



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

September 9, 2019

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

Subject: **Sequoyah Nuclear Plant, Discharge Monitoring Report (DMR), August 2019**

Attached is the August 2019 DMR for Sequoyah Nuclear Plant.

Respectfully,

A handwritten signature in blue ink that reads "Millicent Garland".

Millicent Garland
Environmental Scientist

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

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

MAJOR
 (SUBR 01)
 F - FINAL
 DIFFUSER DISCHARGE
 EFFLUENT

Form Approved.
 OMB No. 2040-0004


TN0026450			101 G		
PERMIT NUMBER			DISCHARGE NUMBER		
MONITORING PERIOD					
YEAR	MO	DAY	YEAR	MO	DAY
From 19	08	01	To 19	08	31

ATTN:Millicent Garland

*** NO DISCHARGE ☐ ***

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	*****	*****	**	*****	*****	42.7	04	0	31 / 31	RCORDR
00010 1 0	PERMIT REQUIREMENT	*****	*****	****	*****	*****	Req. Mon. DAILY MAX	DEG. C.		CONTI NUOUS	CALCTD
EFFLUENT GROSS											
TEMPERATURE, WATER DEG. CENTIGRADE	SAMPLE MEASUREMENT	*****	*****	**	*****	*****	30.1	04	0	31 / 31	MODELD
00010 Z 0	PERMIT REQUIREMENT	*****	*****	****	*****	*****	30.5 DAILY MX	DEG. C.		CONTI NUOUS	CALCTD
INSTREAM MONITORING											
TEMP. DIFF. BETWEEN SAMP. & UPSTRM DEG.C	SAMPLE MEASUREMENT	*****	*****	**	*****	*****	2.0	04	0	31 / 31	CALCTD
00016 1 S	PERMIT REQUIREMENT	*****	*****	****	*****	*****	3.0 DAILY MX	DEG. C.		CONTI NUOUS	CALCTD
EFFLUENT GROSS											
FLOW, IN CONDUIT OR THRU TREATMENT PLANT	SAMPLE MEASUREMENT	*****	1751	03	*****	*****	*****	**	0	31 / 31	RCORDR
50050 1 0	PERMIT REQUIREMENT	*****	Req. Mon. DAILY MAX	MGD	*****	*****	*****	****		CONTI NUOUS	RCORDR
EFFLUENT GROSS											
FLOW, IN CONDUIT OR THRU TREATMENT PLANT	SAMPLE MEASUREMENT	1736	*****	03	*****	*****	*****	03	0	31 / 31	CALCTD
50050 1 0	PERMIT REQUIREMENT	Req. Mon. MO AVG	*****	MGD	*****	*****	*****	MGD		CONTI NUOUS	CALCTD
EFFLUENT GROSS VALUE											
CHLORINE, TOTAL RESIDUAL	SAMPLE MEASUREMENT	*****	*****	**	*****	0.018	0.030	19	0	22 / 31	GRAB
50060 1 0	PERMIT REQUIREMENT	*****	*****	****	*****	0.1 MO AVG	0.1 DAILY MAX	MG/L		FIVE PER WEEK	CALCTD
EFFLUENT GROSS VALUE											
TEMPERATURE - C, RATE OF CHANGE	SAMPLE MEASUREMENT	*****	0.4	62	*****	*****		**	0	31 / 31	CALCTD
82234 1 0	PERMIT REQUIREMENT	*****	2.0 DAILY MX	DEG C/HR	*****	*****	*****	****		CONTI NUOUS	CALCTD
EFFLUENT GROSS											

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER Matthew Rasmussen Site Vice President TYPED OR PRINTED	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 SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT	TELEPHONE		DATE		
			423	843-7001	19	09	05
			AREA CODE	NUMBER	YEAR	MO	DAY

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

No closed mode operation. The following injections occurred: Flogard MS 6236 (mac calc. was 0.02852 mg/L, limit is 0.2 mg/L), Spectrus BD 1500 (max calc. was 0.047 mg/L, limit is 2.0 mg/L), and Spectrus CT 1300 (max calc. was 0.0332 mg/L, limit is 0.05 mg/L).

