19
37	3	-	-	7	12	-	22	14
38	4	-	-	3	13	-	29	25
39	6	-	-	4	6	-	8	-
40	5	-	-	4	12	-	24	23
41	3	-	-	5	-	9	20	20
42	6	-	-	5	-	13	22	21
43	5	-	-	X2				
44	5	-	-	6	13	-	20	23
45	5	-	-	6	-	15	24	24
46	2	-	-	7	-	-	18	22
47	1	-	-	8	14	-	15	22
48	5	-	-	8	-	16	26	21
49	3	-	-	4	-	10	17	25
50	6	-	-	6	14	X-		
51	5	-	-	8	-	13	26	16
52	2	-	-	6	-	13	19	20
53	2	-	-	6	-	13	14	12
54	3	-	-	6	4	1	X-	
55	2	-	-	6	-	15	22	21
56	6	-	-	9	-	X-		
57	6	-	-	1	14	-	17	-
58	2	-	-	6	-	10	19	-
59	1	-	-	6	10	X3		
60	4	-	-	4	8	M		

Toxicity Test Information Sheet

1699

Sponsor: Y-12 Site/Treatment: OF200 Test number:

Test begin date (Day 0)	Test end date	Test duration	Template number
08-09-23	08-16-23	7 ☐ hours ☒ days	☒ NA ☐

Test Organism: ☐ *Ceriodaphnia dubia* ☒ Fathead minnow ☐ Other:

Isolated from: Date: Time:

Hatch date: 08/07/23

Delivery date: 08/08/23

Notes:

Test period	Test purpose	Test stage	Test type
☒ Chronic	☒ Regulatory	☐ Preliminary	☒ Effluent
☐ Acute	☐ Investigative	☒ Analytical	☐ Received waters
		☐ Re-test	☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	DMW 25%.	☒C ☐T	4 =	25%.	☐C ☒T
2 =	6.25%.	☐C ☒T	5 =	50%.	☐C ☒T
3 =	12.50%.	☐C ☒T	6 =	100%.	☐C ☒T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

05/04/23 TMB

☐ Not applicable ☐ Other (describe):

☒ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 970-971

Source of Test Organisms:

☐ ESD cultures: Board numbers: ☐ NA ☐

☒ Vendor: ABS ☐ Other (describe):

Water delivery dates:

☐ Not applicable

Sample ID: 33462 Date: 08/09/23 COC #: 031138

Sample ID: 33463 Date: 08/11/23 COC #: 031139

Sample ID: 33464 Date: 08/14/23 COC #: 031140

Record of Deviations from Method and/or Test Non-Conformities

Date	*Description*	*Initial*
08/16/23	Dilution series were: 0, 6.25, 12.5, 25, 50, and 100%. (Standard dilution)	TMB

Quality Assurance (QA) Record

Procedure	*Name*	*Initial*	*Date*
Test run by:	Trystan A. Bordeau	TMB	08/09/23
Data sheets QA:	Peijia Ku	PK	08/18/23
Data entered:	Trystan A. Bordeau	TMB	08/16/23
Data entry QA:	Peijia Ku	PK	08/18/23

CHRONIC Daily Water/Feeding Log

CHRONIC Daily Water/Feeding Log

Sponsor: Y-12 Test site/treatment: OF 200 Begin Date: 08/09/23 End Date: 08/16/23 Test Number: 1699

Daily Test Info		Temperature Information Therm. #: DD19		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10⁷/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	08/09/23 PK/TMB	25.6 am / pm	24.5 am / pm	B	08/03/23	62	☑Yes	1315 am / pm	1241	1338	334462	970	NA
Day 1	08/10/23 TMB	26.0 am / 26.0 pm	25.5 am / 25.4 pm	B B	08/09/23 08/09/23	68 126	☑Yes	0901 am 1418 pm	1141	1311	334462	970	N/A
Day 2	08/11/23 TMB	26.1 am / 26.0 pm	25.8 am / 25.6 pm	B B	8/10/23 8/10/23	68 83	☑Yes	0839 am 1413 pm	1158	1303	334463	970	N/A
Day 3	08/12/23	26.1 am / 26.0 pm	25.7 am / 25.6 pm	B B	08/11/23 08/11/23	68 50	☑Yes	0841 am 1428 pm	1053	1156	334463	970	N/A
Day 4	08/13/23	26.1 am / 26.0 pm	25.6 am / 25.7 pm	B B	08/12/23 08/12/23	112 69	☑Yes	0832 am 1402 pm	1058	1231	334463	970	N/A
Day 5	08/14/23	26.1 am / 26.0 pm	25.6 am / 25.7 pm	B B	08/13/23 08/13/23	128 80	☑Yes	0530 am 1520 pm	1142	1232	334464	970	N/A
Day 6	08/15/23	26.2 am / 26.1 pm	25.8 am / 25.6 pm	B B	08/14/23 08/14/23	64 44	☑Yes	0830 am 1445 pm	1032	1128	334464	971	N/A
Day 7	08/16/23	25.7 am / — pm	25.5 am / — pm				☐Yes	___ am / ___ pm	1052	1208			N/A

Notes:

08/09/23 TMB

08/28/23 TMB

Fathead Minnow Chronic Daily Survival Log

Sponsor: Y-12 Test site/chemical: OF200 Test Number: 1699

Begin Date: 08/09/23 End Date: 08/16/23

Comment Codes: C = Clear; D = Dead; Fg = Fungus; K = Killed by siphoning; M = Missing; Sk = Sick; SM = Small; SOR = Siphoned and returned; W = Wounded

Treatment Number and Desc.	Replicate Number	Position Number	Day 1 Date TMB 08/10/23	Day 2 Date TMB 08/11/23	Day 3 Date TMB 08/12/23	Day 4 Date TMB 08/13/23	Day 5 Date TMB 08/14/23	Day 6 Date TMB 08/15/23	Day 7 Date TMB 08/16/23
1: 25%. DMW	1	22	10	10	10	10	9 1D	9	9
	2	1	10	10	10	10	10	10	10
	3	23	10	10	10	10	10	10	10
	4	24	10	10	10	10	10	10	10
2: 6.25%.	1	~~15~~ 14	10	10	10	10	10	10	10
	2	5	10	10 1SM	10	10	9 1D	9	9
	3	10	10	10	10	10	10	10	10
	4	21	10	10	10	10	9 1D	9	9
3: 12.50%.	1	4	10	10	10	10	10	10	10
	2	13	10	10	10	10	10	10	10
	3	15	10	10	10	10	10	10	10
	4	19	10	10	10	10	10	10	10
4: 25%.	1	16	10	10	10	10	10	10	10
	2	17	10	10	10	10	10	10	10
	3	3	10	10	10	10	10	10	10
	4	20	10	10	10	10	10	10	10
5: 50%.	1	2	10	10	10	10	10	10	10
	2	8	10	10	10	10	10	10	10
	3	9	10	10	10	10	10	9 1D	9
	4	12	10	10	10	10	10	10	10
6: 100%.	1	6	10	10	10	10	10	10	10
	2	18	10	10	10	10	10	10	10
	3	7	10	10	10	10	10	10	10
	4	11	10	10	10	10	10	10	10

Random assignment of larvae to test chambers

Project: 4-12 Test site/chemical: OP200 Test number: 1699

Treatment	Replicate	Cup 1	Cup 2
Treatment #1 25·1. DMSO			
1	1	21 ✓	41 ✓
1	2	28 ✓	24 ✓
1	3	9 ✓	37 ✓
1	4	34 ✓	19 ✓
Treatment #2 6.25·1.			
2	1	39 ✓	26 ✓
2	2	23 ✓	42 ✓
2	3	46 ✓	4 ✓
2	4	17 ✓	5 ✓
Treatment #3 12.50·1.			
3	1	6 ✓	33 ✓
3	2	43 ✓	14 ✓
3	3	32 ✓	3 ✓
3	4	31 ✓	27 ✓
Treatment #4 25·1.			
4	1	16 ✓	2 ✓
4	2	35 ✓	8 ✓
4	3	7 ✓	15 ✓
4	4	13 ✓	11 ✓
Treatment #5 50·1.			
5	1	22 ✓	18 ✓
5	2	25 ✓	44 ✓
5	3	29 ✓	47 ✓
5	4	36 ✓	40 ✓
Treatment #6 100·1.			
6	1	20 ✓	48 ✓
6	2	38 ✓	10 ✓
6	3	45 ✓	30 ✓
6	4	1 ✓	12 ✓

