**Tennessee Valley Authority**, Sequoyah Nuclear Plant, P.O. Box 2000, Soddy Daisy, TN 37384

June 10, 2020

ATTN: Document Control Desk
U.S. Nuclear Regulatory Commission
Washington, D.C. 20555-0001

Subject: **Sequoyah Nuclear Plant, Discharge Monitoring Report (DMR), May 2020**

Attached is the May 2020 DMR for Sequoyah Nuclear Plant.

Respectfully,

Kelly Robinette
Environmental Technician

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P.O. BOX 2000
(INTEROFFICE OPS-5N-SQN)
SODDY - DAISY, TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

**Info Only**

ATTN:Millicent Garland

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
**DISCHARGE MONITORING REPORT** *(DMR)*

| TN0026450 | 101 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 20 | 05 | 01 | To | 20 | 05 | 31 |

MAJOR
(SUBR 01)
F - FINAL
DIFFUSER DISCHARGE
EFFLUENT

*** NO DISCHARGE ☐ ***

Form Approved.
OMB No. 2040-0004

NOTE: Read instructions before completing this form.

| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| TEMPERATURE, WATER DEG. CENTIGRADE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 36.1 | 04 | 0 | 31 / 31 | RCORDR |
| 00010 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | Req. Mon. DAILY MAX | DEG. C. | | CONTI NUOUS | CALCTD |
| TEMPERATURE, WATER DEG. CENTIGRADE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 23.5 | 04 | 0 | 31 / 31 | MODELD |
| 00010 Z 0 INSTREAM MONITORING | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 30.5 DAILY MX | DEG. C. | | CONTI NUOUS | CALCTD |
| TEMP. DIFF. BETWEEN SAMP. & UPSTRM DEG.C | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 2.2 | 04 | 0 | 31 / 31 | CALCTD |
| 00016 1 S EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 3.0 DAILY MX | DEG. C. | | CONTI NUOUS | CALCTD |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | ******** | | 03 | ******** | ******** | ******** | ** | 0 | 31 / 31 | RCORDR |
| 50050 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | Req. Mon. DAILY MAX | MGD | ******** | ******** | ******** | **** | | CONTI NUOUS | RCORDR |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | 1798 | ******** | 03 | ******** | ******** | ******** | 03 | 0 | 31 / 31 | CALCTD |
| 50050 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | Req. Mon. MO AVG | ******** | MGD | ******** | ******** | ******** | MGD | | CONTI NUOUS | CALCTD |
| CHLORINE, TOTAL RESIDUAL | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | 0.016 | 0.024 | 19 | 0 | 12 / 31 | GRAB |
| 50060 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | 0.1 MO AVG | 0.1 DAILY MAX | MG/L | | FIVE PER WEEK | CALCTD |
| TEMPERATURE - C, RATE OF CHANGE | SAMPLE MEASUREMENT | ******** | 0.4 | 62 | ******** | ******** | | ** | 0 | 31 / 31 | CALCTD |
| 82234 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | 2.0 DAILY MX | DEG C/HR | ******** | ******** | ******** | **** | | CONTI NUOUS | CALCTD |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Matthew Rasmussen<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is , to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations. | Site Vice President<br>SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | 423 | 843-7001 | 20 | 06 | 08 |
| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

No closed mode operation. The following injections occurred: Spectrus BD1500 (max calc. was 0.049 mg/L, max conc. -- 2.0 mg/L), Spectrus CT1300 (max calc. was 0.0332 mg/L, max conc. -- 0.05 mg/L).

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*
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Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN:Millicent Garland

**Info Only**

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
**DISCHARGE MONITORING REPORT** *(DMR)*

| PERMIT NUMBER | DISCHARGE NUMBER |
|---|---|
| TN0026450 | 101 T |

MONITORING PERIOD

| | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 20 | 05 | 01 | To | 20 | 05 | 31 |

MAJOR
(SUBR 01)
F - FINAL
BIOMONITORING FOR OUTFALL 101
EFFLUENT
*** NO DISCHARGE [ ] ***

Form Approved.
OMB No. 2040-0004

NOTE: Read instructions before completing this form.

| PARAMETER | | QUANTITY OR LOADING AVERAGE | QUANTITY OR LOADING MAXIMUM | QUANTITY OR LOADING UNITS | QUALITY OR CONCENTRATION MINIMUM | QUALITY OR CONCENTRATION AVERAGE | QUALITY OR CONCENTRATION MAXIMUM | QUALITY OR CONCENTRATION UNITS | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IC25 STATRE 7DAY CHR CERIODAPHNIA | SAMPLE MEASUREMENT | ******** | ******** | ** | >100.0 | ******** | ******** | 23 | 0 | 3 / 180 | COMPOS |
| TRP3B 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | 42.8 MINIMUM | ******** | ******** | PERCENT | | SEMI ANNUAL | COMPOS |
| IC25 STATRE 7DAY CHR PIMEPHALES | SAMPLE MEASUREMENT | ******** | ******** | ** | >100.0 | ******** | ******** | 23 | 0 | 3 / 180 | COMPOS |
| TRP6C 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | 42.8 MIMINUM | ******** | ******** | PERCENT | | SEMI ANNUAL | COMPOS |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

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| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*
Toxicity was sampled May 4-8, 2020.

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

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SODDY - DAISY, TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

**Info Only**

ATTN:Millicent Garland

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
**DISCHARGE MONITORING REPORT** *(DMR)*

| TN0026450 | 103 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 20 | 05 | 01 | To | 20 | 05 | 31 |

MAJOR
(SUBR 01)
F - FINAL
LOW VOL. WASTE TREATMENT POND
EFFLUENT

*** NO DISCHARGE ☐ ***

Form Approved.
OMB No. 2040-0004

NOTE: Read instructions before completing this form.

| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| PH | SAMPLE MEASUREMENT | ******** | ******** | ** | 7.2 | ******** | 7.6 | 12 | 0 | 4 / 31 | GRAB |
| 00400 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | ** | 6.0 MINIMUM | ******** | 9.0 MAXIMUM | SU | | ONCE/ WEEK | GRAB |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | 7.5 | 7.5 | 19 | 0 | 1 / 31 | GRAB |
| 00530 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | ** | ******** | 30.0 MO AVG | 100.0 DAILY MX | MG/L | | ONCE/ MONTH | GRAB |
| OIL AND GREASE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | <5.0 | <5.0 | 19 | 0 | 1 / 31 | GRAB |
| 00556 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | ** | ******** | 15.0 MO AVG | 20.0 DAILY MX | MG/L | | ONCE/ MONTH | GRAB |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | 0.932 | 1.206 | 03 | ******** | ******** | ******** | ** | 0 | 4 / 31 | INSTAN |
| 50050 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | Req. Mon. MO AVG | Req. Mon DAILY MX | MGD | ******** | ******** | ******** | ** | | ONCE/ WEEK | INSTAN |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
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| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

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Address P.O. BOX 2000
(INTEROFFICE OPS-5N-SQN)
SODDY - DAISY, TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN:Millicent Garland

**Info Only**

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
**DISCHARGE MONITORING REPORT** *(DMR)*

| TN0026450 | 110 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

MONITORING PERIOD

| | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 20 | 05 | 01 | To | 20 | 05 | 31 |

MAJOR
(SUBR 01)
F - FINAL
RECYCLED COOLING WATER
EFFLUENT

*** NO DISCHARGE XX ***

Form Approved.
OMB No. 2040-0004

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| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| TEMPERATURE, WATER DEG. CENTIGRADE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 04 | | | |
| 00010 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | ** | ******** | ******** | REPORT DAILY MX | DEG C | | CONTIN UOUS | CALCTD |
| TEMPERATURE, WATER DEG. CENTIGRADE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 04 | | | |
| 00010 Z 0 INSTREAM MONITORING | PERMIT REQUIREMENT | ******** | ******** | ** | ******** | ******** | 30.5 DAILY MX | DEG C | | CONTIN UOUS | CALCTD |
| TEMP. DIFF. BETWEEN SAMP. & UPSTRM DEG.C | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 04 | | | |
| 00016 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | ** | ******** | ******** | 5 DAILY MX | DEG C | | CONTIN UOUS | CALCTD |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | ******** | | 03 | ******** | ******** | ******** | ** | | | |
| 50050 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | Req. Mon. DAILY MX | MGD | ******** | ******** | ******** | ** | | CONTIN UOUS | RCORDR |
| CHLORINE, TOTAL RESIDUAL | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | | | 19 | | | |
| 50060 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | ** | ******** | 0.1 MO AVG | 0.1 DAILY MX | MG/L | | Five per Week | CALCTD |
| TEMPERATURE - C, RATE OF CHANGE | SAMPLE MEASUREMENT | ******** | | 04 | ******** | ******** | ******** | ** | | | |
| 82234 1 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | 2 DAILY MX | DEG C | ******** | ******** | ******** | ** | | CONTIN UOUS | CALCTD |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
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| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

No Discharge this Period

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P.O. BOX 2000
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Location HAMILTON COUNTY

ATTN:Millicent Garland

**Info Only**

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
**DISCHARGE MONITORING REPORT** *(DMR)*

| TN0026450 | 110 T |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 20 | 05 | 01 | To | 20 | 05 | 31 |

MAJOR
(SUBR 01)
F - FINAL
RECYCLED COOLING WATER
EFFLUENT

*** NO DISCHARGE XX ***

Form Approved.
OMB No. 2040-0004

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| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| IC25 STATRE 7DAY CHR CERIODAPHNIA | SAMPLE MEASUREMENT | ******** | ******** | ** | | ******** | ******** | 23 | | | |
| TRP3B 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 42.8 MINIMUM | ******** | ******** | PERCENT | | SEMI ANNUAL | COMPOS |
| IC25 STATRE 7DAY CHR PIMEPHALES | SAMPLE MEASUREMENT | ******** | ******** | ** | | ******** | ******** | 23 | | | |
| TRP6C 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 42.8 MINIMUM | ******** | ******** | PERCENT | | SEMI ANNUAL | COMPOS |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|
| Matthew Rasmussen<br>Site Vice President<br>TYPED OR PRINTED | | | 423 | 843-7001 | 20 | 06 | 08 |
| | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

No Discharge this Period

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

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Location HAMILTON COUNTY

**Info Only**

ATTN:Millicent Garland

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
**DISCHARGE MONITORING REPORT** *(DMR)*

| TN0026450 | 118 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 20 | 05 | 01 | To | 20 | 05 | 31 |

MAJOR
(SUBR 01)
F - FINAL
WASTEWATER & STORM WATER
EFFLUENT

*** NO DISCHARGE [XX] ***

Form Approved.
OMB No. 2040-0004

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| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| OXYGEN, DISSOLVED (DO) | SAMPLE MEASUREMENT | ******** | ******** | ** | | ******** | ******** | 19 | | | |
| 00300 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | 2 MINIMUM | ******** | ******** | MG/L | | TWICE/ WEEK | GRAB |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00530 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 100 DAILY MX | MG/L | | TWICE/ WEEK | GRAB |
| SOLIDS, SETTLEABLE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 25 | | | |
| 00545 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1 DAILY MX | ML/L | | ONCE/ MONTH | GRAB |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | | | 03 | ******** | ******** | ******** | ** | | | |
| 50050 1 0 EFFLUENT GROSS | PERMIT REQUIREMENT | Req. Mon. MO AVG | Req. Mon. DAILY MX | MGD | ******** | ******** | ******** | * | | ONCE/ BATCH | ESTIMA |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
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| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

During this reporting period, there has been no flow from the Dredge Pond other than that resulting from rainfall. No Discharge this Period

# Environmental Records Processing Form

| Title of File | | |
|---|---|---|
| SQN May 2020 Toxicity Report for DSN101 | | |
| **Site/Plant/Project Name** | **Accession Number (optional)** | **Work Order Number (optional)** |
| SQN | | |
| **Your Name** | **Date Submitted (YYYYMMDD)** | **Document Date (YYYYMMDD)** |
| James W. Osborne, Jr. | 20200605 | 20200605 |

*Show Instructions*

For assistance, please contact the Facility or Site Environmental Contact for your site/project, the Environmental Media Specialists (See Contacts on Environment InsideNet Page), or your Administrative Support Person.

**Document Type**

WATER/WASTEWATER

**Record Type**

NPDES Permit Compliance Records

**TENNESSEE VALLEY AUTHORITY**
**TOXICITY TEST REPORT**

## INTRODUCTION / EXECUTIVE SUMMARY

Report Date: June 05, 2020

1. Facility / Discharger: Sequoyah Nuclear Plant / TVA
2. County / State: Hamilton / Tennessee
3. NPDES Permit #: TN0026450
4. Type of Facility: Nuclear-Fueled Electric Generating Plant
5. Design Flow (MGD): 1,579
6. Receiving Stream: Tennessee River (TRM 483.6)
7. 1Q10: 3,491
8. Outfall Tested: 101
9. Dates Sampled: May 03 – 08, 2020
10. Average Flow on Days Sampled (MGD): 1770, 1745, 1753
11. Pertinent Site Conditions: Production / operation data will be provided upon request.
12. Test Dates: May 05 – 12, 2020
13. Test Type: Short-term Chronic Definitive
14. Test Species: Fathead Minnows (*Pimephales promelas*)
    Daphnids (*Ceriodaphnia dubia*)
15. Concentrations Tested (%):
    *Pimephales promelas*: UV treated Outfall 101: 10.7, 21.4, 42.8, 85.6, 100
    UV treated Intake: 100

    *Ceriodaphnia dubia*: Non-treated Outfall 101: 10.7, 21.4, 42.8, 85.6, 100
    Non-treated Intake: 100
16. Permit Limit Endpoint (%): Outfall 101: $IC_{25}$ = 42.8%
17. Test Results: Outfall 101: *Pimephales promelas*: $IC_{25}$ > 100%
    *Ceriodaphnia dubia*: $IC_{25}$ > 100%

Independent Review by Kelley E. Kisnap:

18. Facility Contact: Millicent Garland Phone #: (423) 843-6714

19. Consulting / Testing Lab: Environmental Testing Solutions, Inc.

20. Lab Contact: Jim Sumner Phone #: (828) 350-9364

21. TVA Contact: Rick Sherrard Phone #: (423) 876-6743

22. Notes: Exposures to samples collected May 03 – 08, 2020 from Outfall 101 resulted in no toxic effects to fathead minnows or daphnids. The resulting IC25 values, for both species, were >100 percent. Exposure of daphnids to intake samples resulted in no significant difference from the control during this study period. Minnow growth in the intake samples was significantly lower than the control.

Independent Review by Kelley E. Keenan:

## METHODS SUMMARY

Samples:

1. Sampling Point: Outfall 101, Intake

2. Sample Type: Composite

3. Sample Information:

| Sample ID | Date (MM-DD-YY) Time (ET) Collected | Date (MM-DD-YY) Time (ET) Received | Arrival Temp. (°C) | Initial TRC* (mg/L) | Date (MM-DD-YY) Time (ET) Last Used By |
|---|---|---|---|---|---|
| 101 | 05-03-20 0730 to 05-04-20 0630 | 05-04-20 1310 | 1.6 | <0.10 | 05-05-20 0958<br>05-06-20 0900 |
| Intake | 05-03-20 0730 to 05-04-20 0630 | 05-04-20 1310 | 1.5 | <0.10 | 05-05-20 0958<br>05-06-20 0900 |
| 101 | 05-05-20 0730 to 05-06-20 0630 | 05-06-20 1250 | 1.2 | <0.10 | 05-07-20 0924<br>05-08-20 0920 |
| Intake | 05-05-20 0730 to 05-06-20 0630 | 05-06-20 1250 | 1.3 | <0.10 | 05-07-20 0924<br>05-08-20 0920 |
| 101 | 05-07-20 0730 to 05-08-20 0630 | 05-08-20 1250 | 1.1, 1.0† | <0.10 | 05-09-20 1018<br>05-10-20 0917<br>05-11-20 0915 |
| Intake | 05-07-20 0730 to 05-08-20 0630 | 05-08-20 1250 | 2.1 | <0.10 | 05-09-20 1018<br>05-10-20 0917<br>05-11-20 0915 |

*TRC = Total Residual Chlorine
†Samples were collected in two 2.5 gallon cubitainers. Temperature was measured in each cubitainer upon arrival.

4. Sample Manipulation: Samples from Outfall 101 and intake were warmed to test temperature (25.0 ± 1.0°C) in a warm water bath.

   Aliquots of Outfall 101 and Intake samples were UV-treated through a 40-watt Smart® UV Sterilizer (manufactured by Emperor Aquatics, Inc.) for 2 minutes.

Independent Review by Kelley E. Keenan: K

| | *Pimephales promelas* | *Ceriodaphnia dubia* |
|---|---|---|
| Test Organisms: | | |
| 1. Source: | In-house Cultures | In-house Cultures |
| 2. Age: | < 24-hours old | < 24-hours old |
| Test Method Summary: | | |
| 1. Test Conditions: | Static, Renewal | Static, Renewal |
| 2. Test Duration: | 7 days | Until at least 60% of control females have 3 broods |
| 3. Control / Dilution Water: | Moderately Hard Synthetic | Moderately Hard Synthetic |
| 4. Number of Replicates: | 4 | 10 |
| 5. Organisms per Replicate: | 10 | 1 |
| 6. Test Initiation: (Date/Time): | 05-05-20 0856 ET | 05-05-20 0958 ET |
| 7. Test Termination: (Date/Time): | 05-12-20 0808 ET | 05-12-20 0911 ET |
| 8. Test Temperature: Outfall 101: | Mean = 24.7°C (24.3 – 25.2°C) | Mean = 24.9°C (24.3 – 25.2°C) |

9. Physical / Chemical Measurements: Alkalinity, hardness, total residual chlorine, and conductivity were measured at the laboratory in each 100% sample. Daily temperatures were measured in one replicate for each test concentration. Pre- and post-exposure test solutions were analyzed daily for pH and dissolved oxygen.

10. Statistics: Statistics were performed according to methods prescribed by EPA using ToxCalc version 5.0 statistical software (Tidepool Scientific Software, McKinneyville, CA).

Independent Review by Kelley E. Keecan:

**TOXICITY TEST RESULTS** (see Appendix C for Bench Sheets)

1. Results of a *Pimephales promelas* Chronic/ 7-day Toxicity Test.
(Genus species) (Type / Duration)

Conducted May 05 – 12, 2020 using effluent from Outfall 101.

| Test Solutions (% Effluent) | Percent Surviving (time interval used – days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control, UV-treated | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10.7% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 21.4% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 42.8% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 85.6% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 100.0% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Intake | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Control, Non-treated | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| Test Solutions (% Effluent) | Mean Dry Weight (mg) (replicate number) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Mean |
| Control, UV-treated | 0.697 | 0.713 | 0.764 | 0.775 | 0.737 |
| 10.7% | 0.757 | 0.726 | 0.689 | 0.748 | 0.730 |
| 21.4% | 0.712 | 0.694 | 0.717 | 0.765 | 0.722 |
| 42.8% | 0.706 | 0.637 | 0.705 | 0.735 | 0.696 |
| 85.6% | 0.738 | 0.686 | 0.693 | 0.676 | 0.698 |
| 100.0% | 0.759 | 0.730 | 0.745 | 0.724 | 0.740 |
| Intake | 0.680 | 0.601 | 0.624 | 0.652 | 0.639 |
| Control, Non-treated | 0.661 | 0.747 | 0.822 | 0.744 | 0.744 |

| | |
|---|---|
| $IC_{25}$ Value: > 100%<br>Permit Limit: 42.8%<br><br>95% Confidence Limits:<br>Upper Limit: NA<br>Lower Limit: NA | Calculated TU Estimates: < 1.0 TUc*<br><br>Permit Limit: 2.3 TUc |

*TUa = 100/$LC_{50}$: TUc = 100/ $IC_{25}$

Independent Review by Kelley E. Keenan:

TOXICITY TEST RESULTS (see Appendix C for Bench Sheets)

2. Results of a *Ceriodaphnia dubia* Chronic/ 7-day Toxicity Test.
(Genus species) (Type / Duration)

Conducted May 05 – 12, 2020 using effluent from Outfall 101.

| Test Solutions (% Effluent) | Percent Surviving (time interval used – days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10.7% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 21.4% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 42.8% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 85.6% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 100.0% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| Test Solutions (% Effluent) | Reproduction (#young/female/7 days) Data (replicate number) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
| Control | 29 | 29 | 30 | 29 | 29 | 28 | 29 | 30 | 31 | 32 | 29.6 |
| 10.7% | 30 | 31 | 29 | 34 | 31 | 29 | 31 | 29 | 32 | 29 | 30.5 |
| 21.4% | 34 | 33 | 33 | 32 | 30 | 33 | 34 | 36 | 34 | 32 | 33.1 |
| 42.8% | 35 | 36 | 34 | 36 | 34 | 33 | 28 | 33 | 32 | 34 | 33.5 |
| 85.6% | 34 | 35 | 36 | 35 | 34 | 35 | 34 | 34 | 35 | 36 | 34.8 |
| 100.0% | 33 | 34 | 38 | 34 | 34 | 34 | 34 | 36 | 36 | 34 | 34.7 |

| | |
|---|---|
| $IC_{25}$ Value: > 100%<br>Permit Limit: 42.8%<br><br>95% Confidence Limits:<br>Upper Limit: NA<br>Lower Limit: NA | Calculated TU Estimates: < 1.0 TUc*<br><br>Permit Limit: 2.3 TUc |

*TUa = 100/$LC_{50}$: TUc = 100/ $IC_{25}$

Independent Review by Kelley E. Keenan:

TOXICITY TEST RESULTS (see Appendix C for Bench Sheets)

2. Results of a *Ceriodaphnia dubia* Chronic/ 7-day Toxicity Test.
(Genus species) (Type / Duration)

Conducted May 05 – 12, 2020 using water from Intake

| Test Solutions (% Effluent) | Percent Surviving (time interval used – days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Intake | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| Test Solutions (% Effluent) | Reproduction (#young/female/7 days) Data (replicate number) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
| Control | 32 | 29 | 31 | 32 | 32 | 29 | 30 | 29 | 29 | 30 | 30.3 |
| Intake | 36 | 32 | 37 | 35 | 36 | 36 | 35 | 36 | 37 | 33 | 35.3 |

| | |
|---|---|
| $IC_{25}$ Value: > 100%<br>Permit Limit: N/A<br><br>95% Confidence Limits:<br>Upper Limit: NA<br>Lower Limit: NA | Calculated TU Estimates: < 1.0 TUc*<br><br>Permit Limit: N/A |

*TUa = 100/$LC_{50}$: TUc = 100/ $IC_{25}$

REFERENCE TOXICANT TEST RESULTS (see Appendix A and D)

| Species | Date | Time | Duration | Toxicant | Results ($IC_{25}$) |
|---|---|---|---|---|---|
| *Pimephales promelas* | May 05 – 12, 2020 | 0822 | 7 days | KCl | 0.67 g/L |
| *Ceriodaphnia dubia* | May 05 – 12, 2020 | 0925 | 7-days | NaCl | 1.10 g/L |

Independent Review by Kelley E. Keenan:

Independent Review by Kelley E. Hansen

## PHYSICAL/CHEMICAL SUMMARY

Water Chemistry Mean Values and Ranges for UV-treated *Pimephales promelas* and Non-treated *Ceriodaphnia dubia*. Sequoyah Nuclear Plant (SQN), Effluent Outfall 101 and Intake performed May 05-12, 2020.

| Test | Sample ID | Temperature (°C) | | Dissolved Oxygen (mg/L) | | pH (S.U.) | | Conductance | Alkalinity | Hardness | *Total Residual |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | (µmhos/cm) | (mg/L $CaCO_3$) | (mg/L $CaCO_3$) | Chlorine (mg/L) |
| *Pimephales promelas* | Control, Non-treated | 24.8<br>24.7 - 24.8 | 24.6<br>24.4 - 24.7 | 7.7<br>7.6 - 7.9 | 7.5<br>7.3 - 7.7 | 7.80<br>7.68 - 7.98 | 7.75<br>7.68 - 7.88 | 314<br>300 - 322 | 60<br>60 - 61 | 91<br>89 - 93 | -<br>- - |
| | Control, UV-treated | 24.8<br>24.8 - 24.9 | 24.7<br>24.6 - 24.8 | 7.8<br>7.6 - 8.0 | 7.5<br>7.2 - 8.0 | 7.98<br>7.92 - 8.05 | 7.83<br>7.67 - 7.93 | 314<br>307 - 323 | 61<br>60 - 62 | 88<br>86 - 90 | -<br>- - |
| | 10.7% | 24.9<br>24.8 - 24.9 | 24.6<br>24.5 - 24.8 | 7.8<br>7.6 - 8.0 | 7.5<br>7.3 - 7.9 | 7.96<br>7.93 - 8.03 | 7.78<br>7.65 - 7.87 | 297<br>288 - 304 | -<br>- - | -<br>- - | -<br>- - |
| | 21.4% | 24.9<br>24.8 - 24.9 | 24.5<br>24.3 - 24.7 | 7.9<br>7.6 - 8.0 | 7.5<br>7.3 - 7.9 | 7.96<br>7.91 - 8.02 | 7.77<br>7.63 - 7.86 | 281<br>274 - 287 | -<br>- - | -<br>- - | -<br>- - |
| | 42.8% | 24.9<br>24.8 - 25.0 | 24.6<br>24.4 - 24.8 | 7.9<br>7.6 - 8.1 | 7.6<br>7.3 - 8.0 | 7.95<br>7.90 - 8.00 | 7.75<br>7.61 - 7.85 | 250<br>240 - 255 | -<br>- - | -<br>- - | -<br>- - |
| | 85.6% | 24.9<br>24.8 - 25.0 | 24.6<br>24.5 - 24.8 | 8.0<br>7.7 - 8.1 | 7.5<br>7.3 - 8.0 | 7.93<br>7.88 - 7.98 | 7.72<br>7.59 - 7.83 | 188<br>179 - 194 | -<br>- - | -<br>- - | -<br>- - |
| | 100% | 25.0<br>24.8 - 25.0 | 24.6<br>24.5 - 24.7 | 8.0<br>7.7 - 8.1 | 7.5<br>7.1 - 8.0 | 7.92<br>7.87 - 7.96 | 7.71<br>7.56 - 7.83 | 165<br>154 - 172 | 65<br>64 - 66 | 70<br>67 - 73 | <0.10<br><0.10 - <0.10 |
| | Intake | 25.0<br>24.9 - 25.2 | 24.6<br>24.3 - 24.8 | 8.0<br>7.6 - 8.2 | 7.4<br>6.5 - 7.9 | 7.92<br>7.88 - 7.96 | 7.70<br>7.49 - 7.83 | 164<br>158 - 176 | 64<br>63 - 65 | 70<br>69 - 71 | <0.10<br><0.10 - <0.10 |
| *Ceriodaphnia dubia* | Control, Non-treated | 24.8<br>24.8 - 24.9 | 25.0<br>24.5 - 25.2 | 7.7<br>7.6 - 7.9 | 7.9<br>7.7 - 8.0 | 7.80<br>7.68 - 7.98 | 7.97<br>7.92 - 8.05 | 314<br>300 - 322 | 60<br>60 - 61 | 91<br>89 - 93 | -<br>- - |
| | 10.7% | 24.8<br>24.8 - 24.9 | 24.9<br>24.6 - 25.1 | 7.9<br>7.8 - 8.0 | 7.8<br>7.7 - 8.0 | 7.98<br>7.91 - 8.04 | 7.96<br>7.90 - 7.99 | 299<br>291 - 307 | -<br>- - | -<br>- - | -<br>- - |
| | 21.4% | 24.9<br>24.8 - 25.0 | 24.9<br>24.6 - 25.2 | 7.9<br>7.8 - 8.0 | 7.9<br>7.7 - 8.0 | 7.97<br>7.92 - 8.02 | 7.95<br>7.90 - 7.99 | 285<br>274 - 289 | -<br>- - | -<br>- - | -<br>- - |
| | 42.8% | 24.9<br>24.8 - 25.0 | 25.0<br>24.3 - 25.2 | 7.9<br>7.8 - 8.0 | 7.9<br>7.8 - 8.0 | 7.96<br>7.92 - 8.01 | 7.95<br>7.88 - 7.98 | 251<br>240 - 257 | -<br>- - | -<br>- - | -<br>- - |
| | 85.6% | 24.9<br>24.8 - 25.0 | 24.9<br>24.6 - 25.2 | 8.0<br>7.9 - 8.0 | 7.9<br>7.8 - 8.0 | 7.93<br>7.89 - 7.98 | 7.93<br>7.87 - 7.98 | 187<br>174 - 193 | -<br>- - | -<br>- - | -<br>- - |
| | 100% | 25.0<br>24.9 - 25.0 | 24.9<br>24.6 - 25.2 | 8.0<br>7.9 - 8.1 | 7.9<br>7.8 - 8.0 | 7.92<br>7.87 - 7.97 | 7.92<br>7.86 - 7.98 | 163<br>151 - 171 | 64<br>62 - 65 | 69<br>67 - 71 | <0.10<br><0.10 - <0.10 |
| | Intake | 25.0<br>24.8 - 25.1 | 25.0<br>24.6 - 25.2 | 8.0<br>7.9 - 8.2 | 7.9<br>7.8 - 8.0 | 7.92<br>7.88 - 7.97 | 7.93<br>7.88 - 7.99 | 164<br>155 - 169 | 64<br>63 - 66 | 69<br>69 - 69 | <0.10<br><0.10 - <0.10 |

*Note: Total residual chlorine was performed on non-treated Outfall 101 and Intake samples.

| Overall temperature (°C) | Average | Minimum | Maximum |
|---|---|---|---|
| *Pimephales promelas* | 24.7 | 24.3 | 25.2 |
| *Ceriodaphnia dubia* | 24.9 | 24.3 | 25.2 |

## SUMMARY / CONCLUSIONS

Exposures to samples collected May 03 – 08, 2020 from Outfall 101 resulted in no toxic effects to fathead minnows or daphnids. The resulting IC25 values, for both species, were >100 percent. Exposure of daphnids to intake samples resulted in no significant difference from the control during this study period. Minnow growth in the intake samples was significantly lower than the control.

Independent Review by Kelley E. Keenan:

## Appendix A

## ADDITIONAL TOXICITY TEST INFORMATION

### SUMMARY OF METHODS

1. *Pimephales promelas*

   Tests were conducted according to EPA-821-R-02-013 (October 2002) using four replicates, each containing ten test organisms, per treatment. Test vessels consisted of 500-mL plastic disposable cups, each containing 250-mL of test solution.

2. *Ceriodaphnia dubia*

   Tests were conducted according to EPA-821-R-02-013 (October 2002) using ten replicates, each containing one test organism, per treatment. Test vessels consisted of 30-mL polypropylene cups, each containing 15-mL of test solution.

### DEVIATIONS / MODIFICATIONS TO TEST PROTOCOL

1. *Pimephales promelas*

   Samples used in the fathead minnow test were exposed to UV light for two minutes prior to introduction of test organisms. UV treatment is used to control interference of fish pathogens. This treatment method was approved on November 23, 2015 by the State of Tennessee in a letter from Jessica Murphy to Terry Cheek, Senior Manager of TVA Water Permits, Compliance, and Monitoring.

2. *Ceriodaphnia dubia*

   None

### DEVIATIONS / MODIFICATIONS TO PRETEST CULTURE OR HOLDING OF TEST ORGANISMS

1. *Pimephales promelas*

   None

2. *Ceriodaphnia dubia*

   None

Independent Review by Kelley E. Keenan:

## PHYSICAL AND CHEMICAL METHODS

1. Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable).

2. Instruments: All identification, service, and calibration information pertaining to laboratory instruments is recorded in calibration and maintenance logbooks.

3. Temperature was measured by SM 2550 B-2010.

4. Dissolved oxygen was measured by SM 4500-O G-2011.

5. The pH was measured by SM 4500-H+ B-2011.

6. Conductance was measured by SM 2510 B-2011.

7. Alkalinity was measured by SM 2320 B-2011.

8. Total hardness was measured by SM 2340 C-2011.

9. Total residual chlorine was measured by ORION 97-70-1977.

## QUALITY ASSURANCE

Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage, glassware preparation, test organism culturing/acquisition and acclimation, test organism handling during test, and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA-821-R-02-013. Any known deviations were noted during the study and are reported herein.

## REFERENCE TOXICANT TESTS (See Appendix D for control chart information)

1. Test Type: 7-day chronic tests with results expressed as $IC_{25}$ values in g/L KCl or NaCl.

2. Standard Toxicant: Potassium Chloride (KCl crystalline) for *Pimephales promelas*.
   Sodium Chloride (NaCl crystalline) for *Ceriodaphnia dubia*.

3. Dilution Water Used: Moderately hard synthetic water.

4. Statistics: ToxCalc software Version 5.0 was used for statistical analyses.

Independent Review by Kelley E. Keenan: K

REFERENCES


1. NPDES Permit No. TN0026450.

2. USEPA. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013 (October 2002).

3. Standard Methods for the Examination of Water and Wastewater. 22nd Edition, 2012.

4. Quality Assurance Program: Standard Operating Procedures, Environmental Testing Solutions, Inc (most current version).


