



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).

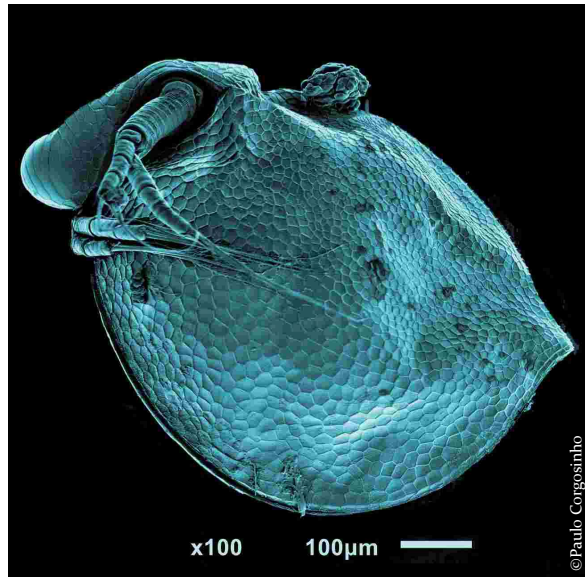
RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted May 3-10, 2023**

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 Concentration₂₅ (IC₂₅) 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 IC₂₅ 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	<i>Ceriodaphnia</i>	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 3×10^6 cells of the green alga *Raphidocelis subcapitata* per 15 mL of test solution every 24 h from an algal stock with a concentration $3.0 - 3.5 \times 10^7$ 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 (\pm SD)
Control	10	10	30.7 \pm 7.3
12.5%	10	10	20.7 \pm 10.7
25%	10	10	24.7 \pm 10.5
50%	10	10	26.9 \pm 11.3
75%	10	10	30 \pm 7.5
100%	10	10	28.9 \pm 10.2

7. STATISTICAL ANALYSES

7.1 Survival

The calculated IC₂₅ for survival was >100% effluent.

7.2 Reproduction

The calculated IC₂₅ for reproduction was >100% effluent.

7.3 Summary of *Ceriodaphnia* toxicity test results:

IC₂₅ for survival: >100%

IC₂₅ 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 (μ S/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 ($\mu\text{S}/\text{cm}$) 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 \pm SD
	1	2	3	4	
Control	0.82	0.78	0.75	0.75	0.78 \pm 0.03
12.5%	0.86	0.67	0.69	0.79	0.75 \pm 0.09
25%	0.74	0.87	0.71	0.77	0.77 \pm 0.07
50%	0.73	0.85	0.91	0.7	0.8 \pm 0.1
75%	0.75	0.7	0.72	0.82	0.75 \pm 0.05
100%	0.71	0.69	0.82	0.78	0.75 \pm 0.06

7. STATISTICAL ANALYSES

7.1 Survival

The calculated IC₂₅ for survival was >100% effluent.

7.2 Growth

The calculated IC₂₅ for growth was >100% effluent.

