



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 Concentration₂₅ (IC₂₅) for survival, growth, and/or reproduction were thus >100% (the highest concentration of effluent tested).

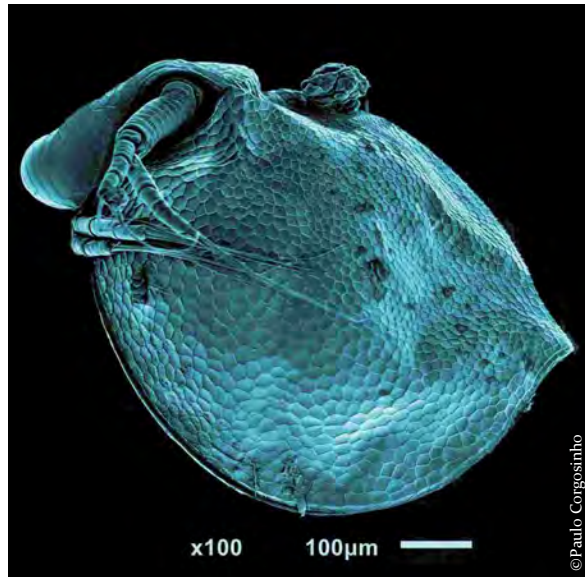
The NPDES permit states that toxicity will be demonstrated if the IC₂₅ is less than or equal to the permit limit (50% effluent for Outfall 200). All of the results for all endpoints were within permit limits.

Outfall	Test Organism	Endpoint	IC ₂₅
Outfall 200	Fathead minnow	Survival	>100%
		Growth	>100%
Outfall 200	<i>Ceriodaphnia dubia</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 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	11/4/2022	11/7/2022
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 \pm 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 \pm 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 (\pm SD)
Control	10	10	38.7 \pm 3.1
12.5%	10	8	30.1 \pm 13.9
25%	10	10	33.6 \pm 12.3
50%	10	10	33.6 \pm 8.4
75%	10	9	31.3 \pm 11.9
100%	10	10	38.6 \pm 5.5

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.)	8.11	8.20	8.08
Conductivity (μ S/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 ($\mu\text{S}/\text{cm}$) 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: 10 November 2022

Report reviewed by: Louise Stevenson *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	11/4/2022	11/7/2022
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 \pm SD)

3.8 Diseases and treatment: None.

4. TEST METHODS

4.1 Toxicity test method: Fathead minnow larval survival and growth test. Reference: EPA Test Method 1000.0, in P.A. Lewis et al., *Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA/821/R/02/013 (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 μ L per beaker twice daily.

5. QUALITY ASSURANCE

- 5.1 Standard toxicant used: Potassium chloride (source: Fisher Scientific).
- 5.2 Date of most recent chronic reference toxicant test: 2-9 Nov 2022.
- 5.3 Dilution water used: 25% DMW [2.5:7.5 (v:v) ratio of degassed mineral water to deionized distilled water augmented with trace metals].
- 5.4 Survival IC_{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 \pm 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 \pm 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 \pm SD
	1	2	3	4	
Control	0.67	0.65	0.73	0.81	0.71 \pm 0.07
12.5%	0.93	0.61	0.54	0.74	0.71 \pm 0.17
25%	0.63	0.75	0.64	0.74	0.69 \pm 0.06
50%	0.64	0.6	0.68	0.59	0.63 \pm 0.04
75%	0.46	0.63	0.61	0.58	0.57 \pm 0.08
100%	0.66	0.74	0.63	0.63	0.66 \pm 0.05

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.)	8.11	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.

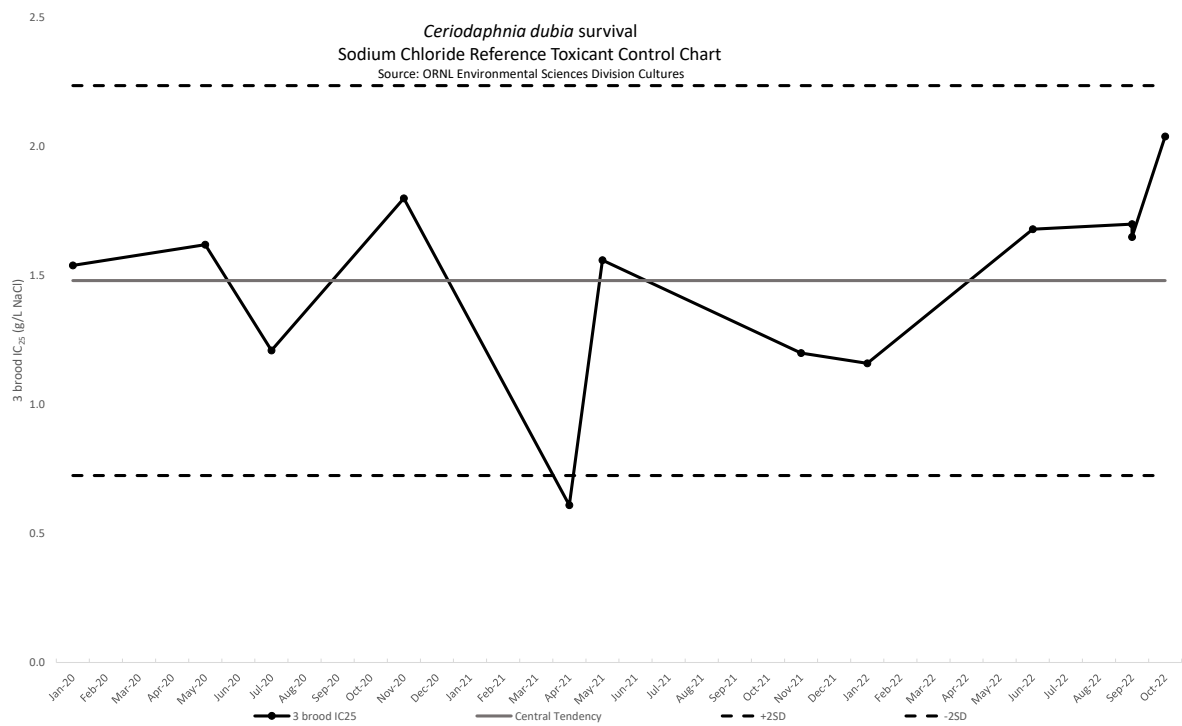
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

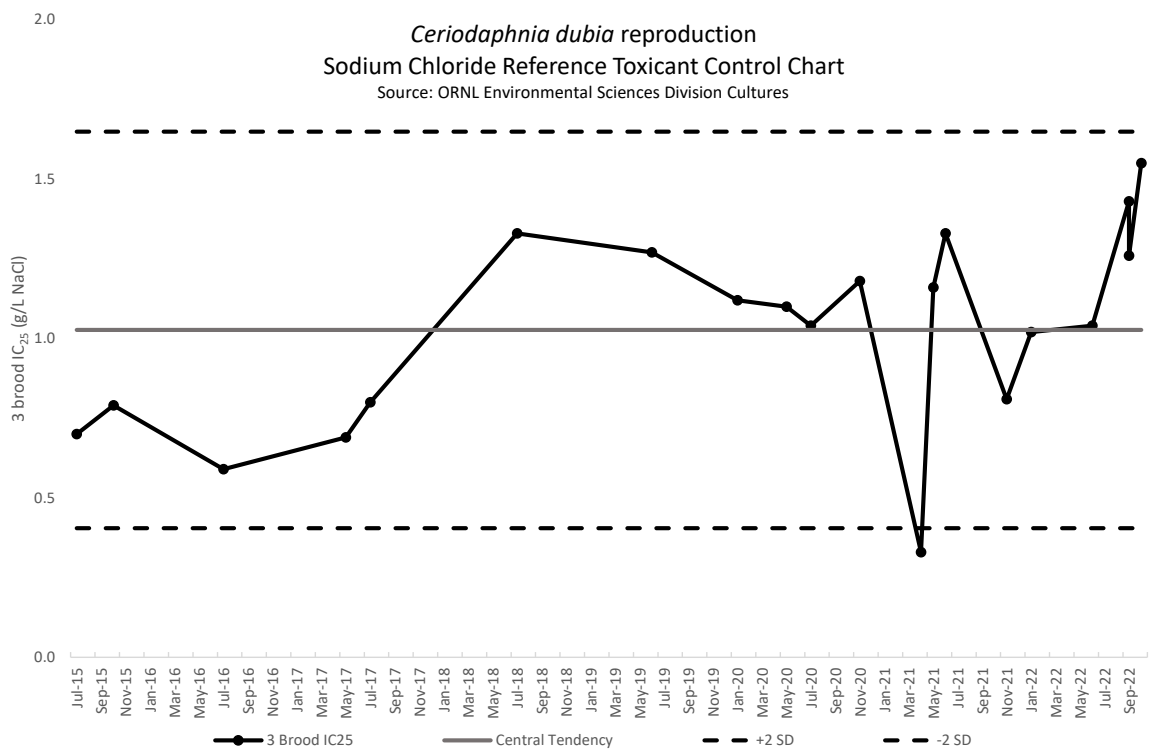
Report prepared by: Peijia Ku

Date: 11 November 2022

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

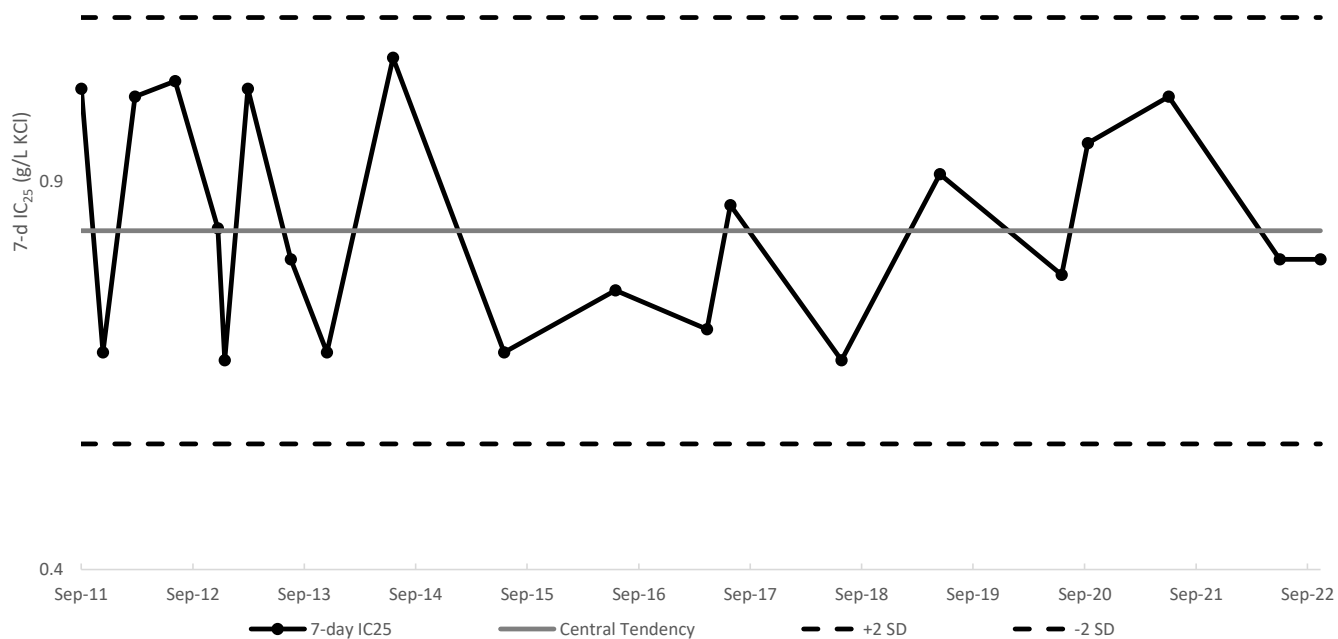
REFERENCE TOXICANT CONTROL CHARTS





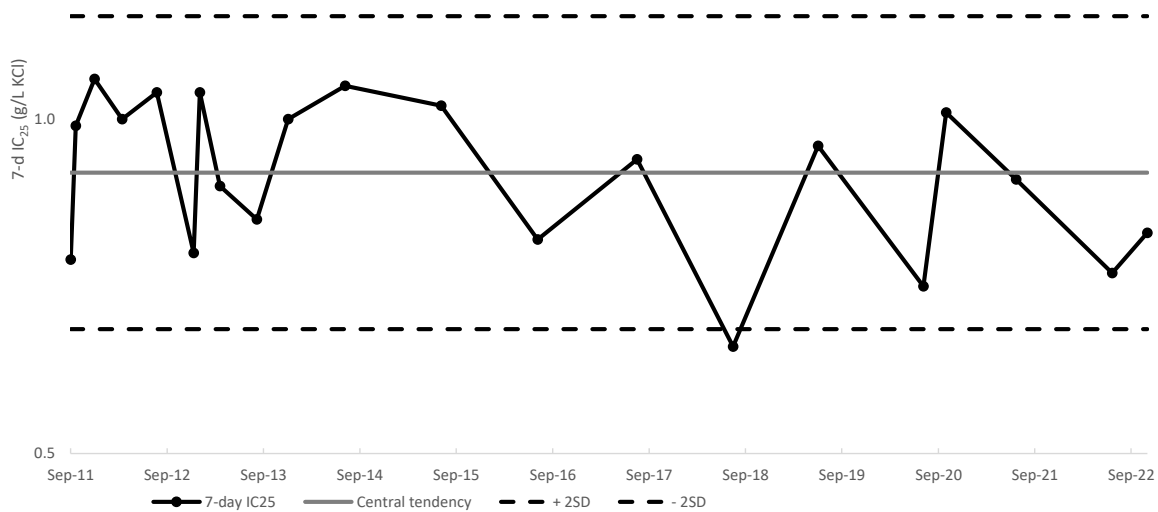
1.4

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

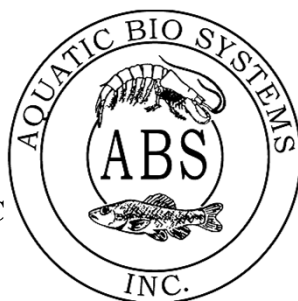


1.5

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

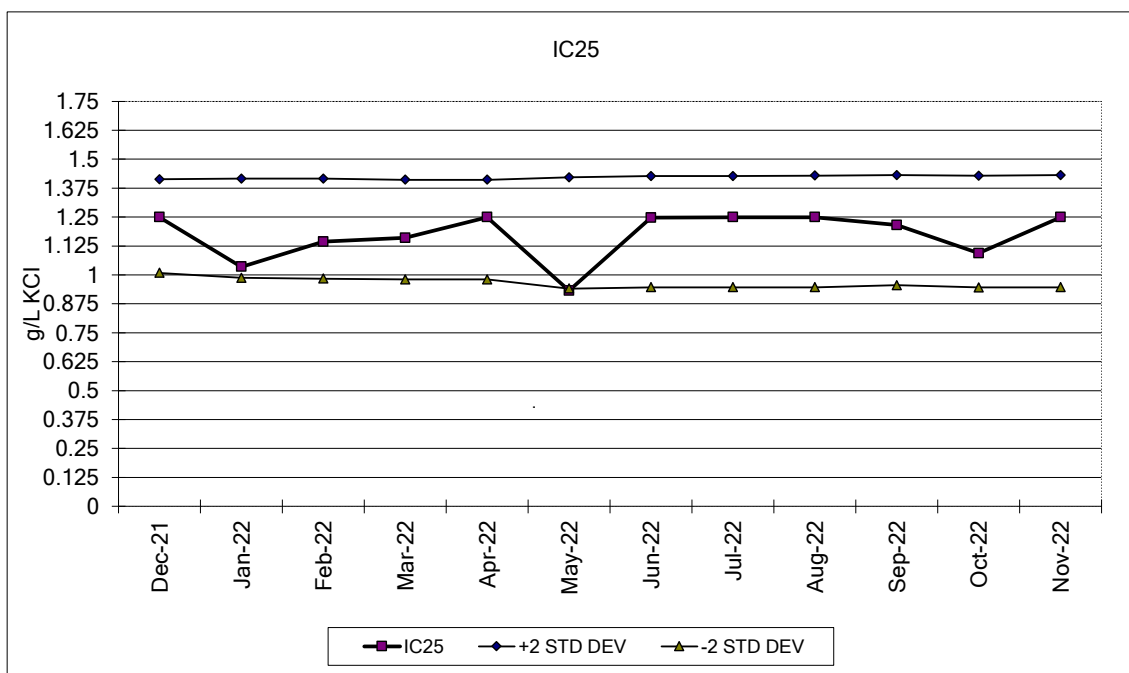


1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



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)
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		Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
		(upper)	(lower)			
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