Independent Review by Kelley E. Keenan

Sequoyah Nuclear Plant Biomonitoring
May 05 – 12, 2020

Appendix B

Diffuser Discharge Concentrations of Total Residual Chlorine,

Diffuser Discharge Concentrations of Chemicals Used to Control Microbiologically Induced Corrosion and Mollusks During Toxicity Test Sampling

| Date | Sodium Hypochlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phosphate | PCL-401 mg/L Copolymer | CL-363 mg/L DMAD | Cuprostat-PF mg/L Azole | H-130M mg/L Quat | Nalco 73551 mg/L EO/PO | H-150M mg/L Quat |
|---|---|---|---|---|---|---|---|---|---|
| 02/06/2005 | - | <0.0042 | 0.028 | 0.010 | - | - | - | - | - |
| 02/07/2005 | - | <0.0116 | 0.028 | 0.010 | - | - | - | 0.007 | - |
| 02/08/2005 | - | <0.0080 | 0.028 | 0.010 | - | - | - | - | - |
| 02/09/2005 | - | 0.0199 | 0.028 | 0.010 | - | - | - | - | - |
| 02/10/2005 | - | <0.0042 | 0.028 | 0.010 | - | - | - | - | - |
| 02/11/2005 | - | 0.0155 | 0.028 | 0.010 | - | - | - | 0.007 | - |
| 06/05/2005 | - | 0.0063 | - | - | - | - | - | - | - |
| 06/06/2005 | - | 0.0043 | - | - | - | - | - | - | 0.037 |
| 06/07/2005 | - | 0.0103 | - | - | - | - | - | - | 0.037 |
| 06/08/2005 | - | 0.0295 | - | - | - | - | - | - | 0.037 |
| 06/09/2005 | - | 0.0129 | - | - | - | - | - | - | - |
| 06/10/2005 | - | 0.0184 | - | - | - | - | - | - | - |
| 07/17/2005 | - | 0.0109 | 0.026 | 0.009 | - | - | - | - | - |
| 07/18/2005 | - | 0.0150 | 0.026 | 0.009 | - | - | - | - | 0.036 |
| 07/19/2005 | - | 0.0163 | 0.026 | 0.009 | - | - | - | - | 0.036 |
| 07/20/2005 | - | 0.0209 | 0.026 | 0.009 | - | - | - | 0.014 | 0.036 |
| 07/21/2005 | - | 0.0242 | 0.026 | 0.009 | - | - | - | - | - |
| 07/22/2005 | - | 0.0238 | 0.054 | 0.018 | - | - | - | 0.014 | - |
| 10/30/2005 | - | 0.0068 | - | - | - | - | - | - | - |
| 10/31/2005 | - | 0.0112 | - | - | - | - | - | - | - |
| 11/01/2005 | - | 0.0104 | - | - | - | - | - | - | 0.035 |
| 11/02/2005 | - | 0.0104 | - | - | - | - | - | - | 0.036 |
| 11/03/2005 | - | 0.0117 | - | - | - | - | - | - | 0.036 |
| 11/04/2005 | - | 0.0165 | - | - | - | - | - | - | 0.035 |
| 11/14/2005 | - | 0.0274 | - | - | - | - | - | - | - |
| 11/15/2005 | - | 0.0256 | - | - | - | - | - | - | - |
| 11/16/2005 | - | 0.0234 | - | - | - | - | - | - | - |
| 11/17/2005 | - | 0.0231 | - | - | - | - | - | - | - |
| 11/18/2005 | - | 0.0200 | - | - | - | - | - | - | - |
| 11/19/2005 | - | 0.0116 | - | - | - | - | - | - | - |

| Date | Sodium Hypochlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phosphate | PCL-401 mg/L Copolymer | CL-363 mg/L DMAD | Cuprostat-PF mg/L Azole | H-130M mg/L Quat | Nalco 73551 mg/L EO/PO | H-150M mg/L Quat | MSW 101 mg/L Phosphate |
|---|---|---|---|---|---|---|---|---|---|---|
| 11/12/2006 | - | 0.0055 | - | - | - | - | - | - | - | - |
| 11/13/2006 | - | 0.0068 | - | - | - | - | - | - | 0.037 | - |
| 11/14/2006 | - | 0.0143 | - | - | - | - | - | - | 0.037 | - |
| 11/15/2006 | - | 0.0068 | - | - | - | - | - | - | 0.037 | - |
| 11/16/2006 | - | 0.0267 | - | - | - | - | - | - | 0.037 | - |
| 11/17/2006 | - | 0.0222 | - | - | - | - | - | - | - | - |
| 11/26/2006 | - | 0.0188 | - | - | - | - | - | - | - | - |
| 11/27/2006 | - | 0.0138 | - | - | - | - | - | - | - | - |
| 11/28/2006 | - | 0.0120 | - | - | - | - | - | - | - | - |
| 11/29/2006 | - | 0.0288 | - | - | - | - | - | - | - | - |
| 11/30/2006 | - | 0.0376 | - | - | - | - | - | - | - | - |
| 12/01/2006 | - | 0.0187 | - | - | - | - | - | - | - | - |
| 05/28/07 | - | - | - | - | - | - | - | - | - | 0.015 |
| 05/29/07 | - | - | - | - | - | - | - | - | 0.036 | 0.015 |
| 05/30/07 | - | 0.0084 | - | - | - | - | - | 0.017 | 0.036 | 0.015 |
| 05/31/07 | - | 0.0103 | - | - | - | - | - | - | 0.036 | 0.015 |
| 06/01/07 | - | 0.0164 | - | - | - | - | - | 0.017 | 0.036 | 0.015 |
| 06/02/07 | - | 0.0305 | - | - | - | - | - | - | - | 0.015 |
| 12/02/07 | - | 0.0241 | - | - | - | - | - | - | - | - |
| 12/03/07 | - | 0.0128 | - | - | - | - | - | - | - | - |
| 12/04/07 | - | 0.0238 | - | - | - | - | - | - | - | - |
| 12/05/07 | - | 0.0158 | - | - | - | - | - | - | - | - |
| 12/06/07 | - | 0.0162 | - | - | - | - | - | - | - | - |
| 12/07/07 | - | 0.0175 | - | - | - | - | - | - | - | |
| 04/13/08 | - | 0.0039 | - | - | - | - | - | - | - | - |
| 04/14/08 | - | 0.0124 | - | - | - | - | - | - | - | - |
| 04/15/08 | - | 0.0229 | - | - | - | - | - | - | - | - |
| 04/16/08 | - | 0.0143 | - | - | - | - | - | - | - | - |
| 04/17/08 | - | 0.0120 | - | - | - | - | - | - | - | - |
| 04/18/08 | - | 0.0149 | - | - | - | - | - | - | - | - |
| 10/26/08 | - | 0.0260 | - | - | - | - | - | - | - | - |
| 10/27/08 | - | 0.0151 | - | - | - | - | - | 0.017 | - | - |
| 10/28/08 | - | 0.0172 | - | - | - | - | - | - | 0.041 | - |
| 10/29/08 | - | 0.0154 | - | - | - | - | - | 0.018 | 0.041 | 0.030 |
| 10/30/08 | - | - | - | - | - | - | - | - | 0.041 | 0.030 |
| 10/31/08 | - | 0.0086 | - | - | - | - | - | - | 0.041 | 0.030 |

| Date | Sodium Hypochlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phosphate | PCL-401 mg/L Copolymer | CL-363 mg/L DMAD | Cuprostat-PF mg/L Azole | H-130M mg/L Quat | Nalco 73551 mg/L EO/PO | Spectrus CT1300 mg/L Quat | H-150M mg/L Quat | MSW 101 mg/L Phosphate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 02/08/09 | - | 0.0197 | - | - | - | - | - | 0.017 | - | - | - |
| 02/09/09 | - | 0.0237 | - | - | - | - | - | 0.017 | - | - | - |
| 02/10/09 | - | 0.0104 | - | - | - | - | - | 0.021 | - | - | - |
| 02/11/09 | - | 0.0155 | - | - | - | - | - | 0.017 | - | - | - |
| 02/12/09 | - | 0.0106 | - | - | - | - | - | 0.017 | - | - | - |
| 02/13/09 | - | - | - | - | - | - | - | - | - | - | - |
| 05/10/09 | - | 0.0129 | - | - | - | - | - | - | - | - | - |
| 05/11/09 | - | 0.0415 | - | - | - | - | - | - | - | 0.0446 | - |
| 05/12/09 | - | 0.0053 | - | - | - | - | - | - | - | 0.0396 | - |
| 05/13/09 | - | 0.0049 | - | - | - | - | - | - | - | 0.0396 | - |
| 05/14/09 | - | <0.0141 | - | - | - | - | - | - | - | 0.0397 | - |
| 05/15/09 | - | <0.0160 | - | - | - | - | - | - | - | - | - |
| 11/15/09 | - | 0.025 | - | - | - | - | - | - | - | - | - |
| 11/16/09 | - | 0.0152 | - | - | - | - | - | - | - | - | - |
| 11/17/09 | - | 0.0255 | - | - | - | - | - | - | - | - | - |
| 11/18/09 | - | 0.0306 | - | - | - | - | - | - | - | - | - |
| 11/19/09 | - | 0.0204 | - | - | | - | - | - | - | - | - |
| 11/20/09 | - | 0.0093 | - | - | - | - | - | - | - | - | - |
| 05/09/10 | - | 0.0192 | - | - | - | - | - | - | - | - | - |
| 05/10/10 | - | 0.0055 | - | - | - | - | - | - | - | - | - |
| 05/11/10 | - | 0.0100 | - | - | - | - | - | - | 0.039 | - | - |
| 05/12/10 | - | 0.0171 | - | - | - | - | - | - | 0.039 | - | - |
| 05/13/10 | - | 0.0041 | - | - | | - | - | - | 0.039 | - | - |
| 05/14/10 | - | 0.0099 | - | - | - | - | - | - | 0.039 | - | - |

| Date | Sodium Hypo-chlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phos-phate | PCL-401 mg/L Copoly-mer | CL-363 mg/L DMAD | Cuprostat -PF mg/L Azole | H-130M mg/L Quat | Nalco 73551 mg/L EO/PO | Spectrus CT1300 mg/L Quat | H-150M mg/L Quat | MSW 101 mg/L Phos-phate | Floguard MS6236 mg/L Phosphate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10/31/10 | - | - | - | - | - | - | - | - | - | - | - | - |
| 11/01/10 | - | 0.0122 | - | - | - | - | - | - | - | - | - | - |
| 11/02/10 | - | 0.0112 | - | - | - | - | - | - | - | - | - | - |
| 11/03/10 | - | 0.0163 | - | - | - | - | - | - | - | - | - | - |
| 11/04/10 | - | 0.0107 | - | - | - | - | - | - | - | - | - | - |
| 11/05/10 | - | 0.0132 | - | - | - | - | - | - | - | - | - | - |
| 05/01/2011 | - | - | - | - | - | - | - | - | - | - | - | - |
| 05/02/2011 | - | - | - | - | - | - | - | - | 0.04 | - | - | - |
| 05/03/2011 | - | - | - | - | - | - | - | - | 0.04 | - | - | - |
| 05/04/2011 | - | 0.0155 | - | - | - | - | - | - | 0.04 | - | - | - |
| 05/05/2011 | - | 0.0179 | - | - | - | - | - | - | 0.04 | - | - | - |
| 05/06/2011 | - | 0.0089 | - | - | - | - | - | - | - | - | - | - |
| 11/06/2011 | - | 0.0168 | - | - | - | - | - | - | - | - | - | - |
| 11/07/2011 | - | 0.0225 | - | - | - | - | - | - | - | - | - | - |
| 11/08/2011 | - | 0.0141 | - | - | - | - | - | - | - | - | - | - |
| 11/09/2011 | - | 0.0239 | - | - | - | - | - | - | - | - | - | - |
| 11/10/2011 | - | 0.0242 | - | - | - | - | - | - | - | - | - | - |
| 11/11/2011 | - | 0.0231 | - | - | - | - | - | - | - | - | - | - |
| 05/06/2012 | - | - | - | - | - | - | - | - | - | - | - | - |
| 05/07/2012 | - | - | - | - | - | - | - | - | - | - | - | - |
| 05/08/2012 | - | - | - | - | - | - | - | - | 0.041 | - | - | - |
| 05/09/2012 | - | 0.0145 | - | - | - | - | - | - | 0.041 | - | - | - |
| 05/10/2012 | - | 0.0298 | - | - | - | - | - | - | 0.041 | - | - | - |
| 05/11/2012 | - | 0.0174 | - | - | - | - | - | - | - | - | - | - |
| 08/12/2012 | - | - | - | - | - | - | - | - | - | - | - | 0.029 |
| 08/13/2012 | - | 0.0256 | - | - | - | - | - | 0.028 | 0.037 | - | - | 0.029 |
| 08/14/2012 | - | 0.0209 | - | - | - | - | - | - | 0.037 | - | - | 0.029 |
| 08/15/2012 | - | 0.0279 | - | - | - | - | - | 0.028 | - | - | - | 0.029 |
| 08/16/2012 | - | 0.0076 | - | - | - | - | - | - | - | - | - | 0.029 |
| 08/17/2012 | - | 0.0446 | - | - | - | - | - | - | - | - | - | 0.032 |
| 05/12/2013 | - | 0.0099 | - | - | - | - | - | - | - | - | - | - |
| 05/13/2013 | - | | - | - | - | - | - | - | - | - | - | 0.064 |
| 05/14/2013 | - | 0.0091 | - | - | - | - | - | 0.039 | - | - | - | 0.064 |
| 05/15/2013 | - | 0.0096 | - | - | - | - | - | 0.039 | - | - | - | 0.064 |
| 05/16/2013 | - | 0.0229 | - | - | - | - | - | - | - | - | - | 0.032 |
| 05/17/2013 | - | 0.0063 | - | - | - | - | - | - | - | - | - | 0.032 |
| 09/15/2013 | - | - | - | - | - | - | - | - | - | - | - | 0.03 |
| 09/16/2013 | - | 0.0072 | - | - | - | - | - | - | 0.0379 | - | - | 0.03 |
| 09/17/2013 | - | 0.0107 | - | - | - | - | - | 0.036 | 0.0379 | - | - | 0.03 |
| 09/18/2013 | - | 0.0217 | - | - | - | - | - | 0.036 | 0.0379 | - | - | 0.03 |
| 09/19/2013 | - | 0.0172 | - | - | - | - | - | - | - | - | - | 0.03 |
| 09/20/2013 | - | 0.0173 | - | - | - | - | - | - | - | - | - | 0.03 |

| Date | Sodium Hypo-chlorite mg/L TRC | Towerbron mg/L TRC | PCL-222 mg/L Phos-phate | PCL-401 mg/L Copoly-mer | CL-363 mg/L DMAD | Cuprostat -PF mg/L Azole | H-130M mg/L Quat | Nalco 73551 mg/L EO/PO | Spectrus CT1300 mg/L Quat | H-150M mg/L Quat | MSW 101 mg/L Phos-phate | Floguard MS6236 mg/L Phosphate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 05/04/2014 | - | 0.0118 | - | - | - | - | - | - | - | - | - | - |
| 05/05/2014 | - | 0.0112 | - | - | - | - | - | - | - | - | - | - |
| 05/06/2014 | - | 0.0096 | - | - | - | - | - | - | - | - | - | - |
| 05/07/2014 | - | 0.0164 | - | - | - | - | - | - | - | - | - | - |
| 05/08/2014 | - | 0.0235 | - | - | - | - | - | - | - | - | - | - |
| 05/09/2014 | - | 0.0110 | - | - | - | - | - | - | - | - | - | - |
| 09/07/2014 | - | - | - | - | - | - | - | - | - | - | - | - |
| 09/08/2014 | - | - | - | - | - | - | - | - | 0.04 | - | - | - |
| 09/09/2014 | - | - | - | - | - | - | - | - | 0.04 | - | - | - |
| 09/10/2014 | - | - | - | - | - | - | - | - | 0.04 | - | - | - |
| 09/11/2014 | - | 0.0070 | - | - | - | - | - | - | - | - | - | - |
| 09/12/2014 | - | 0.0074 | - | - | - | - | - | - | - | - | - | - |
| 08/09/2015 | | - | | | | | | | - | | | - |
| 08/10/2015 | | 0.0195 | | | | | | | 0.03 | | | - |
| 08/11/2015 | | 0.0275 | | | | | | | 0.03 | | | - |
| 08/12/2015 | | 0.0213 | | | | | | | - | | | 0.03 |
| 08/13/2015 | | 0.0192 | | | | | | | - | | | 0.03 |
| 08/14/2015 | | 0.0182 | | | | | | | - | | | 0.03 |
| 10/18/2015 | | 0.0162 | | | | | | | | | | |
| 10/19/2015 | | 0.0125 | | | | | | | | | | |
| 10/20/2015 | | 0.0120 | | | | | | | | | | |
| 10/21/2015 | | 0.0130 | | | | | | | | | | |
| 10/22/2015 | | 0.0174 | | | | | | | | | | |
| 10/23/2015 | | 0.0156 | | | | | | | | | | |
| 05/15/2016 | | - | | | | | | | | | | |
| 05/16/2016 | | 0.0209 | | | | | | | | | | |
| 05/17/2016 | | 0.0210 | | | | | | | | | | |
| 05/18/2016 | | 0.0361 | | | | | | | | | | |
| 05/19/2016 | | 0.0254 | | | | | | | | | | |
| 05/20/2016 | | 0.0261 | | | | | | | | | | |
| 07/31/2016 | | - | | | | | | | - | | | |
| 08/01/2016 | | 0.0091 | | | | | | | 0.03 | | | |
| 08/02/2016 | | 0.0093 | | | | | | | 0.03 | | | |
| 08/03/2016 | | 0.0209 | | | | | | | 0.03 | | | |
| 08/04/2016 | | - | | | | | | | - | | | |
| 08/05/2016 | | - | | | | | | | - | | | |
| 04/30/2017 | | - | | | | | | | | | | |
| 05/01/2017 | | 0.0298 | | | | | | | | | | |
| 05/02/2017 | | 0.0218 | | | | | | | | | | |
| 05/03/2017 | | 0.0260 | | | | | | | | | | |
| 05/04/2017 | | - | | | | | | | | | | |
| 05/05/2017 | | - | | | | | | | | | | |

| Date | Towerbrom mg/L TRC | Floguard MS 6237 mg/L Phosphate/Zinc | Spectrus BD1500 mg/L Quat | Nalco 73551 mg/L EO/PO | Spectrus CT1300 mg/L Quat | Floguard MS6236 mg/L Phosphate |
|---|---|---|---|---|---|---|
| 07/23/2017 | - | - | - | | | |
| 07/24/2017 | 0.0124 | 0.04/0.01 | - | | | |
| 07/25/2017 | 0.0081 | 0.04/0.01 | - | | | |
| 07/26/2017 | 0.0232 | 0.04/0.01 | 0.03 | | | |
| 07/27/2017 | 0.0179 | - | - | | | |
| 07/28/2017 | 0.0296 | - | 0.03 | | | |
| 5/13/2018 | | | | | | |
| 5/14/2018 | | | | | | |
| 5/15/2018 | 0.0229 | | 0.030 | | | 0.0275 |
| 5/16/2018 | 0.0159 | | 0.030 | | | 0.0275 |
| 5/17/2018 | 0.0133 | | 0.030 | | | 0.0275 |
| 5/18/2018 | | | | | | |
| 10/07/2018 | - | | - | | - | - |
| 10/08/2018 | 0.0221 | | - | | - | 0.02852 |
| 10/09/2018 | 0.0098 | | - | | 0.0332 | 0.02852 |
| 10/10/2018 | 0.0187 | | 0.031 | | 0.0332 | 0.02852 |
| 10/11/2018 | 0.0200 | | 0.031 | | - | 0.02852 |
| 10/12/2018 | 0.0187 | | 0.031 | | - | 0.02852 |
| 04/28/2019 | - | | - | | - | - |
| 04/29/2019 | 0.0069 | | 0.047 | | - | - |
| 04/30/2019 | 0.0109 | | 0.047 | | - | - |
| 05/01/2019 | 0.0196 | | - | | - | - |
| 05/02/2019 | 0.0341 | | - | | - | - |
| 05/03/2019 | 0.0281 | | 0.047 | | - | |
| 08/04/2016 | - | | - | | - | - |
| 08/05/2019 | 0.0227 | | 0.047 | | - | 0.02852 |
| 08/06/2019 | 0.0071 | | - | | 0.0332 | 0.02852 |
| 08/07/2019 | 0.0117 | | 0.047 | | 0.0332 | 0.02852 |
| 08/08/2019 | 0.0142 | | - | | 0.0332 | 0.02852 |
| 08/09/2019 | 0.0078 | | 0.047 | | - | 0.02852 |
| 5/3/2020 | - | | - | | - | |
| 5/4/2020 | - | | - | | - | |
| 5/5/2020 | - | | - | | - | |
| 5/6/2020 | 0.0045 | | 0.049 | | 0.0332 | |
| 5/7/2020 | 0.0055 | | - | | 0.0332 | |
| 5/8/2020 | 0.0183 | | 0.049 | | 0.0332 | |

Sequoyah Nuclear Plant Biomonitoring
May 05 – 12, 2020

Appendix C

Chain of Custody Records and
Toxicity Test Bench Sheets

# BIOMONITORING CHAIN OF CUSTODY RECORD



Client: TVA

Project Name: Sequoyah NP Toxicity

PO. Number: N/A

Facility Sampled: Sequoyah NP

NPDES Number: TN0026450

Collected By: Kelly Robinette, Obie Moore

Environmental Testing Solution, Inc.
351 Depot Street.
Asheville, NC
28801
Phone: 828-350-9364
Fax: 828-350-9368

Delivered By (Circle One):
FedEx UPS Bus Client Sonic
Other (specify):

General Comments:
101 Bottle Comp @0720 5-4-20
INT Bottle Comp @ 0800 5-4-20

| Field Identification / Sample Description | Grab/Comp | Collection Date/Time | | Container Number & Volume Collected | Flow (MGD) | Rain Event? (Mark as Appropriate) | | | | Laboratory Use PROJECT # 15061 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | --- | Date (mm/dd/yy) | Time (EST) | --- | --- | Yes | If Yes, Inches | No | Trace | ETS Log Number | Arrival Temp. (°C) | By | Time ET | Appearance |
| SQN-101-TOX | Comp | Start 05/03/20 | 0730 | | | | | | | 200504.04 | 1.6°C | JH | 1310 | * |
| | | End 05/04/20 | 0630 | 1 (2.5gal) | 1769.65 | | | ✓ | | | | | | |
| SQN-INT-TOX | Comp | Start 05/03/20 | 0730 | | | | | | | 200504.05 | 1.5°C | JH | 1310 | * |
| | | End 05/04/20 | 0630 | 1 (2.5 gal) | 1769.65 | | | ✓ | | * CUSTODY SEALS INTACT. SAMPLES RECEIVED IN GOOD CONDITION. JH | | | | |

Sample Custody – Fill In From Top Down

| Relinquished By (Signature): | Date/Time | Received By (Signature): | Date/Time |
|---|---|---|---|
| Kelly Robinette TVA | 5-4-20 @ 0910 ET | B R Shiler Sonic Delivery | 05-04-2020 09:10 ET |
| B R Shiler Sonic Delivery | 05-04-20 13:10 ET | [illegible] ETS | 05-04-20 1310 ET |
| | | | |
| | | | |
| | | | |

# Whole Effluent Sample Receipt Log

Page S1

*Sample temperature performed using verified General Use Thermometer SN: 17065003

| Date Received | Time Received | Received by | Received from | *Sample Temp. (°C) | Project number | Sample number | Sample name and description | State | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 05-04-20 | 1218 | K. Keenan | S. Byers | 2.6 | 15059 | 200504 .01 | Marshall WWTP | NC | |
| 05-04-20 | 1245 | J. Sumner | A. Coates | 1.8, 2.0 | 15060 | 200504 .02 | Eastman Chemical - River Water | TN | |
| 05-04-20 | 1245 | J. Sumner | A. Coates | 1.6, 1.7 | 15060 | 200504 .03 | Eastman Chemical - Blended Effluent | TN | |
| 05-04-20 | 1310 | J. Sumner | Sonic Delivery | 1.6 | 15061 | 200504 .04 | TVA / SQN Outfall 101 | TN | |
| 05-04-20 | 1310 | J. Sumner | Sonic Delivery | 1.5 | 15061 | 200504 .05 | TVA / SQN Intake | TN | |

# BIOMONITORING CHAIN OF CUSTODY RECORD



Client: TVA

Project Name: Sequoyah NP Toxicity

P.O. Number: N/A

Facility Sampled: Sequoyah NP

NPDES Number: TN0026450

Collected By: Kelly Rolinette, Obie Moore

Environmental Testing Solution, Inc.
351 Depot Street.
Asheville, NC
28801
Phone: 828-350-9364
Fax: 828-350-9368

Delivered By (Circle One):
FedEx UPS Bus Client Sonic
Other (specify):

General Comments:
101 Bottle Comp @ 0730
JNT Bottle Comp @ 0800

| Field Identification / Sample Description | Grab/Comp | Collection Date/Time | | Container Number & Volume Collected | Flow (MGD) | Rain Event? (Mark as Appropriate) | | | | Project # 15061 Laboratory Use | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | --- | Date (mm/dd/yy) | Time (EST) | --- | --- | Yes | If Yes, Inches | No | Trace | ETS Log Number | Arrival Temp. (°C) | By | Time ET | Appearance |
| SQN-101-TOX | Comp | Start 05/05/20 | 0730 | | | | | | | 200506.30 | 1.2-C | JH | 1250 | * |
| | | End 05/06/20 | 0630 | 1 (2.5gal) | 1744.89 | | | ✓ | | | | | | |
| SQN-INT-TOX | Comp | Start 05/05/20 | 0730 | | | | | | | 200506.31 | 1.3-C | JH | 1250 | * |
| | | End 05/06/20 | 0630 | 1 (2.5 gal) | 1744.89 | | | ✓ | | * CUSTODY SEALS INTACT. SAMPLES RECEIVED IN GOOD CONDITION. JH | | | | |

Sample Custody – Fill In From Top Down

| Relinquished By (Signature): | Date/Time | Received By (Signature): | Date/Time |
|---|---|---|---|
| Kelly Rolinette TVA | 05-06-20 / 0850 ET | B R Schuler SONIC Delivered | 05-06-20 / 08:50 ET |
| B R Schuler SONIC Delivered | 05-06-20 / 12:50 ET | JH ETS | 05-06-20 1250 ET |
| | | | |
| | | | |
| | | | |

Independent Review by Ashley E. Keenan

# Whole Effluent Sample Receipt Log



*Sample temperature performed using verfied General Use Thermometer SN: 170155003

| Date Received | Time Received | Received by | Received from | *Sample Temp. (°C) | Project number | Sample number | Sample name and description | State | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 05-06-20 | 0945 | K. Keenan | Fed - Ex | 1.1 | 15084 | 200506 .01 | South Cary WWTP | NC | |
| 05-06-20 | 0945 | K. Keenan | Fed - Ex | 1.8 | 15085 | 200506 .02 | PCS Phosphate, Inc. | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.7 | 15086 | 200506 .03 | Allen SS - 006 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.0 | 15087 | 200506 .04 | Apex WRF | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.5 | 15088 | 200506 .05 | Belews Creek SS - 006 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.5 | 15089 | 200506 .06 | Belews Creek SS - 006A | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.6 | 15090 | 200506 .07 | Buck CTCC - 007 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.1 | 15091 | 200506 .08 | Brunswick County | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.5 | 15092 | 200506 .09 | Daikin | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.8 | 15093 | 200506 .10 | Durham County | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.2 | 15094 | 200506 .11 | Hoke County WWTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.2 | 15095 | 200506 .12 | Laurinburg WTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.2 | 15096 | 200506 .13 | Laurinburg WWTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.8 | 15097 | 200506 .14 | James Loughlin WWTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.4 | 15098 | 200506 .15 | Marshall SS - 002 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.4 | 15099 | 200506 .16 | Marshall SS - 005 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.5 | 15100 | 200506 .17 | North Cary WWTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.2 | 15101 | 200506 .18 | Roseboro WWTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.7 | 15102 | 200506 .19 | Scarlett Acres MHP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.5 | 15103 | 200506 .20 | Western Wake | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.2 | 15104 | 200506 .21 | Pender County WTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 4.5 | 15105 | 200506 .22 | Sutton - 001 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 4.5 | 15106 | 200506 .23 | Sutton - 008 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.0 | 15107 | 200506 .24 | Washington WWTP | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 2.0 | 15108 | 200506 .25 | McGuire NS - 001 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.4 | 15109 | 200506 .26 | McGuire NS - 002 | NC | |
| 05-06-20 | 1116 | K. Keenan | Fed - Ex | 1.0 | 15110 | 200506 .27 | Morehead City WWTP | NC | |
| 05-06-20 | 1240 | J. Sumner | A. Coates | 0.6/0.6 | 15060 | 200506 .28 | Eastman Chemical - River Water | TN | |
| 05-06-20 | 1240 | J. Sumner | A. Coates | 0.6/0.5 | 15060 | 200506 .29 | Eastman Chemical - Blended Effluent | TN | |
| 05-06-20 | 1250 | J. Sumner | TVA Courier | 1.2 | 15061 | 200506 .30 | TVA / SQN Outfall 101 | TN | |
| 05-06-20 | 1250 | J. Sumner | TVA Courier | 1.3 | 15061 | 200506 .31 | TVA / SQN Intake | TN | |

# BIOMONITORING CHAIN OF CUSTODY RECORD



Client: TVA

Project Name: Sequoyah NP Toxicity

P.O. Number: N/A

Facility Sampled: Sequoyah NP

NPDES Number: TN0026450

Collected By: Kelly Rainwater, Obie Moore

Environmental Testing Solution, Inc.
351 Depot Street.
Asheville, NC
28801
Phone: 828-350-9364
Fax: 828-350-9368

Delivered By (Circle One):
FedEx UPS Bus Client Sonic
Other (specify):

General Comments:
Bottle 101 comp @ 0715
Bottle INT comp @ 0745

project # 15061

| Field Identification / Sample Description | Grab/Comp | Collection Date/Time | | Container Number & Volume Collected | Flow (MGD) | Rain Event? (Mark as Appropriate) | | | | Laboratory Use | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | — | Date (mm/dd/yy) | Time (EST) | — | — | Yes | If Yes, Inches | No | Trace | ETS Log Number | Arrival Temp. (°C) | By | Time ET | Appearance |
| SQN-101-TOX | Comp | Start 05/07/20 | 0730 | | | | | | | 200508.22 | 1.1, 1.0°C | JA | 1250 | * |
| | | End 05/08/20 | 0630 | 1 (2.5gal) | 1753.36 | | | ✓ | | | | | | |
| SQN-INT-TOX | Comp | Start 05/07/20 | 0730 | | | | | | | 200508.23 | 2.1°C | JA | 1250 | * |
| | | End 05/08/20 | 0630 | 2 (2.5 gal) | 1753.36 | | | ✓ | | * CUSTODY SEALS INTACT. SAMPLES RECEIVED IN GOOD CONDITION. | | | | |

Sample Custody – Fill In From Top Down

| Relinquished By (Signature): | | Date/Time | Received By (Signature): | | Date/Time |
|---|---|---|---|---|---|
| Kelly Rainwater | TVA | 5-8-20 @ 0840 ET | B.R. Shelby | SONIC DELIVERY | 05-08-20 0840 ET |
| B.R. Shelby | SONIC DELIVERY | 05-08-20 / 12:50 ET | [illegible] | ETS | 05-08-20 1250 ET |
| | | | | | |
| | | | | | |
| | | | | | |

# Whole Effluent Sample Receipt Log



*Sample temperature performed using verfied General Use Thermometer SN: 170755003

| Date Received | Time Received | Received by | Received from | *Sample Temp. (°C) | Project number | Sample number | Sample name and description | State | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.0 | 15086 | 200508 .01 | Allen SS - 006 | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.6 | 15087 | 200508 .02 | Apex WRF | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.5 | 15088 | 200508 .03 | Belews Creek SS - 006 | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.5 | 15089 | 200508 .04 | Belews Creek SS - 006A | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.3 | 15090 | 200508 .05 | Buck CTCC - 007 | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.5 | 15091 | 200508 .06 | Brunswick County | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.0 | 15092 | 200508 .07 | Dalkin | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 1.7 | 15093 | 200508 .08 | Durham County | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.4 | 15094 | 200508 .09 | Hoke County WWTP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.4 | 15095 | 200508 .10 | Laurinburg WTP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.4 | 15096 | 200508 .11 | Laurinburg WWTP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.6 | 15097 | 200508 .12 | James Loughlin WWTP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.2 | 15098 | 200508 .13 | Marshall SS - 002 | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.2 | 15099 | 200508 .14 | Marshall SS - 005 | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.4 | 15100 | 200508 .15 | North Cary WWTP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.4 | 15101 | 200508 .16 | Roseboro WWTP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 1.8 | 15102 | 200508 .17 | Scarlett Acres MHP | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 3.0 | 15103 | 200508 .18 | Western Wake | NC | |
| 05-08-20 | 1013 | K. Keenan | Fed - Ex | 2.0 | 15084 | 200508 .19 | South Cary WWTP | NC | |
| 05-08-20 | 1245 | J. Sumner | A. Coates | 2.3/2.1 | 15060 | 200508 .20 | Eastman Chemical - River Water | TN | |
| 05-08-20 | 1245 | J. Sumner | A. Coates | 2.9/3.0 | 15060 | 200508 .21 | Eastman Chemical - Blended Effluent | TN | |
| 05-08-20 | 1250 | J. Sumner | TVA Courier | 1.1/1.0 | 15061 | 200508 .22 | TVA / SQN Outfall 101 | TN | |
| 05-08-20 | 1250 | J. Sumner | TVA Courier | 2.1 | 15061 | 200508 .23 | TVA / SQN Intake | TN | |

Environmental Testing Solutions, Inc.

# Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1000.0)
## Species: *Pimephales promelas*

**Client:** TVA, Sequoyah Nuclear Plant
**NPDES #:** TN 0026450
**Project #:** 15061

**County:** Hamilton
**Outfall #:** 101
**Treatment:** UV (Each concentration was UV-treated for 2-minutes to remove pathogenic interferences.)