08/09/23 TAB

Random assignment of test chambers

Project: 4-12 Test site/chemical: OF200 Test number: 1699

Position	Treatment #	Replicate	Sample ID
1	1	2	25%. DMW
2	5	1	50%.
3	4	3	25%.
4	3	1	12.5%.
5	2	2	6.25%.
6	6	1	100%.
7	6	3	100%.
8	5	2	50%.
9	5	3	25%.
10	2	3	6.25%.
11	6	4	100%.
12	5	4	50%.
13	3	2	12.5%.
14	2	1	6.25%.
15	3	3	12.5%.
16	4	1	25%.
17	4	2	25%.
18	6	2	100%.
19	3	4	12.5%.
20	4	4	25%.
21	2	4	6.25%.
22	1	1	25%. DMW
23	1	3	25%. DMW
24	1	4	25%. DMW

Fathead Minnow Weight and Survival Data

05/09/23 TAB

Sponsor: 4-12	Test number: 1699
Test site/chemical: OF200	Balance ID: A009820
Test Start Date: 08/09/23	Test End Date: 08/10/23
Start Drying Date/Time: 8/16/23 12:15	End Drying Date/time: 8/17/23 08:29

Treatment	Replicate	Pan Wt. (mg) Date: 8/16/23 Balance check: ☑	Pan + Larvae (mg) Date: 8/17/23 Balance check: ☑	Number Surviving
Initial	1	15.3320	16.9070	10
	2	15.4670	17.2095	10
	3	15.4325	16.9080	10
	4	15.4630	16.9900	10
1. 25 µl. DMW	1	15.3395	25.9065	~~10~~ 9 8/17 TAB
	2	15.4315	24.8680	10
	3	15.5055	26.5025	10
	4	15.4665	24.7730	10
2. 6.25 µl.	1	15.3160	25.2715	10
	2	15.5940	25.0230	~~10~~ 9 8/17 TAB
	3	15.3540	25.3725	10
	4	15.2650	25.3600	~~10~~ 9 8/17 TAB
3. 12.50 µl.	1	15.4055	26.0365	10
	2	15.4355	25.5590	10
	3	15.4605	25.9330	10
	4	15.3055	25.5100	10
4. 25 µl.	1	15.3860	25.8730	10
	2	15.2785	25.8920	10
	3	15.4346	25.5745	10
	4	15.4170	25.2915	10
5. 50 µl.	1	15.3875	25.1820	10
	2	15.1525	25.2615	10
	3	15.1495	26.4690	9
	4	15.3305	27.2660	10
6. 100 µl.	1	15.2580	27.0655	10
	2	15.1460	25.6980	10
	3	15.2360	26.2150	10
	4	15.2270	27.2485	10

8/09/23 TAB

05/09/23 TAB

Fathead Minnow Order & Shipment Log

Ordering Information:

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
08/07/23	1699	ABS	400	<1 day Dim Hatch	08/08/23	TMB	N/A

Delivery Information:

Larva source	Approx. number received	Date/time received	Received by (Initials)
ABS	440	08/08/23 12:39	TMB

Monitoring Interval	Hour							
	0	1	2	3	4	5	6	7
Temperature (°C)	12.9	24.2						
Time	12:48	13:59						
Thermometer ID	DD19	DD19						
Initials	TMB	TMB						

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

OAK RIDGE National Laboratory

PO Box 2008
Oak Ridge, TN 37831
(865) 341-0398| stevensonlm@ornl.gov

Date: March 20, 2024

To: K.G. Hanzelka (RC)

c: S. Loveless, J. Stinnett, K. Kinder, T.J. Mathews, P. Ku, T. Bordeau

From: L.M. Stevenson, 1504, MS-6351 (865-341-0398).

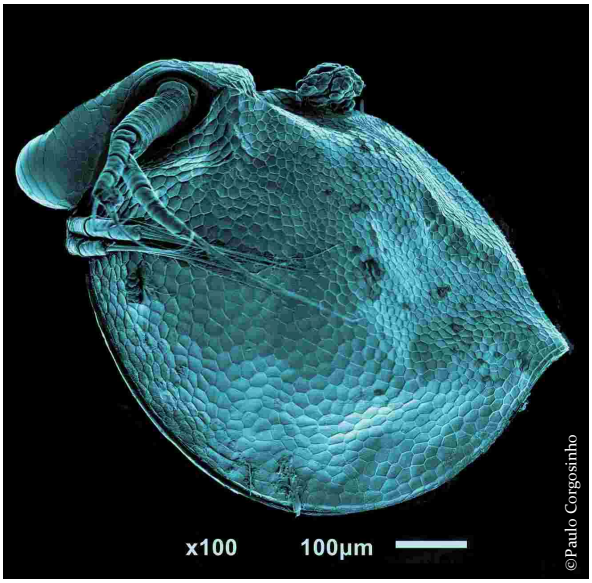
RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted February 21-28, 2024**

Appended are the results of toxicity tests of effluent from Outfall 200 conducted from February 21 to February 28, 2024. 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$_{25}$ (IC_{25}) 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_{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_{25}
Outfall 200	Fathead minnow	Survival	>100%
		Growth	>100%
Outfall 200	*Ceriodaphnia*	Survival	>100%
		Reproduction	>100%

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

Attachment
lms



Ceriodaphnia dubia

TOXICITY TEST REPORT

Test Number 2997 | Y-12 National Security Complex Outfall 200 | 29 February 2024

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 2997 | Start Date: 21 February 2024 | End Date: 28 February 2024

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_{25}) 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: 20 February 2024 to 26 February 2024

2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.

2.5 Samples were used immediately then stored at 4 ± 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	2/20/2024	2/22/2024	2/25/2024
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	2/21/2024	2/23/2024	2/26/2024
Chain-of-Custody Form Number	031143	031144	031145
Sample Temperature (°C)	7.0	8.7	10.5
pH (S.U.)	8.11	8.09	8.16
Conductivity (µS/cm)	556	518	623
Alkalinity (mg/L as $CaCO_3$)	136	115	142
Hardness (mg/L as $CaCO_3$)	219	187	322
Chlorine (Free/Total) (mg/L)	0.01/0.01	0.01/0.01	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 ± 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 (4th 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: No modifications or deviations to Method 1002.0.

4.4 Date and time test started: 2/21/2024, 10:58

4.5 Date and time test terminated: 2/28/2024, 10:12

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 = 25.4 °C; Range = 25.2 – 25.6 °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 10^6 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 10^7 cells/mL (EPA/821/R/02/013; 4th 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: 2/21/2024 – 2/28/2024.

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} = 2.12 g NaCl/L; 95% C.I. = 1.63-2.26 g NaCl/L.
Reproduction IC_{25} = 1.60 g NaCl/L; 95% C.I. = 1.21-1.71 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_{25} for survival: 1.622 ± 0.925 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} for survival: 0.285 g NaCl/L
Central tendency of IC_{25} for reproduction: 1.156 ± 0.679 g NaCl/L (mean ± 2 *SD*).
CV of IC_{25} for reproduction: 0.294 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	29.6 ± 4.5
12.5%	10	10	29.9 ± 7.1
25%	10	9	27.1 ± 12
50%	10	10	29.9 ± 5.5
75%	10	10	29.2 ± 5.1
100%	10	10	29.3 ± 5.7

7. STATISTICAL ANALYSES

7.1 Survival

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

7.2 Reproduction

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

7.3 Summary of *Ceriodaphnia* toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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.)	8.09	8.08	8.09
Conductivity (µS/cm)	235	230	219
Alkalinity (mg/L as $CaCO_3$)	110	110	110
Hardness (mg/L as $CaCO_3$)	120	110	110

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 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 Date: 8 March 2024

Report reviewed by: Louise Stevenson Louise Stevenson Date: 20 March 2024

Fathead Minnow

TOXICITY TEST REPORT

Test Number 1703 | Y-12 National Security Complex Outfall 200 | 29 February 2024

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 1703 | Start Date: 21 February 2024 | End Date: 28 February 2024