Daily Water Chemistry Log

Site/Treatment:

DF100

Associated test numbers:

CD-2978

FHM-1686

PK
11/14/22

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

Observation Day:	0 Nov	1 Nov	2 Nov	3 Nov	4 Nov	5 Nov	6 Nov	7 Nov
Date/Initials:	11/21/22	11/22/22	11/22/22	11/22/22	11/22/22	11/22/22	11/22/22	11/22/22
5-digit ORNL ID	32924	32924	32925	32925	32925	32926	32926	
Rec. temp. (°C) (New ✓)	Sec COC		Sec COC			Sec COC		
DMW Batch #	918	918	918	919	919	919	919	
Conductivity (µS/cm)	237	232	225	239	233	230	220	
Alkalinity (mg/L)	82			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		8.36/7.93	9.19/7.59	8.44/7.57	8.54/7.10	8.82/7.40	8.81/7.47	8.70/7.45
Conductivity (µS/cm)	260	250	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	8.53	8.85	8.89	
Final CD/FHM		9.31/7.83	9.42/7.26	8.53/7.41	8.71/7.15	8.95/7.16	8.96/7.22	8.76/7.13
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	9.40/7.24	8.58/7.37	8.72/7.11	8.98/7.18	8.93/7.16	8.85/7.33
Conductivity (µS/cm)	331	329	313	322	317	324	320 (322)	
Alkalinity (mg/L)								
Hardness (mg/L)								
Chlorine (mg/L)								
pH (S.U.) Initial	8.07	8.06	8.11	8.13	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	9.03	9.17	8.54	9.05	9.41	9.08	
Final CD/FHM		9.13/7.72	9.82/7.38	8.60/7.30	8.76/7.20	8.47/7.13	9.87/7.25	8.80/7.61
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
Conductivity (µS/cm)	413	424	393	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	8.79/7.23	8.88/7.08	8.80/7.16	8.81/7.61

CHAIN OF CUSTODY FORMS

[illegible]

SAMPLES RELINQUISHED BY

SAMPLES RECEIVED BY	
1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
29	30
31	32
33	34
35	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

DATE _____

DATE

TIME

TIME

0810

☐ PM☒ AM

PM

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	HALH C12 #4102
OUTFALL 200	200	0730	C	1	~14 LITERS	3°	10.3°c		<0.05
11/4/22									

SAMPLES RELINQUISHED BY

Q. T. Wilbur

SAMPLES RECEIVED BY

AMP

ORM sample ID = 32925

DATE _____

11-4-22

TIME

0803

☒ AM

PM

DATE _____

11/4/22

TIME

0803

☒ AM☐ PM

[illegible]

A. R. Garland
BPP

DATE	11 / 7 / 22
------	-------------

TIME 0830

☒ AM
☐ PM

SAMPLES RECEIVED BY	
1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
29	30
31	32
33	34
35	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

DATE	11/7/22
------	---------

TIME	0830
------	------

☒ AM
☐ PM

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y12 Site/Treatment: OF200 Test number: CD 2978Test begin date (Day 0) 11/02/2022 Test end date 11/09/2022 Test duration 7 ☐ hours ☒ days ☐ NA ☒ Template number 6Test Organism: ☒ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: _____
Isolated from: _____ Notes: _____Date: 11/01/2022 11/01/2022 Hatch date: _____
Time: 10:00 am 5:50 pm 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%	<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: 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
<u>11/7/22</u>	<u>Noticed late in the day (after running test) that Y12 batch smelled like it had spoiled. Will switch to previous batch (9/13/22) for last day of test.</u>	<u>AMF</u>
<u>11/9/22</u>	<u>1 male in treatment 3 (cup 21)</u>	<u>AMF</u>

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	<u>AMF</u>	<u>AMF</u>	<u>11/9/22</u>
Data sheets QA:	<u>AMF</u>	<u>AMF</u>	<u>11/9/22</u>
Data entered:	<u>AMF</u>	<u>AMF</u>	<u>11/9/22</u>
Data entry QA:	<u>AMF</u>	<u>AMF</u>	<u>11/9/22</u>

Environmental Sciences Division

Rev. 02 2020-01-02

SIGNATURE _____
READ AND UNDERSTOOD _____DATE _____ 20
DATE _____ 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. #: <u>PD19</u>		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R = <i>Raphidocelis</i> , B = Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10 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	11/02/22 PK	25.3 am pm	26.0 am pm	YCT RASU	11/2/22 10/18/22	100 95	<input checked="" type="checkbox"/> 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 11/2/22	100 91	<input checked="" type="checkbox"/> 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 10/18/22	100 8590	<input checked="" type="checkbox"/> Yes	12:56 am pm	12:39	13:28	32925	918	↓
Day 3	11/05/22 AMF	25.9 am pm	25.4 am pm	YCT R	11/2/22 10/18/22	100 83	<input checked="" type="checkbox"/> Yes	10:40 am pm	10:27	11:37	↓	919	↓
Day 4	11/06/22 AMF	25.7 am pm	25.6 am pm	YCT R	11/6/22 10/18/22	100 88	<input checked="" type="checkbox"/> Yes	10:18 am pm	10:04	11:16	↓	919	↓
Day 5	11/07/22 AMF	25.9 am pm	25.6 am pm	YCT R	11/6/22 10/18/22	100 91	<input checked="" type="checkbox"/> Yes	10:42 am pm	10:25	11:28	32926	919	↓
Day 6	11/08/22 AMF	26.0 am pm	25.7 am pm	YCT R	11/6/22 10/18/22	100 89	<input checked="" type="checkbox"/> Yes	10:45 am pm	10:32	11:49	↓	919	↓
Day 7	11/09/22 AMF	26.1 am pm	25.5 am pm				<input type="checkbox"/> Yes	am pm	11:00	12:21			↓

Notes:

Project: Y12

Test site/chemical: OF200

Test Number: 2979

Begin Date: 11/02/2022

End Date: 11/09/2022

Template Number: #6

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

Test Chamber	Treatment Number	Day: 1 PK Date: 11/02/22	2 PK Date: 11/04/22	3 PK Date: 11/05/22	4 PK Date: 11/06/22	5 PK Date: 11/07/22	6 PK Date: 11/08/22	7 PK Date: 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	-	-	1	13	-	21	23
10	4	-	-	6	12	-	17	24
11	2	-	-	6	14	-	20	24
12	3	-	-	6	-	14	20	19
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	-	-	1	-	-	22	- H
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	-	-	16	15	-	6	10
37	2	-	-	7	13	-	20	25
38	1	-	-	8	12	-	18	23
39	6	-	-	7	16	-	19	22
40	5	-	-	5	14	-	21	25
41	4	-	-	6	14	-	20	26
42	6	-	-	5	-	13	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	21
47	4	-	-	7	-	-	11	25
48	5	-	-	1	12	-	11	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	5	-	-	1	X	-	-	-
54	6	-	-	8	13	-	21	18
55	4	-	-	7	14	-	20	25
56	4	-	-	7	16	-	-	2
57	6	-	-	5	13	-	21	25
58	3	-	-	7	10	-	22	21
59	1	-	-	6	14	-	23	3
60	6	-	-	7	-	18	-	19

Toxicity Test Information Sheet

Sponsor: Y12 Site/Treatment: OF200 Test number: **1686**

Test begin date (Day 0) 11-2-22 Test end date 11-9-22 Test duration 7 ☐ hours ☒ days ☒ NA ☐

Template number

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

Isolated from: _____ Date: _____ Hatch date: 10-31-22

Time: _____ Delivery date: 11-1-22 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 =	75%	<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: 918919

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
<u>11-9-22</u>	<u>NONE</u>	<u>MS</u>

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	<u>NATH JONES</u>	<u>MS</u>	<u>11-9-22</u>
Data sheets QA:			
Data entered:	<u>NATH JONES</u>	<u>MS</u>	<u>11-9-22</u>
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: 1686

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	11-2-22 MS	25.5 am 25.5 pm	25.0 am 25.0 pm	B	11-1-22	111	<input checked="" type="checkbox"/> Yes	1532 am 1532 pm	1019	1058	32924	918	N/A
Day 1	11-3-22 MS	24.9 am 25.4 pm	24.5 am 25.1 pm	B	11-2-22	96	<input checked="" type="checkbox"/> Yes	0747 am 1314 pm	0949	1050	32924	918	
Day 2	11-4-22 MS	25.2 am 26.1 pm	25.7 am 25.5 pm	B	11-3-22	103	<input checked="" type="checkbox"/> Yes	0805 am 1441 pm	1108	1208	32925	918	
Day 3	11-5-22 MS	25.1 am 25.4 pm	24.7 am 24.9 pm	B	11-4-22	65	<input checked="" type="checkbox"/> Yes	0852 am 1313 pm	1053	1138	32925	919	
Day 4	11-6-22 MS	25.7 am 25.7 pm	25.0 am 25.3 pm	B	11-5-22	84	<input checked="" type="checkbox"/> Yes	0753 am 1201 pm	0953	1039	32925	919	
Day 5	11-7-22 MS	25.5 am 25.4 pm	25.0 am 24.9 pm	B	11-6-22	102	<input checked="" type="checkbox"/> Yes	0800 am 1508 pm	1016	1113	32926	919	
Day 6	11-8-22 MS	25.3 am 25.3 pm	24.9 am 24.6 pm	B	11-7-22	109	<input checked="" type="checkbox"/> Yes	0800 am 1333 pm	1027	1110	32926	919	
Day 7	11-9-22 MS	25.2 am pm	24.6 am pm				<input type="checkbox"/> Yes	am pm	0939	1143			↓

Notes:

11-2-22
MS

Fathead Minnow Chronic Daily Survival Log

Sponsor: 412 Test site/chemical: OP200 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	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
			Date ^{MS} 11-3-22	Date ^{MS} 11-4-22	Date ^{MS} 11-5-22	Date ^{MS} 11-6-22	Date ^{MS} 11-7-22	Date ^{MS} 11-8-22	Date ^{MS} 11-9-22
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 SM	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 ^{SK}	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

11-2-22
MS

Random Assignment of Test Chambers

Project: <u>V12</u>						
Test site/chemical: <u>OF200</u>						
Test number: <u>1686</u>						
Starting position (on Table of Random Numbers): <u>16</u>						
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-DMW 25%	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-DMW 25%	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-DMW 25%	1	17
18	42	66	90	6-100%	3	18
19	43	67	91	5-75% 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-DMW 25%	2	24

Fathead Minnow Weight and Survival Data

Sponsor: <u>Y12</u>		Test number: <u>1686</u>		
Test site/chemical: <u>OF200</u>		Balance ID: <u>A009820</u>		
Test Start Date: <u>11-2-22</u>		Test End Date: <u>11-9-22</u>		
Start Drying Date/Time: <u>11-9-22e</u>		End Drying Date/Time: <u>11-10-22e</u>		
		<u>1143am</u> <u>0735</u>		
Treatment	Replicate	Pan Wt. (mg) Date: <u>11-8-22</u> Balance check: <input checked="" type="checkbox"/>	Pan + Larvae (mg) Date: <u>11-10-22</u> Balance check: <input checked="" type="checkbox"/>	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	21.5185	10
	3	15.0310	22.3120	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	15.1160	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	15.0260	21.8270	10
	4	15.0745	20.9440	10
5. 75%	1	15.0340	19.6350 *	10
	2	15.1435	21.4260	10
	3	15.0830	21.1510	10
	4	15.0855	20.8780	10
6. 100%	1	15.0915	21.6465	10
	2	15.0665	22.4190	10
	3	15.0550	21.3810	10
	4	15.0670	21.3545	10

Environmental Sciences Division

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

12-22
N8811-2-22
N88

Random Assignment of Larvae to Test Chambers

Project: <u>Y12</u>				Project: <u>ESD</u>			
Test site/chemical: <u>OF200</u>				Test site/chemical: <u>KCl Ref TOX</u>			
Test number: <u>1686</u>				Test number: <u>1687</u>			
Starting position (on Table of Random Numbers): <u>27</u>				Starting position (on Table of Random Numbers): <u>0</u>			
Assigned Numbers				Assigned Numbers			
Sample ID/Treatment				Sample ID/Treatment			
Replicate				Replicate			
1 25	49 73	1. DMW 25%	16 1 23	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		16 3 30	3 27	51 75		8 3 41
4 28	52 76		14 4 43	4 28	52 76		39 4 42
5 29	53 77	2. 125%	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 21	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%	11 1 43	9 33	57 81	3. 0.50g/L	8 1 11
10 34	58 82		14 2 32	10 34	58 82		16 2 46
11 35	59 83		9 3 25	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%	18 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 38	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		28 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 21
22 46	70 94		11 2 31	22 46	70 94		15 2 43
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 41

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	11686 + 11687	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	MS				→			
Comments (e.g. condition of larvae received): Fed 2.18 mL BS to each container @ 1510. MS 11/1/22 Fed 2.29 mL BS to each container @ 0821. MS 11/2/22								

Environmental Sciences Division

Rev. 02 2020-10-28

MS
11-2-22

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*).