### *Dilution preparation:*

| Dilution prep (%) | 10.7 | 21.4 | 42.8 | 85.6 | 100 |
|---|---|---|---|---|---|
| Effluent volume (mL) | 214 | 428 | 856 | 1712 | 2000 |
| Diluent volume (mL) | 1786 | 1572 | 1144 | 288 | 0 |
| Total volume (mL) | 2000 | 2000 | 2000 | 2000 | 2000 |

Sample was not aerated or treated unless otherwise noted on this form. Sample was warmed to 25.0 ± 1.0°C in a warm water bath and then diluted to the test concentrations with moderately hard synthetic water (MHSW).

### *Test organism information:*

| | |
|---|---|
| Organism source: | In-house culture |
| Age: | < 24-hours old |
| Spawn date: | 04-30-20 |
| Hatch dates and times: | 05-04-20 1500 TO 05-05-20 0605 |
| Transfer vessel information: | pH (S.U.) = 8.26<br>Temperature (°C) = 24.1 |
| Average transfer volume (mL): | < 0.25 mL |

### *Test information:*

| | |
|---|---|
| Randomizing template: | GREEN |
| Incubator number and shelf location: | 7E |
| *Artemia* CHM number: | CHM1048 |
| ***Drying information for weight determination:*** | |
| Date / Time in oven: | 05-12-20 0810 |
| *Initial oven temperature: | 60°C |
| Date / Time out of oven: | 05-13-20 0810 |
| *Final oven temperature: | 60°C |
| Total drying time: | 24 HOURS |

*60°C Oven, Thermometer SN: 14-98585

### *Daily feeding and renewal information:*

| Day | Date | Morning feeding | | Afternoon feeding | | Test initiation, renewal, or termination | | Sample numbers used | | MHSW batch used |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Time | Analyst | Outfall 101 | Intake | |
| 0 | 05-05-20 | 0610 | JH | 1230 | JH | 0856 | JH | 200504.04 | 200504.05 | 04-29-20B |
| 1 | 05-06-20 | 0530 | JH | 1130 | JH | 0807 | JH | ↓ | ↓ | ↓ |
| 2 | 05-07-20 | 0600 | JH | 1200 | JH | 0839 | JH | 200505.30 ~~200506.30~~ | 200506.31 ~~200506.31~~ | 04-29-20D |
| 3 | 05-08-20 | 0600 | JH | 1200 | JH | 0833 | JH | ↓ | ↓ | 04-29-20D |
| 4 | 05-09-20 | 0700 | JH | 1300 | JH | 0933 | JH | 200508.22 | 200508.23 | 05-07-20B |
| 5 | 05-10-20 | 0600 | JH | 1200 | JH | 0831 | JH | ↓ | ↓ | ↓ |
| 6 | 05-11-20 | 0600 | JH | 1200 | JH | 0830 | JH | | | |
| 7 | 05-12-20 | | | | | 0808 | JH | | | |

### *Chemical analyses:*

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2011 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2011 | YSI Model 52CE | 18D104324 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2011 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2011 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2011 | Not applicable | Not applicable |
| Chlorine, Total Residual | 0.1 mg/L | ORION 97-70-1977 | Accumet AB250 | 92349123 |
| Temperature | 0.1 °C | SM 2550B-2010 | Digital Thermometer | 13066685 |

| *Control information:* UV-TREATED | | Acceptance criteria |
|---|---|---|
| % Mortality: | 0% | ≤ 20% |
| Average weight per initial larvae: | 0.737 | |
| Average weight per surviving larvae: | 0.737 | ≥ 0.25mg/larvae |

| *Summary of test endpoints:* | |
|---|---|
| 7-day $LC_{50}$ (%) | >100% |
| NOEC (%) | 100% |
| LOEC (%) | >100% |
| ChV (%) | >100% |
| $IC_{25}$ (%) | >100% |

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***
**Client:** **TVA / Sequoyah Nuclear Plant, Outfall 101** **Date:** **05-05-20**

***Survival and Growth Data***

| Day | CONTROL | | | | 10.7% | | | | 21.4% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 7 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| *A = Pan weight (mg)<br>Tray color code: light blue<br>Analyst: TS<br>Date: 04.30.20 | 15.87 | 14.75 | 14.64 | 15.64 | 15.64 | 16.12 | 15.83 | 15.44 | 15.54 | 15.24 | 15.50 | 14.85 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 05-13-20 | 22.84 | 21.88 | 22.28 | 23.39 | 23.21 | 23.38 | 22.72 | 22.92 | 22.66 | 22.18 | 22.67 | 22.50 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 6.97 | 7.13 | 7.64 | 7.75 | 7.57 | 7.26 | 6.89 | 7.48 | 7.12 | 6.94 | 7.17 | 7.65 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0.697 | 0.713 | 0.764 | 0.775 | 0.757 | 0.726 | 0.689 | 0.748 | 0.712 | 0.694 | 0.717 | 0.765 |
| Average weight per initial number of larvae (mg) | 0.737 | | | | 0.730 | | | | 0.722 | | | |
| Percent reduction from control (%) | | | | | 1.0% | | | | 2.1% | | | |

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

| Comments: |
|---|
| |
| |

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***
**Client:** **TVA / Sequoyah Nuclear Plant, Outfall 101** **Date:** **05-05-20**

***Survival and Growth Data***

| Day | 42.8% | | | | 85.6% | | | | 100% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 7 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| *A = Pan weight (mg)<br>Tray color code: light blue<br>Analyst: TS<br>Date: 04.30.20 | 15.79 | 14.56 | 15.87 | 14.69 | 15.66 | 16.15 | 15.19 | 15.21 | 15.03 | 13.95 | 13.63 | 15.99 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JJ<br>Date: 05.13.20 | 22.85 | 20.93 | 22.92 | 22.04 | 23.04 | 23.01 | 22.12 | 21.97 | 22.62 | 21.25 | 21.08 | 23.23 |
| C = Larvae weight (mg) = B – A<br>Analyst: JJ | 7.06 | 6.37 | 7.05 | 7.35 | 7.38 | 6.86 | 6.93 | 6.76 | 7.59 | 7.30 | 7.45 | 7.24 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JJ | 0.706 | 0.637 | 0.705 | 0.735 | 0.738 | 0.686 | 0.693 | 0.676 | 0.759 | 0.730 | 0.745 | 0.724 |
| Average weight per initial number of larvae (mg) | 0.696 | | | | 0.698 | | | | 0.740 | | | |
| Percent reduction from control (%) | | 5.6% | | | | 5.3% | | | | -0.3% | | |

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

***Comments:***

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***
**Client:** **TVA / Sequoyah Nuclear Plant, Outfall 101** **Date:** **05-05-20**

***Survival and Growth Data***

| Day | 100% Intake | | | | Control, Non-treated | | | |
|---|---|---|---|---|---|---|---|---|
| | Y | Z | AA | BB | CC | DD | EE | FF |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 7 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| *A = Pan weight (mg)<br>Tray color code: light blue<br>Analyst: TS<br>Date: 04.30.20 | 15.44 | 15.05 | 15.56 | 14.06 | 14.94 | 14.92 | 15.06 | 15.13 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 05.13.20 | 22.24 | 21.06 | 21.80 | 20.58 | 21.55 | 22.39 | 23.28 | 22.57 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 6.80 | 6.01 | 6.24 | 6.52 | 6.61 | 7.47 | 8.22 | 7.44 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0.680 | 0.601 | 0.624 | 0.652 | 0.661 | 0.747 | 0.822 | 0.744 |
| Average weight per initial number of larvae (mg) / Percent reduction from control (%) | 0.639 | | 13.3 % | | 0.744 | | 06.04.20 JL ~~8.8 %~~ NA | |

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

| ***Comments:*** |
|---|
| |
| |

Independent Review by Kelley E. Keenan: K

Independent Review by: Kelley E. Keenan

# TVA / Sequoyah Nuclear Plant, Outfall 101
## May 05-12, 2020

Environmental Testing Solutions, Inc.

*Pimephales promelas* Chronic Whole Effluent Toxicity Test
EPA-821-R-02-013, Method 1000.0

### Quality Control
### Verification of Data Entry, Calculations, and Statistical Analyses

Project number: 15061

| Concentration (%) | Replicate | Initial number of larvae | Final number of larvae | A = Pan weight (mg) | B = Pan + Larvae weight (mg) | Larvae weight (mg) = B - A | Not for Compliance Assessment, Internal Laboratory QC: Weight / Surviving number of larvae (mg) | Mean weight / Surviving number of larvae (mg) | Coefficient of variation (Mean weight per surviving number of larvae) (%) | Weight / Initial number of larvae (mg) | Mean survival (%) | Mean weight / Initial number of larvae (mg) | Coefficient of variation (Mean weight per initial number of larvae) (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control, Non-treated | CC | 10 | 10 | 14.94 | 21.55 | 6.61 | 0.661 | 0.744 | 8.8 | 0.661 | 100.0 | 0.744 | 8.8 | Not applicable |
| | DD | 10 | 10 | 14.92 | 22.39 | 7.47 | 0.747 | | | 0.747 | | | | |
| | EE | 10 | 10 | 15.06 | 23.28 | 8.22 | 0.822 | | | 0.822 | | | | |
| | FF | 10 | 10 | 15.13 | 22.57 | 7.44 | 0.744 | | | 0.744 | | | | |
| Control, UV-treated | A | 10 | 10 | 15.87 | 22.84 | 6.97 | 0.697 | 0.737 | 5.2 | 0.697 | 100.0 | 0.737 | 5.2 | Not applicable |
| | B | 10 | 10 | 14.75 | 21.88 | 7.13 | 0.713 | | | 0.713 | | | | |
| | C | 10 | 10 | 14.64 | 22.28 | 7.64 | 0.764 | | | 0.764 | | | | |
| | D | 10 | 10 | 15.64 | 23.39 | 7.75 | 0.775 | | | 0.775 | | | | |
| 10.7% | E | 10 | 10 | 15.64 | 23.21 | 7.57 | 0.757 | 0.730 | 4.1 | 0.757 | 100.0 | 0.730 | 4.1 | 1.0 |
| | F | 10 | 10 | 16.12 | 23.38 | 7.26 | 0.726 | | | 0.726 | | | | |
| | G | 10 | 10 | 15.83 | 22.72 | 6.89 | 0.689 | | | 0.689 | | | | |
| | H | 10 | 10 | 15.44 | 22.92 | 7.48 | 0.748 | | | 0.748 | | | | |
| 21.4% | I | 10 | 10 | 15.54 | 22.66 | 7.12 | 0.712 | 0.722 | 4.2 | 0.712 | 100.0 | 0.722 | 4.2 | 2.1 |
| | J | 10 | 10 | 15.24 | 22.18 | 6.94 | 0.694 | | | 0.694 | | | | |
| | K | 10 | 10 | 15.50 | 22.67 | 7.17 | 0.717 | | | 0.717 | | | | |
| | L | 10 | 10 | 14.85 | 22.50 | 7.65 | 0.765 | | | 0.765 | | | | |
| 42.8% | M | 10 | 10 | 15.79 | 22.85 | 7.06 | 0.706 | 0.696 | 6.0 | 0.706 | 100.0 | 0.696 | 6.0 | 5.6 |
| | N | 10 | 10 | 14.56 | 20.93 | 6.37 | 0.637 | | | 0.637 | | | | |
| | O | 10 | 10 | 15.87 | 22.92 | 7.05 | 0.705 | | | 0.705 | | | | |
| | P | 10 | 10 | 14.69 | 22.04 | 7.35 | 0.735 | | | 0.735 | | | | |
| 85.6% | Q | 10 | 10 | 15.66 | 23.04 | 7.38 | 0.738 | 0.698 | 3.9 | 0.738 | 100.0 | 0.698 | 3.9 | 5.3 |
| | R | 10 | 10 | 16.15 | 23.01 | 6.86 | 0.686 | | | 0.686 | | | | |
| | S | 10 | 10 | 15.19 | 22.12 | 6.93 | 0.693 | | | 0.693 | | | | |
| | T | 10 | 10 | 15.21 | 21.97 | 6.76 | 0.676 | | | 0.676 | | | | |
| 100% | U | 10 | 10 | 15.03 | 22.62 | 7.59 | 0.759 | 0.740 | 2.1 | 0.759 | 100.0 | 0.740 | 2.1 | -0.3 |
| | V | 10 | 10 | 13.95 | 21.25 | 7.30 | 0.730 | | | 0.730 | | | | |
| | W | 10 | 10 | 13.63 | 21.08 | 7.45 | 0.745 | | | 0.745 | | | | |
| | X | 10 | 10 | 15.99 | 23.23 | 7.24 | 0.724 | | | 0.724 | | | | |
| 100% Intake | Y | 10 | 10 | 15.44 | 22.24 | 6.80 | 0.680 | 0.639 | 5.4 | 0.680 | 100.0 | 0.639 | 5.4 | 13.3 |
| | Z | 10 | 10 | 15.05 | 21.06 | 6.01 | 0.601 | | | 0.601 | | | | |
| | AA | 10 | 10 | 15.56 | 21.80 | 6.24 | 0.624 | | | 0.624 | | | | |
| | BB | 10 | 10 | 14.06 | 20.58 | 6.52 | 0.652 | | | 0.652 | | | | |

**Outfall 101:**
Dunnett's MSD value: 0.0539
PMSD: 7.3

**Intake:**
Dunnett's MSD value: 0.0498
PMSD: 6.8

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test

Lower PMSD bound determined by USEPA (10th percentile) = 12%
Upper PMSD bound determined by USEPA (90th percentile) = 30%

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b)

USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.

Reviewed and Reviewed by Jim Summer

# TVA / Sequoyah Nuclear Plant, Outfall 101
## May 05-12, 2020

## Statistical Analyses

Environmental Testing Solutions, Inc.

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | PpFRCR | Sample ID: | TVA /SQN Outfall 101 |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date: | May 2020 | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | PP-Pimephales promelas |
| Comments: | UV-treated | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Non-Control | 0.6610 | 0.7470 | 0.8220 | 0.7440 |
| UV-Control | 0.6970 | 0.7130 | 0.7640 | 0.7750 |
| 10.7 | 0.7570 | 0.7260 | 0.6890 | 0.7480 |
| 21.4 | 0.7120 | 0.6940 | 0.7170 | 0.7650 |
| 42.8 | 0.7060 | 0.6370 | 0.7050 | 0.7350 |
| 85.6 | 0.7380 | 0.6860 | 0.6930 | 0.6760 |
| 100 | 0.7590 | 0.7300 | 0.7450 | 0.7240 |
| Intake | 0.6800 | 0.6010 | 0.6240 | 0.6520 |

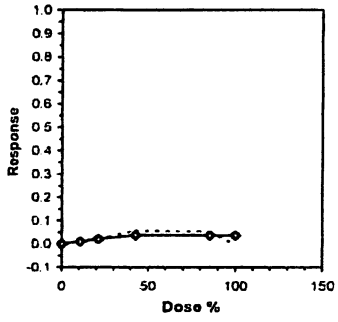
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Non-Control | 0.7435 | 1.0085 | 0.7435 | 0.6610 | 0.8220 | 8.847 | 4 | | | | | |
| UV-Control | 0.7373 | 1.0000 | 0.7373 | 0.6970 | 0.7750 | 5.164 | 4 | | | | 0.7373 | 1.0000 |
| 10.7 | 0.7300 | 0.9902 | 0.7300 | 0.6890 | 0.7570 | 4.147 | 4 | 0.324 | 2.410 | 0.0539 | 0.7300 | 0.9902 |
| 21.4 | 0.7220 | 0.9793 | 0.7220 | 0.6940 | 0.7650 | 4.200 | 4 | 0.681 | 2.410 | 0.0539 | 0.7220 | 0.9793 |
| 42.8 | 0.6958 | 0.9437 | 0.6958 | 0.6370 | 0.7350 | 5.974 | 4 | 1.854 | 2.410 | 0.0539 | 0.7112 | 0.9646 |
| 85.6 | 0.6983 | 0.9471 | 0.6983 | 0.6760 | 0.7380 | 3.925 | 4 | 1.743 | 2.410 | 0.0539 | 0.7112 | 0.9646 |
| 100 | 0.7395 | 1.0031 | 0.7395 | 0.7240 | 0.7590 | 2.125 | 4 | -0.101 | 2.410 | 0.0539 | 0.7112 | 0.9646 |
| Intake | 0.6393 | 0.8671 | 0.6393 | 0.6010 | 0.6800 | 5.358 | 4 | | | | | |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.96763 | 0.884 | -0.1754 | -0.6518 |
| Bartlett's Test indicates equal variances (p = 0.77) | 2.52035 | 15.0863 | | |
| The control means are not significantly different (p = 0.87) | 0.16447 | 2.44691 | | |

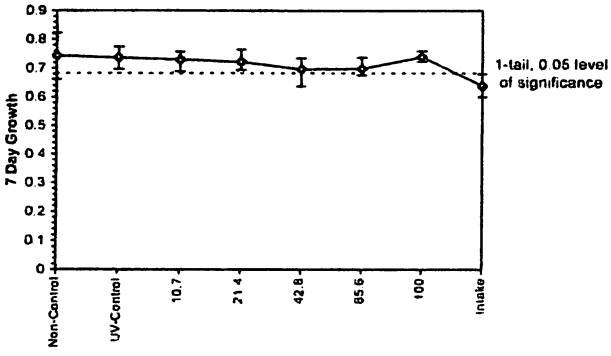
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 100 | >100 | | 1 | 0.05394 | 0.07316 | 0.00147 | 0.001 | 0.24805 | 5, 18 |
| Treatments vs UV-Control | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | Skew |
|---|---|---|---|---|
| IC05 | >100 | | | |
| IC10 | >100 | | | |
| IC15 | >100 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |




**Dose-Response Plot**




Independent Review by Kelley E. Keenan

# TVA / Sequoyah Nuclear Plant, Outfall 101 - Intake
# May 05-12, 2020

## Statistical Analyses

Environmental Testing Solutions, Inc.

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | PpFRCR | Sample ID: | TVA / SQN Intake |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | DMR-Discharge Monitoring Report |
| Sample Date: | May 2020 | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | PP-Pimephales promelas |
| Comments: | UV-treated | | | | |

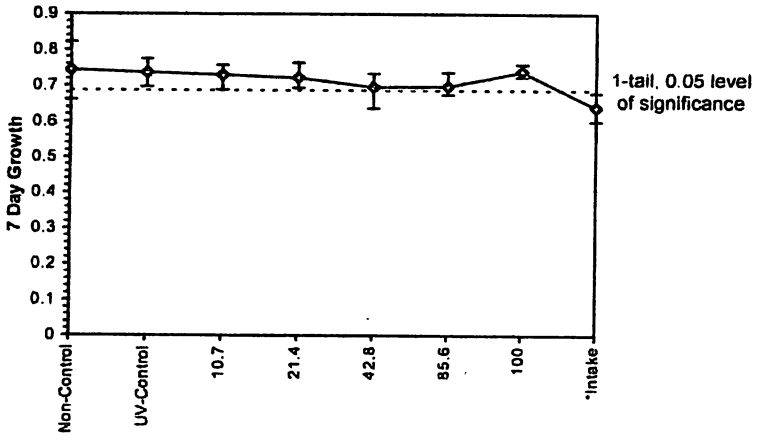
| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Non-Control | 0.6610 | 0.7470 | 0.8220 | 0.7440 |
| UV-Control | 0.6970 | 0.7130 | 0.7640 | 0.7750 |
| 10.7 | 0.7570 | 0.7260 | 0.6890 | 0.7480 |
| 21.4 | 0.7120 | 0.6940 | 0.7170 | 0.7650 |
| 42.8 | 0.7060 | 0.6370 | 0.7050 | 0.7350 |
| 85.6 | 0.7380 | 0.6860 | 0.6930 | 0.6760 |
| 100 | 0.7590 | 0.7300 | 0.7450 | 0.7240 |
| Intake | 0.6800 | 0.6010 | 0.6240 | 0.6520 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| Non-Control | 0.7435 | 1.0085 | 0.7435 | 0.6610 | 0.8220 | 8.847 | 4 | | | |
| UV-Control | 0.7373 | 1.0000 | 0.7373 | 0.6970 | 0.7750 | 5.164 | 4 | | | |
| 10.7 | 0.7300 | 0.9902 | 0.7300 | 0.6890 | 0.7570 | 4.147 | 4 | | | |
| 21.4 | 0.7220 | 0.9793 | 0.7220 | 0.6940 | 0.7650 | 4.200 | 4 | | | |
| 42.8 | 0.6958 | 0.9437 | 0.6958 | 0.6370 | 0.7350 | 5.974 | 4 | | | |
| 85.6 | 0.6983 | 0.9471 | 0.6983 | 0.6760 | 0.7380 | 3.925 | 4 | | | |
| 100 | 0.7395 | 1.0031 | 0.7395 | 0.7240 | 0.7590 | 2.125 | 4 | | | |
| *Intake | 0.6393 | 0.8671 | 0.6393 | 0.6010 | 0.6800 | 5.358 | 4 | 3.827 | 1.943 | 0.0498 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.8868 | 0.749 | 0.01871 | -2.0524 |
| F-Test indicates equal variances (p = 0.87) | 1.23588 | 47.4683 | | |
| The control means are not significantly different (p = 0.87) | 0.16447 | 2.44691 | | |

| Hypothesis Test (1-tail, 0.05) | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|
| Homoscedastic t Test indicates significant differences Treatments vs UV-Control | 0.04976 | 0.06749 | 0.01921 | 0.00131 | 0.00869 | 1, 6 |

**Dose-Response Plot**




Independent Review by Kelley E. Keenan.

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** **Date:** 05-05-20
**Client:** TVA / Sequoyah Nuclear Plant, Outfall 101

### *Daily Chemistry:*

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity, hardness and chlorine (total residual) performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | MS | TS | TS | TS | TS | N |
| Concentration | Parameter | | | | | | |
| UV-Treated CONTROL, MHSW | pH (S.U.) | 7.97 | 7.88 | 7.97 | 7.87 | 7.92 | 7.67 |
| | Dissolved oxygen (mg/L) | 8.0 | 8.0 | 7.7 | 7.6 | 7.6 | 7.5 |
| | Conductivity (µmhos/cm) | 313 | | 316 | | 315 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 62 | |
| | Hardness (mg $CaCO_3$/L) | 90 | | | | 88 | |
| | Temperature (°C) | 24.9 | 24.8 | 24.8 | 24.6 | 24.9 | 24.7 |
| 10.7% | pH (S.U.) | 7.93 | 7.84 | 7.94 | 7.83 | 7.94 | 7.65 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.9 | 7.7 | 7.5 | 7.6 | 7.6 |
| | Conductivity (µmhos/cm) | 289 | | 304 | | 298 | |
| | Temperature (°C) | 24.9 | 24.8 | 24.9 | 24.6 | 24.9 | 24.5 |
| 21.4% | pH (S.U.) | 7.91 | 7.84 | 7.96 | 7.81 | 7.94 | 7.63 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.9 | 7.8 | 7.5 | 7.6 | 7.6 |
| | Conductivity (µmhos/cm) | 274 | | 286 | | 287 | |
| | Temperature (°C) | 24.9 | 24.7 | 24.9 | 24.6 | 24.9 | 24.5 |
| 42.8% | pH (S.U.) | 7.90 | 7.84 | 7.94 | 7.81 | 7.94 | 7.61 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 7.8 | 7.6 | 7.6 | 7.5 |
| | Conductivity (µmhos/cm) | 240 | | 252 | | 255 | |
| | Temperature (°C) | 25.0 | 24.7 | 24.9 | 24.6 | 24.9 | 24.8 |
| 85.6% | pH (S.U.) | 7.88 | 7.80 | 7.92 | 7.74 | 7.91 | 7.59 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 7.8 | 7.4 | 7.7 | 7.5 |
| | Conductivity (µmhos/cm) | 179 | | 189 | | 188 | |
| | Temperature (°C) | 25.0 | 24.7 | 24.9 | 24.5 | 24.9 | 24.8 |
| 100% | pH (S.U.) | 7.87 | 7.78 | 7.90 | 7.74 | 7.90 | 7.56 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 7.8 | 7.4 | 7.7 | 7.6 |
| | Conductivity (µmhos/cm) | 154 | | 163 | | 166 | |
| | Alkalinity (mg $CaCO_3$/L) | 66 | | | | 66 | |
| | Hardness (mg $CaCO_3$/L) | 69 | | | | 67 | |
| | Chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 25.0 | 24.7 | 25.0 | 24.7 | 25.0 | 24.5 |
| 100% Intake | pH (S.U.) | 7.88 | 7.78 | 7.90 | 7.74 | 7.90 | 7.49 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 7.8 | 7.4 | 7.6 | 6.5 |
| | Conductivity (µmhos/cm) | 158 | | 161 | | 167 | |
| | Alkalinity (mg $CaCO_3$/L) | 63 | | | | 65 | |
| | Hardness (mg $CaCO_3$/L) | 69 | | | | 71 | |
| | Chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 25.0 | 24.6 | 25.2 | 24.6 | 25.0 | 24.8 |
| | | Initial | Final | Initial | Final | Initial | Final |

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***
**Client:** **TVA / Sequoyah Nuclear Plant, Outfall 101** **Date:** **05-05-20**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | K | BGL | BGL | TS | TS | TS | TS | MS |
| Concentration | Parameter | | | | | | | | |
| UV-Treated CONTROL, MHSW | pH (S.U.) | 7.96 | 7.76 | ~~7.92~~ 8.04 | 7.83 | 8.05 | 7.88 | 7.93 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.2 | ~~7.8~~ 7.6 | 7.4 | 7.6 | 7.4 | 7.9 | 7.5 |
| | Conductivity (µmhos/cm) | 313 | | 307 | | 323 | | 311 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 61 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 86 | | | | | |
| | Temperature (°C) | 24.8 | 24.6 | 24.8 | 24.8 | 24.8 | 24.6 | 24.8 | 24.6 |
| 10.7% | pH (S.U.) | 7.96 | 7.71 | 8.01 | 7.80 | 8.03 | 7.77 | 7.94 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.3 | 7.9 | 7.4 | 7.8 | 7.5 | 7.9 | 7.5 |
| | Conductivity (µmhos/cm) | 296 | | 290 | | 302 | | 299 | |
| | Temperature (°C) | 24.9 | 24.5 | 24.9 | 24.7 | 24.9 | 24.5 | 24.8 | 24.6 |
| 21.4% | pH (S.U.) | 7.96 | 7.71 | 8.01 | 7.79 | 8.02 | 7.77 | 7.94 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.3 | 7.9 | 7.4 | 7.8 | 7.5 | 7.9 | 7.5 |
| | Conductivity (µmhos/cm) | 284 | | 274 | | 283 | | 282 | |
| | Temperature (°C) | 24.9 | 24.5 | 24.9 | 24.6 | 24.9 | 24.5 | 24.8 | 24.3 |
| 42.8% | pH (S.U.) | 7.95 | 7.62 | 7.98 | 7.77 | 8.00 | 7.75 | 7.94 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.3 | 8.0 | 7.5 | 7.9 | 7.5 | 7.9 | 7.6 |
| | Conductivity (µmhos/cm) | 253 | | 244 | | 253 | | 251 | |
| | Temperature (°C) | 24.9 | 24.4 | 25.0 | 24.6 | 24.9 | 24.7 | 24.8 | 24.6 |
| 85.6% | pH (S.U.) | 7.93 | 7.61 | 7.96 | 7.74 | 7.98 | 7.74 | 7.92 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.3 | 8.0 | 7.5 | 8.0 | 7.5 | 8.0 | 7.6 |
| | Conductivity (µmhos/cm) | 189 | | 188 | | 194 | | 191 | |
| | Temperature (°C) | 25.0 | 24.6 | 25.0 | 24.6 | 24.9 | 24.5 | 24.8 | 24.5 |
| 100% | pH (S.U.) | 7.91 | 7.61 | 7.95 | 7.73 | 7.96 | 7.72 | 7.92 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.1 | 8.0 | 7.4 | 8.0 | 7.6 | 8.0 | 7.6 |
| | Conductivity (µmhos/cm) | 167 | | 167 | | 172 | | 167 | |
| | Alkalinity (mg $CaCO_3$/L) | | | ~~8.0~~ 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 73 | | | | | |
| | Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 24.6 | 25.0 | 24.5 | 25.0 | 24.5 | 24.8 | 24.7 |
| 100% Intake | pH (S.U.) | 7.92 | 7.62 | 7.96 | 7.73 | 7.96 | 7.72 | 7.93 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.2 | 8.0 | 7.3 | 8.0 | 7.6 | 8.1 | 7.6 |
| | Conductivity (µmhos/cm) | 161 | | 161 | | 176 | | 164 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 69 | | | | | |
| | Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 24.3 | 25.0 | 24.6 | 24.9 | 24.5 | 24.9 | 24.6 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

Independent Review by KaDay E. Keenan: K

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*
Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 05-05-20

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | [illegible] | TS | TS | TS | TS | [illegible] |
| Concentration | Parameter | | | | | | |
| Non-treated CONTROL, MHSW | pH (S.U.) | 7.98 | 7.71 | 7.68 | 7.82 | 7.79 | 7.68 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.3 | 7.6 | 7.6 | 7.6 | 7.4 |
| | Conductivity (µmhos/cm) | 321 | | 321 | | 318 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | [illegible] 05-12-20 | | 61 | |
| | Hardness (mg $CaCO_3$/L) | 93 | | | | 89 | |
| | Temperature (°C) | 24.8 | 24.7 | 24.8 | 24.6 | 24.7 | 24.5 |

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | [illegible] | BSL | BSL | TS | TS | TS | TS | MS |
| Concentration | Parameter | | | | | | | | |
| Non-treated CONTROL, MHSW | pH (S.U.) | 7.88 | 7.68 | 7.72 | 7.73 | 7.73 | 7.73 | 7.83 | 7.88 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.4 | 7.6 | 7.7 | 7.7 | 7.6 | 7.6 | 7.6 |
| | Conductivity (µmhos/cm) | 305 | | 300 | | 314 | | 322 | |
| | Alkalinity (mg $CaCO_3$/L) | [illegible] 05-12-20 | | 60 | | [illegible] | | [illegible] 05-12-20 | |
| | Hardness (mg $CaCO_3$/L) | | | 90 | | | | | |
| | Temperature (°C) | 24.8 | 24.4 | 24.7 | 24.5 | 24.8 | 24.5 | 24.8 | 24.7 |

Independent Review by Kelley E. Keenan: [illegible]

Independently Reviewed by Kelly K. Kennell

Environmental Testing Solutions, Inc.

# TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treated
## May 05-12, 2020

### *Pimephales promelas* Chronic Whole Effluent Toxicity Test
### EPA-821-R-02-013, Method 1000.0

### Daily Chemical Analyses

Project number: 15061

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Control, Non-treated | pH (SU) | 7.98 | 7.71 | 7.68 | 7.82 | 7.79 | 7.68 | 7.88 | 7.68 | 7.72 | 7.73 | 7.73 | 7.73 | 7.83 | 7.88 |
| | DO (mg/L) | 7.9 | 7.3 | 7.6 | 7.6 | 7.6 | 7.4 | 7.8 | 7.4 | 7.6 | 7.7 | 7.7 | 7.6 | 7.6 | 7.6 |
| | Conductivity (µmhos/cm) | 321 | | 321 | | 318 | | 305 | | 300 | | 314 | | 322 | |
| | Alkalinity (mg/L $CaCO_3$) | 60 | | | | 61 | | | | 60 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 93 | | | | 89 | | | | 90 | | | | | |
| | Temperature (°C) | 24.8 | 24.7 | 24.8 | 24.6 | 24.7 | 24.5 | 24.8 | 24.4 | 24.7 | 24.5 | 24.8 | 24.5 | 24.8 | 24.7 |
| Control, UV-treated | pH (SU) | 7.97 | 7.88 | 7.97 | 7.87 | 7.92 | 7.67 | 7.96 | 7.76 | 8.04 | 7.83 | 8.05 | 7.88 | 7.93 | 7.93 |
| | DO (mg/L) | 8.0 | 8.0 | 7.7 | 7.6 | 7.6 | 7.5 | 8.0 | 7.2 | 7.6 | 7.4 | 7.6 | 7.4 | 7.9 | 7.5 |
| | Conductivity (µmhos/cm) | 313 | | 316 | | 315 | | 313 | | 307 | | 323 | | 311 | |
| | Alkalinity (mg/L $CaCO_3$) | 60 | | | | 62 | | | | 61 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 90 | | | | 88 | | | | 86 | | | | | |
| | Temperature (°C) | 24.9 | 24.8 | 24.8 | 24.6 | 24.9 | 24.7 | 24.8 | 24.6 | 24.8 | 24.8 | 24.8 | 24.6 | 24.8 | 24.6 |
| 10.7% | pH (SU) | 7.93 | 7.84 | 7.94 | 7.83 | 7.94 | 7.65 | 7.96 | 7.71 | 8.01 | 7.80 | 8.03 | 7.77 | 7.94 | 7.87 |
| | DO (mg/L) | 8.0 | 7.9 | 7.7 | 7.5 | 7.6 | 7.6 | 8.0 | 7.3 | 7.9 | 7.4 | 7.8 | 7.5 | 7.9 | 7.5 |
| | Conductivity (µmhos/cm) | 288 | | 304 | | 298 | | 296 | | 290 | | 302 | | 299 | |
| | Temperature (°C) | 24.9 | 24.8 | 24.9 | 24.6 | 24.9 | 24.5 | 24.9 | 24.5 | 24.9 | 24.7 | 24.9 | 24.5 | 24.8 | 24.6 |
| 21.4% | pH (SU) | 7.91 | 7.84 | 7.96 | 7.81 | 7.94 | 7.63 | 7.96 | 7.71 | 8.01 | 7.79 | 8.02 | 7.77 | 7.94 | 7.86 |
| | DO (mg/L) | 8.0 | 7.9 | 7.8 | 7.5 | 7.6 | 7.6 | 8.0 | 7.3 | 7.9 | 7.4 | 7.8 | 7.5 | 7.9 | 7.5 |
| | Conductivity (µmhos/cm) | 274 | | 286 | | 287 | | 284 | | 274 | | 283 | | 282 | |
| | Temperature (°C) | 24.9 | 24.7 | 24.9 | 24.6 | 24.9 | 24.5 | 24.9 | 24.5 | 24.9 | 24.6 | 24.9 | 24.5 | 24.8 | 24.3 |
| 42.8% | pH (SU) | 7.90 | 7.84 | 7.94 | 7.81 | 7.94 | 7.61 | 7.95 | 7.62 | 7.98 | 7.77 | 8.00 | 7.75 | 7.94 | 7.85 |
| | DO (mg/L) | 8.1 | 8.0 | 7.8 | 7.5 | 7.6 | 7.5 | 8.1 | 7.3 | 8.0 | 7.5 | 7.9 | 7.5 | 7.9 | 7.6 |
| | Conductivity (µmhos/cm) | 240 | | 252 | | 255 | | 253 | | 244 | | 253 | | 251 | |
| | Temperature (°C) | 25.0 | 24.7 | 24.9 | 24.6 | 24.9 | 24.8 | 24.9 | 24.4 | 25.0 | 24.6 | 24.9 | 24.7 | 24.8 | 24.6 |
| 85.6% | pH (SU) | 7.88 | 7.80 | 7.92 | 7.74 | 7.91 | 7.59 | 7.93 | 7.61 | 7.96 | 7.74 | 7.98 | 7.74 | 7.92 | 7.83 |
| | DO (mg/L) | 8.1 | 8.0 | 7.8 | 7.4 | 7.7 | 7.5 | 8.1 | 7.3 | 8.0 | 7.5 | 8.0 | 7.5 | 8.0 | 7.6 |
| | Conductivity (µmhos/cm) | 179 | | 189 | | 188 | | 189 | | 188 | | 194 | | 191 | |
| | Temperature (°C) | 25.0 | 24.7 | 24.9 | 24.5 | 24.9 | 24.8 | 25.0 | 24.6 | 25.0 | 24.6 | 24.9 | 24.5 | 24.8 | 24.5 |
| 100% | pH (SU) | 7.87 | 7.78 | 7.90 | 7.74 | 7.90 | 7.56 | 7.91 | 7.61 | 7.95 | 7.73 | 7.96 | 7.72 | 7.92 | 7.83 |
| | DO (mg/L) | 8.1 | 8.0 | 7.8 | 7.4 | 7.7 | 7.6 | 8.1 | 7.1 | 8.0 | 7.4 | 8.0 | 7.6 | 8.0 | 7.6 |
| | Conductivity (µmhos/cm) | 154 | | 163 | | 166 | | 167 | | 167 | | 172 | | 167 | |
| | Alkalinity (mg/L $CaCO_3$) | 66 | | | | 66 | | | | 64 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 69 | | | | 67 | | | | 73 | | | | | |
| | *Total Residual Chlorine (mg/L | <0.10 | | | | <0.10 | | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 24.7 | 25.0 | 24.7 | 25.0 | 24.5 | 25.0 | 24.6 | 25.0 | 24.5 | 25.0 | 24.5 | 24.8 | 24.7 |
| 100% Intake | pH (SU) | 7.88 | 7.78 | 7.90 | 7.74 | 7.90 | 7.49 | 7.92 | 7.62 | 7.96 | 7.73 | 7.96 | 7.72 | 7.93 | 7.83 |
| | DO (mg/L) | 8.2 | 7.9 | 7.8 | 7.4 | 7.6 | 6.5 | 8.1 | 7.2 | 8.0 | 7.3 | 8.0 | 7.6 | 8.1 | 7.6 |
| | Conductivity (µmhos/cm) | 158 | | 161 | | 167 | | 161 | | 161 | | 176 | | 164 | |
| | Alkalinity (mg/L $CaCO_3$) | 63 | | | | 65 | | | | 64 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 69 | | | | 71 | | | | 69 | | | | | |
| | *Total Residual Chlorine (mg/L | <0.10 | | | | <0.10 | | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 24.6 | 25.2 | 24.6 | 25.0 | 24.8 | 25.0 | 24.3 | 25.0 | 24.6 | 24.9 | 24.5 | 24.9 | 24.6 |

*Note: Total residual chlorine was performed on non-treated Outfall 101 and Intake samples.

File: sqn101_050520chem
Entered by: T. Sleeper
Reviewed by: _____

# Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013 Method 1002.0)

**Species:** *Ceriodaphnia dubia*

**Client:** TVA, Sequoyah Nuclear Plant
**County:** Hamilton
**NPDES #:** TN 0026450
**Outfall #:** 101
**Project #:** 15061
**Treatment:** None

***Dilution preparation:***

| Dilution prep (%) | 10.7 | 21.4 | 42.8 | 85.6 | 100 |
|---|---|---|---|---|---|
| Effluent volume (mL) | 214 | 428 | 856 | 1712 | 2000 |
| Diluent volume (mL) | 1786 | 1572 | 1144 | 288 | 0 |
| Total volume (mL) | 2000 | 2000 | 2000 | 2000 | 2000 |

Sample was not aerated or treated unless otherwise noted on this form. Sample was warmed to 25.0 ± 1.0°C in a warm water bath and then diluted to the test concentrations with moderately hard synthetic water (MHSW).

***Test organism source:***

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Organism age: | < 24-hours old | | | | | | | | | |
| Date and times organisms were born between: | 05-05-20 0645 TO 0912 | | | | | | | | | |
| Culture board: | 04-28-20 B | | | | | 04-28-20 C | | | | |
| Replicate number: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Culture board cup number: | 30 | 31 | 32 | 33 | 40 | 41 | 7 | 8 | 9 | 19 |
| Transfer vessel information: | pH (S.U.): 7.93 Temperature (°C): 24.9 | | | | | | | | | |
| Average transfer volume (mL): | < 0.25 mL | | | | | | | | | |

***Test randomization and location:***

| | |
|---|---|
| Randomizing template color: | RED |
| Incubator number and shelf location: | 2C2 |

***Daily renewal:***

| Day | Date | Test initiation and feeding, renewal and feeding, or termination time | *Feeding Batches Selenastrum | *Feeding Batches YWT | MHSW batch used | Sample numbers used Outfall 101 | Sample numbers used Intake | Analyst |
|---|---|---|---|---|---|---|---|---|
| 0 | 05-05-20 | 0958 | 05-01-20 | 05-01-20 | 04-29-20B | 200504.04 | 200504.05 | JH |
| 1 | 05-06-20 | 0900 | ↓ | ↓ | ↓ | ↓ | ↓ | JH |
| 2 | 05-07-20 | 0924 | ↓ | ↓ | 04-29-20B | 200506.30 ~~200506.30~~ | 200506.31 ~~200506.31~~ | JH |
| 3 | 05-08-20 | 0920 | ↓ | ↓ | ↓ | ↓ | ↓ | JH |
| 4 | 05-09-20 | 1018 | ↓ | ↓ | 05-01-20B | 200508.22 | 200508.23 | JH |
| 5 | 05-10-20 | 0917 | ↓ | ↓ | ↓ | ↓ | ↓ | JH |
| 6 | 05-11-20 | 0915 | ↓ | ↓ | ↓ | ↓ | ↓ | JH |
| 7 | 05-12-20 | 0911 | | | | | | JH |

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354

***Chemical analyses:***

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2011 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2011 | YSI Model 52CE | 18D104324 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2011 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2011 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2011 | Not applicable | Not applicable |
| Chlorine, Total Residual | 0.1 mg/L | ORION 97-70-1977 | Accumet AB250 | 92349123 |
| Temperature | 0.1°C | SM 2550B-2010 | Digital Thermometer | 130664685 |

| ***Control information:*** | Control-1 | Control-2 | Acceptance criteria |
|---|---|---|---|
| % of Male Adults: | 0% | 0% | ≤ 20% |
| % Adults having 3rd Broods: | 100% | 100% | ≥ 60% surviving adults |
| % Mortality: | 0% | 0% | ≤ 20% |
| Mean Offspring/Female: | 29.6 | 30.3 | ≥ 15.0 offspring/female |
| % CV | 4.0% | 4.9% | < 40% |

| ***Summary of test endpoints:*** | |
|---|---|
| 7-day $LC_{50}$ (%) | >100% |
| NOEC (%) | 100% |
| LOEC (%) | >100% |
| ChV (%) | >100% |
| $IC_{25}$ (%) | >100% |

Independent Review by Kelley E. Keenan: K

Species: *Ceriodaphnia dubia*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101 Date: 05-05-20

**CONTROL-1** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 3 | 4 | 5 | 5 | 4 | 4 | 4 | 5 | 6 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 10 | 9 | 10 | 10 | 12 | 10 | 11 | 11 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 13 | 16 | 17 | 14 | 14 | 12 | 15 | 15 | 15 | 16 |
| Total young produced | | 29 | 29 | 30 | 29 | 29 | 28 | 29 | 30 | 31 | 32 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 29.6 |

**CONC: 10.7%** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 3 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 13 | 12 | 13 | 13 | 11 | 10 | 10 | 11 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 16 | 15 | 12 | 16 | 13 | 13 | 17 | 15 | 17 | 14 |
| Total young produced | | 30 | 31 | 29 | 34 | 31 | 29 | 31 | 29 | 32 | 29 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 30.5 |
| % Reduction from Control-1: | -3.0% |

Independent Review by Kelley E. Keenan:

**Species:** ***Ceriodaphnia dubia***

**Client:** **TVA / Sequoyah Nuclear Plant, Outfall 101** **Date:** **05-05-20**

CONC: **21.4%** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 6 | 4 | 4 | 5 | 4 | 6 | 6 | 5 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 12 | 10 | 13 | 11 | 10 | 13 | 12 | 10 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 18 | 17 | 19 | 14 | 15 | 17 | 15 | 19 | 19 | 15 |
| Total young produced | | 34 | 33 | 33 | 32 | 30 | 33 | 34 | 36 | 34 | 32 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note.* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 33.1 |
| % Reduction from Control-1: | -11.8% |

CONC: **42.8%** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 6 | 4 | 6 | 6 | 4 | 5 | 5 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 11 | 12 | 12 | 13 | 12 | 10 | 12 | 12 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 18 | 19 | 18 | 18 | 15 | 17 | 13 | 16 | 15 | 17 |
| Total young produced | | 35 | 36 | 34 | 36 | 34 | 33 | 28 | 33 | 32 | 34 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 33.5 |
| % Reduction from Control-1: | -13.2% |

Independent Review by Kelley E. Keenan: K

**Species:** *Ceriodaphnia dubia*

**Client:** TVA / Sequoyah Nuclear Plant, Outfall 101 **Date:** 05-05-20

**CONC:** 85.6% ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 7 | 4 | 5 | 5 | 4 | 5 | 5 | 6 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 11 | 12 | 12 | 12 | 12 | 13 | 10 | 12 | 12 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 18 | 16 | 20 | 18 | 17 | 18 | 19 | 17 | 17 | 19 |
| Total young produced | | 34 | 35 | 36 | 35 | 34 | 35 | 34 | 34 | 35 | 36 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 34.8 |
| % Reduction from Control-1: | -17.6% |

**CONC:** 100% ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 6 | 6 | 4 | 6 | 5 | 4 | 6 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 10 | 13 | 12 | 13 | 12 | 12 | 13 | 13 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 15 | 18 | 19 | 18 | 15 | 17 | 18 | 17 | 18 | 18 |
| Total young produced | | 33 | 34 | 38 | 34 | 34 | 34 | 34 | 36 | 36 | 34 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 34.7 |
| % Reduction from Control-1: | -17.2% |

Independent Review by Kelley E. Keenan: K

**Species:** ***Ceriodaphnia dubia***

**Client:** TVA / Sequoyah Nuclear Plant, Outfall 101 **Date:** 05-05-20

**CONTROL-2** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 4 | 5 | 4 | 4 | 3 | 5 | 3 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 10 | 10 | 12 | 13 | 10 | 12 | 10 | 11 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 17 | 15 | 16 | 16 | 15 | 16 | 13 | 16 | 14 | 15 |
| Total young produced | | 32 | 29 | 31 | 32 | 32 | 29 | 30 | 29 | 29 | 30 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 30.3 |

**CONC: 100% Intake** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 6 | 4 | 6 | 5 | 5 | 5 | 6 | 4 | 6 | 6 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 13 | 12 | 10 | 13 | 14 | 12 | 12 | 12 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 17 | 15 | 14 | 20 | 18 | 17 | 17 | 20 | 19 | 16 |
| Total young produced | | 36 | 32 | 37 | 35 | 36 | 36 | 35 | 36 | 37 | 33 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 35.3 |
| % Reduction from Control-2: | -16.5% |

Independent Review by Kelley E. Keenan: K

Independent Review by Kelly E. Kenner

# TVA / Sequoyah Nuclear Plant, Outfall 101
## May 05-12, 2020

Environmental Testing Solutions, Inc.

## Verification of *Ceriodaphnia* Reproduction Totals

### Control-1

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 3 | 4 | 5 | 5 | 4 | 4 | 4 | 5 | 6 | 44 |
| 5 | 12 | 10 | 9 | 10 | 10 | 12 | 10 | 11 | 11 | 10 | 105 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 13 | 16 | 17 | 14 | 14 | 12 | 15 | 15 | 15 | 16 | 147 |
| Total | 29 | 29 | 30 | 29 | 29 | 28 | 29 | 30 | 31 | 32 | 296 |

### 10.7%

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 3 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 5 | 44 |
| 5 | 10 | 13 | 12 | 13 | 13 | 11 | 10 | 10 | 11 | 10 | 113 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 16 | 15 | 12 | 16 | 13 | 13 | 17 | 15 | 17 | 14 | 148 |
| Total | 30 | 31 | 29 | 34 | 31 | 29 | 31 | 29 | 32 | 29 | 305 |

### 21.4%

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 6 | 4 | 4 | 5 | 4 | 6 | 6 | 5 | 5 | 5 | 50 |
| 5 | 10 | 12 | 10 | 13 | 11 | 10 | 13 | 12 | 10 | 12 | 113 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 18 | 17 | 19 | 14 | 15 | 17 | 15 | 19 | 19 | 15 | 168 |
| Total | 34 | 33 | 33 | 32 | 30 | 33 | 34 | 36 | 34 | 32 | 331 |

### 42.8%

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 6 | 4 | 6 | 6 | 4 | 5 | 5 | 5 | 5 | 51 |
| 5 | 12 | 11 | 12 | 12 | 13 | 12 | 10 | 12 | 12 | 12 | 118 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 18 | 19 | 18 | 18 | 15 | 17 | 13 | 16 | 15 | 17 | 166 |
| Total | 35 | 36 | 34 | 36 | 34 | 33 | 28 | 33 | 32 | 34 | 335 |

### 85.6%

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 7 | 4 | 5 | 5 | 4 | 5 | 5 | 6 | 5 | 51 |
| 5 | 11 | 12 | 12 | 12 | 12 | 13 | 10 | 12 | 12 | 12 | 118 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 18 | 16 | 20 | 18 | 17 | 18 | 19 | 17 | 17 | 19 | 179 |
| Total | 34 | 35 | 36 | 35 | 34 | 35 | 34 | 34 | 35 | 36 | 348 |

### 100%

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 6 | 6 | 4 | 6 | 5 | 4 | 6 | 5 | 5 | 52 |
| 5 | 13 | 10 | 13 | 12 | 13 | 12 | 12 | 13 | 13 | 11 | 122 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 18 | 19 | 18 | 15 | 17 | 18 | 17 | 18 | 18 | 173 |
| Total | 33 | 34 | 38 | 34 | 34 | 34 | 34 | 36 | 36 | 34 | 347 |

### Control-2

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 4 | 5 | 4 | 4 | 3 | 5 | 3 | 4 | 4 | 41 |
| 5 | 10 | 10 | 10 | 12 | 13 | 10 | 12 | 10 | 11 | 11 | 109 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 17 | 15 | 16 | 16 | 15 | 16 | 13 | 16 | 14 | 15 | 153 |
| Total | 32 | 29 | 31 | 32 | 32 | 29 | 30 | 29 | 29 | 30 | 303 |

### 100% Intake

| Day | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 6 | 4 | 6 | 5 | 5 | 5 | 6 | 4 | 6 | 6 | 53 |
| 5 | 13 | 13 | 12 | 10 | 13 | 14 | 12 | 12 | 12 | 11 | 122 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 17 | 15 | 19 | 20 | 18 | 17 | 17 | 20 | 19 | 16 | 178 |
| Total | 36 | 32 | 37 | 35 | 36 | 36 | 35 | 36 | 37 | 33 | 353 |

Independent Review by Kelley E. Keenan

ETS
Environmental Testing Solutions, Inc.

# TVA / Sequoyah Nuclear Plant, Outfall 101
## May 05-12, 2020
### Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1002.0)
### *Ceriodaphnia dubia* Chronic Whole Effluent Toxicity Test
### EPA-821-R-02-013, Method 1002.0

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

Project number: 15061

| Concentration (%) | Replicate number | | | | | | | | | | Survival (%) | Average reproduction (offspring/female) | Coefficient of variation (%) | Percent reduction from pooled controls (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| Control - 1 | 29 | 29 | 30 | 29 | 29 | 28 | 29 | 30 | 31 | 32 | 100 | 29.6 | 4.0 | Not applicable |
| 10.7% | 30 | 31 | 29 | 34 | 31 | 29 | 31 | 29 | 32 | 29 | 100 | 30.5 | 5.4 | -3.0 |
| 21.4% | 34 | 33 | 33 | 32 | 30 | 33 | 34 | 36 | 34 | 32 | 100 | 33.1 | 4.8 | -11.8 |
| 42.8% | 35 | 36 | 34 | 36 | 34 | 33 | 28 | 33 | 32 | 34 | 100 | 33.5 | 6.9 | -13.2 |
| 85.6% | 34 | 35 | 36 | 35 | 34 | 35 | 34 | 34 | 35 | 36 | 100 | 34.8 | 2.3 | -17.6 |
| 100% | 33 | 34 | 38 | 34 | 34 | 34 | 34 | 36 | 36 | 34 | 100 | 34.7 | 4.3 | -17.2 |
| Control - 2 | 32 | 29 | 31 | 32 | 32 | 29 | 30 | 29 | 29 | 30 | 100 | 30.3 | 4.4 | Not applicable |
| 100% Intake | 36 | 32 | 37 | 35 | 36 | 36 | 35 | 36 | 37 | 33 | 100 | 35.3 | 4.6 | -16.5 |

**Outfall 101:**
**Dunnett's MSD value:** 1.611
**PMSD:** 5.4

**Intake:**
**Dunnett's MSD value:** 1.159
**PMSD:** 3.8

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 13%.
Upper PMSD bound determined by USEPA (90th percentile) = 47%.
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).

USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.

Entered and Reviewed by Jim Sumner

# TVA / Sequoyah Nuclear Plant, Outfall 101
# May 05-12, 2020

## Statistical Analyses

Environmental Testing Solutions, Inc.

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | CdFRCR | Sample ID: | TVA / SQN Outfall 101 |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | DMR-Discharge Monitoring Report |
| Sample Date: | May 2020 | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | Non-treated | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control-1 | 29.000 | 29.000 | 30.000 | 29.000 | 29.000 | 28.000 | 29.000 | 30.000 | 31.000 | 32.000 |
| Control-2 | 32.000 | 29.000 | 31.000 | 32.000 | 32.000 | 29.000 | 30.000 | 29.000 | 29.000 | 30.000 |
| 10.7 | 30.000 | 31.000 | 29.000 | 34.000 | 31.000 | 29.000 | 31.000 | 29.000 | 32.000 | 29.000 |
| 21.4 | 34.000 | 33.000 | 33.000 | 32.000 | 30.000 | 33.000 | 34.000 | 36.000 | 34.000 | 32.000 |
| 42.8 | 35.000 | 36.000 | 34.000 | 36.000 | 34.000 | 33.000 | 28.000 | 33.000 | 32.000 | 34.000 |
| 85.6 | 34.000 | 35.000 | 36.000 | 35.000 | 34.000 | 35.000 | 34.000 | 34.000 | 35.000 | 36.000 |
| 100 | 33.000 | 34.000 | 38.000 | 34.000 | 34.000 | 34.000 | 34.000 | 36.000 | 36.000 | 34.000 |
| Intake | 36.000 | 32.000 | 37.000 | 35.000 | 36.000 | 36.000 | 35.000 | 36.000 | 37.000 | 33.000 |

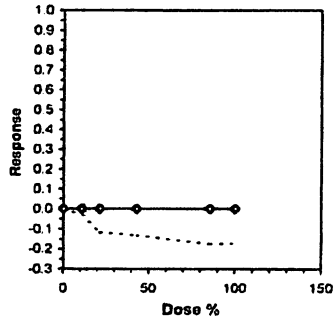
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control-1 | 29.600 | 0.9769 | 29.600 | 28.000 | 32.000 | 3.965 | 10 | * | | | 32.700 | 1.0000 |
| Control-2 | 30.300 | 1.0000 | 30.300 | 29.000 | 32.000 | 4.414 | 10 | | | | | |
| 10.7 | 30.500 | 1.0066 | 30.500 | 29.000 | 34.000 | 5.410 | 10 | -1.278 | 2.287 | 1.611 | 32.700 | 1.0000 |
| 21.4 | 33.100 | 1.0924 | 33.100 | 30.000 | 36.000 | 4.819 | 10 | -4.968 | 2.287 | 1.611 | 32.700 | 1.0000 |
| 42.8 | 33.500 | 1.1056 | 33.500 | 28.000 | 36.000 | 6.930 | 10 | -5.536 | 2.287 | 1.611 | 32.700 | 1.0000 |
| 85.6 | 34.800 | 1.1485 | 34.800 | 34.000 | 36.000 | 2.267 | 10 | -7.381 | 2.287 | 1.611 | 32.700 | 1.0000 |
| 100 | 34.700 | 1.1452 | 34.700 | 33.000 | 38.000 | 4.307 | 10 | -7.239 | 2.287 | 1.611 | 32.700 | 1.0000 |
| Intake | 35.300 | 1.1650 | 35.300 | 32.000 | 37.000 | 4.636 | 10 | | | | | |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates normal distribution (p > 0.01) | 0.8978 | 1.035 | -0.3153 | 2.41185 |
| Bartlett's Test indicates equal variances (p = 0.07) | 10.214 | 15.0863 | | |
| The control means are not significantly different (p = 0.23) | 1.24393 | 2.10092 | | |

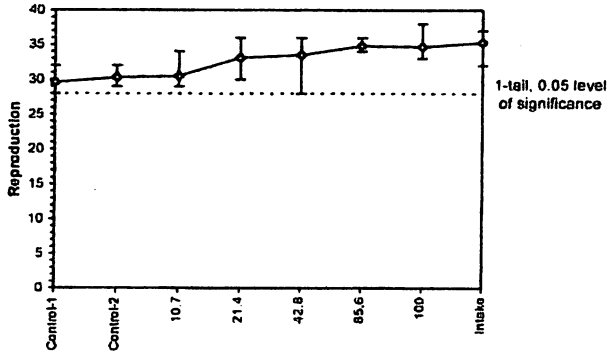
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 100 | >100 | | 1 | 1.61092 | 0.05442 | 47.32 | 2.48148 | 7.0E-11 | 5, 54 |
| Treatments vs Control-1 | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL | Skew |
|---|---|---|---|---|
| IC05 | >100 | | | |
| IC10 | >100 | | | |
| IC15 | >100 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |




**Dose-Response Plot**




Independent Review by Kelley E. Keenan:

# TVA / Sequoyah Nuclear Plant, Outfall 101 - Intake
# May 05-12, 2020

## Statistical Analyses

Environmental Testing Solutions, Inc.

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | CdFRCR | Sample ID: | TVA / SQN Intake |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | DMR-Discharge Monitoring Report |
| Sample Date: | May 2020 | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | Non-treated | | | | |

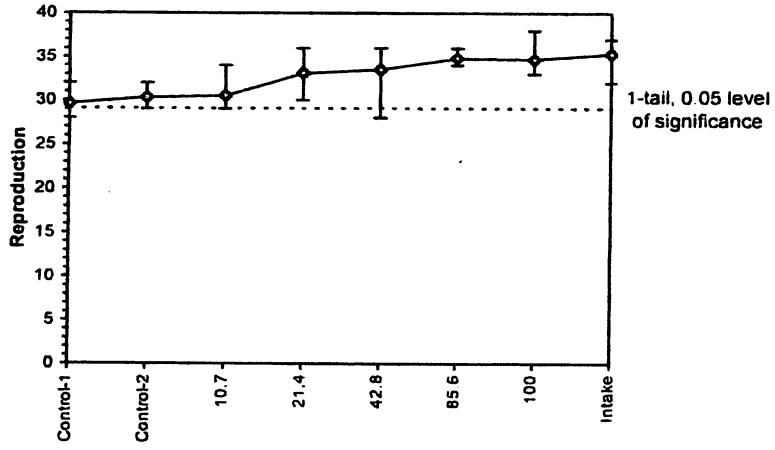
| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control-1 | 29.000 | 29.000 | 30.000 | 29.000 | 29.000 | 28.000 | 29.000 | 30.000 | 31.000 | 32.000 |
| Control-2 | 32.000 | 29.000 | 31.000 | 32.000 | 32.000 | 29.000 | 30.000 | 29.000 | 29.000 | 30.000 |
| 10.7 | 30.000 | 31.000 | 29.000 | 34.000 | 31.000 | 29.000 | 31.000 | 29.000 | 32.000 | 29.000 |
| 21.4 | 34.000 | 33.000 | 33.000 | 32.000 | 30.000 | 33.000 | 34.000 | 36.000 | 34.000 | 32.000 |
| 42.8 | 35.000 | 36.000 | 34.000 | 36.000 | 34.000 | 33.000 | 28.000 | 33.000 | 32.000 | 34.000 |
| 85.6 | 34.000 | 35.000 | 36.000 | 35.000 | 34.000 | 35.000 | 34.000 | 34.000 | 35.000 | 36.000 |
| 100 | 33.000 | 34.000 | 38.000 | 34.000 | 34.000 | 34.000 | 34.000 | 36.000 | 36.000 | 34.000 |
| Intake | 36.000 | 32.000 | 37.000 | 35.000 | 36.000 | 36.000 | 35.000 | 36.000 | 37.000 | 33.000 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| Control-1 | 29.600 | 0.9769 | 29.600 | 28.000 | 32.000 | 3.965 | 10 | | | |
| Control-2 | 30.300 | 1.0000 | 30.300 | 29.000 | 32.000 | 4.414 | 10 | | | |
| 10.7 | 30.500 | 1.0066 | 30.500 | 29.000 | 34.000 | 5.410 | 10 | | | |
| 21.4 | 33.100 | 1.0924 | 33.100 | 30.000 | 36.000 | 4.819 | 10 | | | |
| 42.8 | 33.500 | 1.1056 | 33.500 | 28.000 | 36.000 | 6.930 | 10 | | | |
| 85.6 | 34.800 | 1.1485 | 34.800 | 34.000 | 36.000 | 2.267 | 10 | | | |
| 100 | 34.700 | 1.1452 | 34.700 | 33.000 | 38.000 | 4.307 | 10 | | | |
| Intake | 35.300 | 1.1650 | 35.300 | 32.000 | 37.000 | 4.636 | 10 | -7.481 | 1.734 | 1.159 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.90979 | 0.868 | -0.5837 | -0.2957 |
| F-Test indicates equal variances (p = 0.56) | 1.49689 | 6.54109 | | |
| The control means are not significantly different (p = 0.23) | 1.24393 | 2.10092 | | |

| Hypothesis Test (1-tail, 0.05) | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|
| Homoscedastic t Test indicates no significant differences Treatments vs Control-2 | 1.15893 | 0.03825 | 125 | 2.23333 | 6.3E-07 | 1, 18 |

**Dose-Response Plot**




Independent Review by Kelley E. Keenan:

**Species:** *Ceriodaphnia dubia* **Date:** 05-05-20

**Client:** TVA / Sequoyah Nuclear Plant, Outfall 101

### *Daily Chemistry:*

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity, hardness and chlorine (total residual) performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | MP | TS | TS | TS | TS | M |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.98 | 7.94 | 7.68 | 7.95 | 7.79 | 7.92 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 7.6 | 7.9 | 7.6 | 7.9 |
| | Conductivity (µmhos/cm) | 321 | | 321 | | 318 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 61 | |
| | Hardness (mg $CaCO_3$/L) | 93 | | | | 89 | |
| | Temperature (°C) | 24.8 | 25.1 | 24.8 | 25.2 | 24.8 | 24.5 |
| 10.7% | pH (S.U.) | 7.95 | 7.94 | 7.99 | 7.96 | 8.00 | 7.90 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 8.0 | 7.9 | 8.0 | 7.9 |
| | Conductivity (µmhos/cm) | 291 | | 301 | | 307 | |
| | Temperature (°C) | 24.8 | 24.9 | 24.8 | 25.0 | 24.8 | 24.6 |
| 21.4% | pH (S.U.) | 7.92 | 7.93 | 7.99 | 7.95 | 7.99 | 7.90 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.9 | 8.0 | 8.0 | 8.0 | 7.9 |
| | Conductivity (µmhos/cm) | 274 | | 289 | | 289 | |
| | Temperature (°C) | 24.8 | 24.9 | 24.9 | 25.0 | 24.8 | 24.6 |
| 42.8% | pH (S.U.) | 7.92 | 7.92 | 7.96 | 7.95 | 7.95 | 7.88 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.9 | 8.0 | 8.0 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 240 | | 255 | | 257 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.9 | 24.9 | 24.8 | 24.3 |
| 85.6% | pH (S.U.) | 7.89 | 7.89 | 7.93 | 7.94 | 7.91 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.9 | 8.0 | 8.0 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 174 | | 188 | | 189 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.9 | 24.9 | 24.9 | 24.6 |
| 100% | pH (S.U.) | 7.87 | 7.90 | 7.92 | 7.93 | 7.91 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.9 | 8.0 | 8.0 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 151 | | 161 | | 162 | |
| | Alkalinity (mg $CaCO_3$/L) | 62 | | | | 65 | |
| | Hardness (mg $CaCO_3$/L) | 67 | | | | 69 | |
| | Chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 24.9 | 24.9 | 24.6 |
| 100% Intake | pH (S.U.) | 7.88 | 7.89 | 7.91 | 7.95 | 7.91 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.6 | 8.0 | 8.6 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 155 | | 164 | | 169 | |
| | Alkalinity (mg $CaCO_3$/L) | 63 | | | | 66 | |
| | Hardness (mg $CaCO_3$/L) | 69 | | | | 69 | |
| | Chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.1 | 25.0 | 24.6 |
| | | Initial | Final | Initial | Final | Initial | Final |

Independent Review by Kelley E. Keenan: K

**Species:** *Ceriodaphnia dubia*
**Client:** TVA / Sequoyah Nuclear Plant, Outfall 101 **Date:** 05-05-20

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | K. | BSL | BS | TS | TS | TS | TS | TS |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.88 | 8.05 | 7.72 | 7.98 | 7.73 | 7.96 | 7.83 | 7.99 |
| | Dissolved oxygen (mg/L) | 7.8 | 8.0 | 7.6 | 7.8 | 7.7 | 7.9 | 7.6 | 7.7 |
| | Conductivity (µmhos/cm) | 305 | | 300 | | 314 | | 322 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 60 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 90 | | | | | |
| | Temperature (°C) | 24.8 | 24.9 | 24.9 | 25.1 | 24.8 | 24.9 | 24.8 | 25.2 |
| 10.7% | pH (S.U.) | 7.91 | 7.98 | 8.04 | 7.99 | 7.96 | 7.97 | 7.88 (8.04) | 7.98 |
| | Dissolved oxygen (mg/L) | 7.8 | 8.0 | 8.0 | 7.8 | 8.0 | 7.8 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 301 | | 291 | | 304 | | 301 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.9 | 25.0 | 24.8 | 25.1 | 24.9 | 24.9 |
| 21.4% | pH (S.U.) | 7.92 | 7.98 | 7.99 | 7.99 | 7.97 | 7.97 | 8.02 | 7.96 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.9 | 8.0 | 7.7 | 8.0 | 7.8 | 7.8 | 7.8 |
| | Conductivity (µmhos/cm) | 288 | | 280 | | 288 | | 286 | |
| | Temperature (°C) | 24.8 | 25.2 | 25.0 | 24.9 | 24.8 | 25.1 | 24.9 | 24.9 |
| 42.8% | pH (S.U.) | 7.92 | 7.97 | 7.98 | 7.98 | 7.96 | 7.97 | 8.01 | 7.95 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.8 | 7.9 | 7.8 | 8.0 | 7.8 | 7.8 | 7.8 |
| | Conductivity (µmhos/cm) | 252 | | 248 | | 253 | | 253 | |
| | Temperature (°C) | 24.8 | 25.2 | 25.0 | 25.2 | 24.8 | 24.9 | 24.9 | 25.2 |
| 85.6% | pH (S.U.) | 7.90 | 7.94 | 7.97 | 7.98 | 7.93 | 7.96 | 7.98 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 7.9 | 7.8 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 185 | | 189 | | 193 | | 193 | |
| | Temperature (°C) | 24.9 | 25.0 | 25.0 | 24.9 | 24.8 | 24.9 | 24.9 | 25.0 |
| 100% | pH (S.U.) | 7.89 | 7.93 | 7.96 | 7.98 | 7.92 | 7.95 | 7.97 | 7.92 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 8.0 | 7.8 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 164 | | 164 | | 170 | | 171 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 71 | | | | | |
| | Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 25.2 | 25.0 | 24.9 | 24.9 | 25.0 | 25.0 | 25.0 |
| 100% Intake | pH (S.U.) | 7.89 | 7.93 | 7.97 | 7.99 | 7.93 | 7.93 | 7.97 | 7.92 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.8 | 8.0 | 7.8 | 8.0 | 7.8 | 7.9 | 7.9 |
| | Conductivity (µmhos/cm) | 167 | | 164 | | 168 | | 164 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 69 | | | | | |
| | Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 25.1 | 25.0 | 25.1 | 24.8 | 24.8 | 25.0 | 25.0 | 25.2 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