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_{25}) 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: 21 February 2024 to 26 February 2024

2.4 Sampling method: Three 24-h flow-proportionate composite samples of final effluent.

2.5 Samples were used immediately then stored at 4 ± 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	2/20/2024	2/22/2024	2/25/2024
Composite Duration	24 h	24 h	24 h
Date of Delivery to ESD Tox Lab	11/1/2023	11/3/2023	11/6/2023
Chain-of-Custody Form Number	031104	031141	031142
Sample Temperature (°C)	7.0	8.7	10.5
pH (S.U.)	8.11	8.09	8.16
Conductivity (µS/cm)	556	518	623
Alkalinity (mg/L as $CaCO_3$)	136	115	142
Hardness (mg/L as $CaCO_3$)	219	187	322
Chlorine (Free/Total) (mg/L)	0.01/0.01	0.01/0.01	0.01/0.01

3. TEST ORGANISMS

3.1 Species: Fathead minnow (*Pimephales promelas*).

3.2 Hatch date: 19 February 2024 .

3.3 Life stage: Newly hatched larvae less than 48 h old.

3.4 Incubation water: Dechlorinated tap water.

3.5 Incubation temperature: 25 ± 1 °C.

3.6 Source: Cultures from Aquatic BioSystems, Inc., Fort Collins, CO.

3.7 Mean dry weight at test initiation: 0.136 ± 0.007 mg (mean ± 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 (4th 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, 12.50%, 25%, 50%, 75%, and 100% of full-strength effluent.

4.4 Date and time test started: 2/21/2024, 13:59

4.5 Date and time test terminated: 2/28/2024, 13:48

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 20 February 2024 at 11.6 °C.

4.12 Test temperature: Mean = 25.6 °C; range = 25.1 – 26.0 °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: 02/21/2024 – 02/28/2024.

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} = 0.82 g KCl/L; 95% C.I. = 0.75 – 0.91 g KCl/L.

Growth IC_{25} = 1.01 g KCl/L; 95% C.I. = 0.82 – 1.05 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_{25} for survival: 0.882 ± 0.262 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} for survival: 0.149 g KCl/L

Central tendency of IC_{25} for growth: 0.914 ± 0.227 g KCl/L (mean ± 2 *SD*).

CV of IC_{25} 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

Concentration	Proportion surviving per replicate				Mean
	1	2	3	4	
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

Dry Weight

Concentration	Weight (mg) per replicate				Mean ± SD
	1	2	3	4	
Control	0.65	0.67	0.74	0.82	0.72 ± 0.08
12.5%	0.74	0.71	0.71	0.66	0.71 ± 0.03
25%	0.74	0.66	0.65	0.78	0.71 ± 0.06
50%	0.63	0.77	0.6	0.73	0.68 ± 0.08
75%	0.55	0.73	0.67	0.69	0.66 ± 0.08
100%	0.5	0.75	0.71	0.79	0.69 ± 0.13

7. STATISTICAL ANALYSES

7.1 Survival

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

7.2 Growth

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

7.3 Summary of fathead minnow toxicity test results:

IC_{25} for survival: >100%

IC_{25} 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.)	8.09	8.08	8.09
Conductivity (µS/cm)	235	230	219
Alkalinity (mg/L as $CaCO_3$)	110	110	110
Hardness (mg/L as $CaCO_3$)	120	110	110

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 (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 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.

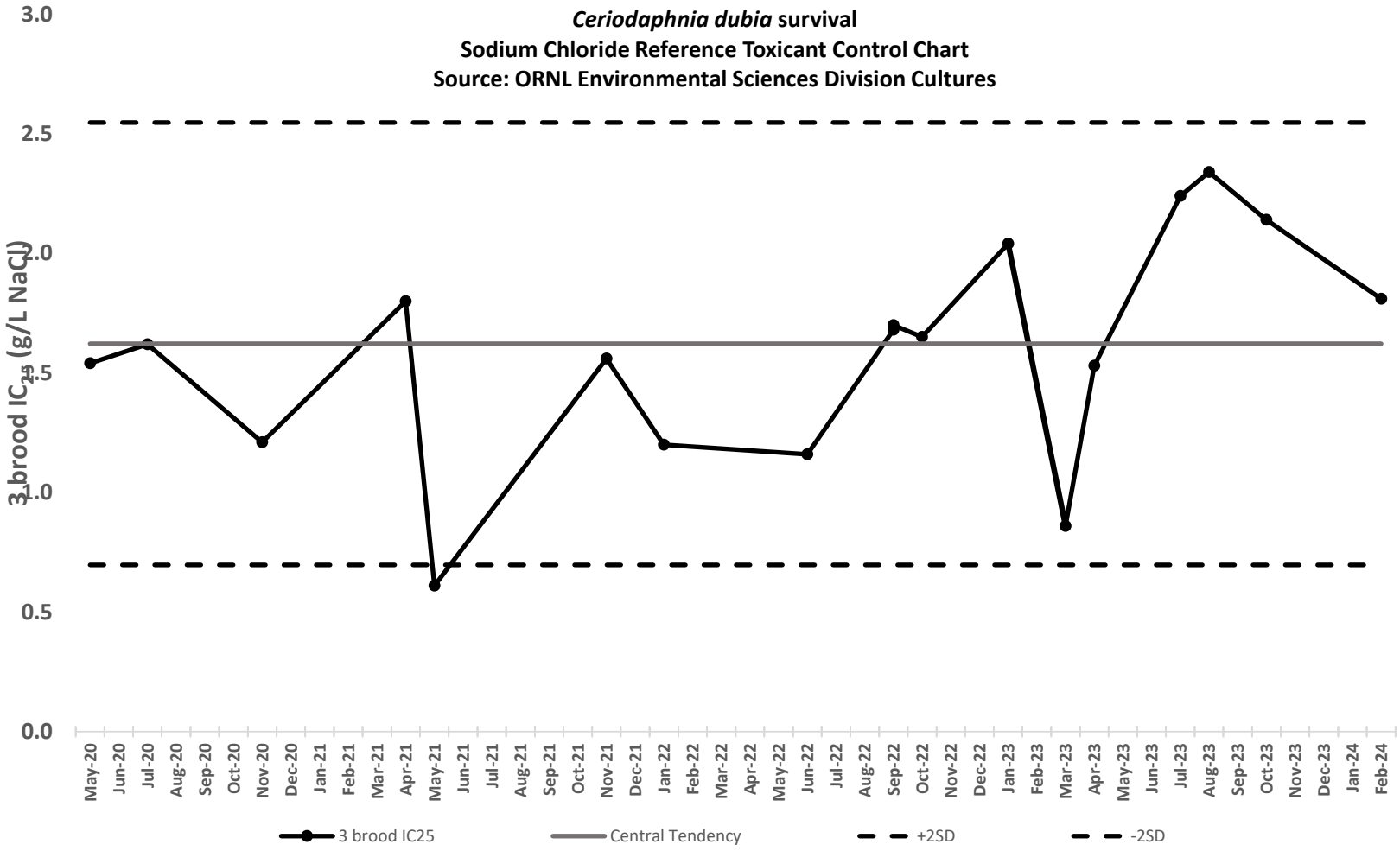
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Trystan A. Bordeau Date: 8 March 2024