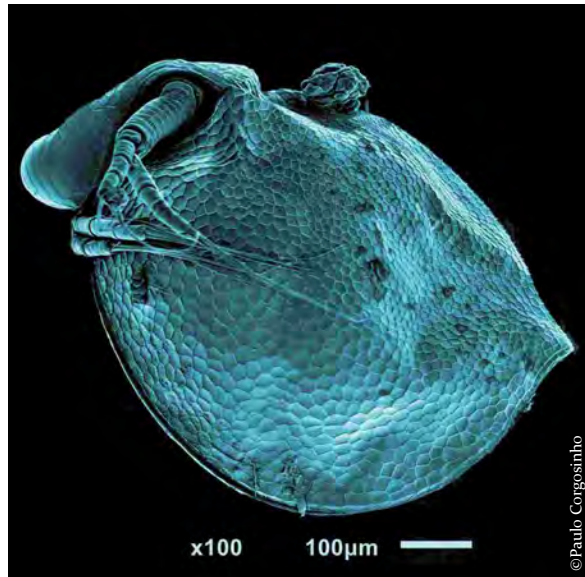
Effluent from Outfall 200 did not reduce fathead minnow survival or growth or *Ceriodaphnia* survival or reproduction by 25% or greater at any of the tested concentrations. For both species, the Inhibition Concentration₂₅ (IC₂₅) for survival, growth, and/or reproduction were thus >100% (the highest concentration of effluent tested).

The NPDES permit states that toxicity will be demonstrated if the 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 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 (IC₂₅) in survival, reproduction, or growth of the test organisms as compared to the controls.

The permit states that toxicity is demonstrated if the IC₂₅ 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 \pm 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 \pm 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 (\pm SD)
Control	10	10	38.7 \pm 4.5
12.5%	10	10	34.8 \pm 4
25%	10	9	32.2 \pm 5.4
50%	10	10	32.3 \pm 2.7
75%	10	10	29.8 \pm 7.2
100%	10	10	30.4 \pm 3

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.)	8.18	8.11	8.22
Conductivity (μ S/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 ($\mu\text{S}/\text{cm}$) 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: 3 February 2023

Report reviewed by: Louise Stevenson *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 \pm SD)

3.8 Diseases and treatment: None.

4. TEST METHODS

4.1 Toxicity test method: Fathead minnow larval survival and growth test. Reference: EPA Test Method 1000.0, in P.A. Lewis et al., *Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA/821/R/02/013 (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 IC₂₅ 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 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.)	8.18	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.

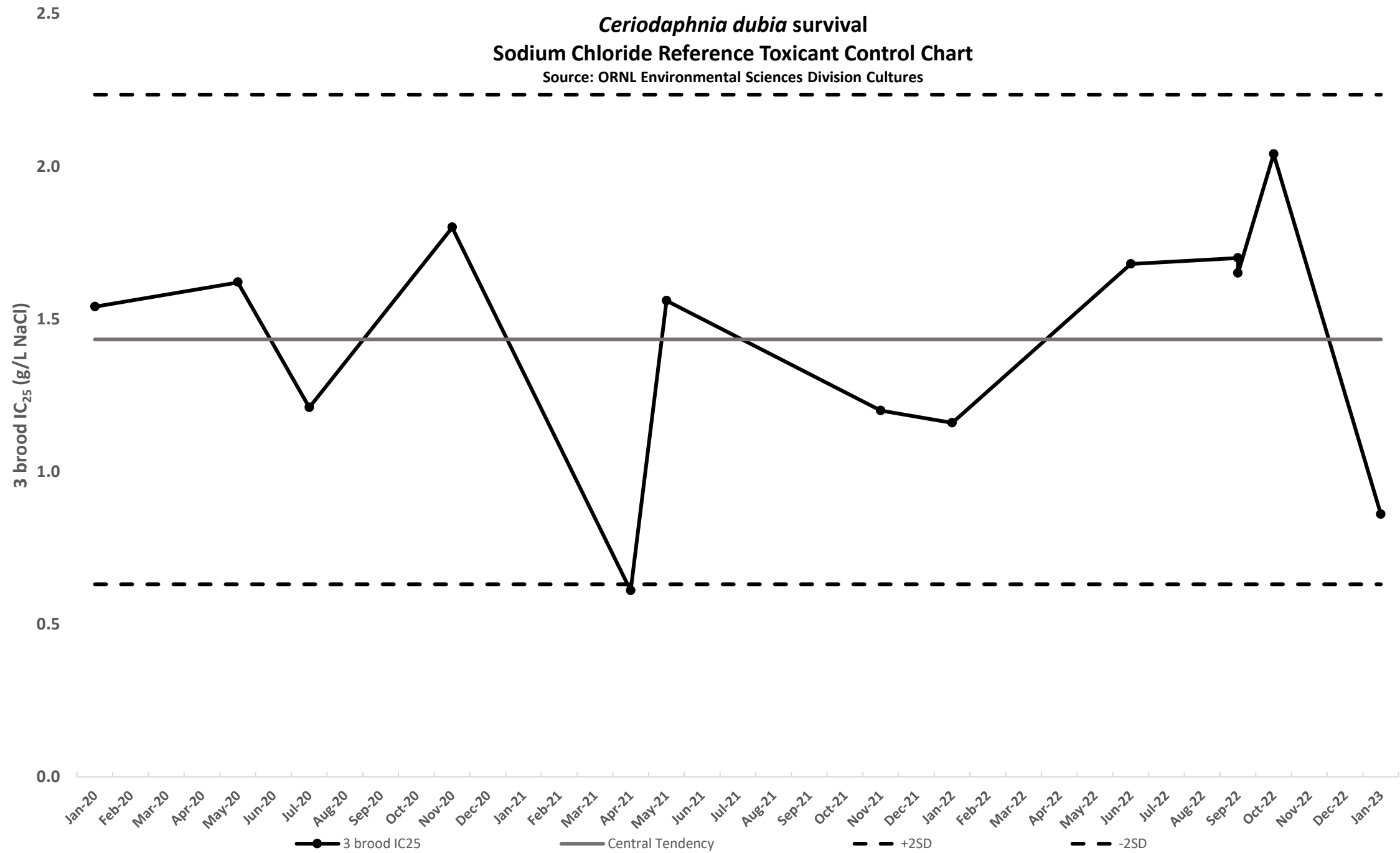
All measurements were made on fresh samples before daily water replacement. In addition, dissolved oxygen and pH were measured on water collected after daily replenishment period.

Report prepared by: Peijia Ku

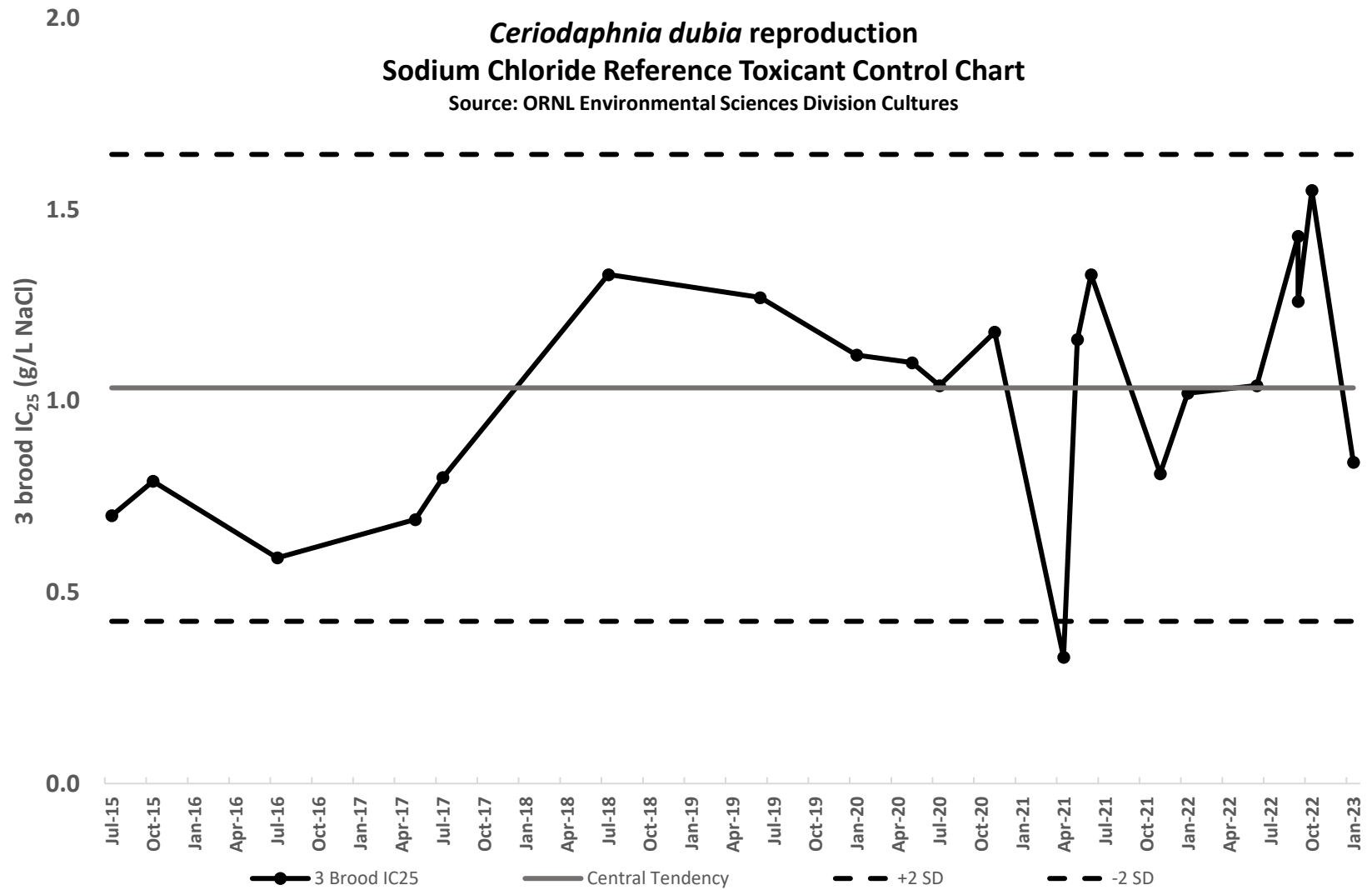
Date: 3 February 2023

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

REFERENCE TOXICANT CONTROL CHARTS

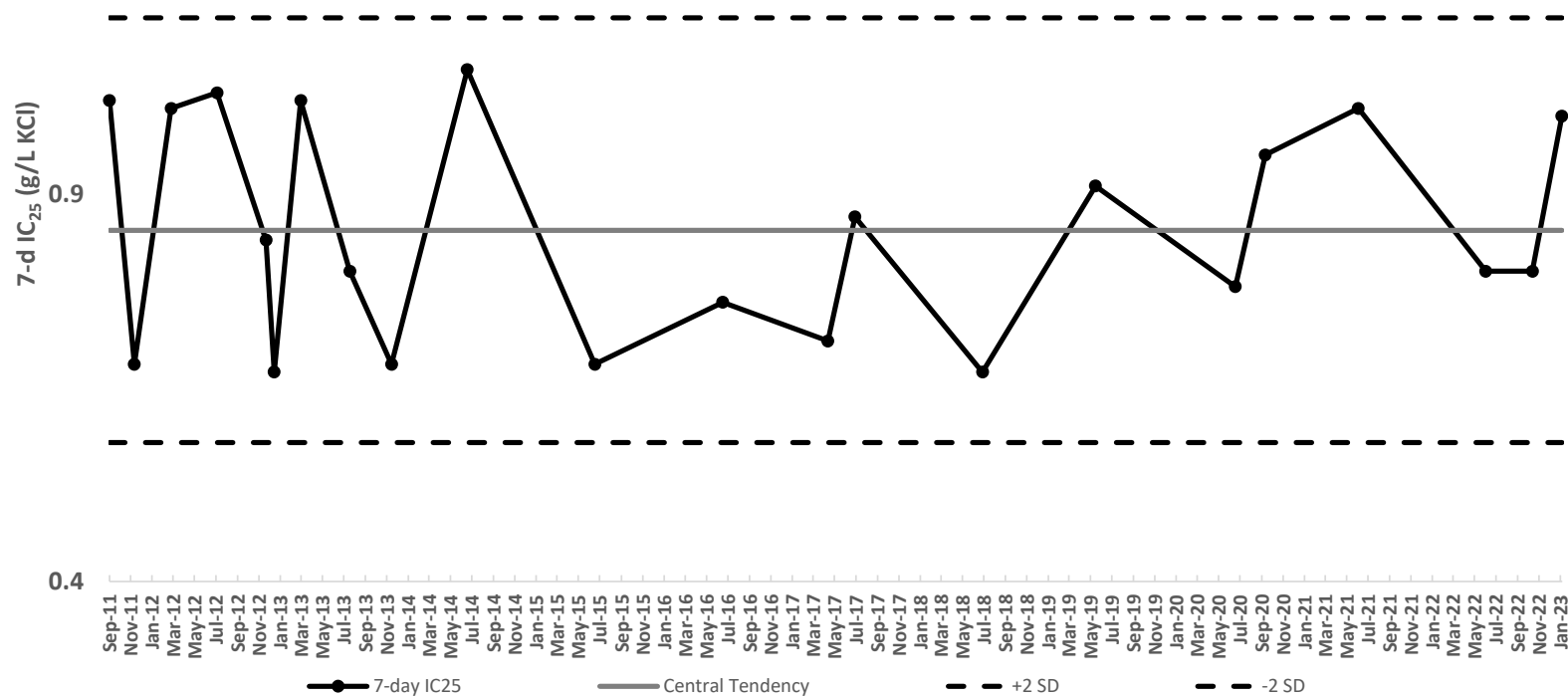


Ceriodaphnia dubia reproduction
Sodium Chloride Reference Toxicant Control Chart
Source: ORNL Environmental Sciences Division Cultures