Independent Review by Kelley E. Keenan:

Independent Review by Kelley E. Keenan:

# TVA / Sequoyah Nuclear Plant, Outfall 101, Non-treated
## May 05-12, 2020

## *Ceriodaphnia dubia* Chronic Whole Effluent Toxicity Test
## EPA-821-R-02-013, Method 1002.0

### Daily Chemical Analyses

Environmental Testing Solutions, Inc

Project number: 15061

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Control | pH (SU) | 7.98 | 7.94 | 7.68 | 7.95 | 7.79 | 7.92 | 7.88 | 8.05 | 7.72 | 7.98 | 7.73 | 7.96 | 7.83 | 7.99 |
| | DO (mg/L) | 7.9 | 7.8 | 7.6 | 7.9 | 7.6 | 7.9 | 7.8 | 8.0 | 7.6 | 7.8 | 7.7 | 7.9 | 7.6 | 7.7 |
| | Conductivity (µmhos/cm) | 321 | | 321 | | 318 | | 305 | | 300 | | 314 | | 322 | |
| | Alkalinity (mg/L $CaCO_3$) | 60 | | | | 61 | | | | 60 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 93 | | | | 89 | | | | 90 | | | | | |
| | Temperature (°C) | 24.8 | 25.1 | 24.8 | 25.2 | 24.8 | 24.5 | 24.8 | 24.9 | 24.9 | 25.1 | 24.8 | 24.9 | 24.8 | 25.2 |
| 10.7% | pH (SU) | 7.95 | 7.94 | 7.99 | 7.96 | 8.00 | 7.90 | 7.91 | 7.98 | 8.04 | 7.99 | 7.96 | 7.97 | 8.04 | 7.98 |
| | DO (mg/L) | 7.9 | 7.8 | 8.0 | 7.9 | 8.0 | 7.9 | 7.8 | 8.0 | 8.0 | 7.8 | 8.0 | 7.8 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 291 | | 301 | | 307 | | 301 | | 291 | | 304 | | 301 | |
| | Temperature (°C) | 24.8 | 24.9 | 24.8 | 25.0 | 24.8 | 24.6 | 24.8 | 25.0 | 24.9 | 25.0 | 24.8 | 25.1 | 24.9 | 24.9 |
| 21.4% | pH (SU) | 7.92 | 7.93 | 7.99 | 7.95 | 7.99 | 7.90 | 7.92 | 7.98 | 7.99 | 7.99 | 7.97 | 7.97 | 8.02 | 7.96 |
| | DO (mg/L) | 7.9 | 7.9 | 8.0 | 8.0 | 8.0 | 7.9 | 7.8 | 7.9 | 8.0 | 7.7 | 8.0 | 7.8 | 7.8 | 7.8 |
| | Conductivity (µmhos/cm) | 274 | | 289 | | 289 | | 288 | | 280 | | 288 | | 286 | |
| | Temperature (°C) | 24.8 | 24.9 | 24.9 | 25.0 | 24.8 | 24.6 | 24.8 | 25.2 | 25.0 | 24.9 | 24.8 | 25.1 | 24.9 | 24.9 |
| 42.8% | pH (SU) | 7.92 | 7.92 | 7.96 | 7.95 | 7.95 | 7.88 | 7.92 | 7.97 | 7.98 | 7.98 | 7.96 | 7.97 | 8.01 | 7.95 |
| | DO (mg/L) | 7.9 | 7.9 | 8.0 | 8.0 | 8.0 | 8.0 | 7.8 | 7.8 | 7.9 | 7.8 | 8.0 | 7.8 | 7.8 | 7.8 |
| | Conductivity (µmhos/cm) | 240 | | 255 | | 257 | | 252 | | 248 | | 253 | | 253 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.9 | 24.9 | 24.8 | 24.3 | 24.8 | 25.2 | 25.0 | 25.2 | 24.8 | 24.9 | 24.9 | 25.2 |
| 85.6% | pH (SU) | 7.89 | 7.89 | 7.93 | 7.94 | 7.91 | 7.87 | 7.90 | 7.94 | 7.97 | 7.98 | 7.93 | 7.96 | 7.98 | 7.93 |
| | DO (mg/L) | 8.0 | 7.9 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 7.8 | 7.9 | 7.8 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 174 | | 188 | | 189 | | 185 | | 189 | | 193 | | 193 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.9 | 24 9 | 24.9 | 24.6 | 24.9 | 25.0 | 25.0 | 24.9 | 24.8 | 24.9 | 24 9 | 25.0 |
| 100% | pH (SU) | 7.87 | 7.90 | 7.92 | 7.93 | 7.91 | 7.86 | 7.89 | 7.93 | 7.96 | 7.98 | 7.92 | 7.95 | 7.97 | 7.92 |
| | DO (mg/L) | 8.0 | 7.9 | 8.0 | 8.0 | 8.1 | 8.0 | 8.0 | 7.8 | 8.0 | 7.8 | 8.0 | 7.8 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 151 | | 161 | | 162 | | 164 | | 164 | | 170 | | 171 | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | | | 65 | | | | 64 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 67 | | | | 69 | | | | 71 | | | | | |
| | Total Residual Chlorine (mg/L) | <0.10 | | | | <0.10 | | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 24.9 | 24.9 | 24.6 | 25.0 | 25.2 | 25.0 | 24.9 | 24.9 | 25.0 | 25.0 | 25.0 |
| 100% Intake | pH (SU) | 7.88 | 7.89 | 7.91 | 7.95 | 7.91 | 7.88 | 7.89 | 7.93 | 7.97 | 7 99 | 7.93 | 7.93 | 7.97 | 7.92 |
| | DO (mg/L) | 8.1 | 8.0 | 8.0 | 8.0 | 8.1 | 8.0 | 8.2 | 7.8 | 8.0 | 7.8 | 8.0 | 7.8 | 7 9 | 7.9 |
| | Conductivity (µmhos/cm) | 155 | | 164 | | 169 | | 167 | | 164 | | 168 | | 164 | |
| | Alkalinity (mg/L $CaCO_3$) | 63 | | | | 66 | | | | 64 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 69 | | | | 69 | | | | 69 | | | | | |
| | Total Residual Chlorine (mg/L) | <0.10 | | | | <0.10 | | | | <0.10 | | | | | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.1 | 25.0 | 24.6 | 25.1 | 25.0 | 25.1 | 24.8 | 24.8 | 25.0 | 25.0 | 25.2 |

File: sqn101_050520chem
Entered by: T.Sleeper
Reviewed by: _____

Environmental Testing Solutions, Inc.



## Total Residual Chlorine (4500-Cl G-2011), Screening Whole Effluent Toxicity Samples

Matrix: Water, RL = 0.10 mg/L

Analyst: TS
Date analyzed: 05.05.20

DPD: CHM 923

***Positive and Negative Control:***

| Control Type | Sample ID | Result (✓) | |
|---|---|---|---|
| | | Positive | Negative |
| Negative control | Deionized water | | ✓ |
| Positive control | Tap water | ✓ | |

***Sample screening:***

| Sample number | Sample ID | Sample characteristics | Result (✓) | |
|---|---|---|---|---|
| | | | Positive | Negative |
| 200504.02 | EASTMAN – RW | light tan, clear, particles | | ✓ |
| 200504.03 | ↓ EFFLUENT | light tan, clear, particles | | ✓ |
| 200504.04 | TVA/SQN 101 | no color, clear, particles | | ✓ |
| 200504.05 | ↓ INTAKE | light yellow, clear, particles | | ✓ |
| 200505.03 | CHATTANOOGA | light yellow, clear, particles | | ✓ |
| 200505.05 | HOKE | no color, clear, particles | | ✓ |
| 200505.06 | LOUGHLIN | light yellow, clear | | ✓ |
| 200505.04 | N. CARY | no color, clear | | ✓ |
| 200505.01 | S. CARY | no color, clear | | ✓ |
| 200505.07 | W. WAKE | light yellow, clear | | ✓ |
| | ~~DAIKIN~~ | | | ~~04-05-20~~ |
| 200505.08 | BOGUE BANKS | light yellow, clear | | ✓ |
| 200505.09 | CAROLINA BEACH | light yellow, clear | | ✓ |
| 200505.10 | ENGELHARD | yellow, clear, sulfur smell | | ✓ |
| 200505.11 | PASQUOTANK | no color, clear | | ✓ |
| 200504.01 ~~200404.01~~ | MARSHALL | light tan, clear | | ✓ |
| 200505.13 | MANTEO | light tan, clear | | ✓ |
| 200505.12 | APEX OIL | light yellow, clear, particles | | ✓ |
| | ~~TS~~ | ~~05.05.20~~ | | |
| | | | | |

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted. A positive result indicates the presence of total residual chlorine above detection (> 0.10 mg/L), which results in a pink to red color change with the addition of the DPD indicator. A negative result indicates the absence of total residual chlorine, which results in no color change with the addition of the DPD indicator.

Reviewed by: [signature]
Date reviewed: 05-05-20

## Total Residual Chlorine (ORION-97-70-1977), Confirmation of Whole Effluent Toxicity Samples

Matrix: Water, RL = 0.05 mg/L, Meter: Accumet Model AB250, SN 92349123

| | | | |
|---|---|---|---|
| Analyst | TS | Iodide reagent: | INR 988 |
| Date analyzed | 05.05.20 | Acid reagent: | INR 939 |

***Calibration:***

| | 0.05 mg/L | 0.50 mg/L | 5.00 mg/L | Slope Values (mV) (suggested range = 26 to 30 mV) | |
|---|---|---|---|---|---|
| Reference standard # | INSS 1889 | INSS 1889 | INSS 1889 | 30 | 27 |

*Note:* For samples with a residual chlorine of > 1.0 mg/L, the samples must be diluted to be within the calibration range.

***Laboratory control sample:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS 1889 | 0.25 | 0.263 | 105.2 % |

***Duplicate sample precision:***

| Sample number | Sample ID | Total residual chlorine (mg/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|
| 200504.04 | TVA \| SQN 101 | S 0.011 | |
| ↓ | Duplicate | D 0.010 | TS 05.05.20 |

***Sample measurements:***

| Sample number | Sample ID | Total residual chlorine (mg/L) |
|---|---|---|
| TV < 0.025 mg/L | Method Blank (MB) | <0.018 |
| 200504.05 | TVA \| SQN INTAKE | <0.009 |

TS 05.05.20

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted.

***Laboratory control sample:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS 1889 | 0.25 | 0.243 | 97.2 % |

Reviewed by [illegible] Date reviewed 05.05.20

Environmental Testing Solutions, Inc.

# Total Residual Chlorine (4500-Cl G-2011), Screening Whole Effluent Toxicity Samples

Matrix: Water, RL = 0.10 mg/L

Analyst: TS

Date analyzed: 05.07.20

DPD: CHM 985

***Positive and Negative Control:***

| Control Type | Sample ID | Result (✓) | |
|---|---|---|---|
| | | Positive | Negative |
| Negative control | Deionized water | | ✓ |
| Positive control | Tap water | ✓ | |

***Sample screening:***

| Sample number | Sample ID | Sample characteristics | Result (✓) | |
|---|---|---|---|---|
| | | | Positive | Negative |
| 200506.28 | EASTMAN - RW | light yellow, clear | | ✓ |
| 200506.29 | ↓ EFFLUENT | light yellow, clear | | ✓ |
| 200506.30 | TVA\|SQN 101 | no color, clear | | ✓ |
| 200506.31 | ↓ INTAKE | light yellow, clear | | ✓ |
| 200507.07 | CHATTANOOGA | light orange, clear | | ✓ |
| 200507.08 | HOKE | no color, clear | | ✓ |
| 200507.09 | LOUGHLIN | light yellow, clear | | ✓ |
| 200507.10 | N. CARY | light yellow, clear | | ✓ |
| 200507.02 | S. CARY | no color, clear, particles | | ✓ |
| 200507.11 | W. WAKE | light yellow, clear | | ✓ |
| 200507.12 | BOGUE BANKS | light yellow, clear | | ✓ |
| 200507.13 | CAROLINA BEACH | light yellow, clear, particles | | ✓ |
| 200507.14 | ENGELHARD | yellow, clear, sulfur smell | | ✓ |
| 200507.15 | PASQUOTANK | no color, clear | | ✓ |
| 200507.16 | CHEMTRADE | no color, clear | | ✓ |
| 200507.03 | ALCOA 005 | no color, clear | | ✓ |
| 200507.04 | ↓ 013 | light tan, clear, particles | | ✓ |
| | | | | |
| | TS 05.07.20 | | | |
| | | | | |

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted. A positive result indicates the presence of total residual chlorine above detection (> 0.10 mg/L), which results in a pink to red color change with the addition of the DPD indicator. A negative result indicates the absence of total residual chlorine, which results in no color change with the addition of the DPD indicator.

Reviewed by: JL

Date reviewed: 05-07-20

## Total Residual Chlorine (ORION-97-70-1977), Confirmation of Whole Effluent Toxicity Samples

Matrix: Water, RL = 0.05 mg/L, Meter: Accumet Model AB250, SN 92349123

| | | | |
|---|---|---|---|
| Analyst | TS | Iodide reagent: | INR ~~939~~ 988 TS 05.07.20 |
| Date analyzed | 05.07.20 | Acid reagent: | INR 939 |

***Calibration:***

| | 0.05 mg/L | 0.50 mg/L | 5.00 mg/L | Slope Values (mV) (suggested range = 26 to 30 mV) | |
|---|---|---|---|---|---|
| Reference standard # | INSS 1889 | INSS 1889 | INSS 1889 | 29 | 30 |

*Note:* For samples with a residual chlorine of > 1.0 mg/L, the samples must be diluted to be within the calibration range.

***Laboratory control sample:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS 1889 | 0.25 | 0.247 | 98.8% |

***Duplicate sample precision:***

| Sample number | Sample ID | Total residual chlorine (mg/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|
| 200506.30 | TVA \| SQN 101 | S 0.005 | |
| ↓ | Duplicate | D 0.006 | TS 05.07.20 |

***Sample measurements:***

| Sample number | Sample ID | Total residual chlorine (mg/L) |
|---|---|---|
| TV < 0.025 mg/L | Method Blank (MB) | <0.010 |
| 200506.31 | TVA \| SQN INTAKE | <0.009 |

TS 05.07.20

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted.

***Laboratory control sample:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS ~~0.235~~ 1889 | 0.25 | 0.235 | 94.0% |

TS 05.07.20

Reviewed by [signature] Date reviewed 05.07.20

Environmental Testing Solutions, Inc.



## Total Residual Chlorine (4500-Cl G-2011), Screening Whole Effluent Toxicity Samples

Matrix: Water, RL = 0.10 mg/L

Analyst: TS
Date analyzed: 05.09.20

DPD: CHM 985

***Positive and Negative Control:***

| Control Type | Sample ID | Result (✓) | |
|---|---|---|---|
| | | Positive | Negative |
| Negative control | Deionized water | | ✓ |
| Positive control | Tap water | ✓ | |

***Sample screening:***

| Sample number | Sample ID | Sample characteristics | Result (✓) | |
|---|---|---|---|---|
| | | | Positive | Negative |
| 200508.20 | EASTMAN - RW | no color, clear | | ✓ |
| 200508.21 | ↓ EFFLUENT | no color, clear | | ✓ |
| 200508.22 | TVA/SQN 101 | light tan, clear, particles | | ✓ |
| 200508.23 | ↓ INTAKE | light tan, clear, particles | | ✓ |
| 200509.01 | CHATTANOOGA | tan, cloudy | | ✓ |
| 200509.02 | HOKE | no color, clear | | ✓ |
| 200509.03 | LOUGHLIN | light yellow, clear | | ✓ |
| 200509.04 | N. CARY | light tan, clear | | ✓ |
| 200509.05 | S. CARY | no color, clear | | ✓ |
| 200509.06 | W. WAKE | light yellow, clear | | ✓ |
| 200509.07 | BOGUE BANKS | light yellow, clear | | ✓ |
| 200509.08 | CAROLINA BEACH | no color, clear | | ✓ |
| 200509.09 | ENGELHARD | yellow, clear, sulfur smell | | ✓ |
| 200509.10 | PASQUOTANK | no color, clear | | ✓ |
| | | | | |
| | | | | |
| | | | | |
| | TS 05.09.20 | | | |
| | | | | |
| | | | | |

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted. A positive result indicates the presence of total residual chlorine above detection (> 0.10 mg/L), which results in a pink to red color change with the addition of the DPD indicator. A negative result indicates the absence of total residual chlorine, which results in no color change with the addition of the DPD indicator.

Reviewed by: JP
Date reviewed: 05-09-20

## Total Residual Chlorine (ORION-97-70-1977), Confirmation of Whole Effluent Toxicity Samples

Matrix: Water, RL = 0.05 mg/L, Meter: Accumet Model AB250, SN 92349123

| | | | |
|---|---|---|---|
| Analyst | TS | Iodide reagent: | INR 988 |
| Date analyzed | 05.09.20 | Acid reagent: | INR 939 |

***Calibration:***

| | 0.05 mg/L | 0.50 mg/L | 5.00 mg/L | Slope Values (mV) (suggested range = 26 to 30 mV) | |
|---|---|---|---|---|---|
| Reference standard # | INSS 1889 | INSS 1889 | INSS 1889 | 30 | 28 |

*Note:* For samples with a residual chlorine of > 1.0 mg/L, the samples must be diluted to be within the calibration range.

***Laboratory control sample:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS 1889 | 0.25 | 0.269 | 107.6 % |

***Duplicate sample precision:***

| Sample number | Sample ID | Total residual chlorine (mg/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|
| 200508.22 | TVA \| SQN 101 | S < 0.003 | |
| ↓ | Duplicate | D < 0.002 | TS 05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Total residual chlorine (mg/L) |
|---|---|---|
| TV < 0.025 mg/L | Method Blank (MB) | < 0.014 |
| 200508.23 | TVA \| SQN INTAKE | < 0.002 |

TS 05.09.20

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted.

***Laboratory control sample:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS 1889 | 0.25 | 0.259 | 103.6 % |

Reviewed by [illegible] Date reviewed 05-09-20

Analyst: N
Date analyzed: 05.01.20

## Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L, Samples are titrated to pH = 4.5 S.U.

pH Meter: Accumet Model AR20, SN 93312452

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of $H_2SO_4$ = (5 mL $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 100 mL sample = N x 500 |
|---|---|---|---|---|---|---|
| INR 912 | INSS 1039 | 0.1 | 12.4 | 12.3 | 0.0203 | 10.2 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 12.4 | 22.0 | 9.6 | 10.2 | 98 | 98.0% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 198934 ~~198030~~ | ETS (alo$H_2O$ sulfur | 100 | 22.0 | 25.2 | 3.2 | 10.2 | S 33 | |
| 1 | Duplicate (D) | 1 | 25.2 | 28.5 | 3.3 | 1 | D 34 | 3.0% |

05100

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| MB (TV < 2.5 mg/L) | Deionized water, pH = 5.2 S.U. | 100 | 0.0 | 0.1 | 0.1 | 10.2 | ND |
| 198938 | ETS Sulfur WER | | 28.5 | 30.1 | 1.6 | | 16 |
| 04.23.20 | MHSW | | 30.1 | 36.1 | 6.0 | | 61 |
| 04.29.20A | | | 36.1 | 42.0 | 5.9 | | 60 |
| B | | | 42.0 | 47.9 | 5.9 | | 60 |
| C | | | 0.0 | 6.0 | 6.0 | | 61 |
| D | | | 6.0 | ~~3.4~~ 12.0 | 6.0 | | 61 |
| E | | | 12.0 | 05/01/20 17.8 | 5.8 | | 59 |
| F | | | 17.8 | 23.5 | 9.7 | | 58 |
| 04.29.20A | Salt SW | | 23.5 | 35.1 | 11.6 | | 120 |
| B | | | 35.1 | 46.1 | 11.0 | | 110 |

05/01/20

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 22.9 | 33.2 | 10.3 | 10.2 | 105 | 105.0% |

Reviewed by: [signature]  Date reviewed: 05.01.20

Analyst: BSL
Date analyzed: 05.09.20

## Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L, Samples are titrated to pH = 4.5 S.U.

pH Meter: Accumet Model AR20, SN 93312452

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of $H_2SO_4$ = (5 mL $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 100 mL sample = N x 500 |
|---|---|---|---|---|---|---|
| INR 969 | INSS 1839 | 0.2 | 12.7 | 12.5 | 0.0200 | 10.0 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 12.7 | 23.1 | 10.4 | 10.0 | 104 | 104.0% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 05.07.20A | MHSW | 100 | 23.1 | 29.4 | 6.3 | 10.0 | S 63 | |
| ↓ | Duplicate (D) | 1 | 29.4 | 35.7 | 6.3 | ↓ | D 63 | BSL — 05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| MB (TV < 2.5 mg/L) | Deionized water, pH = 5.48 s.u. | 100 | 0.0 | 0.2 | 0.2 | 10.0 | ND |
| 05.07.20B | MHSW | 100 | 35.7 | 41.7 | 6.0 | | 60 |
| ↓ C | ↓ | | 41.7 | 47.7 | 6.0 | | 60 |
| ↓ D | ↓ | | 0.0 | 6.1 | 6.1 | | 61 |
| 200504.03 | Eastman 1 | 50 | 6.1 | 11.0 | 4.9 (2) | | 98 |
| 200506.29 | ↓ 2 | ↓ | 11.0 | 15.8 | 4.8 (2) | | 96 |
| 200508.21 | ↓ 3 | | 15.8 | 20.8 | 5.0 (2) | | 100 |
| 200504.03 | Eastman Anti 1 | 50 | 20.8 | 25.4 | 4.6 (2) | | 92 |
| 200506.29 | ↓ 2 | ↓ | 25.4 | 30.3 | 4.9 (2) | | 98 |
| 200508.21 | ↓ 3 | | 30.3 | 35.2 | 4.9 (2) | | 98 |
| 200504.03 04 | Eastman RW 1 | 50 | 35.2 | 39.4 | 4.2 (2) | | 84 |
| 200506.29 28 | ↓ 2 | ↓ | 39.4 | 43.8 | 4.4 (2) | | 88 |
| 200508.21 20 | ↓ 3 | | 0.0 | 4.6 | 4.6 (2) | | 92 |
| 200504.04 | Eastman RW Anti 1 | 50 | 4.6 | 8.9 | 4.3 (2) | | 86 |
| 200506.28 | ↓ 2 | ↓ | 8.9 | 13.3 | 4.4 (2) | | 88 |
| 200508.20 | ↓ 3 | | 13.3 | 17.9 | 4.6 (2) | | 92 |
| 04.29.20B | MHSW VV 1 | 100 | 17.9 | 23.9 | 6.0 | | 60 |
| 04.29.20D | ↓ 2 | ↓ | 23.9 | 30.1 | 6.2 | | 62 |
| 05.07.20B | ↓ 3 | | 30.1 | 36.2 | 6.1 | | 61 |
| 04.29.20B | MHSW Anti 1 | | 36.2 | 42.1 | 5.9 | ↓ | 59 |

05-11-20 K

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS | 100 | 100 | | | | | | BSL 05.09.20 |

Reviewed by: K  Date reviewed: 05.10.20

| | |
|---|---|
| Analyst | BSL |
| Date analyzed | 05.09.20 |

## Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L, Samples are titrated to pH = 4.5 S.U.

pH Meter: Accumet Model AR20, SN 93312452

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of $H_2SO_4$ = (5 mL $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 100 mL sample = N x 500 |
|---|---|---|---|---|---|---|
| INR | INSS | | | | | BSL 05.09.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 0.0 | 10.3 | 10.3 | 10.0 | 103 | 103.0% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 04-29-20D | MHSW Ant. 2 | 100 | 10.3 | 16.4 | 6.1 | 10.0 | S 61 | |
| ↓ | Duplicate (D) | 1 | 16.4 | 22.5 | 6.1 | 1 | D 61 | BSL-05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| ~~MB (TV < 2.5 mg/L)~~ | ~~Deionized water, pH = ____ s.u.~~ | ~~100~~ | | | | | BSL 05.09.20 |
| 05.07.20B | MHSW Ant. 3 | 100 | 22.5 | 28.6 | 6.1 | 10.0 | 61 |
| 200504.04 | TVA SQN 101 1 | | 28.6 | 34.8 | 6.2 | | 62 |
| 200506.30 | 2 | | 34.8 | 41.3 | 6.5 | | 65 |
| 200508.22 | 3 | | 41.3 | 47.7 | 6.4 | | 64 |
| 200504.04 | TVA SQN 101 UV 1 | | 0.0 | 6.6 | 6.6 | | 66 |
| 200506.30 | 2 | | 6.6 | 13.2 | 6.6 | | 66 |
| 200508.22 | 3 | | 13.2 | 19.6 | 6.4 | | 64 |
| 200504.05 | TVA SQN INT 1 | | 19.6 | 25.9 | 6.3 | | 63 |
| 200506.31 | 2 | | 25.9 | 32.5 | 6.6 | | 66 |
| 200508.23 | 3 | | 32.5 | 38.9 | 6.4 | | 64 |
| 200504.05 | TVA SQN INT UV 1 | | 38.9 | 45.2 | 6.3 | | 63 |
| 200506.31 | 2 | | 0.0 | 6.5 | 6.5 | | 65 |
| 200508.23 | 3 | | 6.5 | 12.9 | 6.4 | | 64 |
| 200507.16 | Chem Trade | | 12.9 | 20.0 | 7.1 | | 71 |
| 200505.03 | Chattanooga 1 | 25 | 20.0 | 26.3 | 6.3 (2)(4) | | 130 250 |
| 200507.07 | 2 | | 26.3 | 31.2 | 4.9 (2) | | 98 200 |
| 200509.01 | 3 | | 31.2 | 36.4 | 5.2 (2) | | 100 210 |
| 200505.05 | Hike 1 | 25 | 36.4 | 41.2 | 4.8 (2) | | 96 190 |
| 200507.08 | 2 | | 41.2 | 46.1 | 4.9 (2) | | 98 200 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS | 100 | 100 | | | | | | BSL 05.09.20 |

Reviewed by: [signature] Date reviewed: 05.10.20

| | |
|---|---|
| Analyst | BS |
| Date analyzed | ~~BS~~ 05.09.20 |

## Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L, Samples are titrated to pH = 4.5 S.U.

pH Meter: Accumet Model AR20, SN 93312452

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of $H_2SO_4$ = (5 mL $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 100 mL sample = N x 500 |
|---|---|---|---|---|---|---|
| INR | INSS | | | | | 05.09.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 0.0 | 10.2 | 10.2 | 10.0 | 102 | 102.0% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 200509.02 | Hoke 3 | 25 | 10.2 | 14.6 | 4.4 (4) | 10.0 | S 180 | |
| ↓ | Duplicate (D) | ↓ | 14.6 | 19.0 | 4.4 (4) | ↓ | D 180 | BS — 05.09.20 |

BS 05.09.20

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| MB (TV < 2.5 mg/L) | Deionized water, pH = ____ s.u. | ~~100~~ | | | | | BS 05.09.20 |
| 200505.06 | Loughlin 1 | 50 | 19.0 | 22.4 | 3.4 (2) | 10.0 | 68 |
| 200507.09 | ↓ 2 | ↓ | 22.4 | 25.0 | 2.6 (2) | | 52 |
| 200509.03 | ↓ 3 | ↓ | 25.0 | 27.6 | 2.6 (2) | | 52 |
| 200505.04 | North Cary 1 | 50 | 27.6 | 31.2 | 3.6 (2) | | 72 |
| 200507.10 | ↓ 2 | ↓ | 31.2 | 34.7 | 3.5 (2) | | 70 |
| 200509.04 | ↓ 3 | ↓ | 34.7 | 38.2 | 3.5 (2) | | 70 |
| 200505.01 | South Cary 1 | 50 | 38.2 | 40.8 | 2.6 (2) | | 52 |
| 200507.02 | ↓ 2 | ↓ | 40.8 | 43.1 | 2.3 (2) | | 46 |
| 200509.05 | ↓ 3 | ↓ | 43.1 | 45.4 | 2.3 (2) | | 46 |
| 200505.07 | Western Wake 1 | 100 | 0.0 | 2.3 | 2.3 | | 23 |
| 200507.11 | ↓ 2 | ↓ | 2.3 | 3.3 | 1.0 | | 10 |
| 200509.06 | ↓ 3 | ↓ | 3.3 | 5.1 | 1.8 | | 18 |
| 200505.08 | Bugue Banks 1 | 5 | 5.1 | 9.6 | 4.5 (20) | | 900 |
| 200507.12 | ↓ 2 | ↓ | 9.6 | 14.3 | 4.7 (20) | | 940 |
| 200509.07 | ↓ 3 | ↓ | 14.3 | 19.3 | 5.0 (20) | | 1000 |
| 200505.09 | Carolina Beach 1 | 25 | 19.3 | 21.4 | 2.1 (4) | | 84 |
| 200507.13 | ↓ 2 | ↓ | 21.4 | 23.5 | 2.1 (4) | | 84 |
| 200509.08 | ↓ 3 | ↓ | 23.5 | 25.6 | 2.1 (4) | | 84 |
| 200505.10 | Engel Pond 1 | 2 | 25.6 | 31.5 | 5.9 (50) | | 3000 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS | 100 | 100 | | | | | | 05.09.20 |

Reviewed by: [illegible] Date reviewed: 05.10.20

| | |
|---|---|
| Analyst | BSL |
| Date analyzed | 05.09.20 |

## Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L, Samples are titrated to pH = 4.5 S.U.

pH Meter: Accumet Model AR20, SN 93312452

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of $H_2SO_4$ = (5 mL $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.0180 – 0.0220) | pH Factor or Multiplier = (N x 50000)/ 100 mL sample = N x 500 |
|---|---|---|---|---|---|---|
| INR | INSS | | | | | BSL 05.09.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 31.5 | 41.7 | 10.2 | 10.0 | 102 | 102.5% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 200507.14 | Engelhard 2 | 2 | 41.7 | 47.8 | 6.1 | (50) 10.0 | S 3000 | |
| ↓ | Duplicate (D) | ↓ | 0.0 | 6.1 | 6.1 | (50) ↓ | D 3000 | BSL — 05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| MB (TV < 2.5 mg/L) | ~~Deionized water, pH = 5.0~~ | ~~100~~ | | | | | BSL 05.09.20 |
| 200509.09 | Engelhard 3 | 2 | 6.1 | 12.2 | 6.1 | (50) 10.0 | 3000 |
| 200505.11 | Pasquotank 1 | 2 | 12.2 | 16.6 | 4.4 | (50) | 2200 |
| 200507.15 | ↓ 2 | ↓ | 16.6 | 20.9 | 4.3 | (50) | 2200 |
| 200509.10 | ↓ 3 | ↓ | 20.9 | 25.0 | 4.1 | (50) | 2000 |
| 05.07.20 | Salt SW | 100 | 25.0 | 34.1 | 9.1 | ↓ | 91 |
| | | | | | | | BSL 05.09.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1863 | 100 | 100 | 34.1 | 44.4 | 10.3 | 10.0 | 103 | 103.0% |

Reviewed by: [illegible]  Date reviewed: 05.10.20

Analyst: [illegible]
Date analyzed: 05.01.20

## Hardness (SM 2340 C-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of EDTA = 0.2/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 50 mL sample = N x 1000 |
|---|---|---|---|---|---|---|
| INR 965 | INSS 1033 | 0.0 | 9.7 | 9.7 | 0.0206 | 20.6 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1872 | 40 | 50 | 9.7 | 11.6 | 1.9 | 20.6 | 39 | 97.5% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 198030 | CTS Sutton WEIR | 50 | 11.6 | 13.9 | 2.3 | 20.6 | S 47 | |
| ↓ | Duplicate (D) | ↓ | 13.9 | 16.2 | 2.3 | ↓ | D 47 | 0 [illegible] |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| 050120 MB (TV < 2.5 mg/L) | Deionized water | 50 | 0.0 | 0.0 | 0.0 | 20.6 | ND |
| 199034 ~~198030~~ | CTS Lab H2O - Sutton | ↓ | 16.2 | 18.7 | 2.5 | ↓ | 52 |
| 04.23.20 | MHSW | ↓ | 18.7 | 23.2 | 4.5 | ↓ | 93 |
| 04.29.20 A | ↓ | ↓ | 23.2 | 27.5 | 4.3 | ↓ | 89 |
| B | ↓ | ↓ | 27.5 | 32.0 | 4.5 | ↓ | 93 |
| C | ↓ | ↓ | 32.0 | 36.4 | 4.4 | ↓ | 91 |
| D | ↓ | ↓ | 36.4 | 40.7 | 4.3 | ↓ | ~~86~~ 89 05.01.20 |
| E | ↓ | ↓ | 40.7 | 45.1 | 4.4 | ↓ | 91 |
| F | ↓ | ↓ | 45.1 | 49.3 | 4.2 | ↓ | 86 |