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 Facility **TVA - SEQUOYAH NUCLEAR PLANT**
 Location **HAMILTON COUNTY**

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

MAJOR
 (SUBR 01)

Form Approved.
 OMB No. 2040-0004

TN0026450 **101 T**
PERMIT NUMBER **DISCHARGE NUMBER**

F - FINAL
 BIOMONITORING FOR OUTFALL 101
 EFFLUENT

MONITORING PERIOD
 From **19 08 01** To **19 08 31**

*** NO DISCHARGE ☐ ***

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			
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 MINIMUM	*****	*****	PERCENT		SEMI ANNUAL	COMPOS
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
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	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

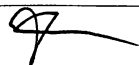
NAME/TITLE PRINCIPAL EXECUTIVE OFFICER

Matthew Rasmussen

Site Vice President

TYPED OR PRINTED

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
 SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT

TELEPHONE

423 843-7001

AREA CODE

NUMBER

DATE

19 09 06

YEAR MO DAY

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

Toxicity was sampled August 4 - 9, 2019. The final report is attached.

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 Location **HAMILTON COUNTY**

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

MAJOR
 (SUBR 01)

Form Approved.
 OMB No. 2040-0004

TN0026450 **103 G**
 PERMIT NUMBER DISCHARGE NUMBER


F - FINAL
 LOW VOL. WASTE TREATMENT POND
 EFFLUENT

MONITORING PERIOD
 From **19 08 01** To **19 08 31**

*** NO DISCHARGE ☐ ***

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 00400 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	6.9	*****	8.6	12	0	5 / 31	GRAB
	PERMIT REQUIREMENT	*****	*****	**	6.0 MINIMUM	*****	9.0 MAXIMUM	SU		ONCE/ WEEK	GRAB
SOLIDS, TOTAL SUSPENDED 00530 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	*****	8.2	8.2	19	0	1 / 31	GRAB
	PERMIT REQUIREMENT	*****	*****	**	*****	30.0 MO AVG	100.0 DAILY MX	MG/L		ONCE/ MONTH	GRAB
OIL AND GREASE 00556 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	*****	<5.0	<5.0	19	0	1 / 31	GRAB
	PERMIT REQUIREMENT	*****	*****	**	*****	15.0 MO AVG	20.0 DAILY MX	MG/L		ONCE/ MONTH	GRAB
FLOW, IN CONDUIT OR THRU TREATMENT PLANT 50050 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	1.102	1.206	03	*****	*****	*****	**	0	4 / 31	INSTAN
	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 Matthew Rasmussen Site Vice President TYPED OR PRINTED	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 SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT	TELEPHONE		DATE		
			423	843-7001	19	09	05
			AREA CODE	NUMBER	YEAR	MO	DAY

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

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

MAJOR
 (SUBR 01)