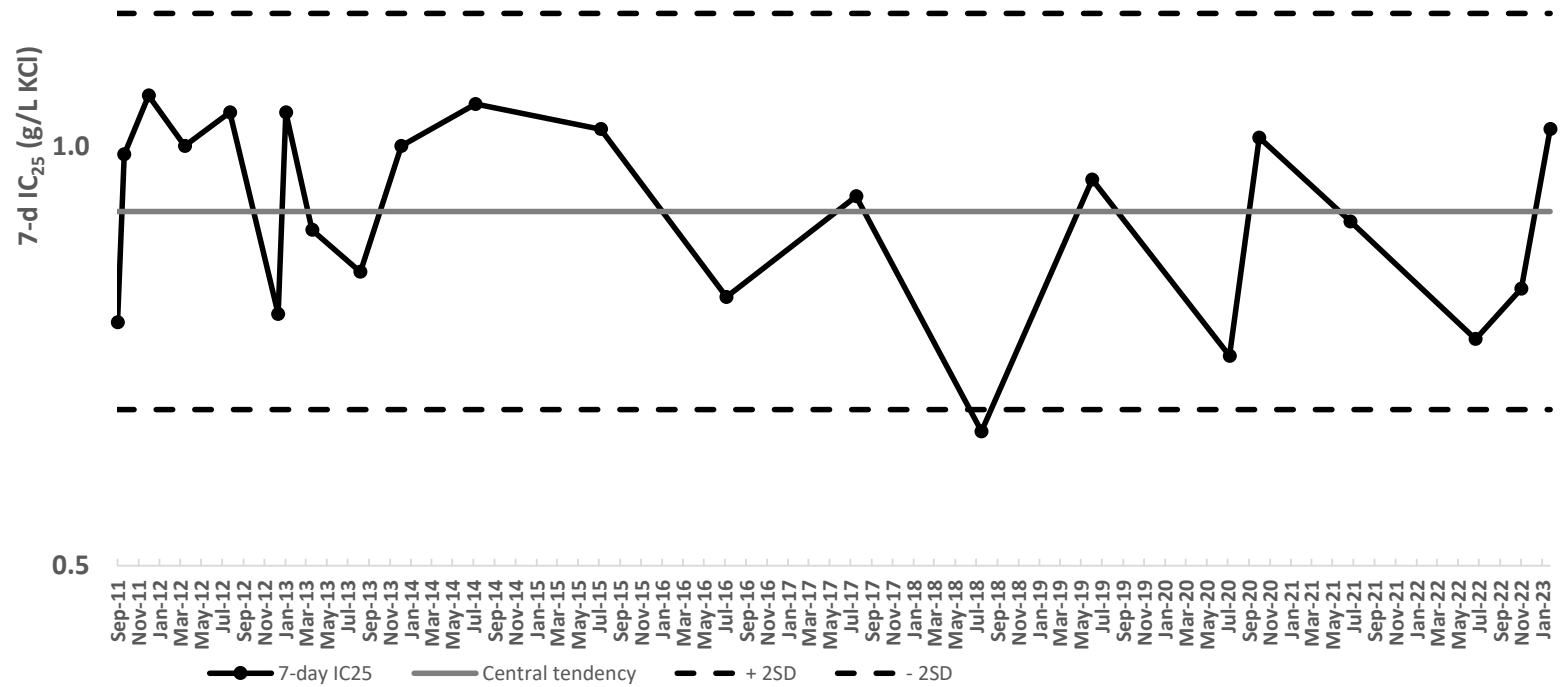
1.4

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

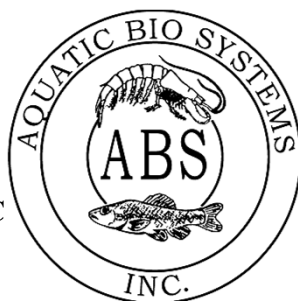


1.5

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

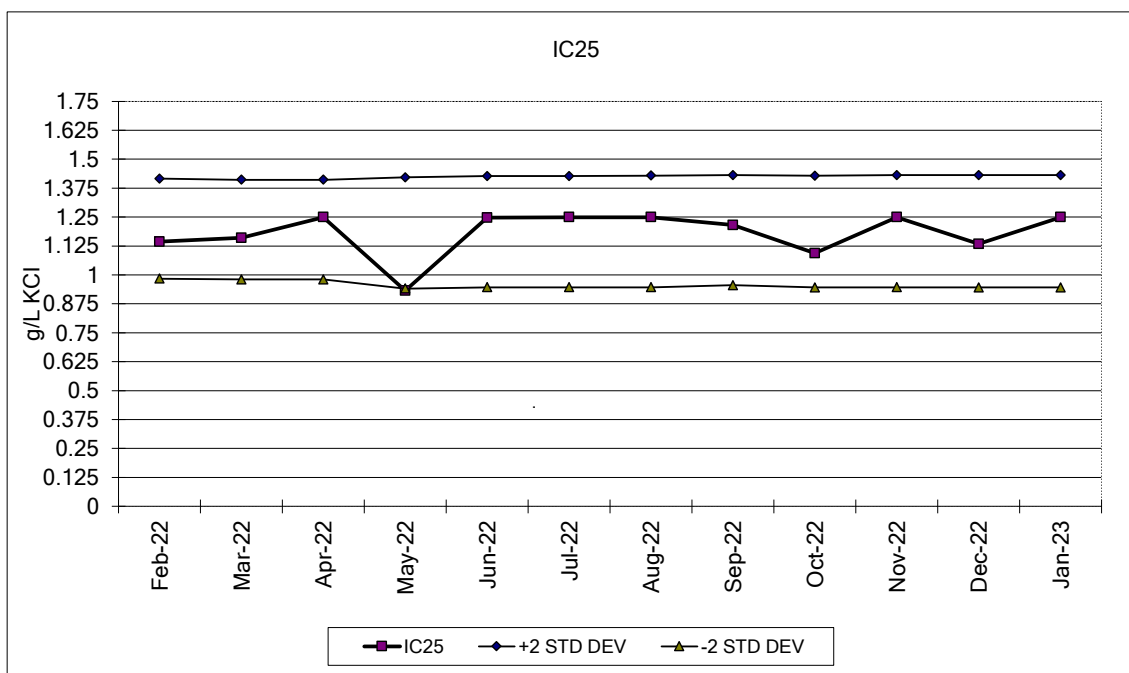


1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



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)
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		Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
		(upper)	(lower)			
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

SOR
SPENOR

Y12

Site/Treatment

OF-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 Date/Initials	0PK/NJ	1PK/NJ	2 PK/NJ	3PK/NJ	4PK/NJ	5PK/NJ	6PK/NJ	7PK/NJ
5-digit ORNL ID	33120	33120	33121	33121	33121	33122	33122	33123
Rec temp (°C) (New ✓)	✓	✓	✓	✓	✓	✓	✓	✓
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	8.131	8.185	8.101	8.114	8.152	8.116	8.122	
Final CD/FHM		8.53/8.08	8.41/8.02	8.49/8.02	8.45/8.02	8.47/8.12	8.42/8.04	8.81/8.30
DO (mg/L) Initial	8.62	8.73	8.75	8.81	8.72	8.71	8.71	
Final CD/FHM		9.08/7.36	8.87/7.00	8.97/7.18	8.83/7.52	8.72/7.51	8.82/7.33	8.57/7.76
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	8.154	8.09	8.120	8.172	8.210	
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	8.82/8.31
DO (mg/L) Initial	8.62	8.77	8.76	8.80	8.67	8.77	8.69	
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
Conductivity (µS/cm)	283	276	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	8.116	8.25	
Final CD/FHM		8.54/8.13	8.55/8.07	8.52/8.10	8.52/8.03	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	8.90	8.64	
Final CD/FHM		8.90/7.33	8.86/7.50	8.95/7.53	8.96/7.37	8.72/7.43	8.91/7.21	8.70/7.73
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	8.74	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	8.91/7.8	8.76/7.65
Conductivity (µS/cm)	367	367	424	425	424	331	333	
Alkalinity (mg/L)								
Hardness (mg/L)								
Chlorine (mg/L)								
pH (S U) Initial	8.102	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	8.67/8.14	8.55/8.22	8.51/8.00	8.87/8.31
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	8.96/7.6	8.81/7.67
Conductivity (µS/cm)	407	407	440	490	488	367	366	
Alkalinity (mg/L)	118		122			108		
Hardness (mg/L)	190		200			150		
Chlorine (mg/L) FHT	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	8.63/8.05	8.84/8.33
DO (mg/L) Initial	9.66	9.60	9.80	10.22	9.53	10.00	9.79	
Final CD/FHM		8.83/7.15	9.02/7.42	8.83/7.54	9.07/7.30	8.75/7.33	9.02/7.16	8.90/7.72

Environmental Sciences Division 4-25-23 OF200 water was very cloudy. NY

Rev 04 2021-02-05

Sponsor

Note: N

Rec. temp

DMW

Con

Alk

Har

pH

DO

Con

Alk

Har

Chl

pH

DO

Con

Alk

Har

Chl

pH

DO

Con

Alk

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Envir

CHAIN OF CUSTODY FORMS

[illegible]

SAMPLES RELINQUISHED BY

BY A. H. Garland

DATE _____

1-25-23

TIME

0852

5 AM

PM

SAMPLES RECEIVED BY	
1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
29	30
31	32
33	34
35	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

Beigta Ku

DATE _____

1-25-23

TIME

0853

☒ AM

PM

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY

CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 01-27-23		ESD TEST NAME TOX TEST		NAME OF SAMPLERS A. GARLAND / J. WILLIAMS			CHAIN-OF-CUSTODY NO. 031073	
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	FRIG TEMP (°C)	#7008 TEMP: REMARKS	#4102 C/2
OUTFALL #200	200	0725	C	1	~14 LITERS	3°	10.8	
<div style="transform: rotate(-30deg); font-size: 2em; opacity: 0.5;"> MOLES 012723 </div>								
ORNL Sample ID = 33121								

THERMOMETER NO.

SAMPLES RELINQUISHED BY

A. Garland

DATE

1/27/23

TIME

0806

☒ AM
☐ PM

SAMPLES RECEIVED BY

Mones

DATE

01-27-23

TIME

0806

☒ AM
☐ PM

[illegible]

SAMPLES RELINQUISHED BY

A. Garland

DATE 1/30/23

TIME	0828
------	------

☒ AM
☐ PM

SAMPLES RECEIVED BY	
1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
29	30
31	32
33	34
35	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

Reijia Km

DATE	1/30/23
------	---------

TIME	0828
------	------

☒ AM
☐ PM

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y12Site/Treatment: OF200Test number: **2979**

Test begin date (Day 0)

Test end date

Test duration

Template number

01/25/202302/01/20237 ☐ hours ☒ days☐ NA ☒ 1

Test

Organism:

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

Isolated from:

Notes:

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

Hatch date: _____

Time: 1020 1750

Delivery date: _____

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%	<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 MetalsBatch 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: 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:	AMF	AMF	2/2/2023
Data entered:	Peijia Ku	PK	02/01/2023
Data entry QA:	AMF	AMF	2/2/2023

Environmental Sciences Division

Rev. 02 2020-01-02

SIGNATURE _____
READ AND UNDERSTOOD _____DATE _____ 20
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		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	01/25/23 PK	25.6 am 14.35 pm	24.8 am 24.8 pm	YCT	12/06/22	100	<input checked="" type="checkbox"/> Yes	12:35 am	11:55	1315	33120 (PK) 031072 14005	928	N/A
Day 1	01/26/23 PK	25.7 am 25.7 pm	24.8 am 24.8 pm	RASU	01/24/23	100	<input checked="" type="checkbox"/> Yes	11:20 am	11:02	11:50	✓	928	
Day 2	01/27/23 PK	25.5 am 25.5 pm	25.0 am 25.0 pm	YCT	01/10/23	100	<input checked="" type="checkbox"/> Yes	12:20 am	11:51	12:40	33121	928	
Day 3	01/28/23 PK	25.5 am 25.5 pm	24.9 am 24.9 pm	YCT	01/10/23	100	<input checked="" type="checkbox"/> Yes	10:04 am	09:35	11:20	↓	929	
Day 4	01/29/23 PK	26.0 am 26.0 pm	24.7 am 24.7 pm	YCT	01/10/23	100	<input checked="" type="checkbox"/> Yes	10:02 am	09:31	10:56	↓	929	
Day 5	01/30/23 PK	25.6 am 25.6 pm	25.0 am 25.0 pm	YCT	01/10/23	100	<input checked="" type="checkbox"/> Yes	10:50 am	10:25	11:38	33122	929	
Day 6	01/31/23 PK	25.5 am 25.5 pm	25.0 am 25.0 pm	YCT	01/10/23	100	<input checked="" type="checkbox"/> Yes	10:55 am	10:30	12:10	↓	930	
Day 7	02/01/23 PK	25.7 am 25.7 pm	25.0 am 25.0 pm	YCT			<input type="checkbox"/> Yes		11:40	13:10			N/A

Notes:

Environmental Sciences Division

Rev. 03 2020-06-05

01/24/23 PK

SIGNATURE
READ AND UNDERSTOOD

DATE

20

PROJECT NAME

10

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

 PK
 01/24/23

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

Toxicity Test Information Sheet

Sponsor: Y12Site/Treatment: OF200Test number: **1688**

Test begin date (Day 0)

01-25-23

Test end date

02-01-23

Test duration

7☐ hours☒ days

Template number

☒ NA ☐

Test Organism:

☐ *Ceriodaphnia dubia*

Isolated from:

Date:

Time:

☒ Fathead minnow☐ Other:

Notes:

Hatch date: 01-23-23Delivery date: 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%	<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 =	15%	<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 MetalsBatch number: 928-930

Source of Test Organisms:

☐ ESD cultures: Board numbers: ☐ NA ☐☒ Vendor: APS☐ Other (describe):

Water delivery dates:

☐ Not applicableSample ID: 33120Date: 01-25-23COC #: 031072Sample ID: 33121Date: 01-27-23COC #: 031073Sample ID: 33122Date: 01-30-23COC #: 031074MSX
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.	MSX
01-26-23	Trt 5, replicate 4 beaker overturned + was lost.	MSX

Quality Assurance (QA) Record

Procedure	Name	Initial	Date
Test run by:	Nikki Jones	MSX	02-01-23
Data sheets QA:	MSX	MSX	2/3/23
Data entered:	Nikki Jones	MSX	02-01-23
Data entry QA:	MSX	MSX	2/3/23

CHRONIC Daily Water/Feeding Log

Sponsor: U12 Test site/treatment: DF200 Begin Date: 01-25-23 End Date: 02-01-23 Test Number: 1688

Daily Test Info		Temperature Information		Feeding Information					Test Initiation, Water Change, or Test Termination				Sample Info
		Therm. #: <u>DD19</u>		(Food codes: YCT = yeast-cerophyl-trout, R = <i>Raphidocelis</i> , B = Brine shrimp) Acceptable algal cell density range = $3.0 - 3.5 \times 10^7$ /mL									
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 M8	— am 26.4 pm	— am 25.9 pm	—	—	—	<input checked="" type="checkbox"/> Yes	— am 1516 pm	1108	1129	33120	928	N/A
Day 1	01-26-23 M8	26.2 am 26.2 pm	25.5 am 25.6 pm	B	01-25-23	59	<input checked="" type="checkbox"/> Yes	0830 am 1607 pm	1031	1136	33120	928	
Day 2	01-27-23 M8	24.1 am 25.3 pm	23.8 am 24.8 pm	B	01-26-23	67	<input checked="" type="checkbox"/> Yes	0837 am 1513 pm	1114	1212	33121	928	
Day 3	01-28-23 M8	25.6 am 25.7 pm	25.2 am 25.4 pm	B	01-27-23	98	<input checked="" type="checkbox"/> Yes	0744 am 1222 pm	0954	1038	33121	929	
Day 4	01-29-23 M8	25.8 am 25.8 pm	25.4 am 25.4 pm	B	01-28-23	083	<input checked="" type="checkbox"/> Yes	0730 am 1130 pm	0930	1009	33121	929	
Day 5	01-30-23 M8	26.0 am 26.2 pm	25.6 am 25.8 pm	B	01-29-23	100	<input checked="" type="checkbox"/> Yes	0816 am 1310 pm	1047	1138	33122	929	
Day 6	01-31-23 M8	26.1 am 24.8 pm	25.7 am 24.9 pm	B	01-30-23	72	<input checked="" type="checkbox"/> Yes	0827 am 1441 pm	1031	1116	33122	930	
Day 7	02-01-23 M8	24.1 am — pm	23.9 am — pm				<input type="checkbox"/> Yes	— am — pm	1055	1139			↓