[illegible] 05.01.20

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1872 | 40 | 50 | 46.3 | 48.2 | 1.9 | 20.6 | 39 | 97.5% |

Reviewed by: [illegible] Date reviewed: 05.15.20

Analyst: BSL
Date analyzed: ~~05-10-20~~ BSL 05-09-20

## Hardness (SM 2340 C-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of EDTA = 0.2/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 50 mL sample = N x 1000 |
|---|---|---|---|---|---|---|
| INR 965 | INSS 1872 | 0.0 | 9.8 | 9.8 | 0.0204 | 20.4 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS ~~1872~~ 1833 BSL ~~05-10-20~~ 05-09-20 | 40 | 50 | 9.8 | 11.7 | 1.9 | 20.4 | 39 | 97.5% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| | Marcellus | 50 | 11.7 | 15.8 | 4.1 | 20.4 | S 84 | |
| | Duplicate (D) | ↓ | 15.8 | 19.9 | 4.1 | ↓ | D 84 | BSL — 05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| MB (TV < 2.5 mg/L) | Deionized water | 50 | 0.0 | 0.0 | 0.0 | 20.4 | ND |
| | 1000 hr e/o | ↓ | 19.9 | 20.2 | 0.3 | ↓ | 6.1 |
| | 1 UP | ↓ | 20.2 | 20.6 | 0.4 | ↓ | 8.0 |
| 199006 | Flotty RS e/o | 10 | 20.6 | 25.3 | 4.7 (5) | ↓ | 480 |
| 199007 | 1 UP | 50 | 25.3 | 25.9 | 0.6 | ↓ | 12 |
| 04-29-20B | MHSW UV 1 | ↓ | 25.9 | 30.3 | 4.4 | ↓ | 90 |
| 04-29-20D | 2 | ↓ | 30.3 | 34.6 | 4.3 | ↓ | 88 |
| 05-01-20B | ↓ 3 | ↓ | 34.6 | 38.8 | 4.2 | ↓ | 86 |
| 200504.03 | Eastman 1 | 25 | 38.8 | 41.4 | 2.6 (2) | ↓ | 110 |
| 200506.29 | ↓ 2 | ↓ | 41.4 | 44.0 | 2.6 (2) | ↓ | 110 |
| 200508.21 | ↓ 3 | ↓ | 44.0 | 46.6 | 2.6 (2) | ↓ | 110 |
| 200504.03 | Eastman Ant. 1 | ↓ | 0.0 | 2.6 | 2.6 (2) | ↓ | 110 |
| 200506.29 | ↓ 2 | ↓ | 2.6 | 5.4 | 2.8 (2) | ↓ | 110 |
| 200508.21 | ↓ 3 | ↓ | 5.4 | 8.2 | 2.8 (2) | ↓ | 110 |
| 04-29-20B | MHSW Anti 1 | 50 | 8.2 | ~~13.5~~ BSL 05-09-20 (12.5) | 4.3 | ↓ | 88 |
| 04-29-20D | ↓ 2 | ↓ | ~~13.5~~ BSL | 16.9 | 4.4 | ↓ | 90 |
| 05-01-20B | ↓ 3 | ↓ | 16.9 | 21.2 | 4.3 | ↓ | 88 |
| 200504.02 | Eastman RW 1 | 25 | 21.2 | 23.4 | 2.2 (2) | ↓ | 90 |
| 200506.28 | ↓ 2 | ↓ | 23.4 | 25.7 | 2.3 (2) | ↓ | 94 |
| 200508.20 | ↓ 3 | ↓ | 25.7 | 28.1 | 2.4 (2) | ↓ | 98 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS | 40 | 50 | | | | | | BSL ~~05-10-20~~ 05-09-20 |

Reviewed by: [signature] Date reviewed: 05-09-20

Analyst: BSL
Date analyzed: ~~05.10.20~~ BSL 05.09.20

## Hardness (SM 2340 C-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of EDTA = 0.2/E (acceptable range = 0.0180–0.0220) | pH Factor or Multiplier = (N x 50000)/ 50 mL sample = N x 1000 |
|---|---|---|---|---|---|---|
| INR | INSS | | | | | BSL 05.09.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1833 | 40 | 50 | 28.1 | 30.1 | 2.0 | 20.4 | 41 | 102.5% |

***Duplicate sample precision:***

| Sample number | Sample ID Eastman | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 200504.02 | RW Anti 1 | 25 | 30.1 | 32.3 | 2.2 (2) | 20.4 | S 90 | |
| ↓ | Duplicate (D) | ↓ | 32.3 | 34.5 | 2.2 (2) | ↓ | D 90 | BSL 05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| ~~MB (TV < 2.5 mg/L)~~ | ~~Deionized water~~ | 50 | | | | | 05.09.20 BSL |
| 200506.28 | Eastman RW Anti 2 | 25 | 34.5 | 37.0 | 2.5 (2) | 20.4 | 100 |
| 200508.20 | ↓ ↓ 3 | ↓ | 37.0 | 39.3 | 2.3 (2) | | 94 |
| 200504.04 | TVASQN 101 1 | 50 | 39.3 | 42.6 | 3.3 | | 67 |
| 200506.30 | 2 | | 42.6 | 46.0 | 3.4 | | 69 |
| 200508.22 | ↓ 3 | | 0.0 | 3.5 | 3.5 | | 71 |
| 200504.04 | TVASQN 101 UV 1 | | 3.5 | 6.9 | ~~6.4~~ 3.4 BSL 05.09.20 | | 69 |
| 200506.30 | 2 | | 6.9 | 10.2 | 3.3 | | 67 |
| 200508.22 | ↓ 3 | | 10.2 | 13.8 | 3.6 | | 73 |
| 200504.05 | TVA SQN INT 1 | | 13.8 | 17.2 | 3.4 | | 69 |
| 200506.31 | 2 | | 17.2 | 20.6 | 3.4 | | 69 |
| 200508.23 | ↓ 3 | | 20.6 | 24.0 | 3.4 | | 69 |
| 200504.05 | TVASQN INT UV 1 | | 24.0 | 27.4 | 3.4 | | 69 |
| 200506.31 | 2 | | 27.4 | 30.9 | 3.5 | | 71 |
| 05-12 ~~200504~~ 200508.23 | ↓ 3 | | 30.9 | 34.3 | 3.4 | | 69 |
| 200507.16 | Chemtrade | | 34.3 | 37.3 | 3.0 | | 61 |
| 200505.03 | Chattanooga 1 | 25 | 37.3 | 41.2 | 3.9 (2) | | ~~80~~ 160 BSL 05.20 |
| 200507.07 | ↓ 2 | | 41.2 | 44.8 | 3.6 (2) | | 150 |
| 200509.01 | ↓ 3 | | 0.0 | 4.6 | 4.6 (2) | | 190 |
| 200505.05 | Hoke 1 | | 4.6 | 9.5 | 4.9 (2) | | 200 |

***Laboratory control standard (LCS):***

| ~~Reference standard~~ number | ~~True value (TV)~~ (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS | 40 | 50 | | | | | | 05.09.20 |

Reviewed by: [illegible] Date reviewed: 05.09.20

Analyst: BSL
Date analyzed: 05.09.20

## Hardness (SM 2340 C-2011)

Matrix: Water, RL = 5.0 mg $CaCO_3$/L

***Titrant normality and multiplier determination:***

| Titrant reference number | Normality check standard number | Begin mL | End mL | Total mL (E) | Normality (N) of EDTA = 0.2/E (acceptable range = 0.0180 - 0.0220) | pH Factor or Multiplier = (N x 50000)/ 50 mL sample = N x 1000 |
|---|---|---|---|---|---|---|
| INR | INSS | | | | | BSL 05.09.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1833 | 40 | 50 | 9.5 | 11.4 | 1.9 | 20.4 | 39 | 97.5% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 200507.08 | Hole 2 | 25 | 11.4 | 16.3 | 4.9 (2) | 20.4 | S 200 | |
| ↓ | Duplicate (D) | ↓ | 16.3 | 21.2 | 4.9 (2) | ↓ | D 200 | BSL 05.09.20 |

***Sample measurements:***

| Sample number | Sample ID | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| ~~MB (TV< 2.5 mg/L)~~ | ~~Deionized water~~ | ~~50~~ | | | | | 05.09.20 BSL |
| 200509.02 | Hole 3 | 25 | 21.2 | 25.6 | 4.4 (2) | 20.4 | 180 |
| 200505.06 | Loughlin 1 | 25 | 25.6 | 28.2 | 2.6 (2) | | 110 |
| 200507.09 | ↓ 2 | ↓ | 28.2 | 31.3 | 3.1 (2) | | 130 |
| 200509.03 | ↓ 3 | ↓ | 31.3 | 34.7 | 3.4 (2) | | 140 |
| 200505.04 | North Cuy 1 | 50 | 34.7 | 37.8 | 3.1 | | 63 |
| 200507.10 | ↓ 2 | ↓ | 37.8 | 40.6 | 2.8 | | 57 |
| 200509.04 | ↓ 3 | ↓ | 40.6 | 43.6 | 3.0 | | 61 |
| 200505.01 | South Cuy 1 | 50 | 43.6 | 46.7 | 3.1 | | 63 |
| 200507.02 | ↓ 2 | ↓ | 0.0 | 2.6 | 2.6 | | 53 |
| 200509.05 | ↓ 3 | ↓ | 2.6 | 5.5 | 2.9 | | 59 |
| 200505.07 | Western Weave 1 | 25 | 5.5 | 7.0 | 1.5 (2) | | 61 |
| 200507.11 | ↓ 2 | ↓ | 7.0 | 8.4 | 1.4 (2) | | 57 |
| 200509.06 | ↓ 3 | ↓ | 8.4 | 9.9 | 1.5 (2) | | 61 |
| 05.07.20 A | MHSW | 50 | 9.9 | 14.2 | 4.3 | | 89 |
| ↓ B | ↓ | ↓ | 14.2 | 18.6 | 4.4 | | 90 |
| ↓ C | ↓ | ↓ | 18.6 | 22.8 | 4.2 | | 86 |
| ↓ D | ↓ | ↓ | 22.8 | 26.9 | 4.1 | ↓ | 84 |
| | | | | | | | |
| | | | | | | | BSL 0509.20 |

***Laboratory control standard (LCS):***

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (mL) | Begin mL | End mL | Total mL | Multiplier | Hardness (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS 1833 | 40 | 50 | 26.9 | 28.9 | 2.0 | 20.4 | 41 | 102.5% |

Reviewed by: [illegible] Date reviewed: 05.09.20

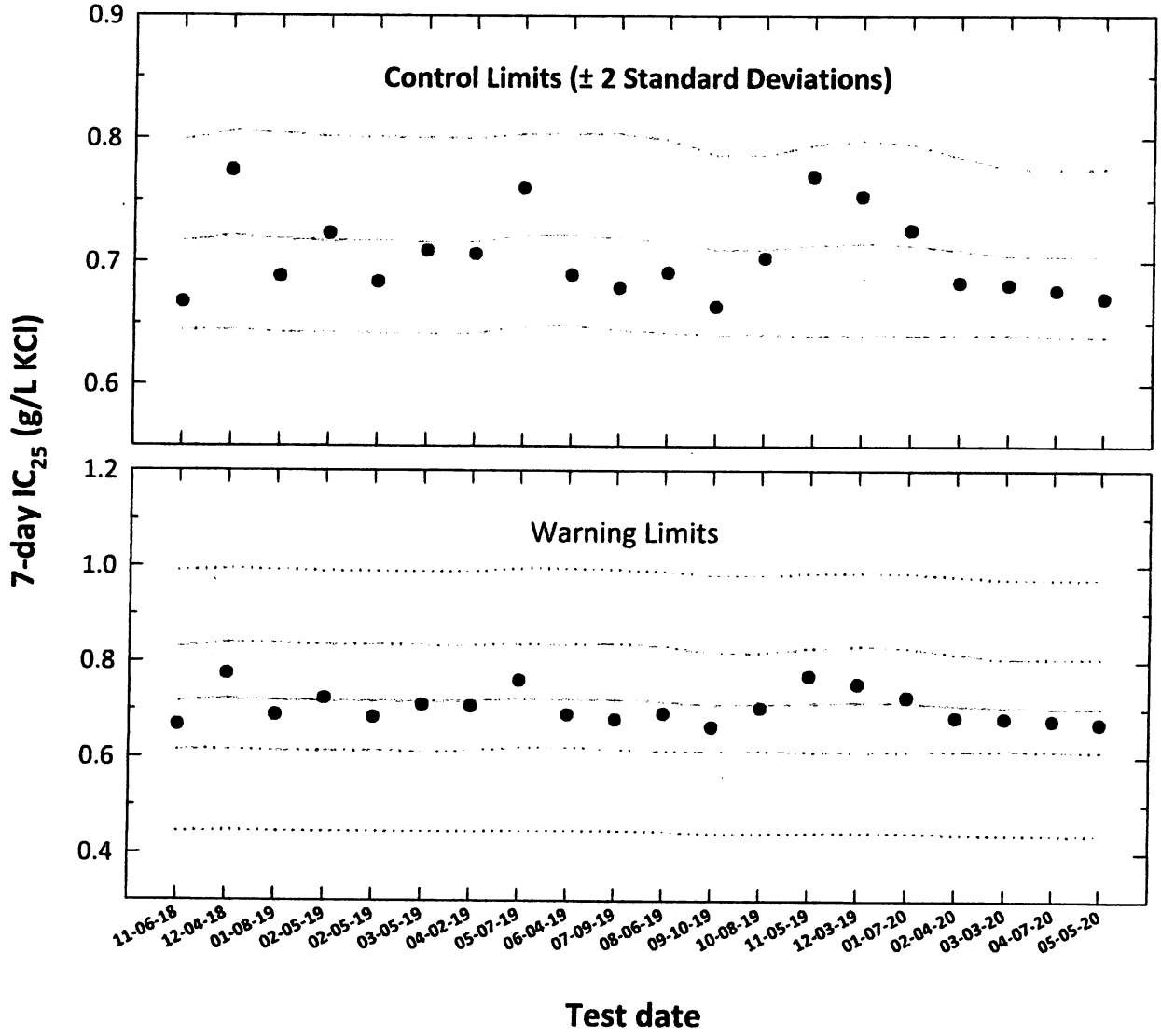
Sequoyah Nuclear Plant Biomonitoring
May 05 – 12, 2020

Appendix D

Reference Toxicant Test and
Control Chart

Environmental Testing Solutions, Inc.

# *Pimephales promelas*
# Chronic Reference Toxicant Control Chart
# Source: In-house Culture




- ● **7-day $IC_{25}$** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic $IC_{25}$ converted to anti-logarithmic values)
- **Control Limits** (mean logarithmic $IC_{25}$ ± 2 standard deviations converted to anti-logarithmic values)
- **Laboratory Warning Limits** (mean logarithmic $IC_{25}$ ± 2 coefficent of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25}$ ± $S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Independent Review by Kelley E. Keenan:



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Independently Reviewed by Kelley E. Hansen

# *Pimephales promelas*
## Chronic Reference Toxicant Control Chart
## Source: In-house Culture

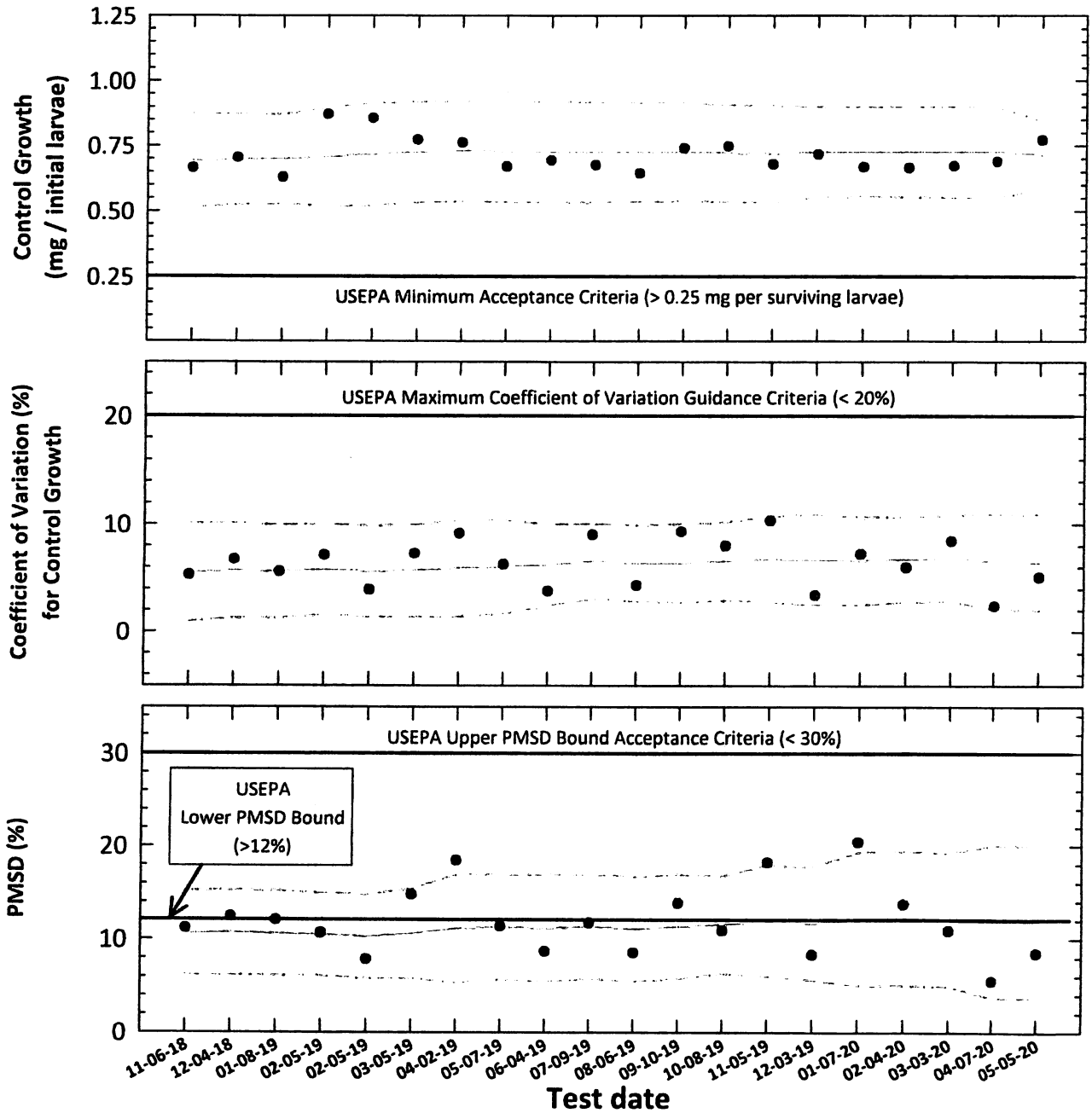
| Test number | Test date | 7-day $IC_{25}$ ToxCal Determination (g/L KCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L KCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7-day $IC_{25}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 75th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.75}$ | CT + $S_{A.75}$ |
| 1 | 11-06-18 | 0.6673 | -0.1757 | -0.1444 | 0.0234 | 0.7171 | 0.6440 | 0.7986 | 0.6152 | 0.8307 | 0.4446 | 0.9896 |
| 2 | 12-04-18 | 0.7740 | -0.1113 | -0.1419 | 0.0241 | 0.7212 | 0.6454 | 0.8060 | 0.6160 | 0.8388 | 0.4472 | 0.9953 |
| 3 | 01-08-19 | 0.6882 | -0.1623 | -0.1434 | 0.0245 | 0.7188 | 0.6422 | 0.8045 | 0.6123 | 0.8380 | 0.4456 | 0.9919 |
| 4 | 02-05-19 | 0.7232 | -0.1407 | -0.1442 | 0.0240 | 0.7174 | 0.6422 | 0.8013 | 0.6126 | 0.8344 | 0.4448 | 0.9900 |
| 5 | 02-05-19 | 0.6837 | -0.1651 | -0.1442 | 0.0240 | 0.7174 | 0.6423 | 0.8013 | 0.6128 | 0.8343 | 0.4448 | 0.9901 |
| 6 | 03-05-19 | 0.7090 | -0.1494 | -0.1448 | 0.0240 | 0.7165 | 0.6416 | 0.8002 | 0.6119 | 0.8333 | 0.4442 | 0.9888 |
| 7 | 04-02-19 | 0.7064 | -0.1509 | -0.1446 | 0.0239 | 0.7167 | 0.6419 | 0.8003 | 0.6123 | 0.8333 | 0.4444 | 0.9891 |
| 8 | 05-07-19 | 0.7600 | -0.1192 | -0.1418 | 0.0234 | 0.7214 | 0.6478 | 0.8035 | 0.6193 | 0.8352 | 0.4473 | 0.9956 |
| 9 | 06-04-19 | 0.6892 | -0.1616 | -0.1417 | 0.0233 | 0.7217 | 0.6484 | 0.8032 | 0.6201 | 0.8347 | 0.4474 | 0.9959 |
| 10 | 07-09-19 | 0.6789 | -0.1682 | -0.1429 | 0.0240 | 0.7196 | 0.6443 | 0.8037 | 0.6150 | 0.8365 | 0.4462 | 0.9931 |
| 11 | 08-06-19 | 0.6912 | -0.1604 | -0.1449 | 0.0237 | 0.7163 | 0.6422 | 0.7989 | 0.6129 | 0.8317 | 0.4441 | 0.9885 |
| 12 | 09-10-19 | 0.6635 | -0.1782 | -0.1488 | 0.0223 | 0.7099 | 0.6407 | 0.7865 | 0.6124 | 0.8179 | 0.4401 | 0.9796 |
| 13 | 10-08-19 | 0.7029 | -0.1531 | -0.1487 | 0.0222 | 0.7100 | 0.6409 | 0.7866 | 0.6126 | 0.8179 | 0.4402 | 0.9798 |
| 14 | 11-05-19 | 0.7693 | -0.1139 | -0.1466 | 0.0235 | 0.7135 | 0.6404 | 0.7950 | 0.6111 | 0.8277 | 0.4424 | 0.9846 |
| 15 | 12-03-19 | 0.7531 | -0.1232 | -0.1456 | 0.0240 | 0.7151 | 0.6401 | 0.7988 | 0.6103 | 0.8322 | 0.4434 | 0.9868 |
| 16 | 01-07-20 | 0.7257 | -0.1392 | -0.1464 | 0.0236 | 0.7139 | 0.6404 | 0.7957 | 0.6110 | 0.8285 | 0.4426 | 0.9851 |
| 17 | 02-04-20 | 0.6831 | -0.1655 | -0.1493 | 0.0220 | 0.7091 | 0.6406 | 0.7849 | 0.6126 | 0.8160 | 0.4396 | 0.9786 |
| 18 | 03-03-20 | 0.6811 | -0.1668 | -0.1519 | 0.0207 | 0.7048 | 0.6407 | 0.7754 | 0.6138 | 0.8050 | 0.4370 | 0.9726 |
| 19 | 04-07-20 | 0.6768 | -0.1696 | -0.1523 | 0.0210 | 0.7042 | 0.6393 | 0.7757 | 0.6121 | 0.8057 | 0.4366 | 0.9718 |
| 20 | 05-05-20 | 0.6704 | -0.1737 | -0.1524 | 0.0211 | 0.7040 | 0.6389 | 0.7758 | 0.6115 | 0.8060 | 0.4365 | 0.9716 |

*Note:*
7-day $IC_{25}$ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
CT = Central tendency of the $IC_{25}$ values.
S = Standard deviation of the $IC_{25}$ values.
Control Limits = Mean logarithmic $IC_{25}$ ± 2 standard deviations converted to anti-logarithmic values.
Warning Limits = Mean logarithmic $IC_{25}$ ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).
CV = Coefficient of variation.

Entered and Reviewed by Jim Summer

Environmental Testing Solutions, Inc.

# *Pimephales promelas*
## Chronic Reference Toxicant Testing, Test Acceptability Criteria
## Organism Source: In-house Culture




- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
  PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

**Central Tendency** (mean Control Growth, CV or PMSD)

**95% Confidence Interval** (mean Control Growth, CV or PMSD ± 2 Standard Deviations)

Independent Review by Kelley E. Keenan:

Entered and Reviewed by Jim Sumner

Independent Review by Walter E. Kosnitz

Environmental Testing Solutions, Inc.

# *Pimephales promelas*
## Chronic Reference Toxicant Testing, Test Acceptability Criteria
## Source: In-house Culture

| Test number | Test date | ToxCal Determination | | | | | Control Growth (mg/initial larvae) | | | Control Growth CV (%) | | | Test PMSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control Survival (%) | Control Growth Mean (mg/initial larvae) | Control Growth CV (%) | Test MSD | Test PMSD (%) | CT | 95% Confidence Interval CT - 2S | CT + 2S | CT | 95% Confidence Interval CT - 2S | CT + 2S | CT | 95% Confidence Interval CT - 2S | CT + 2S |
| 1 | 11-06-18 | 100 | 0.666 | 5.3 | 0.0744 | 11.2 | 0.693 | 0.513 | 0.873 | 5.5 | 0.9 | 10.1 | 10.6 | 6.2 | 15.1 |
| 2 | 12-04-18 | 100 | 0.705 | 6.7 | 0.0876 | 12.4 | 0.698 | 0.524 | 0.872 | 5.7 | 1.3 | 10.1 | 10.7 | 6.2 | 15.3 |
| 3 | 01-08-19 | 100 | 0.629 | 5.6 | 0.0758 | 12.1 | 0.699 | 0.528 | 0.871 | 5.6 | 1.3 | 10.0 | 10.6 | 6.1 | 15.1 |
| 4 | 02-05-19 | 100 | 0.871 | 7.1 | 0.0929 | 10.7 | 0.707 | 0.519 | 0.895 | 5.8 | 1.6 | 10.1 | 10.5 | 6.1 | 14.9 |
| 5 | 02-05-19 | 100 | 0.856 | 3.9 | 0.0671 | 7.8 | 0.718 | 0.522 | 0.914 | 5.6 | 1.4 | 9.8 | 10.3 | 5.8 | 14.7 |
| 6 | 03-05-19 | 100 | 0.773 | 7.3 | 0.1141 | 14.8 | 0.725 | 0.532 | 0.919 | 5.7 | 1.4 | 10.0 | 10.6 | 5.8 | 15.4 |
| 7 | 04-02-19 | 100 | 0.762 | 9.1 | 0.1406 | 18.4 | 0.730 | 0.539 | 0.922 | 5.9 | 1.4 | 10.4 | 11.1 | 5.3 | 16.9 |
| 8 | 05-07-19 | 100 | 0.670 | 6.3 | 0.0763 | 11.4 | 0.727 | 0.534 | 0.920 | 6.1 | 1.7 | 10.5 | 11.3 | 5.7 | 16.9 |
| 9 | 06-04-19 | 100 | 0.694 | 3.8 | 0.0602 | 8.7 | 0.725 | 0.532 | 0.919 | 6.2 | 2.4 | 10.0 | 11.2 | 5.4 | 16.9 |
| 10 | 07-09-19 | 100 | 0.676 | 9.0 | 0.0794 | 11.7 | 0.725 | 0.532 | 0.919 | 6.5 | 3.0 | 10.1 | 11.3 | 5.8 | 16.9 |
| 11 | 08-06-19 | 100 | 0.645 | 4.3 | 0.0551 | 8.5 | 0.727 | 0.537 | 0.917 | 6.3 | 2.8 | 9.9 | 11.1 | 5.5 | 16.7 |
| 12 | 09-10-19 | 100 | 0.741 | 9.3 | 0.1027 | 13.9 | 0.726 | 0.536 | 0.915 | 6.4 | 2.7 | 10.1 | 11.4 | 5.8 | 16.9 |
| 13 | 10-08-19 | 100 | 0.748 | 8.0 | 0.0817 | 10.9 | 0.722 | 0.537 | 0.908 | 6.5 | 2.9 | 10.2 | 11.5 | 6.3 | 16.7 |
| 14 | 11-05-19 | 100 | 0.679 | 10.3 | 0.1238 | 18.2 | 0.719 | 0.533 | 0.905 | 6.8 | 2.7 | 10.8 | 12.0 | 6.0 | 17.9 |
| 15 | 12-03-19 | 100 | 0.717 | 3.4 | 0.0599 | 8.4 | 0.726 | 0.553 | 0.900 | 6.7 | 2.5 | 10.9 | 11.7 | 5.6 | 17.7 |
| 16 | 01-07-20 | 100 | 0.668 | 7.2 | 0.1365 | 20.4 | 0.728 | 0.556 | 0.899 | 6.6 | 2.5 | 10.7 | 12.2 | 5.0 | 19.3 |
| 17 | 02-04-20 | 100 | 0.666 | 6.0 | 0.0914 | 13.7 | 0.727 | 0.555 | 0.899 | 6.7 | 2.8 | 10.7 | 12.3 | 5.1 | 19.4 |
| 18 | 03-03-20 | 100 | 0.672 | 8.5 | 0.0734 | 10.9 | 0.726 | 0.553 | 0.899 | 6.8 | 2.8 | 10.8 | 12.1 | 4.9 | 19.2 |
| 19 | 04-07-20 | 100 | 0.689 | 2.4 | 0.0381 | 5.5 | 0.725 | 0.551 | 0.899 | 6.5 | 2.1 | 10.9 | 11.9 | 3.7 | 20.1 |
| 20 | 05-05-20 | 100 | 0.772 | 5.1 | 0.0656 | 8.5 | 0.715 | 0.583 | 0.846 | 6.4 | 2.0 | 10.9 | 11.9 | 3.7 | 20.1 |

*Note:* **Control Survival =** USEPA minimum test acceptability criteria ≥ 80% survival.

**Control Mean Growth =** USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

**CV =** Coefficient of variation for control growth.

USEPA maximum CV guidance criteria (90th percentile) < 20%

**MSD =** Minimum significant difference.

**PMSD =** Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) > 12%.

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

**CT =** Central tendency of the growth, CV or PMSD values.

**S =** Standard deviation of the growth, CV or PMSD values.

Reviewed and Approved by ETS Laboratory

Environmental Testing Solutions, Inc.