Report reviewed by: Louise Stevenson Date: 20 March 2024

Louise Stevenson

REFERENCE TOXICANT CONTROL CHARTS


Ceriodaphnia dubia survival
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
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May-21
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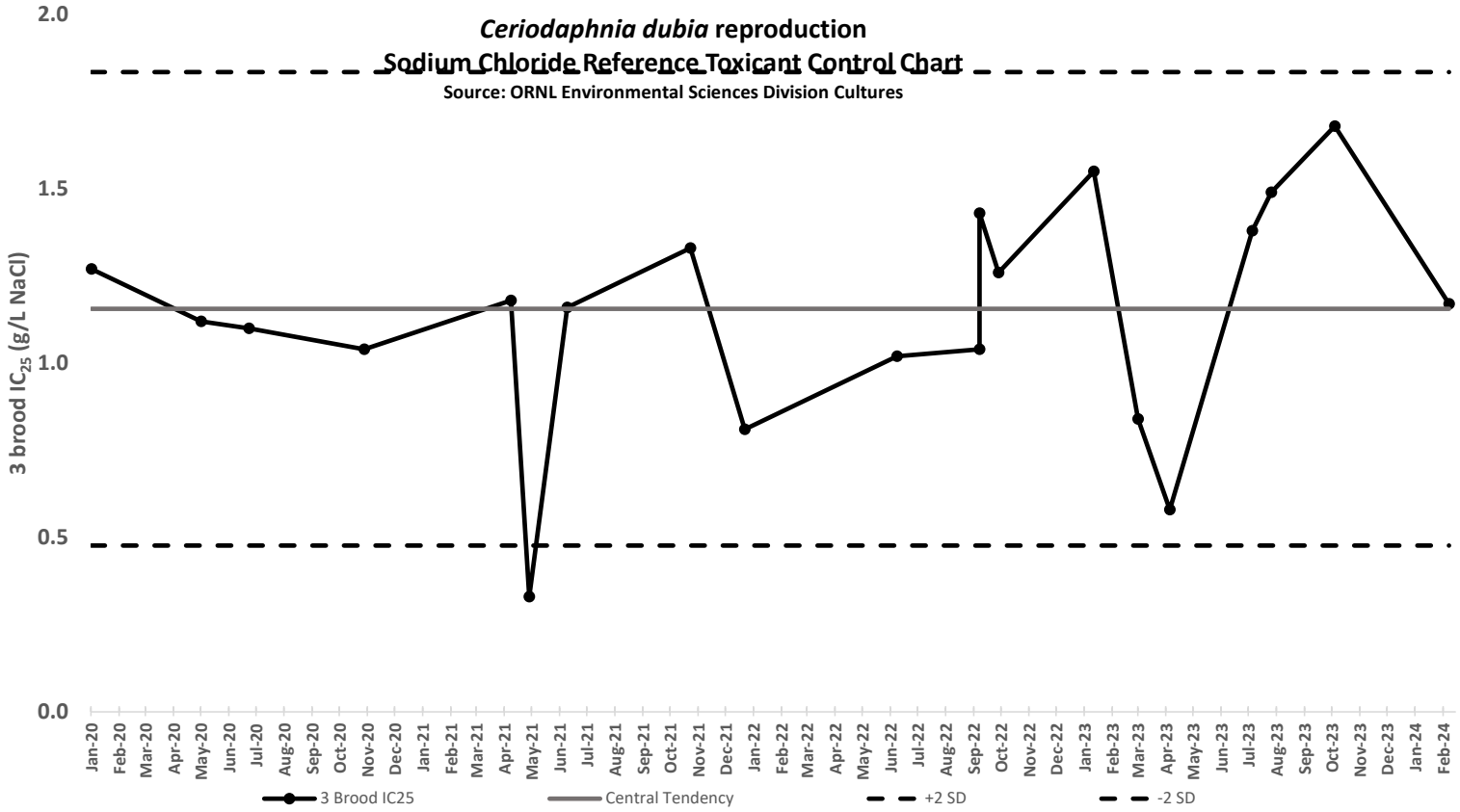
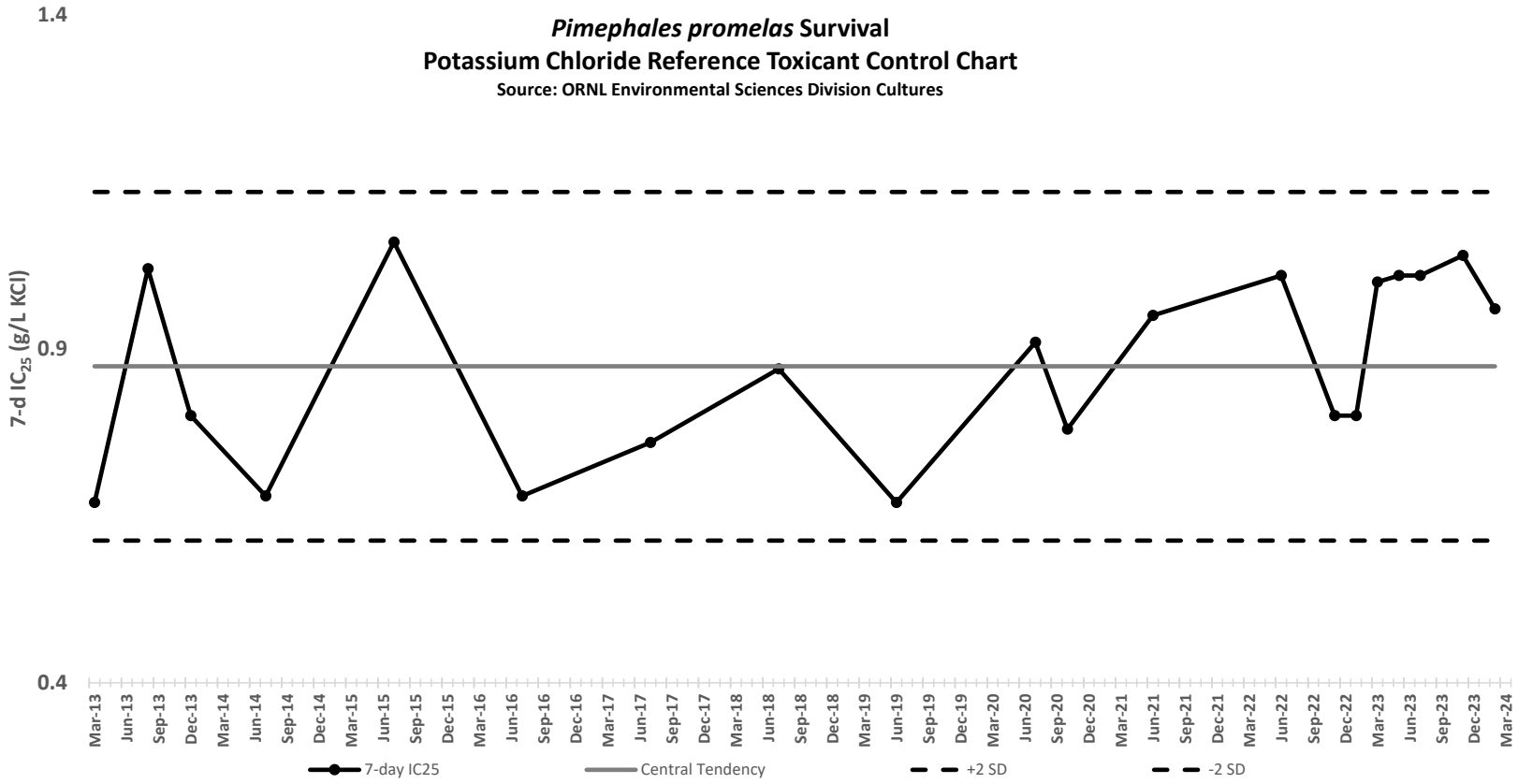
Jan-24
Feb-24
3 brood IC25
Central Tendency
+2SD
-2SD


Ceriodaphnia dubia reproduction
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
3 brood IC25 (g/L NaCl)
2.0
1.5
1.0
0.5
0.0
Jan-20 Feb-20 Mar-20 Apr-20 May-20 Jun-20 Jul-20 Aug-20 Sep-20 Oct-20 Nov-20 Dec-20 Jan-21 Feb-21 Mar-21 Apr-21 May-21 Jun-21 Jul-21 Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 May-22 Jun-22 Jul-22 Aug-22 Sep-22 Oct-22 Nov-22 Dec-22 Jan-23 Feb-23 Mar-23 Apr-23 May-23 Jun-23 Jul-23 Aug-23 Sep-23 Oct-23 Nov-23 Dec-23 Jan-24 Feb-24
3 Brood IC25
Central Tendency
+2 SD
-2 SD