Notes:

Environmental Sciences Division

Rev. 03 2020-06-05

M8
01-25-23

Fathead Minnow Chronic Daily Survival Log

Sponsor: Y12

Test site/chemical: DF200

Test Number: 1488

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	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
			Date	Date	Date	Date	Date	Date	Date
			01-26-23	01-27-23	01-28-23	01-29-23	01-30-23	01-31-23	02-01-23
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	10	10	10	10	10	10	10
	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	10	10	10	10	10	10	10
6: 100%	1	3	10	10	10	10	10	10	10
	2	12	10	10	10	10	10	10	10
	3	19	10	10	10	10	10	10	10
	4	17	10	10	10	10	10	10	10

Random Assignment of Test Chambers

Project: <u>412</u>						
Test site/chemical: <u>DF200</u>						
Test number: <u>1688</u>						
Starting position (on Table of Random Numbers): <u>7</u>						
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	10-100%	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	10-100%	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	10-100%	4	17
18	42	66	90	2-12.5%	4	18
19	43	67	91	10-100%	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
NPT

Fathead Minnow Weight and Survival Data

Sponsor: <u>U12</u>		Test number: <u>1688</u>		
Test site/chemical: <u>OF100</u>		Balance ID: <u>A009810</u>		
Test Start Date: <u>01-25-23</u>		Test End Date: <u>02-01-23</u>		
Start Drying Date/Time: <u>02-01-23 @ 1:39</u>		End Drying Date/time: <u>02-02-23 @ 10:17</u>		
Treatment	Replicate	Pan Wt. (mg) Date: <u>02-01-23</u> Balance check: <input checked="" type="checkbox"/>	Pan + Larvae (mg) Date: <u>02-02-23</u> Balance check: <input checked="" type="checkbox"/>	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	20.3665	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. 7.5%	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	15.1465	20.7540	10
	2	15.1295	21.0865	10
	3	15.0595	20.8555	10
	4	15.1510	20.5655	10
5. 7.5%	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

Environmental Sciences Division

Rev. 03 2020-10-28

01-25-23
MS01-25-23
MS

Random Assignment of Larvae to Test Chambers

Project: <u>Y12</u>				Project: <u>ESD</u>			
Test site/chemical: <u>OF100</u>				Test site/chemical: <u>LCI Ref Tox</u>			
Test number: <u>1688</u>				Test number: <u>1689</u>			
Starting position (on Table of Random Numbers): <u>1</u>				Starting position (on Table of Random Numbers): <u>19</u>			
Assigned Numbers				Assigned Numbers			
Sample ID/Treatment				Sample ID/Treatment			
Replicate				Replicate			
1	25	49	73	1.	DMW	25%	1
2	26	50	74	2	26	50	74
3	27	51	75	3	27	51	75
4	28	52	76	4	28	52	76
5	29	53	77	5	29	53	77
6	30	54	78	6	30	54	78
7	31	55	79	7	31	55	79
8	32	56	80	8	32	56	80
9	33	57	81	9	33	57	81
10	34	58	82	10	34	58	82
11	35	59	83	11	35	59	83
12	36	60	84	12	36	60	84
13	37	61	85	13	37	61	85
14	38	62	86	14	38	62	86
15	39	63	87	15	39	63	87
16	40	64	88	16	40	64	88
17	41	65	89	17	41	65	89
18	42	66	90	18	42	66	90
19	43	67	91	19	43	67	91
20	44	68	92	20	44	68	92
21	45	69	93	21	45	69	93
22	46	70	94	22	46	70	94
23	47	71	95	23	47	71	95
24	48	72	96	24	48	72	96

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	660	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								

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).

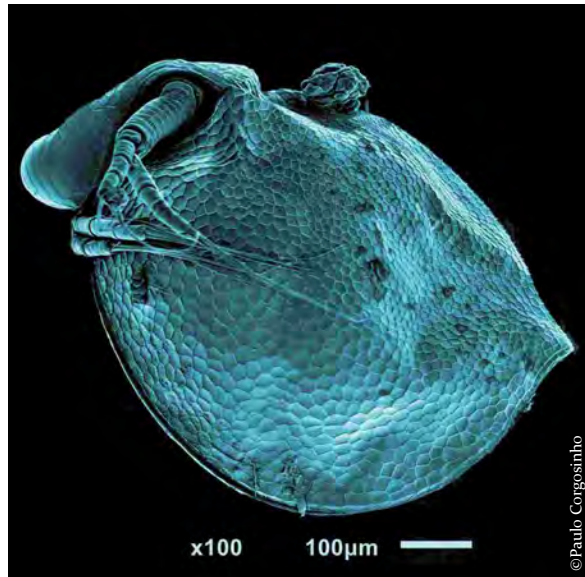
RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted May 3-10, 2023**