# Potassium Chloride Chronic Reference Toxicant Test (EPA-822-R-02-013, Method 1000.0)

## Species: *Pimephales promelas*

PpKClCR Test Number: 64

| Dilution preparation information: | | | | | | | Comments: |
|---|---|---|---|---|---|---|---|
| KCl Stock INSS number: | | | INSS 1886 | | | | |
| Stock preparation: | | | 50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water. | | | | |
| Dilution prep (mg/L) | 300 | 450 | 600 | 750 | 900 | 1050 | |
| Stock volume (mL) | 12 | 18 | 24 | 30 | 36 | 42 | |
| Diluent volume (mL) | 1988 | 1982 | 1976 | 1970 | 1964 | 1958 | |
| Total volume (mL) | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | |

| Test organism information: | | Test information: | |
|---|---|---|---|
| Organism source: | In-house culture | Randomizing template: | Yellow |
| Age: | < 24-hours old | Incubator number and shelf location: | 7B |
| Spawn date: | 04-30-20 | *Artemia* CHM number: | CHM1048 |
| Hatch dates and times: | 05-04-20 1500 TO 05-05-20 0605 | *Drying information for weight determination:* | |
| Transfer vessel information: | pH = 8.26 S.U.<br>Temperature = 24.1 °C | Date / Time in oven: | 05-12-20 0810 |
| | | *Initial oven temperature: | 60°C |
| Average transfer volume: | < 0.25 mL | Date / Time out of oven: | 05-13-20 0810 |
| | | *Final oven temperature: | 60°C |
| | | Total drying time: | 24 HOURS |

*60°C Oven, Thermometer SN: 14-98585

### Daily feeding and renewal information:

| Day | Date | Morning feeding | | Afternoon feeding | | Test initiation, renewal, or termination | | MHSW batch used |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Time | Analyst | |
| 0 | 05-05-20 | 0610 | JH | 1230 | JH | 0822 | JH | 04-29-20B |
| 1 | 05-06-20 | 0530 | JH | 1130 | JH | 0730 | JH | ↓ |
| 2 | 05-07-20 | 0600 | JH | 1200 | JH | 0800 | JH | 04-29-20D |
| 3 | 05-08-20 | 0600 | JH | 1200 | JH | 0800 | JH | ↓ |
| 4 | 05-09-20 | 0700 | JH | 1300 | JH | 0900 | JH | 05-07-20B |
| 5 | 05-10-20 | 0600 | JH | 1200 | JH | 0800 | JH | ↓ |
| 6 | 05-11-20 | 0600 | JH | 1200 | JH | 0800 | JH | ↓ |
| 7 | 05-12-20 | | | | | 0722 | JH | |

### Chemical analyses:

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2011 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2011 | YSI Model 52CE | 18D104324 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2011 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2011 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2011 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2550B-2010 | Digital Thermometer | 13066485 |

| Control information: | | Acceptance criteria | Summary of test endpoints: | |
|---|---|---|---|---|
| % Mortality: | 0% | ≤ 20% | 7-day $LC_{50}$ (mg/L KCl) | 797.9 |
| Average weight per initial larvae: | 0.772 | | NOEC (mg/L KCl) | 600 |
| Average weight per surviving larvae: | 0.772 | ≥ 0.25 mg/larvae | LOEC (mg/L KCl) | 750 |
| | | | ChV (mg/L KCl) | 670.8 |
| | | | $IC_{25}$ (mg/L KCl) | 670.4 |

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***

PpKClCR Test Number: 64

***Survival and Growth Data***

| Day | Control | | | | 300 mg KCl/L | | | | 450 mg KCl/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 7 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| *A = Pan weight (mg)<br>Tray color code: grey<br>Analyst: TS<br>Date: 04.30.20 | 15.29 | 14.40 | 15.09 | 13.96 | 14.60 | 15.42 | 14.84 | 15.24 | 14.20 | 14.83 | 16.31 | 15.93 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 05.13.20 | 23.13 | 22.15 | 23.19 | 21.13 | 23.02 | 22.98 | 22.98 | 23.51 | 21.72 | 22.61 | 24.15 | 23.96 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 7.84 | 7.75 | 8.10 | 7.17 | 8.42 | 7.56 | 8.14 | 8.27 | 7.52 | 7.78 | 7.84 | 8.03 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0.784 | 0.775 | 0.810 | 0.717 | 0.842 | 0.756 | 0.814 | 0.827 | 0.752 | 0.778 | 0.784 | 0.803 |
| Average weight per initial number of larvae (mg) | 0.772 | | | | 0.810 | | | | 0.779 | | | |
| Percent reduction from control (%) | | | | | | | -5.0% | | | | -1.0% | |

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

***Comments:***

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***

PpKCICR Test Number: 64

***Survival and Growth Data***

| Day | 600 mg KCl/L | | | | 750 mg KCl/L | | | | 900 mg KCl/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 2d | 8 2d | 8 2d | 8 2d |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 8 | 8 | 8 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 6 2d | 8 | 7 1d | 7 1d |
| 4 | 10 | 10 | 10 | 10 | 7 3d | 8 2d | 8 2d | 9 1d | 5 1d | 5 3d | 4 3d | 4 3d |
| 5 | 10 | 10 | 10 | 10 | 7 | 8 | 7 1d | 8 1d | 3 2d | 4 1d | 3 1d | 4 |
| 6 | 10 | 10 | 10 | 10 | 7 | 8 | 7 | 7 1d | 2 1d | 3 1d | 2 1d | 3 1d |
| 7 | 10 | 10 | 10 | 9 1d | 6 1d | 6 2d | 5 2d | 6 1d | 2 2b | 3 | 2 2b | 3 |
| *A = Pan weight (mg)<br>Tray color code: Grey<br>Analyst: TS<br>Date: 04.30.20 | 15.24 | 15.14 | 14.78 | 15.72 | 13.13 | 14.86 | 14.72 | 15.38 | 15.09 | 13.63 | 15.85 | 14.56 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 05.13.20 | 22.90 | 22.66 | 22.77 | 22.39 | 17.22 | 19.43 | 18.52 | 19.71 | 19.79 | 16.11 | 17.86 | 17.22 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 7.66 | 7.52 | 7.99 | 6.67 | 4.09 | 4.57 | 3.80 | 4.33 | 4.70 | 2.48 | 2.01 | 2.66 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0.766 | 0.752 | 0.799 | 0.667 | 0.409 | 0.457 | 0.380 | 0.433 | 0.470 | 0.248 | 0.201 | 0.266 |
| Average weight per initial number of larvae (mg) | 0.746 | | | | 0.420 | | | | 0.296 | | | |
| Percent reduction from control (%) | | 3.37. | | | | 45.67. | | | | 61.67. | | |

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

***Comments:***

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **64**

***Survival and Growth Data***

| Day | 1050 mg KCl/L | | | |
|---|---|---|---|---|
| | Y | Z | AA | BB |
| 0 | 10 | 10 | 10 | 10 |
| 1 | 4 d | 4 d | 5 d | 4 d |
| 2 | 4 | 4 | 5 | 2 d |
| 3 | 3 d | 4 | 4 d | 2 |
| 4 | 1 d | 2 d | 2 d | 2 |
| 5 | 1 | 1 d | 1 d | 2 |
| 6 | 1 | 1 | 1 | 2 |
| 7 | 0 d | 1 | 1 w | 1 d |
| *A = Pan weight (mg)<br>Tray color code: grey<br>Analyst: TS<br>Date: 04.30.20 | 14.06 | 14.88 | 14.51 | 14.82 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 05.13.20 | → | 15.66 | 15.41 | 15.60 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | → | 0.78 | 0.90 | 0.78 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0 | 0.078 | 0.090 | 0.078 |
| Average weight per initial number of larvae (mg) / Percent reduction from control (%) | 0.062 | | 92.07 | |

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

| ***Comments:*** |
|---|
| |
| |
| |

Independent Review by KaDey E. Keenan: K

Independently Reviewed by Kelley E. Kessler:

Environmental Testing Solutions, Inc.

# *Pimephales promelas* Chronic Reference Toxicant Test
## EPA-821-R-02-013, Method 1000.0

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

Test number: 64

Test dates: May 05-12, 2020

| Concentration (mg/L KCl) | Replicate | Initial number of larvae | Final number of larvae | A = Pan weight (mg) | B = Pan + Larvae weight (mg) | Larvae weight (mg) = B - A | Weight / Surviving number of larvae (mg) | Mean weight/ Surviving number of larvae (mg) | Coefficient of variation (mean weight per surviving number of larvae) (%) | Weight / Initial number of larvae (mg) | Mean survival (%) | Mean weight/ Initial number of larvae (mg) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | A | 10 | 10 | 15.29 | 23.13 | 7.84 | 0.784 | 0.772 | 5.1 | 0.784 | 100.0 | 0.772 | 5.1 | Not applicable |
| | B | 10 | 10 | 14.40 | 22.15 | 7.75 | 0.775 | | | 0.775 | | | | |
| | C | 10 | 10 | 15.09 | 23.19 | 8.10 | 0.810 | | | 0.810 | | | | |
| | D | 10 | 10 | 13.96 | 21.13 | 7.17 | 0.717 | | | 0.717 | | | | |
| 300 | E | 10 | 10 | 14.60 | 23.02 | 8.42 | 0.842 | 0.810 | 4.6 | 0.842 | 100.0 | 0.810 | 4.6 | -5.0 |
| | F | 10 | 10 | 15.42 | 22.98 | 7.56 | 0.756 | | | 0.756 | | | | |
| | G | 10 | 10 | 14.84 | 22.98 | 8.14 | 0.814 | | | 0.814 | | | | |
| | H | 10 | 10 | 15.24 | 23.51 | 8.27 | 0.827 | | | 0.827 | | | | |
| 450 | I | 10 | 10 | 14.20 | 21.72 | 7.52 | 0.752 | 0.779 | 2.7 | 0.752 | 100.0 | 0.779 | 2.7 | -1.0 |
| | J | 10 | 10 | 14.83 | 22.61 | 7.78 | 0.778 | | | 0.778 | | | | |
| | K | 10 | 10 | 16.31 | 24.15 | 7.84 | 0.784 | | | 0.784 | | | | |
| | L | 10 | 10 | 15.93 | 23.96 | 8.03 | 0.803 | | | 0.803 | | | | |
| 600 | M | 10 | 10 | 15.24 | 22.90 | 7.66 | 0.766 | 0.765 | 3.3 | 0.766 | 97.5 | 0.746 | 7.5 | 3.3 |
| | N | 10 | 10 | 15.14 | 22.66 | 7.52 | 0.752 | | | 0.752 | | | | |
| | O | 10 | 10 | 14.78 | 22.77 | 7.99 | 0.799 | | | 0.799 | | | | |
| | P | 10 | 9 | 15.72 | 22.39 | 6.67 | 0.741 | | | 0.667 | | | | |
| 750 | Q | 10 | 6 | 13.13 | 17.22 | 4.09 | 0.682 | 0.731 | 5.2 | 0.409 | 57.5 | 0.420 | 7.9 | 45.6 |
| | R | 10 | 6 | 14.86 | 19.43 | 4.57 | 0.762 | | | 0.457 | | | | |
| | S | 10 | 5 | 14.72 | 18.52 | 3.80 | 0.760 | | | 0.380 | | | | |
| | T | 10 | 6 | 15.38 | 19.71 | 4.33 | 0.722 | | | 0.433 | | | | |
| 900 | U | 10 | 2 | 15.09 | 19.79 | 4.70 | 2.350 | 1.267 | 57.3 | 0.470 | 25.0 | 0.296 | 40.2 | 61.6 |
| | V | 10 | 3 | 13.63 | 16.11 | 2.48 | 0.827 | | | 0.248 | | | | |
| | W | 10 | 2 | 15.85 | 17.86 | 2.01 | 1.005 | | | 0.201 | | | | |
| | X | 10 | 3 | 14.56 | 17.22 | 2.66 | 0.887 | | | 0.266 | | | | |
| 1050 | Y | 10 | 0 | 0.00 | 0.00 | 0.00 | 0.000 | 0.820 | 8.4 | 0.000 | 7.5 | 0.062 | 67.3 | 92.0 |
| | Z | 10 | 1 | 14.88 | 15.66 | 0.78 | 0.780 | | | 0.078 | | | | |
| | AA | 10 | 1 | 14.51 | 15.41 | 0.90 | 0.900 | | | 0.090 | | | | |
| | BB | 10 | 1 | 14.82 | 15.60 | 0.78 | 0.780 | | | 0.078 | | | | |

Dunnett's MSD value: 0.0656 MSD = Minimum Significant Difference

PMSD: 8.5 PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) = 12%.
Upper PMSD bound determined by USEPA (90th percentile) = 30%.
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.

Checked and Reviewed by the Supervisor

# Statistical Analyses

Environmental Testing Solutions, Inc.

**Larval Fish Growth and Survival Test-7 Day Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | PpKCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | KCL-Potassium chloride |
| Sample Date: | | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | PP-Pimephales promelas |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1 0000 | 1.0000 |
| 300 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 450 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 600 | 1.0000 | 1.0000 | 1.0000 | 0.9000 |
| 750 | 0.6000 | 0.6000 | 0.5000 | 0.6000 |
| 900 | 0.2000 | 0.3000 | 0 2000 | 0.3000 |
| 1050 | 0.0000 | 0.1000 | 0.1000 | 0 1000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1 0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | | | 0 | 40 |
| 300 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 | 0 | 40 |
| 450 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 | 0 | 40 |
| 600 | 0.9750 | 0.9750 | 1.3713 | 1.2490 | 1.4120 | 5.942 | 4 | 16.00 | 10.00 | 1 | 40 |
| *750 | 0.5750 | 0.5750 | 0.8609 | 0.7854 | 0.8861 | 5.847 | 4 | 10.00 | 10.00 | 17 | 40 |
| *900 | 0.2500 | 0.2500 | 0.5216 | 0.4636 | 0.5796 | 12.838 | 4 | 10.00 | 10.00 | 30 | 40 |
| *1050 | 0.0750 | 0.0750 | 0.2810 | 0.1588 | 0.3218 | 28.997 | 4 | 10.00 | 10.00 | 37 | 40 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.81969 | 0.896 | -1.3514 | 1.56422 |
| Equality of variance cannot be confirmed | | | | |

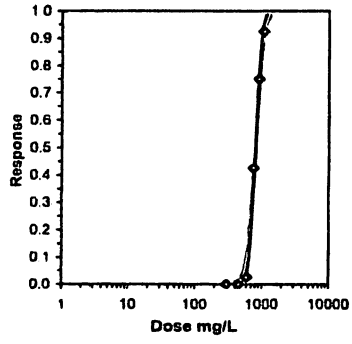
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 600 | 750 | 670.82 | |
| Treatments vs D-Control | | | | |

**Maximum Likelihood-Probit**

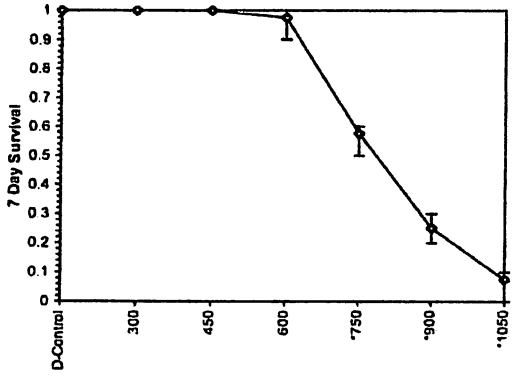
| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 13.1841 | 1.64636 | 9.95727 | 16.411 | 0 | 1.50588 | 9.48773 | 0.8256 | 2.90194 | 0.07585 | 3 |
| Intercept | -33.26 | 4.79052 | -42.649 | -23.87 | | | | | | | |

TSCR

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 531.487 | 459.659 | 582.356 |
| EC05 | 3.355 | 598.664 | 536.54 | 642.674 |
| EC10 | 3.718 | 637.88 | 582.107 | 677.976 |
| EC15 | 3.964 | 665.78 | 614.622 | 703.335 |
| EC20 | 4.158 | 688.822 | 641.403 | 724.561 |
| EC25 | 4.326 | 709.225 | 664.957 | 743.671 |
| EC40 | 4.747 | 763.356 | 725.878 | 796.651 |
| EC50 | 5.000 | 797.89 | 762.772 | 832.948 |
| EC60 | 5.253 | 833.987 | 799.187 | 873.465 |
| EC75 | 5.674 | 897.64 | 858.369 | 950.993 |
| EC80 | 5.842 | 924.228 | 881.621 | 985.231 |
| EC85 | 6.036 | 956.215 | 908.786 | 1027 53 |
| EC90 | 6.282 | 998.038 | 943.298 | 1084.33 |
| EC95 | 6.645 | 1063.42 | 995.635 | 1175.8 |
| EC99 | 7.326 | 1197.83 | 1099.31 | 1371.77 |




**Dose-Response Plot**




Independent Review by Kelley E. Keensen

# Statistical Analyses

Environmental Testing Solutions, Inc.

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | PpKClCR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | KCL-Potassium chloride |
| Sample Date: | | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | PP-Pimephales promelas |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0.7840 | 0.7750 | 0.8100 | 0.7170 |
| 300 | 0.8420 | 0.7560 | 0.8140 | 0.8270 |
| 450 | 0.7520 | 0.7780 | 0.7840 | 0.8030 |
| 600 | 0.7660 | 0.7520 | 0.7990 | 0.6670 |
| 750 | 0.4090 | 0.4570 | 0.3800 | 0.4330 |
| 900 | 0.4700 | 0.2480 | 0.2010 | 0.2660 |
| 1050 | 0.0000 | 0.0780 | 0.0900 | 0.0780 |

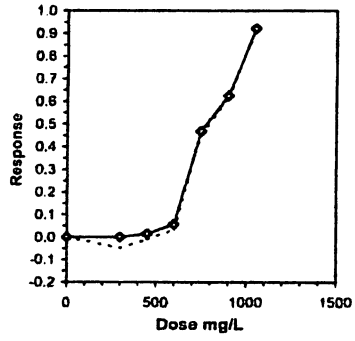
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 0.7715 | 1.0000 | 0.7715 | 0.7170 | 0.8100 | 5.087 | 4 | | | | 0.7906 | 1.0000 |
| 300 | 0.8098 | 1.0496 | 0.8098 | 0.7560 | 0.8420 | 4.645 | 4 | -1.336 | 2.290 | 0.0656 | 0.7906 | 1.0000 |
| 450 | 0.7793 | 1.0100 | 0.7793 | 0.7520 | 0.8030 | 2.703 | 4 | -0.271 | 2.290 | 0.0656 | 0.7793 | 0.9856 |
| 600 | 0.7460 | 0.9669 | 0.7460 | 0.6670 | 0.7990 | 7.538 | 4 | 0.890 | 2.290 | 0.0656 | 0.7460 | 0.9436 |
| 750 | 0.4198 | 0.5441 | 0.4198 | 0.3800 | 0.4570 | 7.852 | 4 | | | | 0.4198 | 0.5309 |
| 900 | 0.2963 | 0.3840 | 0.2963 | 0.2010 | 0.4700 | 40.179 | 4 | | | | 0.2963 | 0.3747 |
| 1050 | 0.0615 | 0.0797 | 0.0615 | 0.0000 | 0.0900 | 67.298 | 4 | | | | 0.0615 | 0.0778 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.9106 | 0.844 | -0.9009 | 0.25121 |
| Bartlett's Test indicates equal variances (p = 0.52) | 2.27034 | 11.3449 | | |

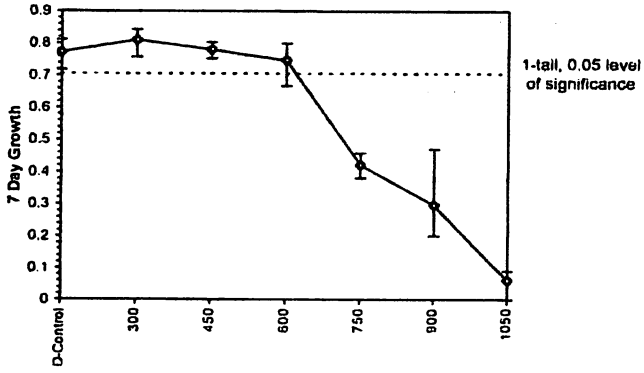
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test Treatments vs D-Control | 600 | >600 | | | 0.06558 | 0.085 | 0.00276 | 0.00164 | 0.22369 | 3, 12 |

**Linear Interpolation (200 Resamples)**

| Point | mg/L | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 577.02 | 47.50 | 390.05 | 634.23 | -0.7814 |
| IC10 | 615.83 | 17.87 | 518.03 | 638.68 | -2.3313 |
| IC15 | 634.01 | 9.77 | 591.73 | 654.53 | -0.9399 |
| IC20 | 652.18 | 8.49 | 617.40 | 670.95 | -0.8268 |
| IC25 | 670.36 | 7.43 | 640.30 | 687.90 | -0.6947 |
| IC40 | 724.89 | 6.15 | 706.22 | 744.01 | 0.1163 |
| IC50 | 779.68 | 36.01 | 730.69 | 982.42 | 2.1299 |




**Dose-Response Plot**




Independent Review by Kelley E. Keenan

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKCICR Test Number: 64

## *Daily Chemistry:*

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | MS | TS | TS | TS | TS | K |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.98 | 7.84 | 7.68 | 7.80 | 7.79 | 7.60 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | TS 05.06.20 7.0 7.6 | 7.7 | 7.6 | 6.4 |
| | Conductivity (µmhos/cm) | 321 | | 321 | | 318 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 61 | |
| | Hardness (mg $CaCO_3$/L) | 93 | | K 05-12-20 | | 89 | |
| | Temperature (°C) | 24.9 | 24.5 | 24.8 | 24.7 | 24.8 | 24.6 |
| 300 mg KCl/L | pH (S.U.) | 7.99 | 7.82 | 7.87 | 7.80 | 7.96 | 7.57 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.9 | 8.0 | 7.7 | 7.9 | 6.5 |
| | Conductivity (µmhos/cm) | 884 | | 860 | | 866 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.9 | 24.5 | 24.7 | 24.5 |
| 450 mg KCl/L | pH (S.U.) | 7.99 | 7.83 | 7.86 | 7.80 | 7.95 | 7.57 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.9 | 8.0 | 7.6 | 8.0 | 6.4 |
| | Conductivity (µmhos/cm) | 1110 | | 1130 | | 1140 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.9 | 24.5 | 24.8 | 24.5 |
| 600 mg KCl/L | pH (S.U.) | 7.99 | 7.83 | 7.87 | 7.80 | 7.98 | 7.56 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.9 | 8.0 | 7.6 | 8.0 | 6.5 |
| | Conductivity (µmhos/cm) | 1370 | | 1390 | | 1400 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.9 | 24.5 | 24.8 | 24.7 |
| 750 mg KCl/L | pH (S.U.) | 7.99 | 7.83 | 7.87 | 7.79 | 7.97 | 7.56 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.9 | 8.0 | 7.6 | 8.0 | 6.5 |
| | Conductivity (µmhos/cm) | 1640 | | 1660 | | 1660 | |
| | Temperature (°C) | 24.9 | 24.4 | 24.8 | 24.7 | 24.7 | 24.7 |
| 900 mg KCl/L | pH (S.U.) | 7.99 | 7.82 | 7.86 | 7.78 | 7.97 | 7.54 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.0 | 7.6 | 8.0 | 6.3 |
| | Conductivity (µmhos/cm) | 1940 | | 1940 | | 1930 | |
| | Temperature (°C) | 24.9 | 24.4 | 24.8 | 24.3 | 24.7 | 24.5 |
| 1050 mg KCl/L | pH (S.U.) | 7.99 | 7.85 | 7.88 | 7.86 | 7.96 | 7.58 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.0 | 7.7 | 8.0 | 7.0 |
| | Conductivity (µmhos/cm) | 2260 | | 2220 | | 2230 | |
| | Temperature (°C) | 24.9 | 24.6 | 24.8 | 24.7 | 24.7 | 24.7 |
| | | Initial | Final | Initial | Final | Initial | Final |

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

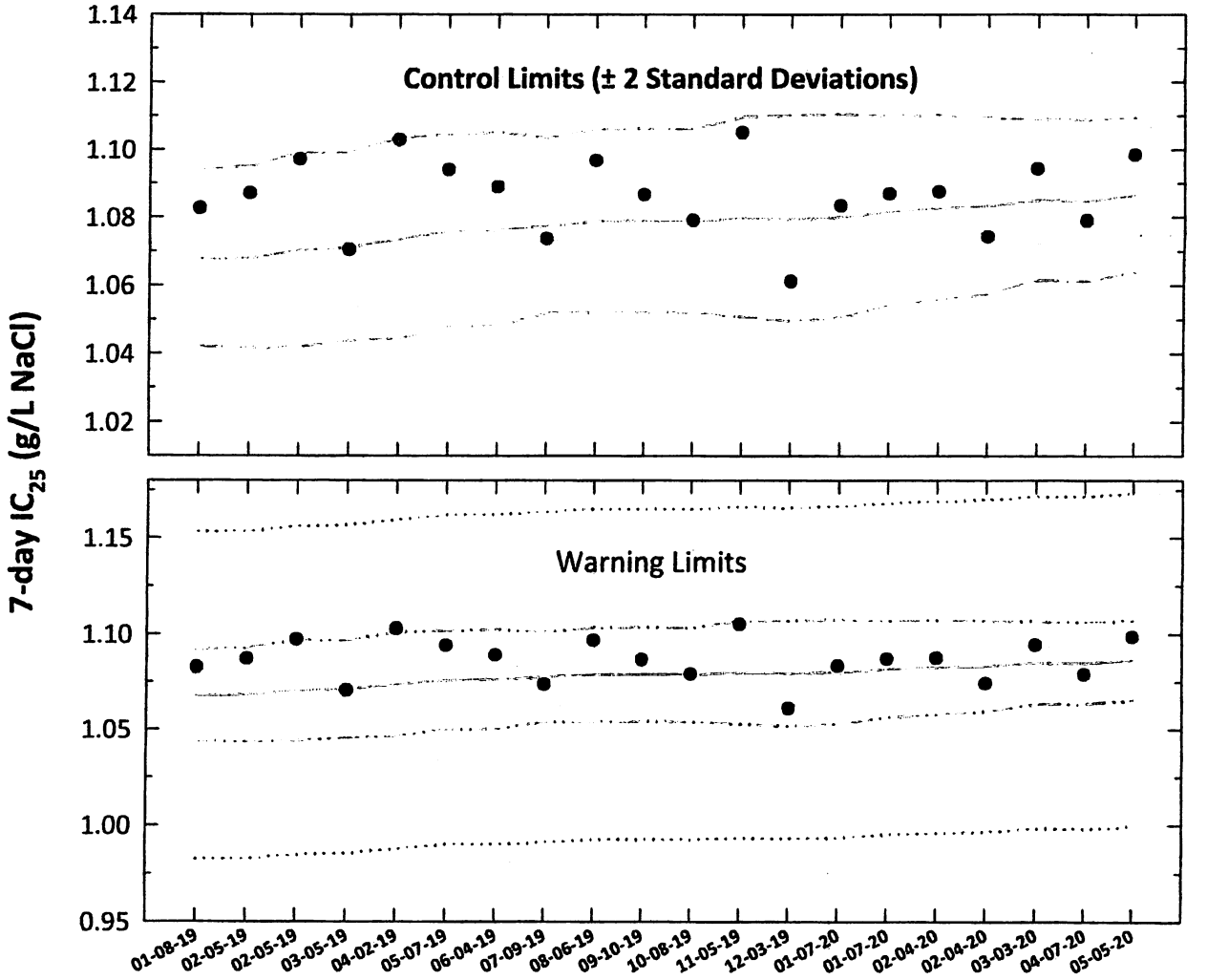
**Species:** ***Pimephales promelas*** PpKClCR Test Number: 64

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | N | BSL | BSL | TS | TS | TS | TS | MS |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.88 | 7.70 | 7.72 | 7.75 | 7.73 | 7.67 | 7.83 | 7.61 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.3 | 7.4 | 7.5 | 7.7 | 7.6 | 7.6 | 7.1 |
| | Conductivity (µmhos/cm) | 305 | | 300 | | 314 | | 322 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 60 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | 12-20 | 90 | | | | | 12-20 |
| | Temperature (°C) | 24.8 | 24.5 | 24.7 | 24.6 | 24.7 | 24.7 | 24.7 | 24.4 |
| 300 mg KCl/L | pH (S.U.) | 7.96 | 7.73 | 7.91 | 7.80 | 7.87 | 7.72 | 8.01 | 7.68 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.2 | 7.7 | 7.5 | 7.8 | 7.5 | 7.7 | 7.2 |
| | Conductivity (µmhos/cm) | 850 | | 844 | | 886 | | 859 | |
| | Temperature (°C) | 24.9 | 24.5 | 24.8 | 24.3 | 24.7 | 24.5 | 24.8 | 24.4 |
| 450 mg KCl/L | pH (S.U.) | 7.98 | 7.72 | 7.96 | 7.81 | 7.90 | 7.74 | 8.00 | 7.78 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.2 | 7.8 | 7.6 | 7.9 | 7.5 | 7.8 | 7.2 |
| | Conductivity (µmhos/cm) | 1100 | | 1100 | | 1150 | | 1100 | |
| | Temperature (°C) | 24.9 | 24.4 | 24.8 | 24.5 | 24.7 | 24.5 | 24.8 | 24.4 |
| 600 mg KCl/L | pH (S.U.) | 7.99 | 7.72 | 7.97 | 7.80 | 7.91 | 7.74 | 8.00 | 7.79 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.2 | 7.7 | 7.5 | 7.9 | 7.5 | 7.8 | 7.3 |
| | Conductivity (µmhos/cm) | 1380 | | 1380 | | 1420 | | 1370 | |
| | Temperature (°C) | 24.9 | 24.6 | 24.8 | 24.5 | 24.7 | 24.5 | 24.8 | 24.7 |
| 750 mg KCl/L | pH (S.U.) | 8.00 | 7.66 | 8.00 | 7.80 | 7.93 | 7.76 | 8.00 | 7.78 |
| | Dissolved oxygen (mg/L) | 8.0 | 6.9 | 7.8 | 7.3 | 7.9 | 7.6 | 7.9 | 7.3 |
| | Conductivity (µmhos/cm) | 1630 | | 1630 | | 1670 | | 1630 | |
| | Temperature (°C) | 24.9 | 24.6 | 24.7 | 24.5 | 24.7 | 24.6 | 24.7 | 24.6 |
| 900 mg KCl/L | pH (S.U.) | 7.99 | 7.65 | 7.99 | 7.83 | 7.94 | 7.76 | 8.00 | 7.78 |
| | Dissolved oxygen (mg/L) | 8.0 | 6.9 | 7.9 | 7.3 | 8.0 | 7.6 | 7.9 | 7.3 |
| | Conductivity (µmhos/cm) | 1910 | | 1930 | | 2020 | | 1920 | |
| | Temperature (°C) | 24.8 | 24.3 | 24.7 | 24.5 | 24.7 | 24.6 | 24.7 | 24.6 |
| 1050 mg KCl/L | pH (S.U.) | 8.00 | 7.75 | 8.01 | 7.90 | 7.85 | 7.78 | 8.00 | 7.80 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.4 | 7.9 | 7.5 | 8.0 | 7.6 | 7.9 | 7.3 |
| | Conductivity (µmhos/cm) | 2140 | | 2140 | | 2210 | | 2170 | |
| | Temperature (°C) | 24.8 | 24.7 | 24.8 | 24.6 | 24.7 | 24.7 | 24.8 | 24.7 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

Independent Review by Kelley E. Keenan: N

Environmental Testing Solutions, Inc.

# *Ceriodaphnia dubia*
# Chronic Reference Toxicant Control Chart
# Source: In-house Culture




- ● **7-day $IC_{25}$** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic $IC_{25}$ converted to anti-logarithmic values)
- **Control Limits** (mean logarithmic $IC_{25}$ ± 2 standard deviations converted to anti-logarithmic values)
- **Laboratory Warning Limits** (mean logarithmic $IC_{25}$ ± 2 coefficent of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Independent Review by Kelley E. Keenan:



Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Kennedy

Environmental Testing Solutions, Inc.

# *Ceriodaphnia dubia*
## Chronic Reference Toxicant Control Chart
## Source: In-house Culture

| Test number | Test date | 7-day $IC_{25}$ ToxCal Determination (g/L NaCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L NaCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7-day $IC_{25}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 10th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.10}$ | CT + $S_{A.10}$ |
| 1 | 01-08-19 | 1.0826 | 0.0345 | 0.0284 | 0.0053 | 1.0677 | 1.0420 | 1.0940 | 1.0437 | 1.0917 | 0.9823 | 1.1531 |
| 2 | 02-05-19 | 1.0871 | 0.0363 | 0.0286 | 0.0054 | 1.0680 | 1.0416 | 1.0950 | 1.0433 | 1.0927 | 0.9825 | 1.1534 |
| 3 | 02-05-19 | 1.0971 | 0.0403 | 0.0295 | 0.0058 | 1.0702 | 1.0419 | 1.0992 | 1.0438 | 1.0966 | 0.9846 | 1.1558 |
| 4 | 03-05-19 | 1.0705 | 0.0296 | 0.0298 | 0.0056 | 1.0710 | 1.0438 | 1.0990 | 1.0456 | 1.0965 | 0.9853 | 1.1567 |
| 5 | 04-02-19 | 1.1029 | 0.0425 | 0.0308 | 0.0060 | 1.0735 | 1.0443 | 1.1035 | 1.0463 | 1.1007 | 0.9876 | 1.1594 |
| 6 | 05-07-19 | 1.0940 | 0.0390 | 0.0318 | 0.0057 | 1.0759 | 1.0481 | 1.1044 | 1.0501 | 1.1017 | 0.9898 | 1.1620 |
| 7 | 06-04-19 | 1.0889 | 0.0370 | 0.0319 | 0.0058 | 1.0762 | 1.0480 | 1.1052 | 1.0500 | 1.1024 | 0.9901 | 1.1623 |
| 8 | 07-09-19 | 1.0737 | 0.0309 | 0.0324 | 0.0052 | 1.0774 | 1.0519 | 1.1035 | 1.0538 | 1.1010 | 0.9912 | 1.1636 |
| 9 | 08-06-19 | 1.0967 | 0.0401 | 0.0329 | 0.0054 | 1.0787 | 1.0522 | 1.1060 | 1.0541 | 1.1034 | 0.9924 | 1.1650 |
| 10 | 09-10-19 | 1.0866 | 0.0361 | 0.0330 | 0.0054 | 1.0789 | 1.0522 | 1.1063 | 1.0541 | 1.1037 | 0.9926 | 1.1652 |
| 11 | 10-08-19 | 1.0791 | 0.0331 | 0.0329 | 0.0054 | 1.0787 | 1.0521 | 1.1061 | 1.0540 | 1.1034 | 0.9924 | 1.1650 |
| 12 | 11-05-19 | 1.1051 | 0.0434 | 0.0334 | 0.0059 | 1.0799 | 1.0508 | 1.1097 | 1.0530 | 1.1067 | 0.9935 | 1.1662 |
| 13 | 12-03-19 | 1.0612 | 0.0258 | 0.0332 | 0.0061 | 1.0794 | 1.0496 | 1.1101 | 1.0518 | 1.1071 | 0.9931 | 1.1658 |
| 14 | 01-07-20 | 1.0834 | 0.0348 | 0.0335 | 0.0060 | 1.0801 | 1.0506 | 1.1105 | 1.0528 | 1.1075 | 0.9937 | 1.1665 |
| 15 | 01-07-20 | 1.0870 | 0.0362 | 0.0341 | 0.0056 | 1.0817 | 1.0543 | 1.1098 | 1.0564 | 1.1070 | 0.9952 | 1.1683 |
| 16 | 02-04-20 | 1.0875 | 0.0364 | 0.0345 | 0.0054 | 1.0827 | 1.0559 | 1.1101 | 1.0580 | 1.1074 | 0.9961 | 1.1693 |
| 17 | 02-04-20 | 1.0743 | 0.0311 | 0.0347 | 0.0052 | 1.0832 | 1.0575 | 1.1095 | 1.0595 | 1.1069 | 0.9965 | 1.1699 |
| 18 | 03-03-20 | 1.0944 | 0.0392 | 0.0354 | 0.0047 | 1.0850 | 1.0616 | 1.1089 | 1.0634 | 1.1066 | 0.9982 | 1.1718 |
| 19 | 04-07-20 | 1.0790 | 0.0330 | 0.0353 | 0.0048 | 1.0848 | 1.0612 | 1.1089 | 1.0630 | 1.1065 | 0.9980 | 1.1715 |
| 20 | 05-05-20 | 1.0985 | 0.0408 | 0.0360 | 0.0045 | 1.0864 | 1.0639 | 1.1094 | 1.0657 | 1.1071 | 0.9995 | 1.1733 |

*Note:* 7-day $IC_{25}$ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).

CT = Central tendency of the $IC_{25}$ values.

S = Standard deviation of the $IC_{25}$ values.