1.4
Pimephales promelas Survival
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
7-d IC25 (g/L KCl)
0.9
0.4
Mar-13
Jun-13
Sep-13
Dec-13
Mar-14
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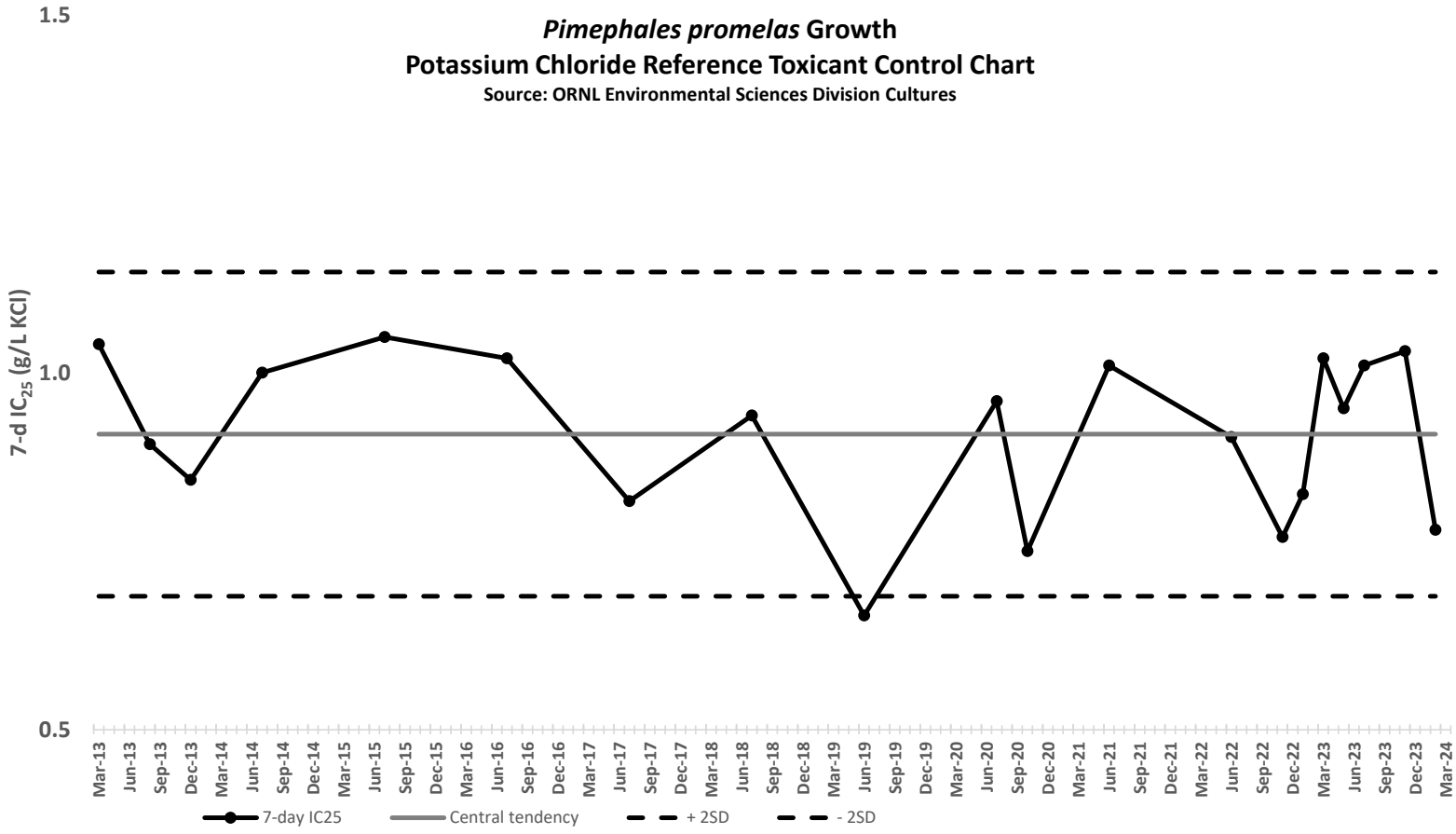
Jun-23
Sep-23
Dec-23
Mar-24
7-day IC25
Central Tendency
+2 SD
-2 SD


Pimephales promelas Growth
Potassium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures
1.5
1.0
0.5
7-d IC25 (g/L KCl)
Mar-13
Jun-13
Sep-13
Dec-13
Mar-14
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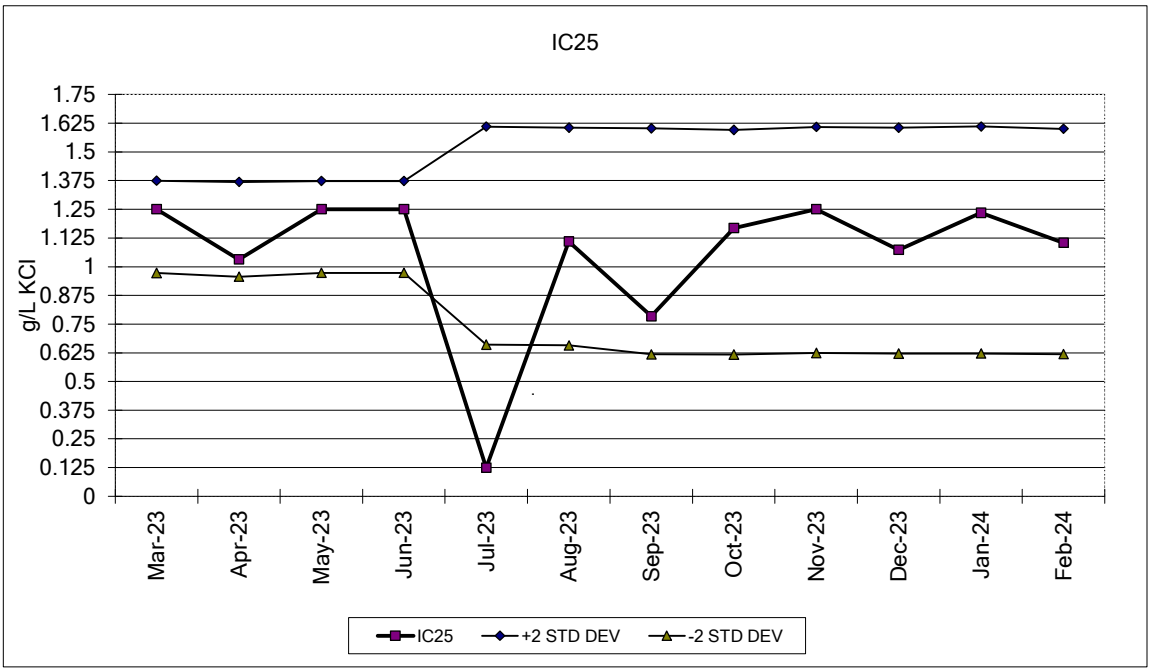
Dec-21
Mar-22
Jun-22
Sep-22
Dec-22
Mar-23
Jun-23
Sep-23
Dec-23
Mar-24
7-day IC25
Central tendency
+ 2SD
- 2SD

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

Date	NOEC (g/L KCl)	LOEC (g/L KCl)
Sep-23	0.50	1.0
Oct-23	0.50	1.0
Nov-23	0.50	1.0
Dec-23	0.50	1.0
Jan-24	0.50	1.0
Feb-24	0.50	1.0

IC 25 for Growth Test

Date	IC25 g/L KCl	95% Confidence (upper)	95% Confidence (lower)	Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
Sep-23	0.785	0.868	0.709	1.110	1.603	0.618
Oct-23	1.169	1.287	0.796	1.107	1.596	0.617
Nov-23	1.250	1.250	1.142	1.116	1.608	0.624
Dec-23	1.074	1.244	-0.446	1.113	1.605	0.621
Jan-24	1.235	1.259	1.048	1.117	1.611	0.622
Feb-24	1.104	1.338	-0.448	1.110	1.601	0.619

**Current Test Dates: 1/30-2/6/2024

WATER CHEMISTRY DATA LOGSHEETS

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Daily Water Chemistry Log

Sponsor: Y-12 Site/Treatment: OF200 Associated test numbers: FHM 1703 , CD 2097

Note: Not all parameters are required for all tests. All unused cells should be lined through or marked "NA."