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

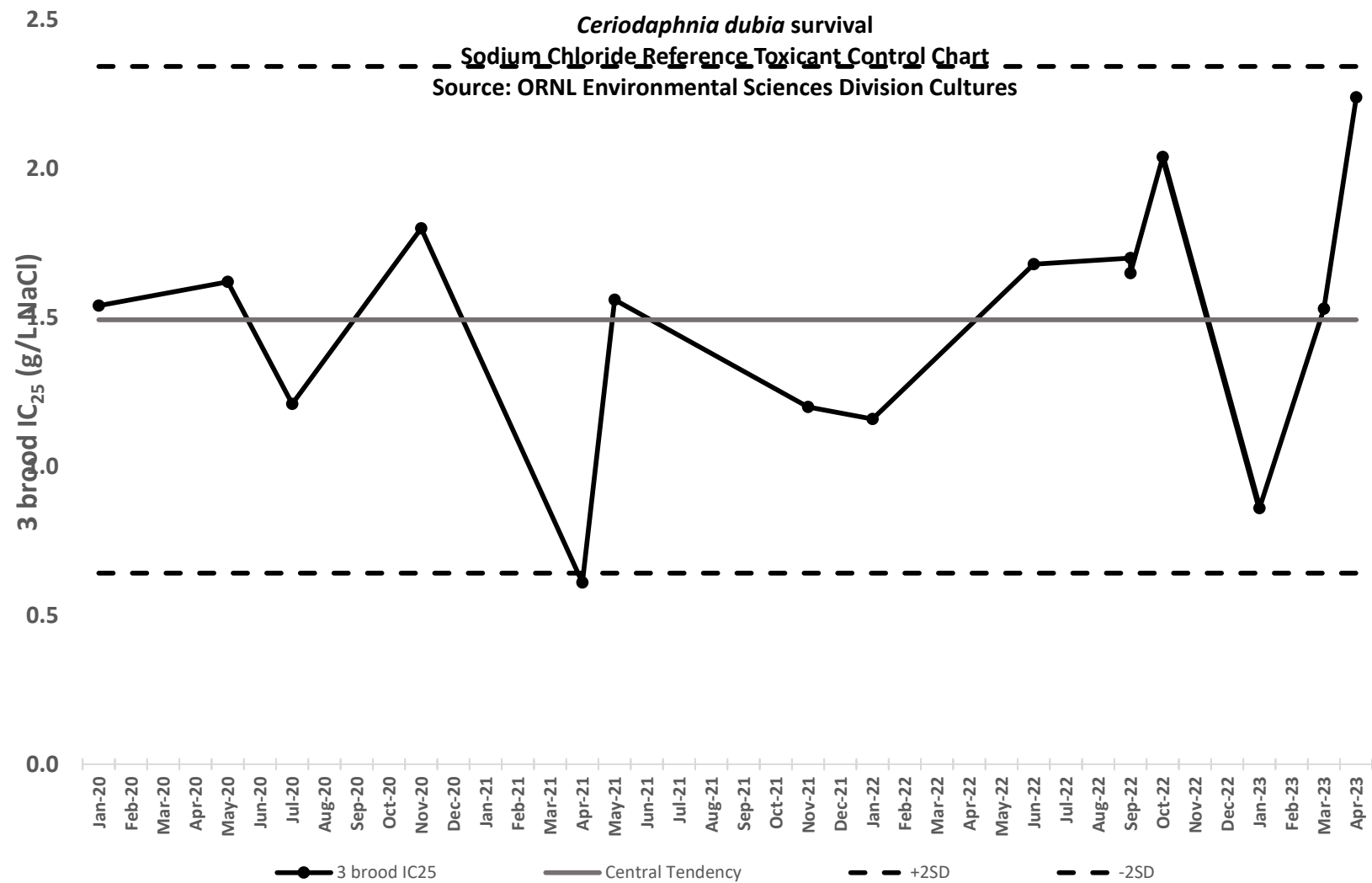
Date: 18 May 2023

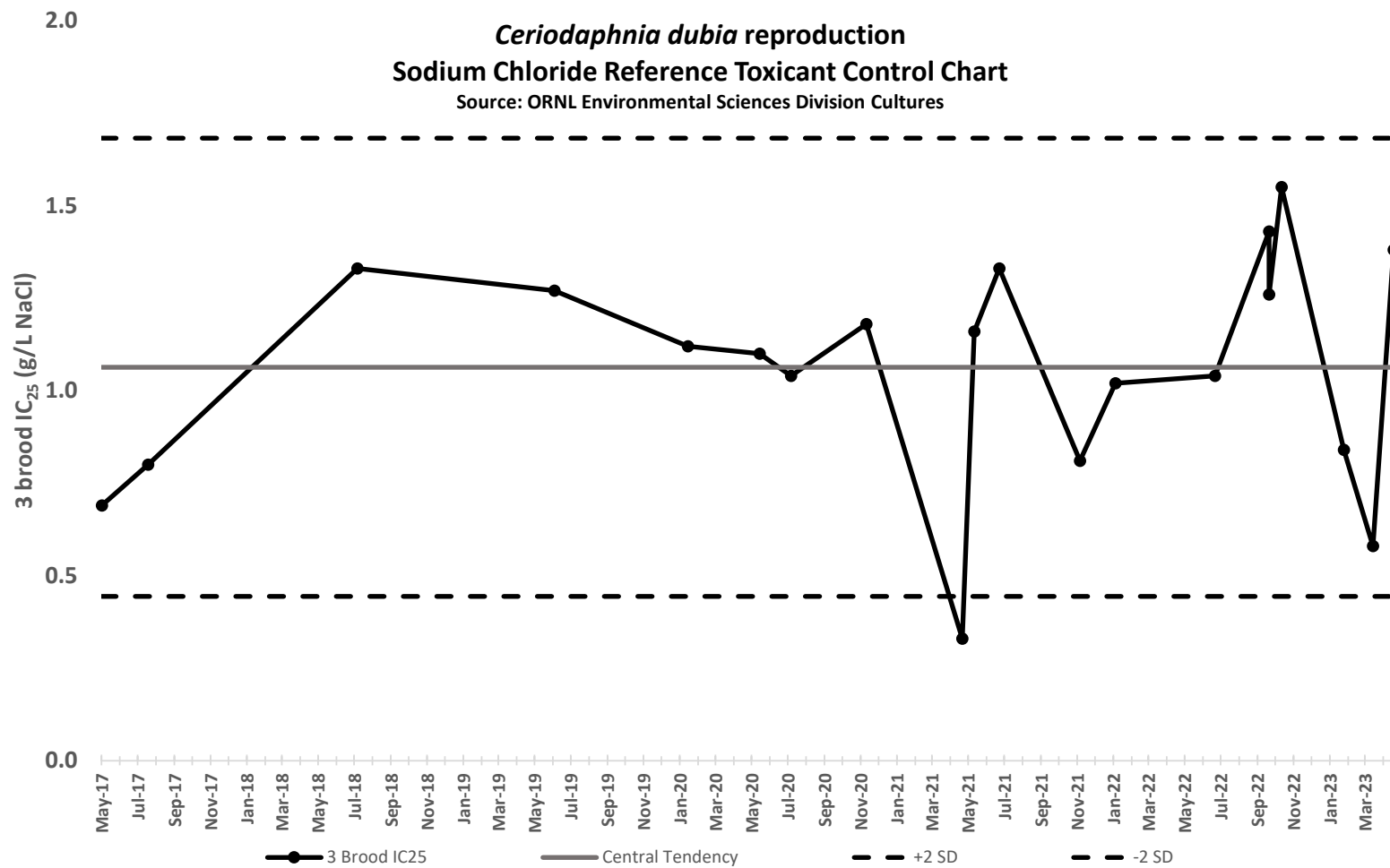
Report reviewed by: Louise Stevenson

Date: 22 May 2023



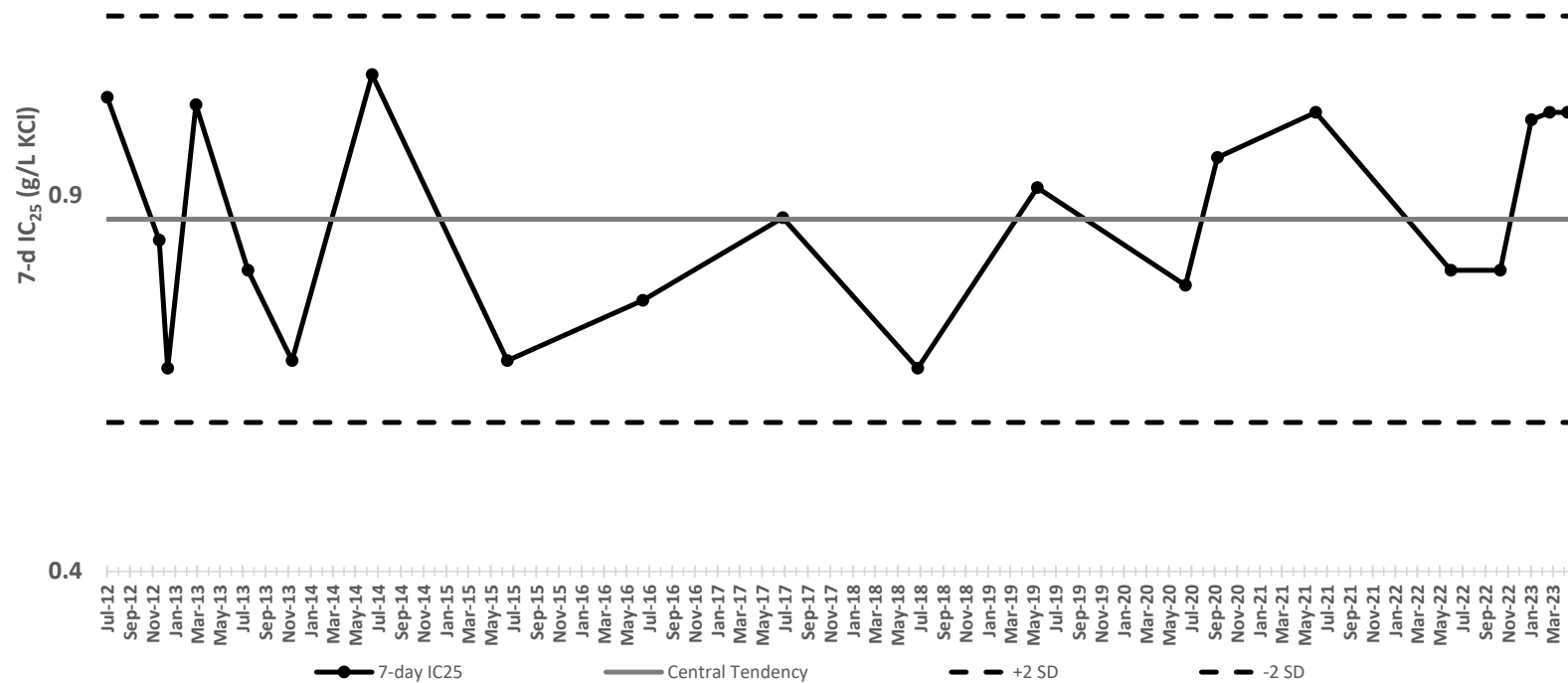
REFERENCE TOXICANT CONTROL CHARTS





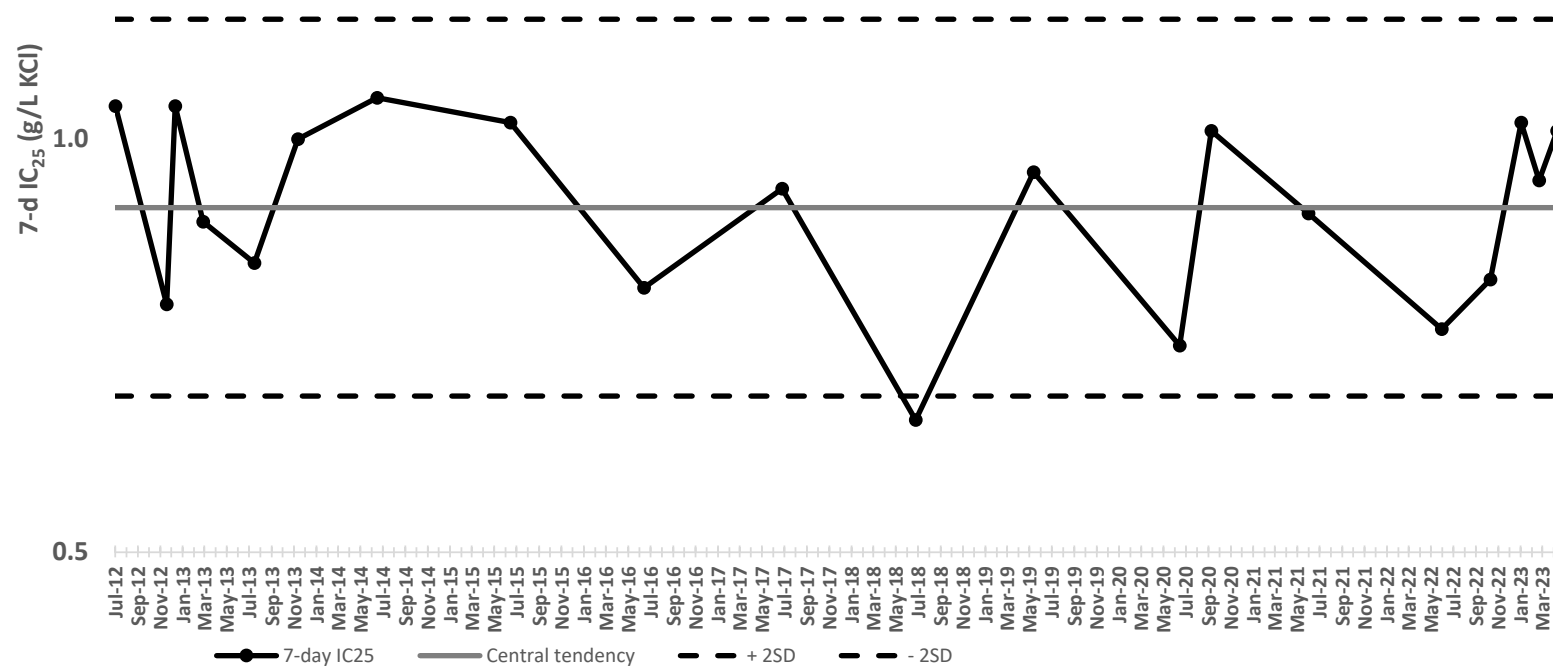
1.4

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

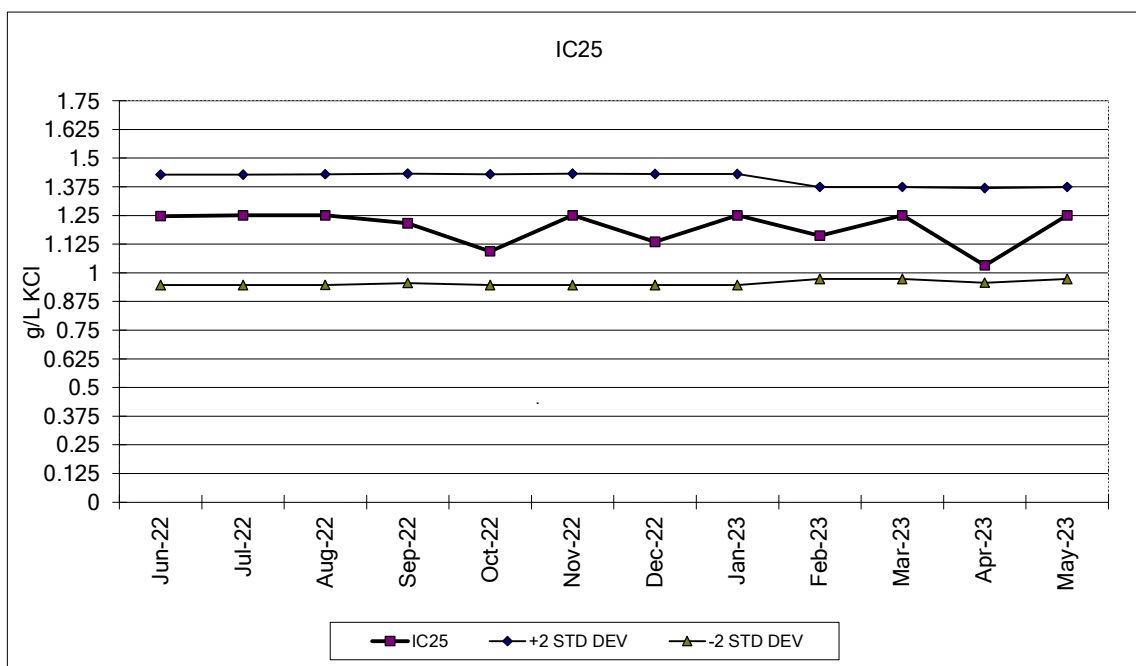


1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



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 ✓)	selected	selected	selected			selected		
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) FIT	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. S. Garland

DATE

5/3/23

[illegible]

1308

AM

☒ PM

SAMPLES RECEIVED BY	
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100	100

Peigia Ku

DATE

5/3/23

TIME

1308

☐ AM

☒ PM

ENVIRONMENTAL SCIENCES DIVISION TOXICOLOGY LABORATORY

CHAIN-OF-CUSTODY

DATE (MM/DD/YY) 05/05/23		ESD TEST NAME TOX TEST		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)	#17009 TEMP	REMARKS #4102 C ₁									
TOX TEST	200	0710	C	1	~17 LITERS	4°	11.6°	<0.05									
<div style="transform: rotate(-45deg); font-size: 2em; opacity: 0.5;"> A.S. 5/5/23 </div>																	

THERMOMETER NO.

SAMPLES RELINQUISHED BY A.S. Garland		DATE 5/5/23	TIME 0802	<input checked="" type="checkbox"/> AM <input type="checkbox"/> PM
SAMPLES RECEIVED BY Peijia Ku		DATE 5/5/23	TIME 0802	<input checked="" type="checkbox"/> AM <input type="checkbox"/> PM

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

[illegible]

THERMOMETER NO.

SAMPLES RELINQUISHED BY

A. L. Garland

DATE

5/8/23

TIME

0823

☒ AM
☐ PM

SAMPLES RECEIVED BY	
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85	86
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Peijia Ku

DATE _____

05/08/23

TIME

0823

☒ AM
☐ PM

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y12Site/Treatment: OF200

Test number:

2985

PK- Test begin date (Day 0)

05/04/2305/03/2023

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:

☒ ESD 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
		Therm. #: <u>PD20</u>		(Food codes: YCT = yeast-cerophyl-trout, R = <i>Raphidocelis</i> , B = Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x 10 ⁷ /mL									
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 3.5E7	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	am 25.7 pm	am 25.3 pm	YCT R	3/21/23 5/3/23	100 91	✓ Yes 3.28E+07	am pm	1610	1700	↓	947	
Day 4	05/07/23 PK	am 25.7 pm	am 25.5 pm	YCT R	3/21/23 5/3/23	100 92	✓ Yes 3.25E+07	am pm	1450	1800	↓	950	
Day 5	05/08/23 PK	am 25.8 pm	am 25.3 pm	YCT R	3/21/23 5/3/23	100 92	✓ Yes 3.25E+07	am pm	1515	1635	33288	950	
Day 6	05/09/23 PK	am 25.6 pm	am 25.3 pm	YCT R	3/21/23 5/3/23	100 90	✓ Yes 3.34E+07	am pm	1504	1630	↓	950	
Day 7	05/10/23 PK	am 25.3 pm	am 25.0 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: Y12 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

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 Therm. #: <u>DD19</u>		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, S = Selenestrum, B = Brine shrimp) Acceptable cell density range = 3.0 to 3.5 x 10 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 count	Feed Time	Start Time	End Time	Sample ID	Control Water Batch Number	Analyte
Day 0	05/03/23 PK4/NNJ	26.4 am 26.4 pm	25.3 am 25.3 pm	B	05/02/23	195	<input type="checkbox"/> NA <input checked="" type="checkbox"/> Yes	1752 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	05/04/23	79	<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	05/04/23	109	<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	05/05/23	78	<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	05/06/23	91	<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	05/07/23	80	<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	05/08/23	91	<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	210 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 SK	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	22.3345	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 ; 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	33,289 ✓
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 ✓

Fathead Minnow Order & Shipment Log

pk
05/02/23

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):

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).

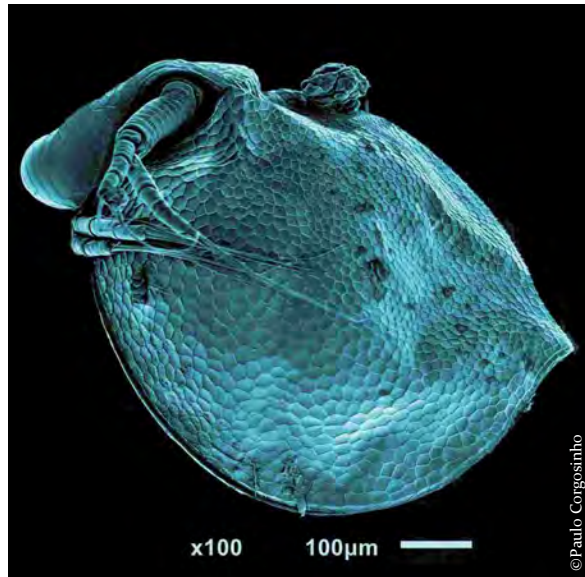
RE: **Toxicity Tests of Effluent from the Y-12 National Security Complex Outfall 200 Conducted August 9-16, 2023**

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 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 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 10⁶ 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 10⁷ 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 IC₂₅ = 2.34 g NaCl/L; 95% C.L. = 1.98-2.47 g NaCl/L.
Reproduction IC₂₅ = 1.49 g NaCl/L; 95% C.L. = 1.34-1.63 g NaCl/L.
The IC₂₅s 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 IC₂₅ for survival: 1.55 ± 0.925 g NaCl/L (mean ± 2 SD).

CV of IC₂₅ for survival: 0.299 g NaCl/L

Central tendency of IC₂₅ for reproduction: 1.10 ± 0.621 g NaCl/L (mean ± 2 SD).

CV of IC₂₅ 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 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.)	8.14	8.03	8.15
Conductivity ($\mu\text{S}/\text{cm}$)	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 ($\mu\text{S}/\text{cm}$) 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 \pm 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 IC₂₅ = 1.04 g KCl/L; 95% C.I. = 0.80 – 1.09 g KCl/L.
Growth IC₂₅ = 1.03 g KCl/L; 95% C.I. = 0.65 – 1.08 g KCl/L.
The IC₂₅s 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 IC₂₅ for survival: 0.868 ± 0.275 g KCl/L (mean ± 2 SD).
CV of IC₂₅ for survival: 0.158 g KCl/L
Central tendency of IC₂₅ for growth: 0.916 ± 0.227 g KCl/L (mean ± 2 SD).

CV of IC₂₅ 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 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.)	8.14	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.