7.3 Summary of fathead minnow toxicity test results:

IC₂₅ for survival: >100%

IC₂₅ 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

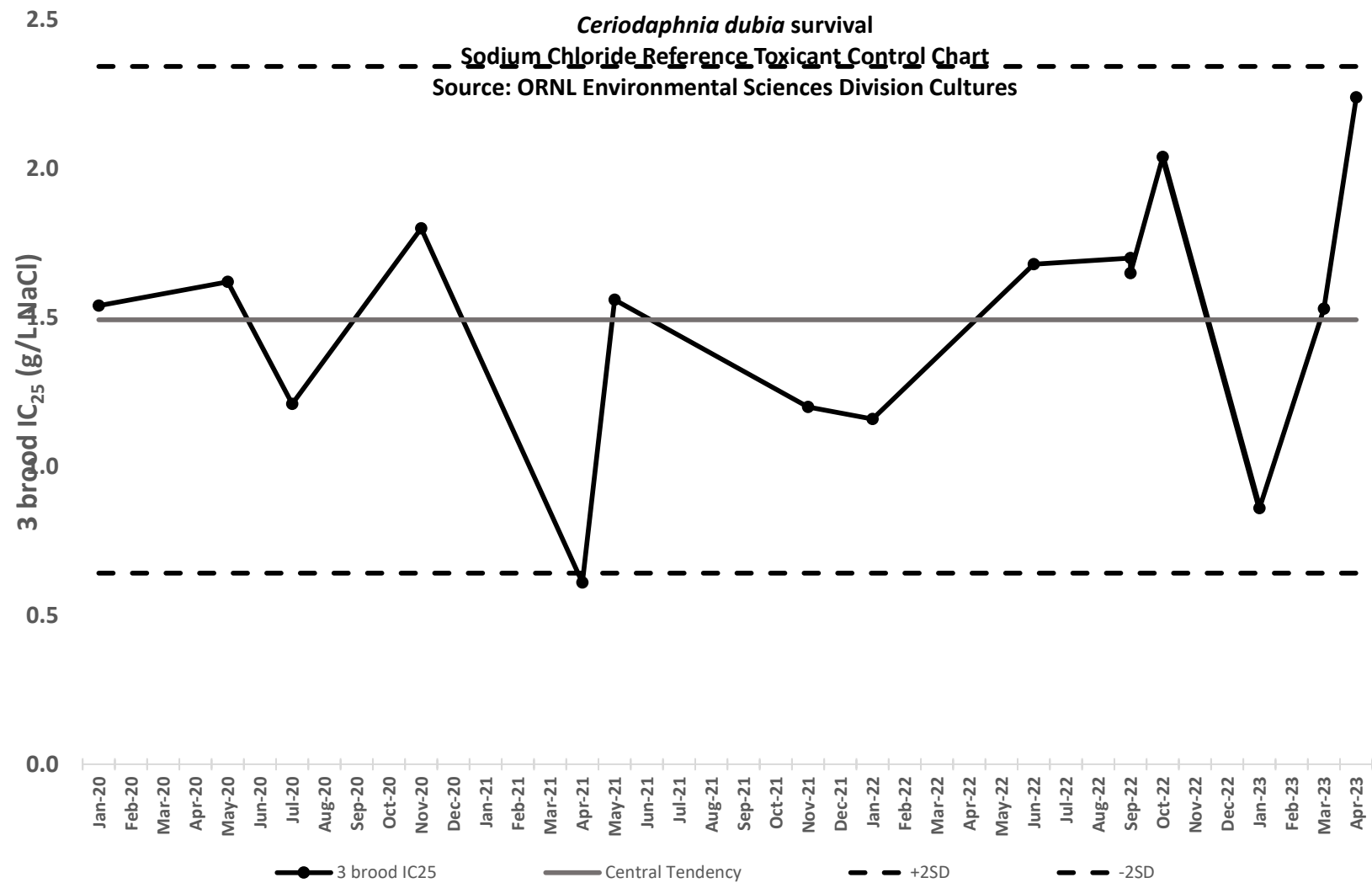
Date: 18 May 2023

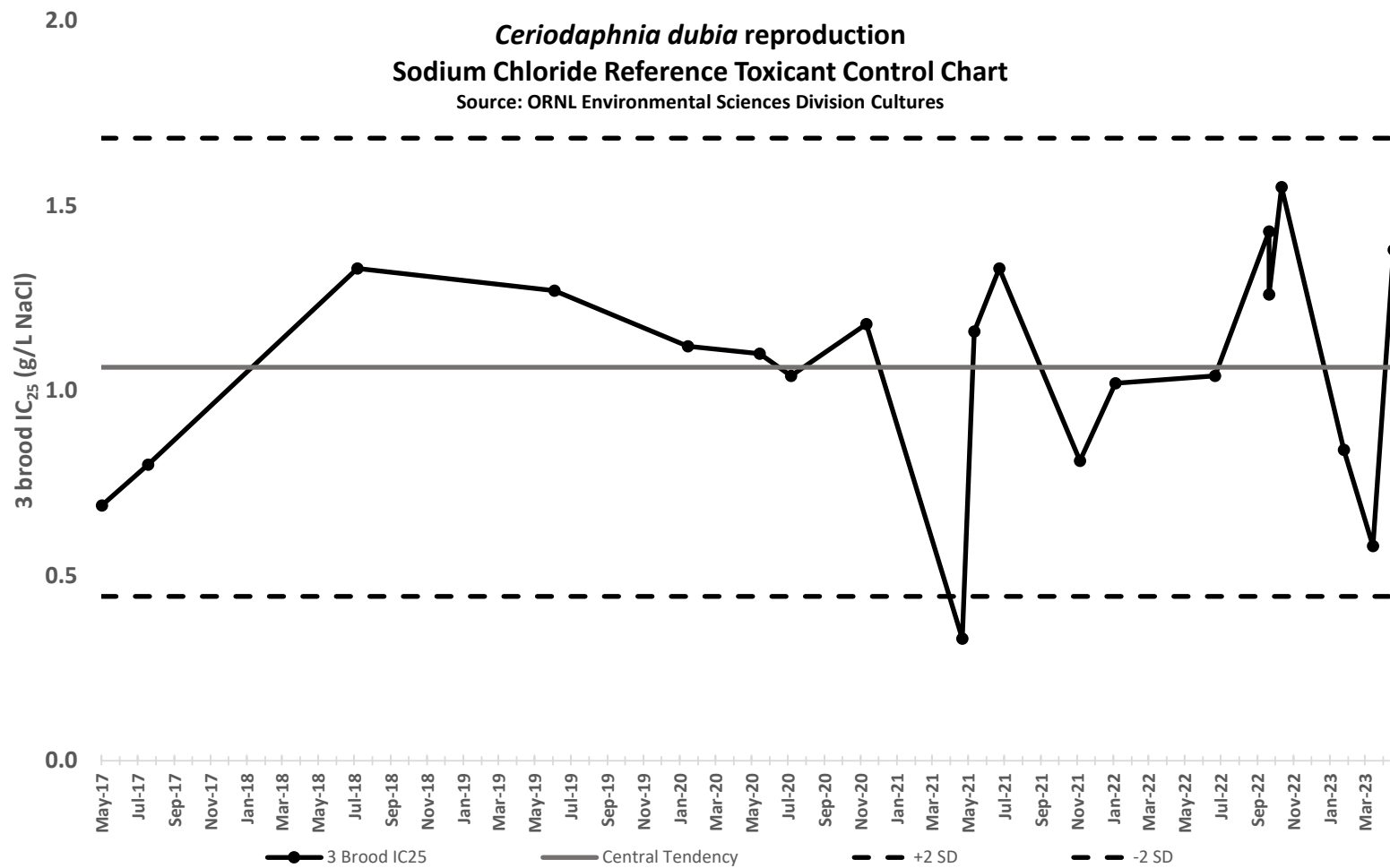
Report reviewed by: Louise Stevenson

Date: 22 May 2023

Louise Stevenson

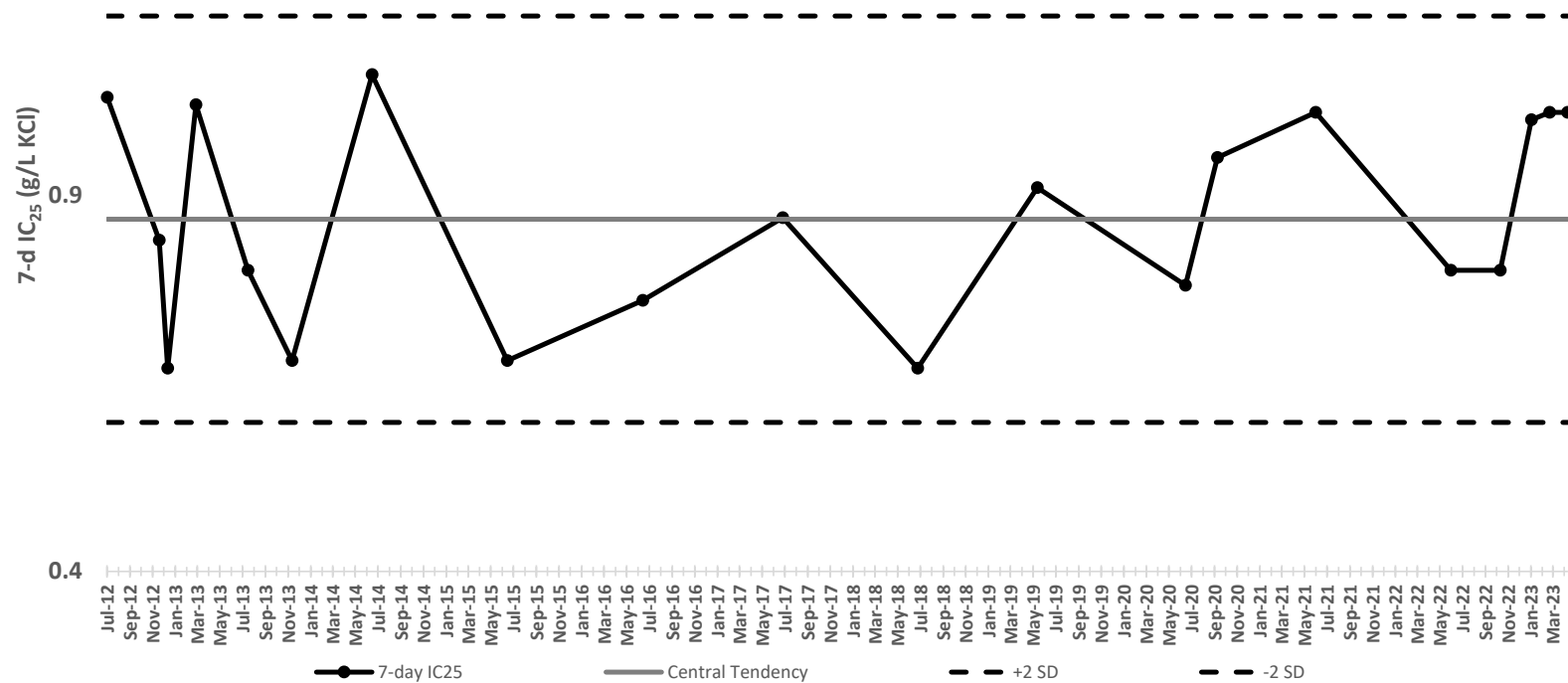
REFERENCE TOXICANT CONTROL CHARTS





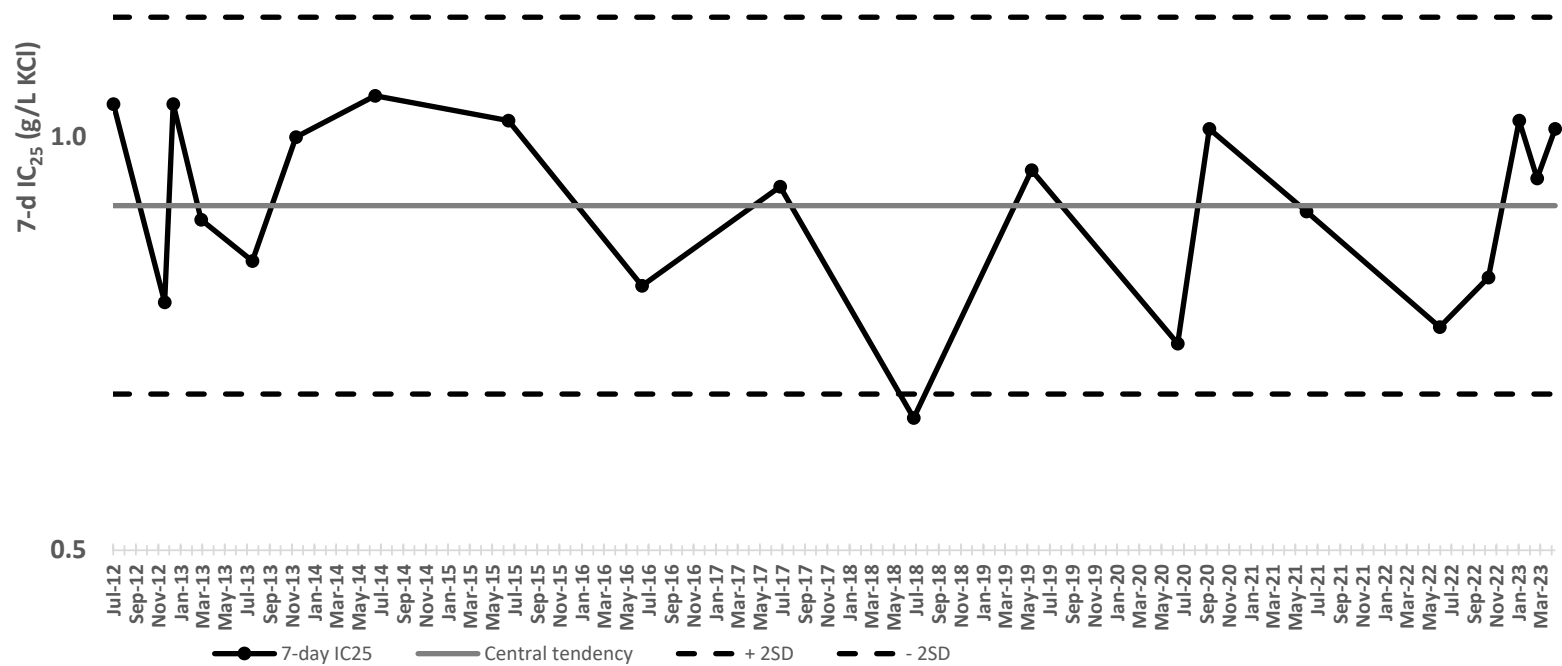
1.4