		Observation Day:	0	1	2	3 TMB	4	5	6 TMB	7 TMB
		Date/Initials:	2/21/24	2/22/24	2/23/24	2/24/24	2/25/24	2/26/24	2/27/24	2/28/24
		5-digit ORNL ID	33822	33822	33823	33823	33823	33824	33824	
		Rec. temp. (°C) (New ✓)	SecCoc ☐	SecCoc ☐	SecCoc ☐	SecCoc ☐	SecCoc ☐	SecCoc ☐	SecCoc ☐	☐
①	Control: 25% DMW	DMW Batch #	1005	1005	1005	1005	1005	1005	1005	
		Conductivity (µS/cm)	235	222	284	230	225	219	232	
		Alkalinity (mg/L)	110			110			110	
		Hardness (mg/L)	120			110			110	
		pH (S.U.) Initial	8.09	8.03	8.02	8.08	8.00	8.09	8.00	
		Final CD/FHM		8.36/8.02	8.47/7.99	8.42/7.92	8.30/7.97	8.46/7.86	8.48/7.84	8.47/7.87
		DO (mg/L) Initial	8.80	8.67	8.54	8.57	8.74	8.72	8.56	
		Final CD/FHM		8.86/7.93	8.83/6.94	8.83/7.04	8.69/7.13	8.97/7.07	8.68/6.30	8.38/6.23
②	12.5%	Conductivity (µS/cm)	275	262	244	267	263	269	290	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	7.89	7.94	8.00	8.10	7.94	8.08	8.20	
		Final CD/FHM		8.39/7.92	8.56/7.97	8.49/7.92	8.43/7.97	8.48/7.92	8.50/7.86	8.47/7.95
		DO (mg/L) Initial	8.83	8.84	8.63	8.71	9.09	8.86	8.71	
		Final CD/FHM		8.90/6.50	8.79/6.74	9.03/6.97	8.85/6.96	9.01/6.92	8.68/6.36	8.41/6.41
③	25%	Conductivity (µS/cm)	316	306	284	295	301	322	338	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.02	8.03	8.01	8.10	7.96	8.09	8.20	
		Final CD/FHM		8.45/8.00	8.51/8.01	8.50/7.95	8.47/7.98	8.52/7.96	8.53/7.91	8.52/7.99
		DO (mg/L) Initial	9.13	9.06	8.78	8.94	9.21	9.08	8.89	
		Final CD/FHM		9.03/6.66	8.77/6.75	9.06/6.96	8.98/6.88	9.17/6.95	8.73/6.37	8.60/6.65
④	50%	Conductivity (µS/cm)	397	393	365	371	376	427	434	
		Alkalinity (mg/L)								
		Hardness (mg/L)								
		Chlorine (mg/L)								
		pH (S.U.) Initial	8.05	8.07	8.03	8.08	7.99	8.10	8.19	
		Final CD/FHM		8.50/8.09	8.65/8.10	8.52/8.00	8.51/8.01	8.54/8.03	8.57/8.01	8.54/8.04
		DO (mg/L) Initial	9.50	9.07	9.27	9.38	9.70	9.50	9.36	
		Final CD/FHM		9.01/6.93	8.84/6.72	9.03/6.88	9.05/6.81	9.21/6.95	8.75/6.44	8.61/6.83
⑤	75%	Conductivity (µS/cm)	485	474	443	447	448	522	526	
		Alkalinity (mg/L)						*142		
		Hardness (mg/L)						322		
		Chlorine (mg/L) F/T						0.01/0.01		
		pH (S.U.) Initial	8.07	8.09	8.03	8.10	8.02	8.12	8.19	
		Final CD/FHM		8.53/8.15	8.58/8.25	8.54/8.09	8.51/8.04	8.55/8.07	8.60/8.05	8.58/8.08
		DO (mg/L) Initial	10.01	10.15	9.80	9.92	10.24	10.07	10.05	
		Final CD/FHM		9.03/7.00	8.83/6.94	9.07/6.97	9.03/6.74	9.21/7.16	8.77/6.48	8.63/7.01
⑥	100%	Conductivity (µS/cm)	556	553	518	519	518	623	619	
		Alkalinity (mg/L)	136		115					
		Hardness (mg/L)	219	223	187					
		Chlorine (mg/L) T/F	0.01/0.01		0.01/0.01					
		pH (S.U.) Initial	8.11	8.16	8.09	8.14	8.07	8.16	8.20	
		Final CD/FHM		8.54/8.20	8.58/8.23	8.54/8.15	8.51/8.07	8.55/8.13	8.64/8.08	8.58/8.11
		DO (mg/L) Initial	10.45	10.65	10.40	10.39	11.04	10.49	10.65	
		Final CD/FHM		9.03/7.00	8.79/6.86	9.10/6.99	9.05/6.85	9.23/7.31	8.80/6.53	8.65/7.09

Environmental Sciences Division * OF200 Effluent (Treatment 6) water parameters Rev. 04 2021-02-05

CHAIN OF CUSTODY FORMS

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

DATE (MM/DD/YY): 02/21/24 | ESD TEST NAME: TOX TEST | NAME OF SAMPLERS: A. GARLAND / J. WILLIAMS | CHAIN-OF-CUSTODY NO.: 031143

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C) FRIG	REMARKS #7009 TEMP	REMARKS #5102 Cl2
OUTFALL #200	200	0730	C	1	~17L	4°	7°	<0.05

2/21/24

THERMOMETER NO.

SAMPLES RELINQUISHED BY: A. L. Garland — DATE: 2/21/24 — TIME: 0810 ☒ AM ☐ PM

SAMPLES RECEIVED BY: [signature] — DATE: 2/21/24 — TIME: 0810 ☒ AM ☐ 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)	ESD TEST NAME	NAME OF SAMPLERS	CHAIN-OF-CUSTODY NO.
02/23/24	TOX TEST	D. CRAZE / J. WILLIAMS	031144

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG. TEMP (°C)	YSI # 7009 Temp / REMARKS / ORION # 5102 Cl_2
OUTFALL #200	200	0700	C	1	~17L	3°	8.7° / <0.05

QTW 2/23/24

THERMOMETER NO.

SAMPLES RELINQUISHED BY: J. T. Williams	DATE: 2/23/24	TIME: 0745	☑ AM ☐ PM
SAMPLES RECEIVED BY: I. Rose	DATE: 2/23/24	TIME: 0745	☒ AM ☐ 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): 02/26/24 | ESD TEST NAME: TOX TEST | NAME OF SAMPLERS: A. GARLAND/J. WILLIAMS | CHAIN-OF-CUSTODY NO.: 031145

FRIG

SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	REMARKS #7009 TEMP	REMARKS #5102 C/2
OUTFALL # 200	200	0730	C	1	~17L	3°	10.5	<0.05

A&Y. 2/26/24

THERMOMETER NO.

SAMPLES RELINQUISHED BY: C. L. Garland — DATE: 2/26/24 — TIME: 0810 ☒ AM ☐ PM

SAMPLES RECEIVED BY: [signature] — DATE: 2/26/24 — TIME: 0810 ☒ AM ☐ PM

UCN-18631 (3 3-92)

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

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Test number: 2997

Sponsor: Y-12 **Site/Treatment:** OF200

Test begin date (Day 0)	Test end date	Test duration	Template number
02/21/24	02/28/24	7 ☐ hours ☒ days	TAB 2/21/24 ~~☒~~ NA ☒ 4

Test Organism: ☒ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: _____

Isolated from:

Date:	02/20/24	Hatch date:	
Time:	0745	1545	Delivery date:

Notes:

Test period	Test purpose	Test stage	Test type
☒ Chronic	☒ Regulatory	☐ Preliminary	☒ Effluent
☐ Acute	☐ Investigative	☒ Analytical	☐ Received waters
		☐ Re-test	☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	25% DMW	☒C ☐T	4 =	TAB 2/21/24 ~~50%~~ 50%	☐C ☒T
2 =	12.5%	☐C ☒T	5 =	75%	☐C ☒T
3 =	25%	☐C ☒T	6 =	100%	☐C ☒T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☒ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 1005, 1007-1008

Source of Test Organisms:

☒ ESD cultures: Board numbers: TAB, 2/21/24 ~~☒~~ NA ☒ 4823 - 4824

☐ Vendor: _____ ☐ Other (describe): _____

TAB 02/21/24

Water delivery dates:

☐ Not applicable

Sample ID: 33822 Date: 2/21/24 COC #: 031143
Sample ID: 33823 Date: 2/23/24 COC #: 031144
Sample ID: 33824 Date: 2/26/24 COC #: 031145

Record of Deviations from Method and/or Test Non-Conformities

Date	Description	Initial
03/06/24	No deviations observed	TAB

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	Trystan A. Bordlee	TAB	03/05/24
Data sheets QA:	Nikki Jones	NJS	03-05-24
Data entered:	Trystan A. Bordlee	TAB	03/05/24
Data entry QA:	Nikki Jones	NJS	03-05-24

TAB 2/21/24

CHRONIC Daily Water/Feeding Log

Sponsor: Y-12 Test site/treatment: OF200 Begin Date: 2/21/24 End Date: 2/28/24 Test Number: 2997

Daily Test Info		Temperature Information Therm. #: DD20		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10^7/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	2/21/24 TAB	26.2 am / — pm	25.3 am / — pm	YCT R	100 ⇔ 2/16/24 92 ⇔ 2/20/24		☒Yes 3.25E7	1110 am / — pm	1058	1123	33822	1005	NA
Day 1	2/22/24 TAB	26.2 am / — pm	25.2 am / — pm	YCT R	2/16/24 2/20/24	100 92	☒Yes 3.25E7	1102 am / — pm	1050	1102	33822	1005	
Day 2	2/23/24 TAB	26.1 am / — pm	25.6 am / — pm	YCT R	2/16/24 2/20/24	100 95	☒Yes 3.14E7	1106 am / — pm	1058	1131	33823	1005	
Day 3	2/24/24 TAB	26.1 am / — pm	25.5 am / — pm	YCT R	2/16/24 2/20/24	100 96	☒Yes 3.18E7	1111 am / — pm	1100	1156	33823	1007 ~~1006~~ *	
Day 4	2/25/24 TAB	26.1 am / — pm	25.2 am / — pm	YCT R	2/16/24 2/20/24	100 95	☒Yes 3.16E7	1113 am / — pm	1101	1144	33823	1007 1006 *	
Day 5	2/26/24 TAB	26.2 am / — pm	25.3 am / — pm	YCT R	2/16/24 2/20/24	100 91	☒Yes 3.30E7	1121 am / — pm	1105	1204	33824	1007 ~~1006~~ *	
Day 6	2/27/24 TAB	26.1 am / — pm	25.4 am / — pm	YCT R	2/16/24 2/20/24	100 91	☒Yes 3.28E7	1120 am / — pm	1109	1201	33824	1008	
Day 7	2/28/24 TAB	___ am / ___ pm	___ am / ___ pm				☐Yes	___ am / ___ pm	0931	1012			

Notes:

* 1007, not 1006. TAB 2/27/24

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Ceriodaphnia Chronic Daily Survival & Reproduction Log

Project: Y-12 Test site/chemical: OF200 Test Number: 2997

Begin Date: 02/21/24 End Date: 02/28/24 Template Number: 4

Codes: (-) Alive and No Reproduction; (*N*) Alive and Reproduction; (*xN*) Dead and Reproduction; (M) Male

TAB 2/21/24

Test Chamber	Treatment Number	Day: 1 TAB Date: 2/22/24	2 TAB 2/23/24	3 TAB 2/24/24	4 TAB 2/25/24	5 TAB 2/26/24	6 TAB 2/27/24	7 TAB 2/28/24
1	3	—	x (Kinod)					
2	6	—	—	4	—	9	18	—
3	3	—	—	2	—	—	4	—
4	4	—	—	5	—	11	17	—
5	6	—	—	4	8	—	17	21
6	6	—	—	5	7	—	12	16
7	3	—	—	6	—	14	17	—
8	4	—	—	7	—	9	18	14
9	1	—	—	6	—	4	19	—
10	2	—	—	7	—	2	19	16
11	2	—	—	7	1	—	21	18
12	3	—	—	8	—	13	17	—
13	4	—	—	—	—	3	5	—
14	1	—	—	3	—	11	18	—
15	3	—	—	6	—	11	17	—
16	1	—	—	6	5	—	17	17
17	4	—	—	5	—	9	18	15
18	5	—	—	4	5	—	20	22
19	3	—	—	6	—	2	16	18
20	1	—	—	1	—	—	21	6
21	6	—	—	4	6	—	17	21
22	5	—	—	6	—	14	19	12
23	5	—	—	—	—	—	10	2
24	5	—	—	5	—	11	17	14
25	2	—	—	7	—	10	21	—
26	4	—	—	6	7	—	21	16
27	1	—	—	5	—	12	17	16
28	6	—	—	4	—	11	18	16
29	4	—	—	4	—	4	15	17
30	4	—	—	5	—	—	17	20
31	1	—	—	8	—	—	18	14
32	1	—	—	8	—	8	20	—
33	1	—	—	2	—	2	14	—
34	2	—	—	4	—	9	10	—
35	1	—	—	—	11	3	12	18
36	3	—	—	7	—	7	18	16
37	5	—	—	6	—	10	15	15
38	3	—	—	6	—	—	20	19
39	5	—	—	3	—	8	19	11
40	5	—	—	5	—	—	18	22
41	4	—	—	5	2	—	17	20
42	4	—	—	8	—	11	18	12
43	2	—	—	1	—	—	—	—
44	3	—	—	4	—	12	18	16
45	5	—	—	4	—	—	18	17
46	2	—	—	6	6	—	19	—
47	6	—	—	5	—	14	18	17
48	1	—	—	6	10	—	17	21
49	2	—	—	3	—	2	11	—
50	3	—	—	6	—	—	13	17
51	5	—	—	5	4	—	18	21
52	2	—	—	5	—	7	22	18
53	6	—	—	3	—	1	1	11
54	6	—	—	4	—	14	16	17
55	4	—	—	5	10	—	15	15
56	5	—	—	5	5	—	19	18
57	2	—	—	6	—	13	19	13
58	2	—	—	5	—	9	18	—
59	6	—	—	5	—	2	11	11
60	6	—	—	4	—	10	17	—

Toxicity Test Information Sheet

Sponsor: Y-12 **Site/Treatment:** OF200 **Test number:** 1703

Test begin date (Day 0)	Test end date	Test duration	Template number
02/21/24	02/28/24	7 ☐ hours ☒ days	☒ NA ☐ _____

TAB 2/24/24

Test Organism: ☐ *Ceriodaphnia dubia* ☒ Fathead minnow ☐ Other: _____

Isolated from: Date: _____ Time: _____

Hatch date: 02/19/24

Delivery date: 02/20/24

Notes:

Test period	Test purpose	Test stage	Test type
☒ Chronic	☒ Regulatory	☐ Preliminary	☒ Effluent
☐ Acute	☐ Investigative	☒ Analytical	☐ Received waters
		☐ Re-test	☐ Substance

Treatment descriptions:

Number	Treatment Description*	Type**	Number	Treatment Description*	Type**
1 =	25% DMW	☒ C ☐ T	4 =	50%	☐ C ☒ T
2 =	12.5%	☐ C ☒ T	5 =	75%	☐ C ☒ T
3 =	25%	☐ C ☒ T	6 =	100%	☐ C ☒ T

*If DMW, include Batch number **C = Control, T= Treatment

Dilution Water Type:

☐ Not applicable ☐ Other (describe): _____

☒ 25% Dilute Mineral Water (DMW) + Trace Metals Batch number: 1005

Source of Test Organisms:

☐ ESD cultures: Board numbers: ☐ NA ☐ _____

☒ Vendor: ABS ☐ Other (describe): _____

Water delivery dates:

☐ Not applicable

Sample ID: 33822 Date: 2/21/24 COC #: 031143

Sample ID: 33823 Date: 2/23/24 COC #: 031144

Sample ID: 33824 Date: 2/26/24 COC #: 031145

Record of Deviations from Method and/or Test Non-Conformities		
Date	*Description*	*Initial*
03/06/24	No Deviations observed	TAB

Quality Assurance (QA) Record			
Procedure	*Name*	*Initial*	*Date*
Test run by:	Trystan A. Bordeau	TAB	3/5/24
Data sheets QA:	[illegible]	[illegible]	3-6-24
Data entered:	Trystan A. Bordeau	TAB	3/5/24
Data entry QA:	[illegible]	[illegible]	3-6-24