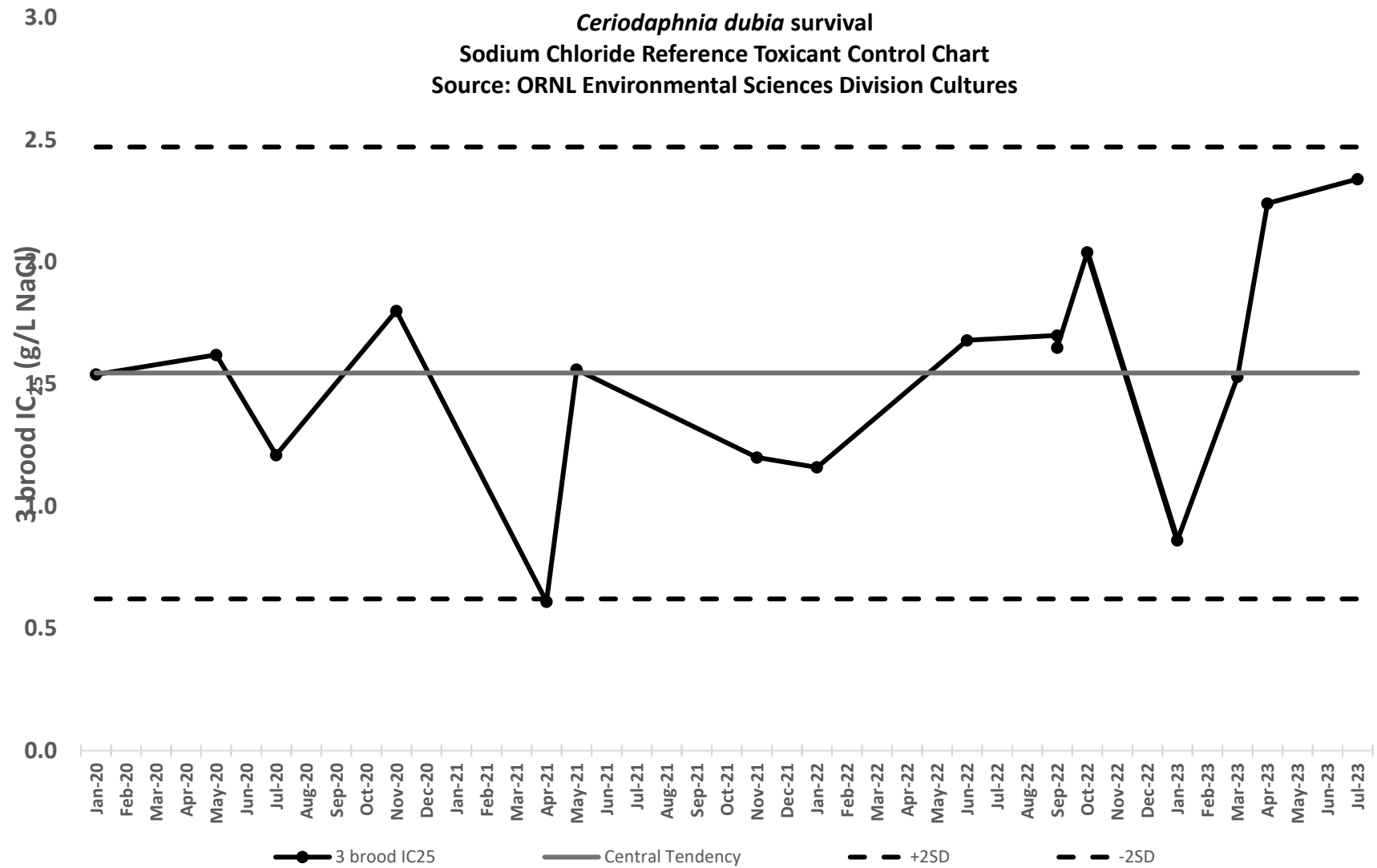
Report prepared by: Trystan A. Bordeaux

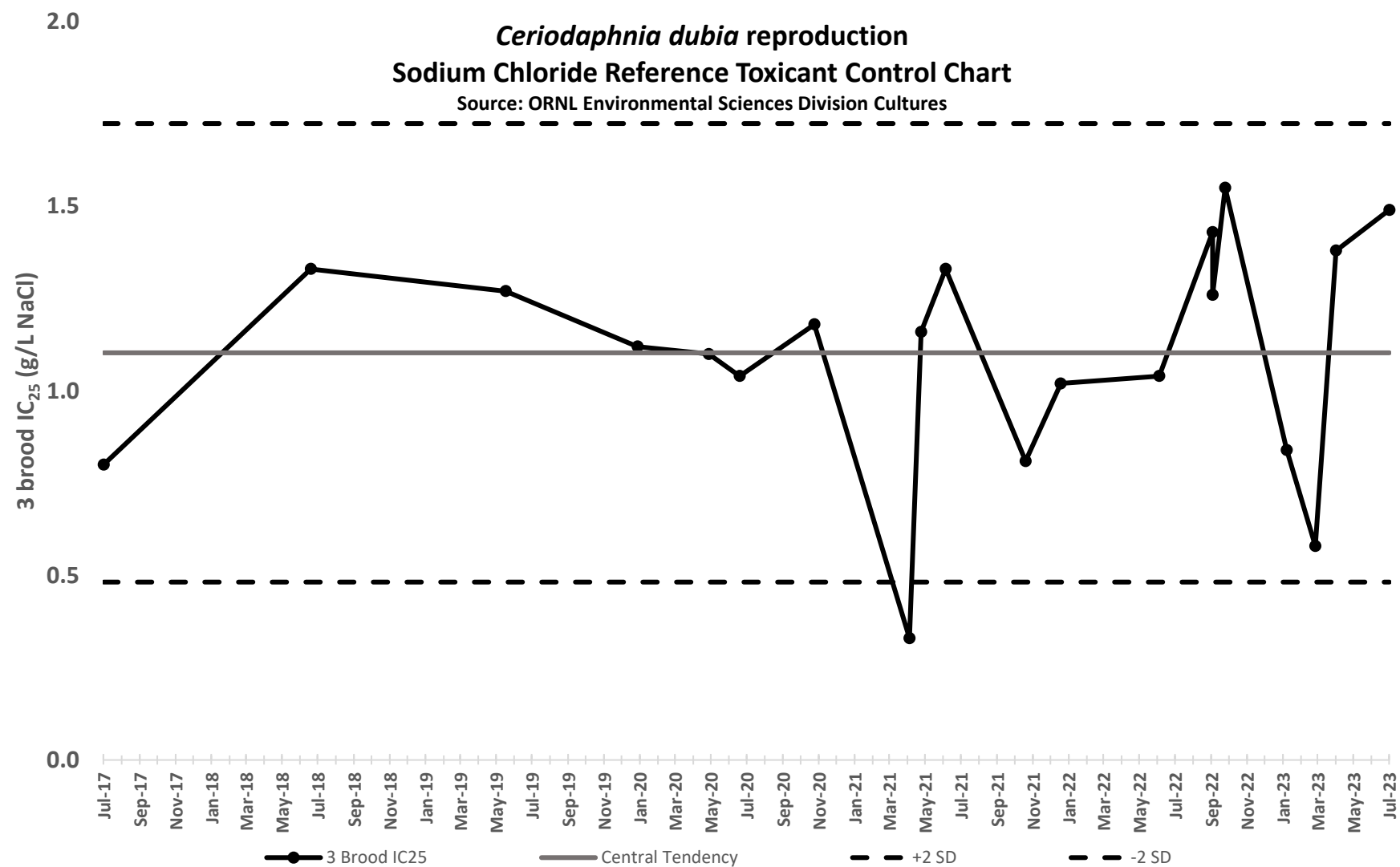
Date: 17 August 2023

Report reviewed by: Louise Stevenson *Louise Stevenson*

Date: 28 August 2023

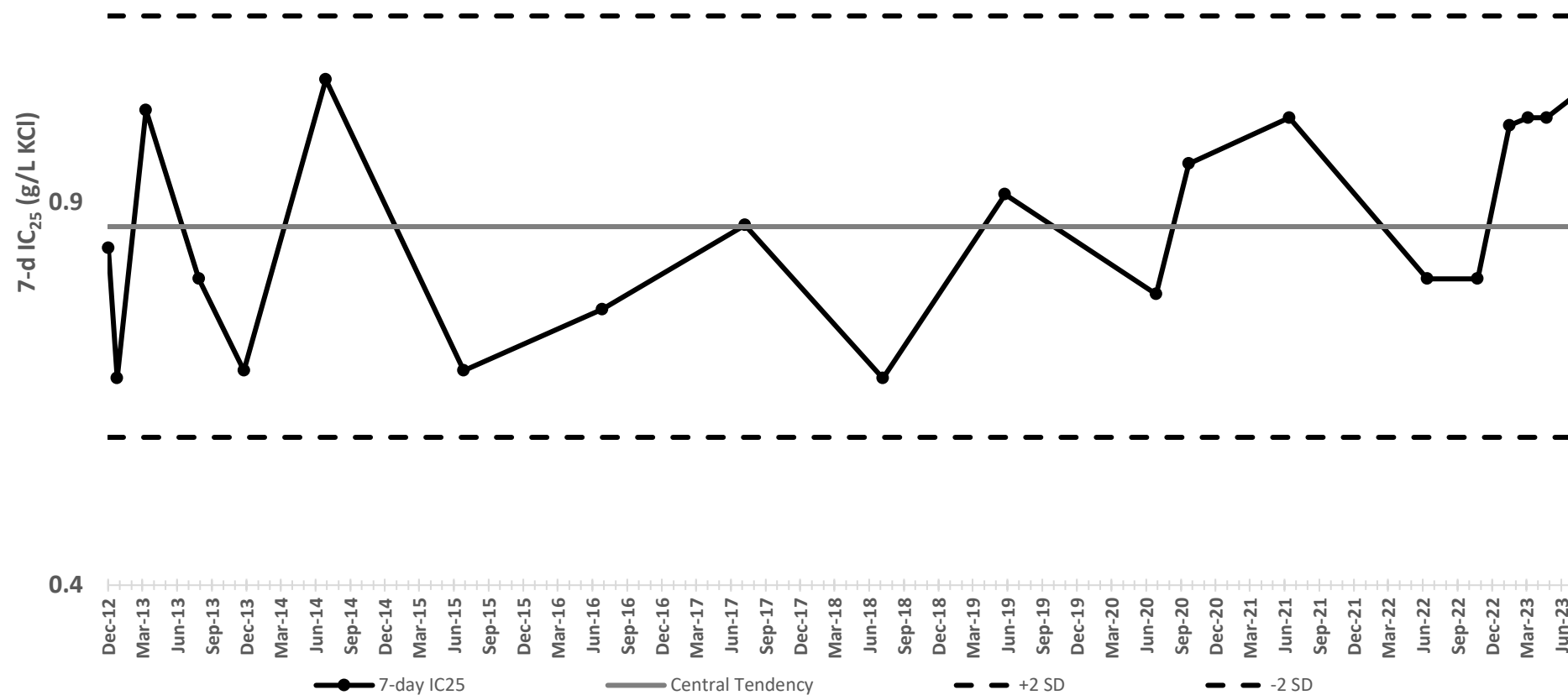
REFERENCE TOXICANT CONTROL CHARTS





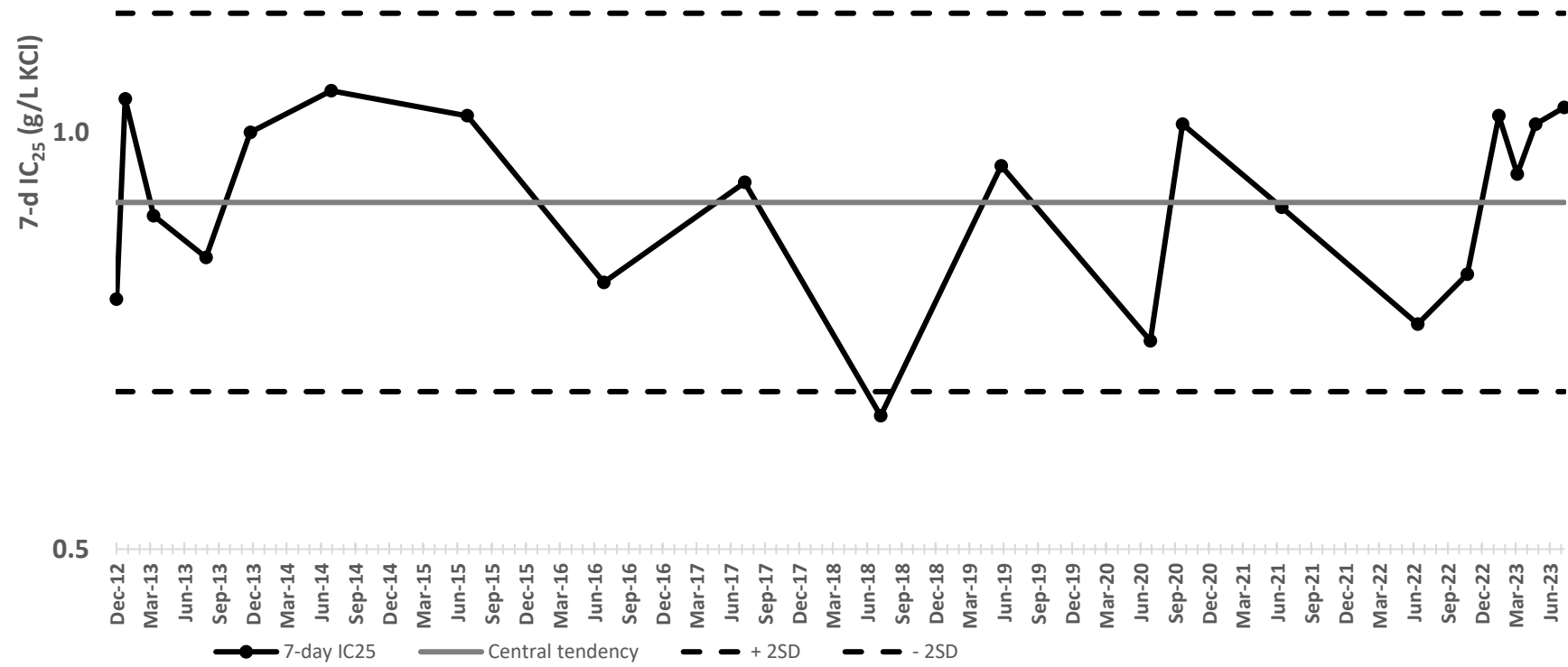
1.4

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

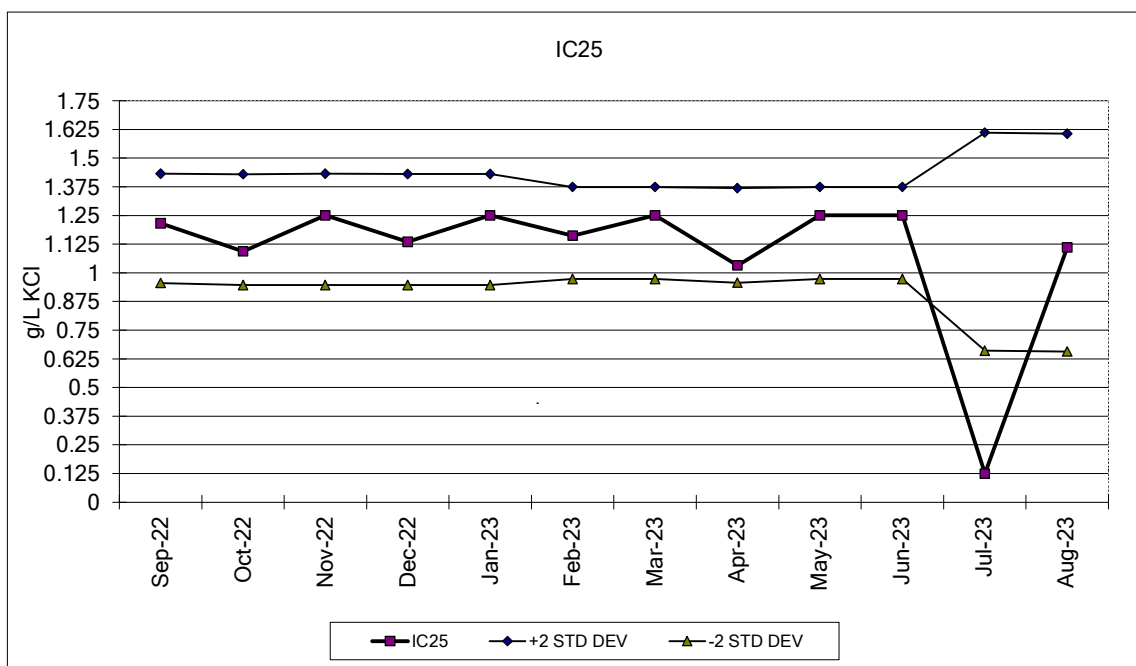


1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



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)
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		Avg. IC25 g/L KCl	+2 STD DEV	-2 STD DEV
		(upper)	(lower)			
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