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



1.5

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

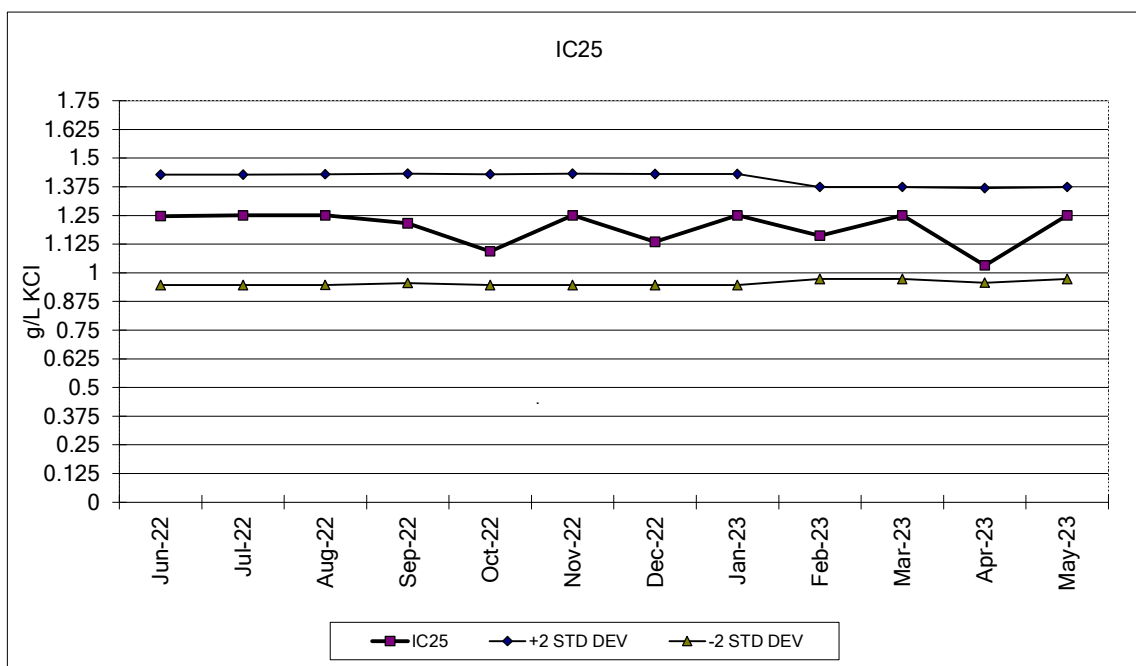


1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel:970/484-5091 Fax:970/484-2514

Pimephales promelas



Chronic 7 Day Survival Test Data

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) (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: OF200 Associated test numbers: CD2985; FHM1692

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

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	33287			33288		
Rec. temp. (°C) (New ✓)	sercol ✓	sercol ✓	sercol ✓			sercol ✓		
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	8.57/7.91	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
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
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	8.63/7.98	8.36/8.04
DO (mg/L) Initial	8.77	8.76	8.72	8.76	8.54	8.36	8.74	
Final CD/FHM		8.70/7.45	8.96/7.30	8.83/7.30	8.98/7.41	8.89/6.55	9.03/7.09	8.52/7.50
Conductivity (µS/cm)	399	396	381	375	382	327	322	
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
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
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	8.52/8.12	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		8.78/7.51	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

[illegible]

THERMOMETER NO.