CHRONIC Daily Water/Feeding Log

Sponsor: Y-12 Test site/treatment: OF200 Begin Date: 02/21/24 End Date: 02/28/24 Test Number: 1703

Daily Test Info		Temperature Information Therm. #: DD-20		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R= *Raphidocelis*, B=Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10^7/mL					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 cell density	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	2/21/24 TAB	— am 26.2 pm	— am 25.6 pm	B	2/20/24	88	☒Yes	— am 1609 pm	1359	1446	33822	1005	N/A
Day 1	2/22/24 TAB	26.1 am 26.2 pm	25.7 am 25.2 pm	B B	2/21/24 2/21/24	79 68	☒Yes	0956 am 1601 pm	1334	1425	33822	1005	
Day 2	2/23/24 TAB	26.3 am 26.2 pm	26.0 am 25.3 pm	B B	2/22/24 2/22/24	70 55	☒Yes	1002 am 1510 pm	1352	1438	33823	1005	
Day 3	2/24/24 TAB	26.4 am 25.9 pm	25.9 am 25.3 pm	B B	2/23/24 2/23/24	68 54	☒Yes	1100 am 1602 pm	1427	1501	33823	1007 * ~~1006~~	
Day 4	2/25/24 TAB	26.1 am 26.0 pm	25.7 am 25.1 pm	B B	2/24/24 2/24/24	60 84	☒Yes	1055 am 1604 pm	1345	1427	33823	1007 * ~~1006~~	
Day 5	2/26/24 TAB	26.1 am 26.2 pm	25.5 am 25.8 pm	B B	2/25/24 2/25/24	68 75	☒Yes	1107 am 1642 pm	1348	1421	33824	1007 * ~~1006~~	
Day 6	2/27/24 TAB	26.2 am 26.1 pm	25.9 am 25.7 pm	B B	2/26/24 2/26/24	66 71	☒Yes	1100 am 1404 pm	1356	1423	33824	1008	
Day 7	2/28/24 TAB	26.1 am — pm	25.8 am — pm				☐Yes	— am — pm	1348	1522			

Notes:

* 1007, not 1006. TAB 2/27/24

TAB 2/20/24

Fathead Minnow Chronic Daily Survival Log

Sponsor: Y-12 Test site/chemical: 6F200 Test Number: 1703

Begin Date: 02/21/24 End Date: 02/28/24

Comment Codes: C = Clear; D = Dead; Fg = Fungus; K = Killed by siphoning; M = Missing; Sk = Sick; SM = Small; SOR = Siphoned and returned; W = Wounded

Treatment Number and Desc.	Replicate Number	Position Number	Day 1 Date TAB 2/22/24	Day 2 Date TAB 2/23/24	Day 3 Date TAB 2/24/24	Day 4 Date TAB 2/25/24	Day 5 Date TAB 2/26/24	Day 6 Date TAB 2/27/24	Day 7 Date TAB 2/28/24
1: Control 25% Dilw	1	23	10	10	10	10	10	10	10
	2	11	10	10	10	10	10	10	10
	3	9	10	10	10	10	10	10	10
	4	13	10	10	10	10	10	10	10
2: 12.5%	1	16	10	10	10	10	10	10	10
	2	12	10	10	10	10	10	10	10
	3	18	10	10	10	10	10	10	10
	4	17	10	10	10	10	10	10	10
3: 25%	1	14	10 1SM	10 1SM	10 1SM	10 1SM	10 1SM	10 1SM	10
	2	22	10	10	10	10	10	10	10
	3	19	10	10	10	10	10	10	10
	4	15	10	10	10	10	10	10	10
4: 50%	1	21	10	10	10	10	10	10	10
	2	4	10	10	10	10	10	10	10
	3	24	10	10	10	10	10	10	10
	4	6	10	10	10	10	10	10	10
5: 75%	1	8	10	10	10	10	10	10	10
	2	20	10	10	10	10	10	10	10
	3	3	10	10	10	10	10	10	10
	4	5	10	10	10	10	10	10	10
6: 100%	1	7	10	10	10	10	10	10	10
	2	2	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10	10
	4	1	10	10	10	10	10	10	10

TAB 2/26/24

Random assignment of test chambers

Project: 4-12 OF200 Test site/chemical: OF200 Test number: 1703

Position	Treatment #	Replicate	Sample ID
1	6	4	100%.
2	6	2	100%.
3	5	3	75%.
4	4	2	50%.
5	5	4	75%.
6	4	4	50%.
7	6	1	100%.
8	5	1	75%.
9	1	3	25%. DMW
10	6	3	100%.
11	1	2	25%. DMW
12	2	2	12.5%.
13	1	4	25%. DMW
14	3	1	25%.
15	3	4	25%.
16	2	1	12.5%.
17	2	4	12.5%.
18	2	3	12.5%.
19	3	3	25%.
20	5	2	75%.
21	4	1	50%.
22	3	2	25%.
23	1	1	25%. DMW
24	4	3	50%.

TAB 2/10/17

Random assignment of larvae to test chambers

Project: 4-12 Test site/chemical: OF200 Test number: 1703

TAB 2/20/24

Treatment	Replicate	Cup 1	Cup 2
Treatment #1 TMB 2/21/24 ~~12.5 l.~~ 25 l. DMSO control			
1	1	11 ✓	17 ✓
1	2	13 ✓	9 ✓
1	3	48 ✓	2 ✓
1	4	6 ✓	36 ✓
Treatment #2 TMB 2/21/24 ~~25 l.~~ 12.5 l.			
2	1	29 ✓	41 ✓
2	2	7 ✓	8 ✓
2	3	1 ✓	37 ✓
2	4	27 ✓	45 ✓
Treatment #3 TMB 2/21/24 ~~50 l.~~ 25 l.			
3	1	28 ✓	15 ✓
3	2	14 ✓	43 ✓
3	3	40 ✓	25 ✓
3	4	22 ✓	16 ✓
Treatment #4 50 l.			
4	1	42 ✓	3 ✓
4	2	38 ✓	39 ✓
4	3	19 ✓	21 ✓
4	4	33 ✓	24 ✓
Treatment #5 75 l.			
5	1	10 ✓	46 ✓
5	2	20 ✓	35 ✓
5	3	30 ✓	34 ✓
5	4	44 ✓	31 ✓
Treatment #6 100 l.			
6	1	5 ✓	18 ✓
6	2	12 ✓	4 ✓
6	3	47 ✓	23 ✓
6	4	26 ✓	32 ✓

Fathead Minnow Weight and Survival Data

TMB
2/20/24

Sponsor: Y-12	Test number: 1703
Test site/chemical: OF200	Balance ID: A009820
Test Start Date: 2/21/24	Test End Date: 2/28/24
Start Drying Date/Time: 2/28/24 1530	End Drying Date/time: 2/29/24 1015

Treatment	Replicate	Pan Wt. (mg) Date: 2/25/24 Balance check: ☒	Pan + Larvae (mg) Date: 2/29/24 Balance check: ☒	Number Surviving
Initial	1	15.2465		10
	2	15.5390		10
	3	15.4340		10
	4	15.3980		10
1. 25 l. DMW	1	15.4545	21.9275	10
	2	15.4000	22.1175	10
	3	15.4385	22.8150	10
	4	15.3935	23.6390	10
2. 12.5 l.	1	15.3615	22.7440	10
	2	15.3670	22.4485	10
	3	15.3935	22.5430	10
	4	15.5375	22.1555	10
3. 25 l.	1	15.5310	22.9125	10
	2	15.4810	22.0895	10
	3	15.5425	22.0080	10
	4	15.4775	23.3125	10
4. 50 l.	1	15.5080	21.7845	10
	2	15.5485	23.2135	10
	3	15.5015	21.4520	10
	4	14.9360	22.2380	10
5. 75 l.	1	14.4455	19.9545	10
	2	14.1345	21.4220	10
	3	14.7470	21.4195	10
	4	14.7145	21.6025	10
6. 100 l.	1	14.7265	19.7085	10
	2	14.7140	22.1780	10
	3	14.7500	21.8575	10
	4	14.8705	22.7655	10