05/08/23
TPO

Daily Water Chemistry Log

Sponsor: Y-12

Site/Treatment: OF-200

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 PM/17B	1 PM/17B	2 PM/17B	3 PM/17B	4 PM/17B	5 PM/17B	6 PM/17B	7 PM/17B
Date/Initials:		08/09/23	08/10/23	08/11/23	08/12/23	08/12/23	08/14/23	08/15/23	08/16/23
5-digit ORNL ID		33462	33463	33463	33463	33463	33464	33464	33464
Rec. temp. (°C) (New ✓)		12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Control: 25% DMW	DMW Batch #	970	970	970	970	970	971	971	971
	Conductivity (µS/cm)	242	231	230	211	201	214	225	243
	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	8.43	8.38	8.35
	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
Control: 6.25%	DO (mg/L) Initial	8.43	8.42	8.40	8.34	8.30	8.38	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	8.47/7.25
	Conductivity (µS/cm)	266	235	235	234	232	232	245	
	Alkalinity (mg/L)								
	Hardness (mg/L)								
	Chlorine (mg/L)								
Control: 12.5%	pH (S.U.) Initial	8.20	8.10	8.03	7.99	8.03	8.09	8.31	
	Final CD/FHM		8.36/7.94	8.34/7.90	8.27/7.94	8.44/7.91	8.19/7.88	8.39/7.83	8.37/7.92
	DO (mg/L) Initial	8.45	8.34	8.37	8.41	8.25	8.46	8.34	
	Final CD/FHM		8.53/6.78	8.53/7.02	8.50/7.40	8.65/6.94	8.28/7.08	8.28/7.27	8.48/7.11
	Conductivity (µS/cm)	239	279	280	244	252	262	260	
	Alkalinity (mg/L)								
Control: 25%	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	8.36/7.95
	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	8.36/6.75	8.44/6.51
Control: 50%	Conductivity (µS/cm)	339	330	329	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
Control: 100%	DO (mg/L) Initial	8.43	8.71	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	8.71/7.12	8.57/7.04	8.31/6.72	8.47/6.58
	Conductivity (µS/cm)	434	431	341	340	337	369	363	
	Alkalinity (mg/L)								
	Hardness (mg/L)								
	Chlorine (mg/L)								
Control: 100%	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	8.61/7.90	8.45/7.95
	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	8.41/7.36	8.58/6.60	8.53/7.01	8.36/6.58	8.46/6.51
	Conductivity (µS/cm)	601	624	463	465	466	498	497	
	Alkalinity (mg/L)	168		138			162		
Control: 100%	Hardness (mg/L)	260		230			200		
	Chlorine (mg/L) F/T	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	8.39/8.10	8.41/7.98	8.40/8.02	8.42/8.04	8.46/8.10
	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	8.47/7.39	8.23/6.97	8.40/6.72	8.39/7.00	7.95/6.52	8.41/6.58

Environmental Sciences Division

Rev. 04 2021-02-05

CHAIN OF CUSTODY FORMS

[illegible]

SAMPLES RELINQUISHED BY

BY Anthony Garland
Peijia Ku

SAMPLES RECEIVED BY	
1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
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65	66
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73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

DATE	8/9/23
DATE	8/9/23

TIME	0810	<input checked="" type="checkbox"/> AM
		<input type="checkbox"/> PM
TIME	0811	<input checked="" type="checkbox"/> AM
		<input type="checkbox"/> PM

DATE (MM/DD/YY)		ESD TEST NAME		NAME OF SAMPLERS			CHAIN-OF-CUSTODY NO.	
08/11/23		TOX TEST		A. GARLAND/D. CRAZE			031139	
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	#7009 TEMP	#5102 REMARKS
TOX TEST	200	0735	C	1	~ 16L	3°	12.9	Ch ≤0.05
<div style="transform: rotate(-30deg);"> A.S.H. 8/11/23 </div>								

SAMPLES RELINQUISHED BY

by A. G. Garland
Moore

DATE 8/11/23

TIME 0810

☒ AM
☐ PM

SAMPLES RECEIVED BY	
1	2
3	4
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21	22
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57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

DATE 8/11/22

TIME	0810
------	------

☒ AM
☐ PM

DATE (MM/DD/YY)		ESD TEST NAME		NAME OF SAMPLERS			CHAIN-OF-CUSTODY NO.	
08/14/23		TOX TEST		A. GARLAND / J. WILLIAMS			031140	
SAMPLE NAME	OUTFALL NUMBER	SAMPLING TIME	SAMPLE TYPE *	NO. OF CONTAINERS	TOTAL VOLUME	TEMP (°C)	#	REMARKS
TOX TEST	200	0730	C	1	~17 L	3°	7009	5102
							TEMP	C/L
							10.8	<0.05
<div style="transform: rotate(-45deg);"> R. G. G. 8/14/23 </div>								

SAMPLES RELINQUISHED BY

A. R. Garland

DATE 8/14/23

TIME 0827

☒ AM
☐ PM

SAMPLES RECEIVED BY	
1	2
3	4
5	6
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45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

Peçija ku

DATE 08/14/23

TIME	0827
------	------

☒ AM
☐ PM

TOXICITY TEST LOGSHEETS

Toxicity Test Information Sheet

Sponsor: Y-12 Site/Treatment: OF 200 Test number **2992**
 Test begin date (Day 0) 08/09/23 Test end date 08/16/23 Test duration 7 ☐ hours ☒ days ☐ NA ☒ 1
 Template number

Test Organism: ☒ *Ceriodaphnia dubia* ☐ Fathead minnow ☐ Other: _____
 Isolated from: _____ Notes: _____
 Date: 08/08/23 Hatch date: _____
 Time: 0838 1620 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 =	<u>25% DMW</u>	<input checked="" type="checkbox"/> C <input type="checkbox"/> T	4 =	<u>25%</u>	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
2 =	<u>6.25%</u>	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	5 =	<u>50%</u>	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
3 =	<u>12.5%</u>	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	6 =	<u>100%</u>	<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: 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
<u>08/16/23</u>	<u>Dilution series were 0, 6.25, 12.5, 25, 50, 100% (standard dilution)</u>	<u>PK</u>

Quality Assurance (QA) Record			
Procedure	Name	Initial	Date
Test run by:	<u>Peijia Ku</u>	<u>PK</u>	<u>08/16/2023</u>
Data sheets QA:	<u>Tristan A. Boreau</u>	<u>TAB</u>	<u>08/17/2023</u>
Data entered:	<u>Peijia Ku</u>	<u>PK</u>	<u>08/16/2023</u>
Data entry QA:	<u>Tristan A. Boreau</u>	<u>TAB</u>	<u>08/17/2023</u>

Environmental Sciences Division

Rev. 02 2020-01-21

READ AND UNDERSTOOD

DATE

20

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		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R = <i>Raphidocelis</i> , B = Brine shrimp) Acceptable algal cell density range = $3.0 - 3.5 \times 10^7/\text{mL}$					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	08/07/23 PK	25.5 am pm	25.0 am pm	YCT RASU	07/25/23 08/08/23	100 95	<input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> Yes	1000 am pm	0940	1145	33464	971	
Day 7	08/16/23	am pm	am pm	PK			<input type="checkbox"/> Yes	am pm	0831	0940 PK			↓

Notes:

Environmental Sciences Division

Rev. 03 2020-06-05

08/08/23
TAD

Project: Y12 Test site/chemical: JP-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	-	16	14
19	2	-	-	4	15	-	-	14
20	3	-	-	X2	-	-	-	-
21	4	-	-	4	-	9	12	-
22	4	-	-	6	-	17	19	18
23	4	-	-	5	12	-	25	26
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 24	-
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	01	-	-

Toxicity Test Information Sheet

1699

Sponsor: Y-12 Site/Treatment: OF200 Test number: 1699
 Test begin date (Day 0): 08-09-23 Test end date: 08-16-23 Test duration: 7 ☐ hours ☒ days ☒ NA ☐
 Template number: _____

Test Organism: ☐ *Ceriodaphnia dubia* ☒ Fathead minnow ☐ Other: _____
 Isolated from: _____ Hatch date: 08/07/23 Notes: _____
 Date: _____ Delivery date: 08/08/23
 Time: _____

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 =	25%.	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
2 =	6.25%.	<input type="checkbox"/> C <input checked="" type="checkbox"/> T	5 =	50%.	<input type="checkbox"/> C <input checked="" type="checkbox"/> T
3 =	12.50%.	<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: 970-971

Source of Test Organisms:

☐ BSD 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)	TAB

Quality Assurance (QA) Record

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

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. #: <u>DD19</u>		Feeding Information (Food codes: YCT = yeast-cerophyl-trout, R = <i>Raphidocelis</i> , B = Brine shrimp) Acceptable algal cell density range = 3.0 - 3.5 x10 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	<u>08/09/23</u> <u>PK/TPB</u>	<u>25.6</u> am pm	<u>24.5</u> am pm	<u>B</u>	<u>08/09/23</u>	<u>62</u>	<input checked="" type="checkbox"/> Yes	<u>1315</u> am pm	<u>1241</u>	<u>1338</u>	<u>33462</u>	<u>970</u>	<u>NA</u>
Day 1	<u>08/10/23</u> <u>TPB</u>	<u>26.0</u> am <u>26.0</u> pm	<u>25.5</u> am <u>25.4</u> pm	<u>B</u> <u>B</u>	<u>08/10/23</u> <u>08/10/23</u>	<u>68</u> <u>126</u>	<input checked="" type="checkbox"/> Yes	<u>0901</u> am <u>1418</u> pm	<u>1141</u>	<u>1311</u>	<u>33462</u>	<u>970</u>	<u>N/A</u>
Day 2	<u>08/11/23</u> <u>TPB</u>	<u>26.1</u> am <u>26.0</u> pm	<u>25.8</u> am <u>25.6</u> pm	<u>B</u> <u>B</u>	<u>08/11/23</u> <u>8/16/23</u>	<u>68</u> <u>83</u>	<input checked="" type="checkbox"/> Yes	<u>0839</u> am <u>1413</u> pm	<u>1158</u>	<u>1303</u>	<u>33463</u>	<u>970</u>	<u>N/A</u>
Day 3	<u>08/12/23</u>	<u>26.1</u> am <u>26.0</u> pm	<u>25.7</u> am <u>25.6</u> pm	<u>B</u> <u>B</u>	<u>08/11/23</u> <u>08/11/23</u>	<u>68</u> <u>50</u>	<input checked="" type="checkbox"/> Yes	<u>0841</u> am <u>1428</u> pm	<u>1053</u>	<u>1156</u>	<u>33463</u>	<u>970</u>	<u>N/A</u>
Day 4	<u>08/13/23</u>	<u>26.1</u> am <u>26.0</u> pm	<u>25.6</u> am <u>25.7</u> pm	<u>B</u> <u>B</u>	<u>08/12/23</u> <u>08/12/23</u>	<u>112</u> <u>69</u>	<input checked="" type="checkbox"/> Yes	<u>0832</u> am <u>1402</u> pm	<u>1058</u>	<u>1231</u>	<u>33463</u>	<u>970</u>	<u>N/A</u>
Day 5	<u>08/14/23</u>	<u>26.1</u> am <u>26.0</u> pm	<u>25.6</u> am <u>25.7</u> pm	<u>B</u> <u>B</u>	<u>08/13/23</u> <u>08/13/23</u>	<u>125</u> <u>80</u>	<input checked="" type="checkbox"/> Yes	<u>0530</u> am <u>1520</u> pm	<u>1142</u>	<u>1232</u>	<u>33464</u>	<u>970</u>	<u>N/A</u>
Day 6	<u>08/15/23</u>	<u>26.2</u> am <u>26.1</u> pm	<u>25.8</u> am <u>25.6</u> pm	<u>B</u> <u>B</u>	<u>08/14/23</u> <u>08/14/23</u>	<u>64</u> <u>44</u>	<input checked="" type="checkbox"/> Yes	<u>0830</u> am <u>1445</u> pm	<u>1032</u>	<u>1128</u>	<u>33464</u>	<u>971</u>	<u>N/A</u>
Day 7	<u>08/16/23</u>	<u>25.7</u> am pm	<u>25.5</u> am pm				<input type="checkbox"/> Yes	am pm	<u>1052</u>	<u>1208</u>			<u>N/A</u>

Notes:

08/09/23
TPB

08/29/23
TAS

Fathead Minnow Chronic Daily Survival Log

Sponsor: Y-12 Test site/chemical: OF200 Test Number: 1699Begin 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	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
			Date ^{TMS} 08/10/23	Date ^{TMS} 08/11/23	Date ^{TMS} 08/12/23	Date ^{TMS} 08/13/23	Date ^{TMS} 08/14/23	Date ^{TMS} 08/15/23	Date ^{TMS} 08/16/23
1: 25%. DMW	1	22	10	10	10	10	9 10	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	14	10	10	10	10	10	10	10
	2	5	10	10 SM	10	10	9 10	9	9
	3	10	10	10	10	10	10	10	10
	4	21	10	10	10	10	9 10	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 10	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

121

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

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

09/09/23
TRG

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

08/09/23
TAB

Sponsor: 4-12		Test number: 1699		
Test site/chemical: OF200		Balance ID: A 009820		
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: <input checked="" type="checkbox"/>	Pan + Larvae (mg) Date: 8/17/23 Balance check: <input checked="" type="checkbox"/>	Number Surviving
Initial	1	15.3320	16.9070	10
	2	15.4676	17.2095	10
	3	15.4325	16.9080	10
	4	15.4630	16.9900	10
1. 25%. DMW	1	15.3395	25.9065	10 9 8/17
	2	15.4315	24.8680	10
	3	15.5055	26.5025	10
	4	15.4665	24.7730	10
2. 6.25%.	1	15.3160	25.2715	10
	2	15.5940	25.0230	10 9 8/17
	3	15.3540	25.3725	10
	4	15.2650	25.3600	10 9 8/17
3. 12.50%.	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%.	1	15.3860	25.8730	10
	2	15.2785	25.8920	10
	3	15.4246	25.5745	10
	4	15.4170	25.2915	10
5. 50%.	1	15.3875	25.1820	10
	2	15.1525	25.2615	10
	3	15.1495	26.4690	9
	4	15.3305	27.2460	10
6. 100%.	1	15.2580	27.0655	10
	2	15.1460	25.6980	10
	3	15.2360	26.2150	10
	4	15.2270	27.2485	10

Fathead Minnow Order & Shipment Log

Ordering Information:

Date Ordered	Test #(s)	Vendor	Quantity ordered	Description (larval age, etc.)	Expected delivery	Ordered by	Comments
08/08/23	1699	ABS	400	< 1 day PM 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):								