Control Limits = Mean logarithmic $IC_{25}$ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic $IC_{25}$ ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

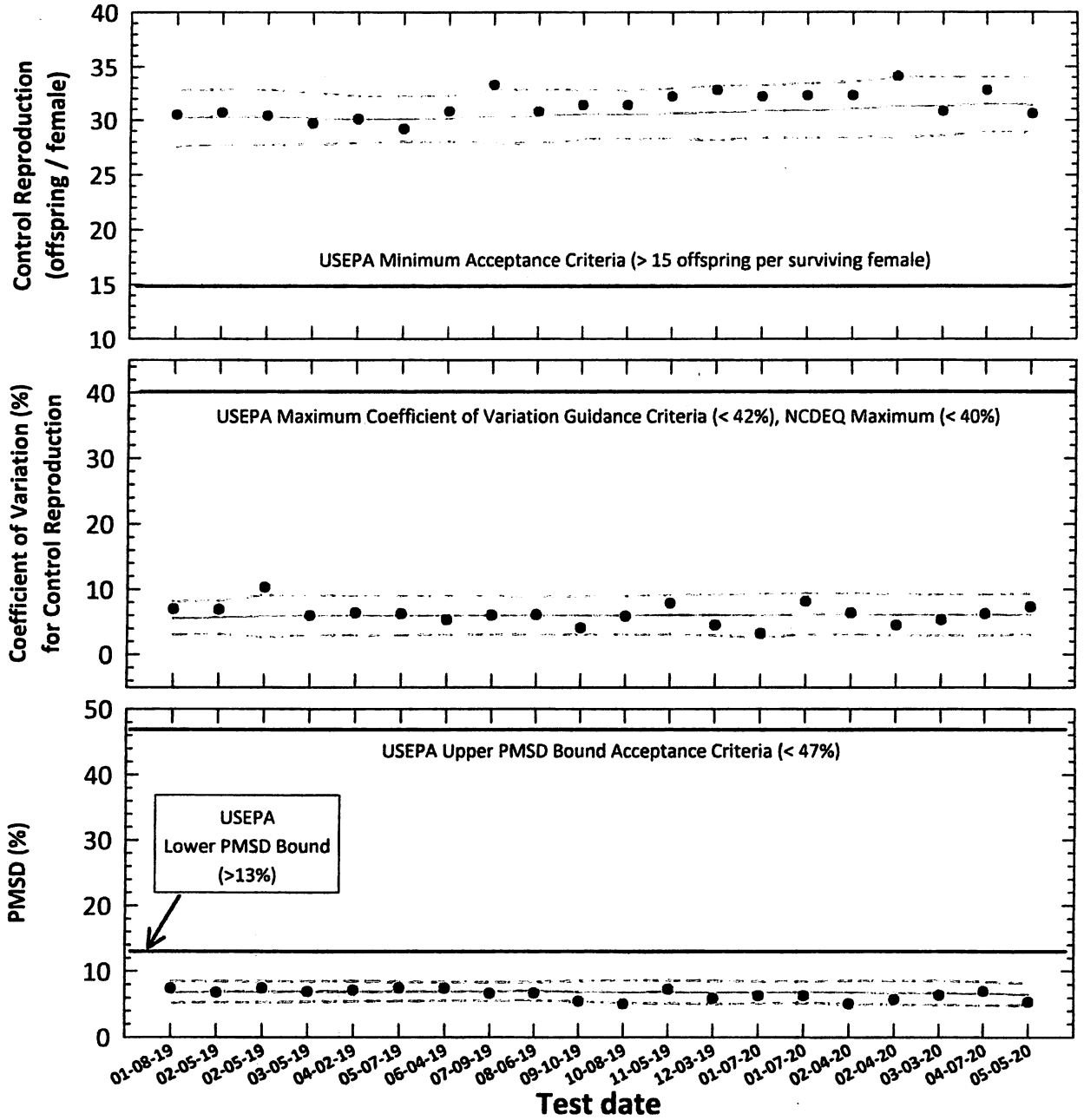
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Summer

Environmental Testing Solutions, Inc.

# *Ceriodaphnia dubia*
## Chronic Reference Toxicant Testing, Test Acceptability Criteria
## Organism Source: In-house Culture




- **Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
  PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

**Central Tendency** (mean Control Reproduction, CV or PMSD)

**95% Confidence Interval** (mean Control Reproduction, CV or PMSD ± 2 Standard Deviations)

Independent Review by Kelley E. Koenen

Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Bonstemi

Environmental Testing Solutions, Inc.

# *Ceriodaphnia dubia*
## Chronic Reference Toxicant Testing, Test Acceptability Criteria
## Source: In-house Culture

| Test number | Test date | ToxCal Determination: Control Survival (%) | ToxCal Determination: Control Reproduction: Mean (offspring/female) | ToxCal Determination: Control Reproduction: CV (%) | ToxCal Determination: Test: MSD | ToxCal Determination: Test: PMSD (%) | Control Reproduction (offspring/female): CT | Control Reproduction (offspring/female): 95% Confidence Interval CT - 2S | Control Reproduction (offspring/female): 95% Confidence Interval CT + 2S | Control Reproduction CV (%): CT | Control Reproduction CV (%): 95% Confidence Interval CT - 2S | Control Reproduction CV (%): 95% Confidence Interval CT + 2S | Test PMSD (%): CT | Test PMSD (%): 95% Confidence Interval CT - 2S | Test PMSD (%): 95% Confidence Interval CT + 2S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 01-08-19 | 100 | 30.5 | 7.0 | 2.266 | 7.4 | 30.2 | 27.6 | 32.8 | 5.6 | 3.0 | 8.1 | 6.8 | 5.2 | 8.5 |
| 2 | 02-05-19 | 100 | 30.7 | 6.9 | 2.090 | 6.8 | 30.3 | 27.8 | 32.9 | 5.7 | 3.1 | 8.2 | 6.9 | 5.3 | 8.4 |
| 3 | 02-05-19 | 100 | 30.4 | 10.3 | 2.273 | 7.5 | 30.3 | 27.8 | 32.9 | 5.9 | 2.6 | 9.1 | 6.9 | 5.3 | 8.5 |
| 4 | 03-05-19 | 100 | 29.7 | 5.9 | 2.054 | 6.9 | 30.2 | 27.8 | 32.6 | 6.0 | 2.9 | 9.1 | 6.9 | 5.4 | 8.5 |
| 5 | 04-02-19 | 100 | 30.1 | 6.4 | 2.152 | 7.1 | 30.1 | 27.9 | 32.3 | 5.9 | 2.9 | 8.9 | 7.0 | 5.4 | 8.5 |
| 6 | 05-07-19 | 100 | 29.2 | 6.2 | 2.188 | 7.5 | 30.1 | 28.0 | 32.2 | 5.9 | 2.9 | 8.9 | 6.9 | 5.5 | 8.3 |
| 7 | 06-04-19 | 100 | 30.8 | 5.3 | 2.287 | 7.4 | 30.2 | 28.0 | 32.3 | 6.0 | 3.0 | 8.9 | 6.9 | 5.5 | 8.4 |
| 8 | 07-09-19 | 100 | 33.3 | 6.0 | 2.214 | 6.6 | 30.4 | 27.9 | 32.9 | 6.0 | 3.1 | 8.9 | 7.0 | 5.6 | 8.4 |
| 9 | 08-06-19 | 100 | 30.8 | 6.1 | 2.060 | 6.7 | 30.4 | 27.9 | 32.9 | 6.0 | 3.1 | 8.9 | 7.0 | 5.6 | 8.4 |
| 10 | 09-10-19 | 100 | 31.4 | 4.0 | 1.708 | 5.4 | 30.5 | 28.3 | 32.8 | 5.9 | 3.0 | 8.9 | 6.9 | 5.4 | 8.5 |
| 11 | 10-08-19 | 100 | 31.4 | 5.9 | 1.581 | 5.0 | 30.5 | 28.3 | 32.8 | 6.0 | 3.0 | 8.9 | 6.9 | 5.1 | 8.6 |
| 12 | 11-05-19 | 100 | 32.2 | 7.9 | 2.349 | 7.3 | 30.6 | 28.3 | 33.0 | 6.1 | 3.0 | 9.1 | 6.9 | 5.1 | 8.6 |
| 13 | 12-03-19 | 100 | 32.8 | 4.5 | 1.927 | 5.9 | 30.7 | 28.1 | 33.2 | 6.0 | 2.9 | 9.2 | 6.8 | 5.0 | 8.5 |
| 14 | 01-07-20 | 100 | 32.2 | 3.2 | 2.023 | 6.3 | 30.9 | 28.4 | 33.3 | 5.9 | 2.6 | 9.3 | 6.8 | 5.1 | 8.5 |
| 15 | 01-07-20 | 100 | 32.3 | 8.1 | 2.030 | 6.3 | 30.9 | 28.4 | 33.4 | 6.2 | 3.0 | 9.4 | 6.8 | 5.2 | 8.4 |
| 16 | 02-04-20 | 100 | 32.3 | 6.4 | 1.632 | 5.1 | 31.0 | 28.5 | 33.6 | 6.2 | 3.0 | 9.4 | 6.8 | 5.0 | 8.6 |
| 17 | 02-04-20 | 100 | 34.1 | 4.5 | 1.948 | 5.7 | 31.3 | 28.4 | 34.1 | 6.0 | 2.9 | 9.2 | 6.7 | 4.9 | 8.6 |
| 18 | 03-03-20 | 100 | 30.8 | 5.3 | 1.960 | 6.4 | 31.3 | 28.6 | 34.0 | 6.0 | 2.9 | 9.2 | 6.7 | 4.8 | 8.5 |
| 19 | 04-07-20 | 100 | 32.8 | 6.2 | 2.287 | 7.0 | 31.5 | 28.9 | 34.0 | 6.0 | 2.9 | 9.2 | 6.6 | 4.9 | 8.4 |
| 20 | 05-05-20 | 100 | 30.6 | 7.3 | 1.633 | 5.3 | 31.4 | 28.9 | 34.0 | 6.2 | 3.0 | 9.3 | 6.5 | 4.8 | 8.1 |

*Note:* Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 13%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 47%.

CT = Central tendency of the reproduction, CV or PMSD values.

S = Standard deviation of the reproduction, CV or PMSD values.

Entered and Reviewed By Jim Surbaugh

Environmental Testing Solutions, Inc.

# Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)
## Species: *Ceriodaphnia dubia*

CdNaClCR #: 243

| *Dilution preparation information:* | | | | | | *Comments:* |
|---|---|---|---|---|---|---|
| NaCl Stock INSS number: | INSS ~~1886~~ 1887 | | | | | |
| Stock preparation: | 100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water. | | | | | |
| Dilution prep (mg/L) | 600 | 800 | 1000 | 1200 | 1400 | |
| Stock volume (mL) | 9 | 12 | 15 | 18 | 21 | |
| Diluent volume (mL) | 1491 | 1488 | 1485 | 1482 | 1479 | |
| Total volume (mL) | 1500 | 1500 | 1500 | 1500 | 1500 | |

### *Test organism source:*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Organism age: | < 24-hours old | | | | | | | | | |
| Date and times organisms were born between: | 05-05-20 0645 TO 0912 | | | | | | | | | |
| Culture board: | 04-26-20 A | | | | | | | | | |
| Replicate number: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Culture board cup number: | 2 | 3 | 8 | 9 | 10 | 14 | 20 | 22 | 30 | 33 |
| Transfer vessel information: | pH (S.U.): 7.93 Temperature (°C): 24.9 | | | | | | | | | |
| Average transfer volume (mL): | < 0.25 mL | | | | | | | | | |

### *Test randomization and location:*

| | |
|---|---|
| Randomizing template color: | BLACK |
| Incubator number and shelf location: | 2B2 |

### *Daily renewal:*

| Day | Date | Test initiation and feeding, renewal and feeding, or termination time | *Feeding Batches *Selenastrum* | *Feeding Batches YWT | MHSW batch used | Analyst |
|---|---|---|---|---|---|---|
| 0 | 05-05-20 | 0925 | 05-01-20 | 05-01-20 | 04-29-20B | JH |
| 1 | 05-06-20 | 0826 | ↓ | ↓ | ↓ | JH |
| 2 | 05-07-20 | 0850 | ↓ | ↓ | 04-29-20D | JH |
| 3 | 05-08-20 | 0846 | ↓ | ↓ | ↓ | JH |
| 4 | 05-09-20 | 0945 | ↓ | ↓ | 05-07-20B | JH |
| 5 | 05-10-20 | 0846 | ↓ | ↓ | ↓ | JH |
| 6 | 05-11-20 | 0845 | ↓ | ↓ | ↓ | JH |
| 7 | 05-12-20 | 0825 | | | | JH |

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

### *Chemical analyses:*

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2011 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2011 | YSI Model 52CE | 18D104324 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2011 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2011 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2011 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2550B-2010 | Digital Thermometer | 130664685 |

| *Control information:* | | Acceptance criteria | *Summary of test endpoints:* | |
|---|---|---|---|---|
| % of Male Adults: | 0% | ≤ 20% | 7-day $LC_{50}$ (mg/L NaCl) | >1400 |
| % Adults having 3rd Broods: | 100% | ≥ 80% | NOEC (mg/L NaCl) | 1000 |
| % Mortality: | 0% | ≤ 20% | LOEC (mg/L NaCl) | 1200 |
| Mean Offspring/Female: | 30.6 | ≥ 15.0 offspring/female | ChV (mg/L NaCl) | 1095.5 |
| % CV: | 7.37% | < 40.0 % | $IC_{25}$ (mg/L NaCl) | 1098.5 |

Independent Review by Kelley E. Keenan. K

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 243**

**CONTROL** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 3 | 3 | 6 | 4 | 5 | 5 | 4 | 5 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 10 | 10 | 11 | 9 | 12 | 12 | 12 | 10 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 18 | 15 | 15 | 13 | 16 | 14 | 13 | 16 | 18 | 16 |
| Total young produced | | 35 | 28 | 28 | 30 | 29 | 31 | 30 | 32 | 33 | 30 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 30.6 |

**600 mg NaCl/L** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 3 | 4 | 5 | 4 | 5 | 5 | 5 | 4 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 10 | 11 | 10 | 13 | 10 | 13 | 12 | 10 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 15 | 19 | 14 | 17 | 16 | 17 | 15 | 15 | 15 | 18 |
| Total young produced | | 32 | 32 | 29 | 32 | 33 | 32 | 33 | 32 | 29 | 33 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 31.7 |
| % Reduction from Control: | -3.6% |

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 243**

**800 mg NaCl/L** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 3 | 5 | 5 | 4 | 5 | 5 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 13 | 13 | 11 | 12 | 10 | 10 | 10 | 12 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 16 | 15 | 17 | 18 | 14 | 17 | 17 | 15 | 14 | 17 |
| Total young produced | | 30 | 33 | 33 | 34 | 31 | 31 | 32 | 30 | 31 | 32 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 31.7 |
| % Reduction from Control: | -3.6% |

**1000 mg NaCl/L** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 3 | 5 | 4 | 6 | 4 | 5 | 5 | 3 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 10 | 13 | 12 | 10 | 10 | 10 | 13 | 11 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 16 | 17 | 14 | 14 | 13 | 14 | 16 | 12 | 15 | 13 |
| Total young produced | | 32 | 32 | 31 | 32 | 27 | 29 | 31 | 28 | 31 | 28 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 30.1 |
| % Reduction from Control: | 1.6% |

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 243**

**1200 mg NaCl/L** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 3 | 2 | 2 | 4 | 1 | 4 | 3 | 3 | 3 | 2 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 8 | 5 | 5 | 7 | 9 | 6 | 4 | 5 | 7 | 7 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 7 | 9 | 10 | 4 | 8 | 6 | 10 | 10 | 4 | 9 |
| Total young produced | | 18 | 16 | 17 | 15 | 18 | 16 | 17 | 18 | 14 | 18 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note:* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 16.7 |
| % Reduction from Control: | 45.4% |

**1400 mg NaCl/L** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 1 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 2 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 0 | 2 | 0 | 0 | 0 | 1 | 3 | 1 | 1 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total young produced | | 1 | 2 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 3 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

*Note.* Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | ~~1.0~~ 1.7 |
| % Reduction from Control: | ~~94.8%~~ 94.4% |

Independent Review by Kelley E. Keenan: K

Independent Review by Kelley E. Kaarsar

Environmental Testing Solutions, Inc.

# Verification of *Ceriodaphnia* Reproduction Totals

## Control

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 3 | 3 | 6 | 4 | 5 | 5 | 4 | 5 | 4 | 44 |
| 5 | 12 | 10 | 10 | 11 | 9 | 12 | 12 | 12 | 10 | 10 | 108 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 18 | 15 | 15 | 13 | 16 | 14 | 13 | 16 | 18 | 16 | 154 |
| Total | 35 | 28 | 28 | 30 | 29 | 31 | 30 | 32 | 33 | 30 | 306 |

## 1000 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 3 | 5 | 4 | 6 | 4 | 5 | 5 | 3 | 5 | 5 | 45 |
| 5 | 13 | 10 | 13 | 12 | 10 | 10 | 10 | 13 | 11 | 10 | 112 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 16 | 17 | 14 | 14 | 13 | 14 | 16 | 12 | 15 | 13 | 144 |
| Total | 32 | 32 | 31 | 32 | 27 | 29 | 31 | 28 | 31 | 28 | 301 |

## 600 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 3 | 4 | 5 | 4 | 5 | 5 | 5 | 4 | 5 | 45 |
| 5 | 12 | 10 | 11 | 10 | 13 | 10 | 13 | 12 | 10 | 10 | 111 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 19 | 14 | 17 | 16 | 17 | 15 | 15 | 15 | 18 | 161 |
| Total | 32 | 32 | 29 | 32 | 33 | 32 | 33 | 32 | 29 | 33 | 317 |

## 1200 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 3 | 2 | 2 | 4 | 1 | 4 | 3 | 3 | 3 | 2 | 27 |
| 5 | 8 | 5 | 5 | 7 | 9 | 6 | 4 | 5 | 7 | 7 | 63 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 7 | 9 | 10 | 4 | 8 | 6 | 10 | 10 | 4 | 9 | 77 |
| Total | 18 | 16 | 17 | 15 | 18 | 16 | 17 | 18 | 14 | 18 | 167 |

## 800 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 5 | 3 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 46 |
| 5 | 10 | 13 | 13 | 11 | 12 | 10 | 10 | 10 | 12 | 10 | 111 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 16 | 15 | 17 | 18 | 14 | 17 | 17 | 15 | 14 | 17 | 160 |
| Total | 30 | 33 | 33 | 34 | 31 | 31 | 32 | 30 | 31 | 32 | 317 |

## 1400 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 1 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 2 | 8 |
| 5 | 0 | 2 | 0 | 0 | 0 | 1 | 3 | 1 | 1 | 0 | 8 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Total | 1 | 2 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 17 |

Entered and Reviewed by [illegible]

Independent Review by Kelley E. Keesee:

Environmental Testing Solutions, Inc.

# *Ceriodaphnia dubia* Chronic Reference Toxicant Test
## EPA-821-R-02-013, Method 1002.0

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

Test number: CdNaClCR #243

Test dates: May 05-12, 2020

| Concentration (mg/L NaCl) | Replicate number | | | | | | | | | | Survival (%) | Average reproduction (offspring/female) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| Control | 35 | 28 | 28 | 30 | 29 | 31 | 30 | 32 | 33 | 30 | 100 | 30.6 | 7.3 | Not applicable |
| 600 | 32 | 32 | 29 | 32 | 33 | 32 | 33 | 32 | 29 | 33 | 100 | 31.7 | 4.7 | -3.6 |
| 800 | 30 | 33 | 33 | 34 | 31 | 31 | 32 | 30 | 31 | 32 | 100 | 31.7 | 4.2 | -3.6 |
| 1000 | 32 | 32 | 31 | 32 | 27 | 29 | 31 | 28 | 31 | 28 | 100 | 30.1 | 6.4 | 1.6 |
| 1200 | 18 | 16 | 17 | 15 | 18 | 16 | 17 | 18 | 14 | 18 | 100 | 16.7 | 8.5 | 45.4 |
| 1400 | 1 | 2 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 100 | 1.7 | 48.4 | 94.4 |

Dunnett's MSD value: 1.633

PMSD: 5.3

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 13%.

Upper PMSD bound determined by USEPA (90th percentile) = 47%.

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.

Entered and Reviewed by Jim Somoar

# Statistical Analyses

Environmental Testing Solutions, Inc.

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | CdNaClCR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| D-Control | 35.000 | 28.000 | 28.000 | 30.000 | 29.000 | 31.000 | 30.000 | 32.000 | 33.000 | 30.000 |
| 600 | 32.000 | 32.000 | 29.000 | 32.000 | 33.000 | 32.000 | 33.000 | 32.000 | 29.000 | 33.000 |
| 800 | 30.000 | 33.000 | 33.000 | 34.000 | 31.000 | 31.000 | 32.000 | 30.000 | 31.000 | 32.000 |
| 1000 | 32.000 | 32.000 | 31.000 | 32.000 | 27.000 | 29.000 | 31.000 | 28.000 | 31.000 | 28.000 |
| 1200 | 18.000 | 16.000 | 17.000 | 15.000 | 18.000 | 16.000 | 17.000 | 18.000 | 14.000 | 18.000 |
| 1400 | 1.000 | 2.000 | 2.000 | 2.000 | 1.000 | 1.000 | 3.000 | 1.000 | 1.000 | 3.000 |

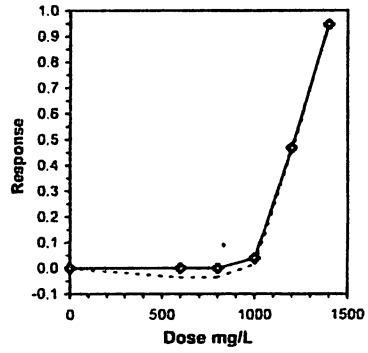
| | | | Transform: Untransformed | | | | | Rank | 1-Tailed | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Mean | N-Mean |
| D-Control | 30.600 | 1.0000 | 30.600 | 28.000 | 35.000 | 7.259 | 10 | | | 31.333 | 1.0000 |
| 600 | 31.700 | 1.0359 | 31.700 | 29.000 | 33.000 | 4.714 | 10 | 123.00 | 75.00 | 31.333 | 1.0000 |
| 800 | 31.700 | 1.0359 | 31.700 | 30.000 | 34.000 | 4.219 | 10 | 124.50 | 75.00 | 31.333 | 1.0000 |
| 1000 | 30.100 | 0.9837 | 30.100 | 27.000 | 32.000 | 6.352 | 10 | 101.50 | 75.00 | 30.100 | 0.9606 |
| *1200 | 16.700 | 0.5458 | 16.700 | 14.000 | 18.000 | 8.492 | 10 | 55.00 | 75.00 | 16.700 | 0.5330 |
| *1400 | 1.700 | 0.0556 | 1.700 | 1.000 | 3.000 | 48.428 | 10 | 55.00 | 75.00 | 1.700 | 0.0543 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | 1.13376 | 1.035 | -0.0271 | 0.04608 |
| Bartlett's Test indicates equal variances (p = 0.11) | 8.88134 | 15.0863 | | |

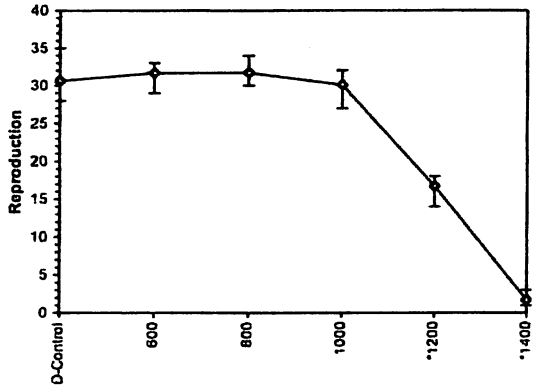
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1000 | 1200 | 1095.45 | |
| Treatments vs D-Control | | | | |

**Linear Interpolation (200 Resamples)**

| Point | mg/L | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05 | 1004.98 | 29.8289 | 915.717 | 1020.12 | -1.7672 |
| IC10 | 1028.36 | 8.30365 | 1010.46 | 1042.38 | -0.1710 |
| IC15 | 1051.74 | 7.49304 | 1035.03 | 1064.44 | -0.1325 |
| IC20 | 1075.12 | 6.79639 | 1060.27 | 1086.39 | -0.0978 |
| IC25 | 1098.51 | 6.25191 | 1085.64 | 1109.29 | -0.0764 |
| IC40 | 1168.66 | 5.90541 | 1157.44 | 1179.7 | -0.0976 |
| IC50 | 1213.78 | 5.32573 | 1203.3 | 1222.89 | -0.3410 |




**Dose-Response Plot**




Independent Review by Kelsey E. Keenan:

# Statistical Analyses

Environmental Testing Solutions, Inc.

**Used for PMSD calculation only.**

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

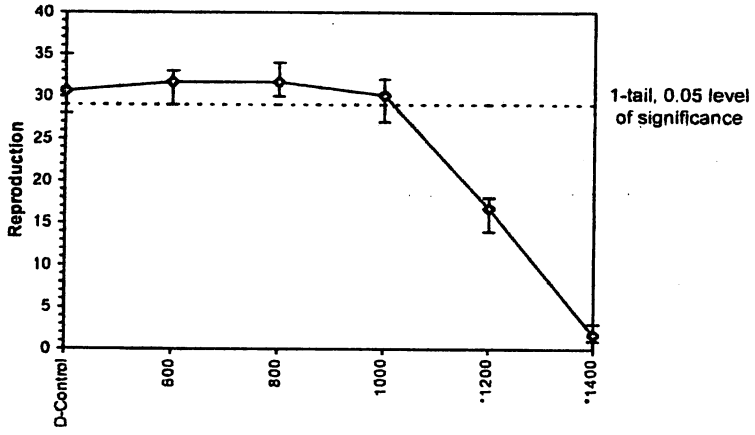
| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/5/2020 | Test ID: | CdNaClCR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/12/2020 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | FWCHR-EPA-821-R-02-013 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| D-Control | 35.000 | 28.000 | 28.000 | 30.000 | 29.000 | 31.000 | 30.000 | 32.000 | 33.000 | 30.000 |
| 600 | 32.000 | 32.000 | 29.000 | 32.000 | 33.000 | 32.000 | 33.000 | 32.000 | 29.000 | 33.000 |
| 800 | 30.000 | 33.000 | 33.000 | 34.000 | 31.000 | 31.000 | 32.000 | 30.000 | 31.000 | 32.000 |
| 1000 | 32.000 | 32.000 | 31.000 | 32.000 | 27.000 | 29.000 | 31.000 | 28.000 | 31.000 | 28.000 |
| 1200 | 18.000 | 16.000 | 17.000 | 15.000 | 18.000 | 16.000 | 17.000 | 18.000 | 14.000 | 18.000 |
| 1400 | 1.000 | 2.000 | 2.000 | 2.000 | 1.000 | 1.000 | 3.000 | 1.000 | 1.000 | 3.000 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| D-Control | 30.600 | 1.0000 | 30.600 | 28.000 | 35.000 | 7.259 | 10 | | | |
| 600 | 31.700 | 1.0359 | 31.700 | 29.000 | 33.000 | 4.714 | 10 | -1.540 | 2.287 | 1.633 |
| 800 | 31.700 | 1.0359 | 31.700 | 30.000 | 34.000 | 4.219 | 10 | -1.540 | 2.287 | 1.633 |
| 1000 | 30.100 | 0.9837 | 30.100 | 27.000 | 32.000 | 6.352 | 10 | 0.700 | 2.287 | 1.633 |
| *1200 | 16.700 | 0.5458 | 16.700 | 14.000 | 18.000 | 8.492 | 10 | 19.464 | 2.287 | 1.633 |
| *1400 | 1.700 | 0.0556 | 1.700 | 1.000 | 3.000 | 48.428 | 10 | 40.468 | 2.287 | 1.633 |

| Auxiliary Tests | | | | | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | | | | | 1.13376 | | 1.035 | | -0.0271 | 0.04608 |
| Bartlett's Test indicates equal variances (p = 0.11) | | | | | 8.88134 | | 15.0863 | | | |
| **Hypothesis Test (1-tail, 0.05)** | **NOEC** | **LOEC** | **ChV** | **TU** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Dunnett's Test | 1000 | 1200 | 1095.45 | | 1.63301 | 0.05337 | 1499.11 | 2.55 | 1.4E-45 | 5, 54 |
| Treatments vs D-Control | | | | | | | | | | |

**Dose-Response Plot**




Independent Review by Kelley E. Asenau

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 243**

***Daily Chemistry:***

**Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | MS | TS | TS | TS | TS | K |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.98 | 7.92 | 7.68 | 7.89 | 7.79 | 7.83 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 7.6 | 7.7 | 7.6 | 7.7 |
| | Conductivity (µmhos/cm) | 321 | | 321 | | 318 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | 03-11-20 | | 61 | |
| | Hardness (mg $CaCO_3$/L) | 93 | | | | 89 | |
| | Temperature (°C) | 24.9 | 25.1 | 24.8 | 25.2 | 24.8 | 25.1 |
| 600 mg NaCl/L | pH (S.U.) | 8.03 | 7.93 | 7.82 | 7.94 | 7.91 | 7.88 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.8 | 7.6 | 7.7 | 7.6 | 7.7 |
| | Conductivity (µmhos/cm) | 1480 | | 1460 | | 1460 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.9 | 25.2 | 24.9 | 24.7 |
| 800 mg NaCl/L | pH (S.U.) | 8.02 | 7.92 | 7.84 | 7.92 | 7.91 | 7.87 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.8 | 7.6 | 7.7 | 7.6 | 7.7 |
| | Conductivity (µmhos/cm) | 1860 | | 1870 | | 1880 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.0 | 24.9 | 24.9 |
| 1000 mg NaCl/L | pH (S.U.) | 8.01 | 7.92 | 7.84 | 7.93 | 7.91 | 7.88 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.8 | 7.6 | 7.7 | 7.7 | 7.8 |
| | Conductivity (µmhos/cm) | 2250 | | 2220 | | 2260 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.0 | 24.9 | 24.9 |
| 1200 mg NaCl/L | pH (S.U.) | 8.00 | 7.92 | 7.84 | 7.93 | 7.93 | 7.88 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.7 | 7.7 | 7.7 | 7.7 | 7.9 |
| | Conductivity (µmhos/cm) | 2670 | | 2262 | | 2660 | |
| | Temperature (°C) | 25.0 | 25.0 | 25.0 | 24.9 | 24.7 | 24.9 |
| 1400 mg NaCl/L | pH (S.U.) | 7.99 | 7.92 | 7.84 | 7.92 | 7.93 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.7 | 7.8 | 7.7 | 7.7 | 7.9 |
| | Conductivity (µmhos/cm) | 3140 | | 2990 | | 3000 | |
| | Temperature (°C) | 25.0 | 25.1 | 25.0 | 24.9 | 24.7 | 24.8 |
| | | Initial | Final | Initial | Final | Initial | Final |

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #:** 243

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | K | BSL | BSL | TS | TS | TS | TS | TS |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.88 | 7.87 | 7.72 | 7.96 | 7.73 | 8.02 | 7.83 | 7.81 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.7 | 7.6 | 7.7 | 7.7 | 7.5 | 7.6 | 7.4 |
| | Conductivity (µmhos/cm) | 305 | | 300 | | 314 | | 322 | |
| | Alkalinity (mg $CaCO_3$/L) | 05-11-20 | | 60 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 90 | | | | K 05-11-20 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.8 | 25.0 | 24.8 | 25.2 | 24.8 | 25.2 |
| 600 mg NaCl/L | pH (S.U.) | 7.96 | 7.94 | 7.98 | 7.96 | 7.90 | 8.03 | 7.94 | 7.86 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.8 | 7.7 | 7.7 | 7.6 | 7.5 | 7.7 | 7.4 |
| | Conductivity (µmhos/cm) | 1470 | | 1450 | | 1500 | | 1490 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.8 | 25.2 | 24.7 | 24.9 | 24.9 | 25.0 |
| 800 mg NaCl/L | pH (S.U.) | 7.95 | 7.95 | 7.98 | 7.96 | 7.90 | 8.03 | 7.93 | 7.87 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.8 | 7.7 | 7.7 | 7.6 | 7.5 | 7.7 | 7.4 |
| | Conductivity (µmhos/cm) | 1860 | | 1840 | | 1910 | | 1870 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.8 | 25.2 | 24.7 | 24.9 | 24.9 | 25.0 |
| 1000 mg NaCl/L | pH (S.U.) | 7.95 | 7.94 | 7.98 | 7.95 | 7.93 | 8.03 | 7.96 | 7.88 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 7.7 | 7.8 | 7.7 | 7.5 | 7.7 | 7.5 |
| | Conductivity (µmhos/cm) | 2220 | | 2230 | | 2200 | | 2250 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.9 | 24.8 | 24.9 | 24.9 | 25.0 | 25.0 |
| 1200 mg NaCl/L | pH (S.U.) | 7.95 | 7.95 | 7.97 | 7.94 | 7.92 | 8.04 | 7.96 | 7.87 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 7.8 | 7.9 | 7.7 | 7.5 | 7.8 | 7.5 |
| | Conductivity (µmhos/cm) | 2500 | | 2570 | | 2620 | | 2610 | |
| | Temperature (°C) | 24.8 | 25.1 | 24.9 | 24.8 | 24.9 | 25.1 | 24.9 | 25.2 |
| 1400 mg NaCl/L | pH (S.U.) | 7.96 | 7.93 | 7.98 | 7.94 | 7.94 | 8.04 | 7.98 | 7.89 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 7.8 | 7.9 | 7.7 | 7.6 | 7.8 | 7.5 |
| | Conductivity (µmhos/cm) | 2950 | | 2940 | | 3020 | | 3020 | |
| | Temperature (°C) | 24.9 | 25.0 | 24.8 | 24.9 | 24.8 | 24.8 | 24.9 | 24.9 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

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