SAMPLES RELINQUISHED BY

A. L. Garland

DATE

5/3/23

TIME

1308

□ AM

☒ PM

SAMPLES RECEIVED BY

Peigra Kn

DATE

5/3/22

TIME

1308

☐ AM☒ PM

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

DATE (MM/DD/YY)		ESD TEST NAME		NAME OF SAMPLERS			CHAIN-OF-CUSTODY NO.		
05/05/23		TOX TEST		GARLAND / WILLIAMS			031102		
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	#7009 TEMP	REMARKS	#4102 C/L
TOX TEST	200	0710	C	1	~ 17 LITERS	4°	11.6°		<0.05
A.S.G. 5/5/23									

THERMOMETER NO.

SAMPLES RELINQUISHED BY

A. S. Garland

DATE _____

5/5/23

TIME

0802

□ PM

SAMPLES RECEIVED BY

Prigla kv

DATE

5/5/23

TIME

0802

☒ AM

PM

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY
CHAIN-OF-CUSTODY

[illegible]

THERMOMETER NO.

SAMPLES RELINQUISHED BY	<i>A. L. Garland</i>	DATE	<i>5/8/23</i>	TIME	<i>0823</i>	<input checked="" type="checkbox"/> AM <input type="checkbox"/> PM
SAMPLES RECEIVED BY	<i>Prigita Km</i>	DATE	<i>05/08/23</i>	TIME	<i>0823</i>	<input checked="" type="checkbox"/> AM <input type="checkbox"/> PM

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y12Site/Treatment: OF200

Test number:

2985

PK- Test begin date (Day 0)

05/03/2023

Test end date

05/10/2023

Test duration

7☐ hours ☒ days

Template number

☐ NA ☒ 3 PK

Test

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

Organism:

Isolated from:

Date: 5/3/235/4/23

Hatch date: _____

Time: 10:00pm5:55 am

Delivery date: _____

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%	<input checked="" type="checkbox"/> C <input type="checkbox"/> T	4 =	50% OF200	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
2 =	12.5% OF200	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	5 =	75% OF200	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
3 =	25% OF200	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	6 =	100% OF200	<input type="checkbox"/> C <input checked="" type="checkbox"/> T

*If DMW, include Batch number

**C = Control, T = Treatment

Dilution Water Type:

☐ Not applicable☐ Other (describe): _____☒ 25% Dilute Mineral Water (DMW) + Trace MetalsBatch number: 947, 950

Source of Test Organisms:

☒ BSD cultures: Board numbers: ☐ NA ☐ 4762☐ Vendor: _____☐ Other (describe): _____

Water delivery dates:

☐ Not applicableSample ID: 33286Date: 05/03/23COC #: 031101Sample ID: 33287Date: 05/05/23COC #: 031102Sample ID: 33288Date: 05/08/23COC #: 031103

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

Date	Description	Initial
<u>05/10/23</u>	<u>None</u>	<u>PK</u>

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	<u>Rejia Ku</u>	<u>PK</u>	<u>05/10/23</u>
Data sheets QA:	<u>Rejia Ku</u>	<u>PK</u>	<u>05-11-23</u>
Data entered:	<u>Rejia Ku</u>	<u>PK</u>	<u>05/10/23</u>
Data entry QA:	<u>Rejia Ku</u>	<u>PK</u>	<u>05-11-23</u>

Environmental Sciences Division

Rev. 02 2020-01-02

READ AND UNDERSTOOD

DATE

20

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		Feeding Information					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	26.0 am pm	25.0 am pm	YCT R	3/21/23 5/3/23	100 86	✓ Yes 3.5E7	1830 am pm	1803	1900	33286	947	NA
Day 1	05/04/23 PK	26.1 am pm	25.0 am pm	YCT R	3/21/23 5/3/23	100 91	✓ Yes 3.30E+07	1650 am pm	1630	1725	↓	947	
Day 2	05/05/23 PK	25.7 am pm	25.3 am pm	YCT R	3/21/23 5/3/23	100 90	✓ Yes 3.33E+07	1525 am 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	1800	↓	950	
Day 5	05/08/23 PK	25.8 am pm	25.3 am 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: 04-200 Test Number: 2985
 Begin Date: 05/03/2023 End Date: 05/10/2023 Template Number: A-3 PK
 Codes: (-) Alive and No Reproduction; (N) Alive and Reproduction; (xN) Dead and Reproduction; (M) Male PK

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	-	16
2	6	-	-	-	5	13	18 PK	18
3	5	-	-	-	6	13	13	-
4	3	-	-	-	7	1	-	2
5	5	-	-	-	5	17	17	-
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	-	-
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: 412 Site/Treatment: OT-200 Test number: **1692**Test begin date (Day 0) 05/03/2023 Test end date 05/10/2023 Test duration 7 ☐ hours ☒ days ☒ NA ☐ Template number PKTest Organism: ☐ *Ceriodaphnia dubia* ☒ Fathead minnow ☐ Other: 05/02/23
Isolated from: _____ Hatch date: 05/04/2023
Date: _____ Time: _____ 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%	<input checked="" type="checkbox"/> C <input type="checkbox"/> T	4 =	50%	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
2 =	12.5%	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	5 =	75%	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
3 =	25%	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	6 =	100%	<input type="checkbox"/> C <input checked="" type="checkbox"/> 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:

☐ BSD 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: 05/05/23 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	NA	PK

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	Rejia Hu	PK	05/10/23
Data sheets QA:	Rejia Hu	PK	05-12-23
Data entered:	Rejia Hu	PK	05/10/23
Data entry QA:	Rejia Hu	PK	05-12-23

05/10/23
PK

CHRONIC Daily Water/Feeding Log

Sponsor: Y12 Test site/treatment: OF200 Begin Date: 05/03/2023 End Date: 05/10/2023 Test Number: 1692

Daily Test Info		Temperature Information		Feeding Information					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 count	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	05/03/23 PK/ANJ	am 26.4 pm	am 25.3 pm	B	05/02/23	195	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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	05/04/23 05/03/23	79 94	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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	91 161	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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	<input type="checkbox"/> NA <input checked="" type="checkbox"/> 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

Sponsor: Y12 Test site/chemical: DF200 Test Number: 1692

PK
05/02/23

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	Day 2 Date	Day 3 Date	Day 4 Date	Day 5 Date PK	Day 6 Date PK	Day 7 Date PK
			05/04/23 PK	05/05/23 PK	05/06/23 PK	05/07/23 PK	05/08/23	05/09/23	05/10/23
1: 25% DMW	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% DF200	1	13	10	10	10	10	10	10	9 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% DF200	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% DF200	1	7	10	10	10	9 ID	9	9	9
	2	20	10	10	10 SK	10	10	10	10
	3	1	10	10	10	10	10	10	10
	4	22	10 PK 15	10	10	10	10	10	10
5: 75% DF200	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% DF200	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: V12 Test site/chemical: OF200 Test Number: 1692Starting Position Number (on Table of Random Numbers): 29PK
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

Fathead Minnow Weight and Survival Data

PK
05/02/23

Sponsor: Y12		Test number: 1692		
Test site/chemical: OF-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: <input checked="" type="checkbox"/>	Pan + Larvae (mg) Date: 05/11/23 Balance check: <input checked="" type="checkbox"/>	Number Surviving
Initial	1	15.1175	16.4060	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	22.8305	10
	4	15.2245	22.7680	10
2. 12.5% OF-200	1	15.1305	23.7075	10
	2	15.1785	21.8920	10
	3	15.0895	PK 20 21.9525	9
	4	15.2905	23.1545	10
3. 25% OF-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% OF-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% OF-200	1	15.3705	22.8755	10
	2	15.3250	22.3335	9
	3	15.1295	22.3390	10
	4	15.3605	23.5665	10
6. 100% OF-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: DF-200 3 KCl Test Number: 1692; 1693Starting Position Number (on Table of Random Numbers): 3, 11PK
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	2,441 ✓
2	26	50	74		3,82 ✓	2	26	50	74		18,352 ✓
3	27	51	75		10,403 ✓	3	27	51	75		29,443 ✓
4	28	52	76		34,344 ✓	4	28	52	76		1,324 ✓
5	29	53	77	2. 12.5% DF200	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% DF200	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% DF200	25,301 ✓	13	37	61	85	4. 1.0g/L KCl	34,471 ✓
14	38	62	86		28,352 ✓	14	38	62	86		5,82 ✓
15	39	63	87		16,443 ✓	15	39	63	87		19,443 ✓
16	40	64	88		7,334 ✓	16	40	64	88		26,404 ✓
17	41	65	89	5. 75% DF200	4,321 ✓	17	41	65	89	5. 1.25g/L KCl	33,279 ✓
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% DF200	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):								