Form Approved.
 OMB No. 2040-0004

TN0026450 **110 G**
PERMIT NUMBER **DISCHARGE NUMBER**

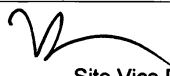
F - FINAL
 RECYCLED COOLING WATER
 EFFLUENT

MONITORING PERIOD
 From **19 08 01** To **19 08 31**

*** NO DISCHARGE ☒ ***

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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	PERMIT REQUIREMENT	*****	*****	**	*****	*****	REPORT DAILY MX	DEG C		CONTINUOUS	CALCTD
EFFLUENT GROSS VALUE											
TEMPERATURE, WATER DEG. CENTIGRADE	SAMPLE MEASUREMENT	*****	*****	**	*****	*****		04			
00010 Z 0	PERMIT REQUIREMENT	*****	*****	**	*****	*****	30.5 DAILY MX	DEG C		CONTINUOUS	CALCTD
INSTREAM MONITORING											
TEMP. DIFF. BETWEEN SAMP. & UPSTRM DEG.C	SAMPLE MEASUREMENT	*****	*****	**	*****	*****		04			
00016 1 0	PERMIT REQUIREMENT	*****	*****	**	*****	*****	5 DAILY MX	DEG C		CONTINUOUS	CALCTD
EFFLUENT GROSS VALUE											
FLOW, IN CONDUIT OR THRU TREATMENT PLANT	SAMPLE MEASUREMENT	*****		03	*****	*****	*****	**			
50050 1 0	PERMIT REQUIREMENT	*****	Req. Mon. DAILY MX	MGD	*****	*****	*****	**		CONTINUOUS	RCORDR
EFFLUENT GROSS VALUE											
CHLORINE, TOTAL RESIDUAL	SAMPLE MEASUREMENT	*****	*****	**	*****			19			
50060 1 0	PERMIT REQUIREMENT	*****	*****	**	*****	0.1 MO AVG	0.1 DAILY MX	MG/L		Five per Week	CALCTD
EFFLUENT GROSS VALUE											
TEMPERATURE - C, RATE OF CHANGE	SAMPLE MEASUREMENT	*****		04	*****	*****	*****	**			
82234 1 0	PERMIT REQUIREMENT	*****	2 DAILY MX	DEG C	*****	*****	*****	**		CONTINUOUS	CALCTD
EFFLUENT GROSS VALUE											
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

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Matthew Rasmussen			423	843-7001	19	09	05
Site Vice President			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
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 Location HAMILTON COUNTY

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES)

DISCHARGE MONITORING REPORT (DMR)

MAJOR
(SUBR 01)

F - FINAL

RECYCLED COOLING WATER

EFFLUENT

Form Approved.
OMB No. 2040-0004

TN0026450

PERMIT NUMBER

110 T

DISCHARGE NUMBER

MONITORING PERIOD

From

YEAR	MO	DAY
19	08	01


 To

YEAR	MO	DAY
19	08	31

*** NO DISCHARGE ☒ ***

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			
IC25 STATRE 7DAY CHR CERIODAPHNIA	SAMPLE MEASUREMENT	*****	*****	**		*****	*****	23			
TRP3B 1 0 0	PERMIT REQUIREMENT	*****	*****	****	42.8	*****	*****	PERCENT		SEMI	COMPOS
EFFLUENT GROSS VALUE					MINIMUM					ANNUAL	
IC25 STATRE 7DAY CHR PIMEPHALES	SAMPLE MEASUREMENT	*****	*****	**		*****	*****	23			
TRP6C 1 0 0	PERMIT REQUIREMENT	*****	*****	****	42.8	*****	*****	PERCENT		SEMI	COMPOS
EFFLUENT GROSS VALUE					MINIMUM					ANNUAL	
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
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	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

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Matthew Rasmussen			423	843-7001	19	09	05	
Site Vice President			AREA CODE	NUMBER	YEAR	MO	DAY	
TYPED OR PRINTED	SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT							

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

MAJOR
 (SUBR 01)

Form Approved.
 OMB No. 2040-0004

TN0026450 **118 G**
 PERMIT NUMBER DISCHARGE NUMBER

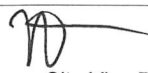
F - FINAL
 WASTEWATER & STORM WATER
 EFFLUENT

MONITORING PERIOD
 From **19 08 01** To **19 08 31**

*** NO DISCHARGE ☒ ***

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			
OXYGEN, DISSOLVED (DO) 00300 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**		*****	*****	19			
	PERMIT REQUIREMENT	*****	*****	****	2 MINIMUM	*****	*****	MG/L		TWICE/ WEEK	GRAB
SOLIDS, TOTAL SUSPENDED 00530 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	*****	*****		19			
	PERMIT REQUIREMENT	*****	*****	****	*****	*****	100 DAILY MX	MG/L		TWICE/ WEEK	GRAB
SOLIDS, SETTLEABLE 00545 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	*****	*****		25			
	PERMIT REQUIREMENT	*****	*****	****	*****	*****	1 DAILY MX	ML/L		ONCE/ MONTH	GRAB
FLOW, IN CONDUIT OR THRU TREATMENT PLANT 50050 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT			03	*****	*****	*****	**			
	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										

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			423	843-7001	19	09	05
			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 August 2019 Semiannual Whole Effluent Toxicity Report for DSN101

Site/Plant/Project Name

Sequoyah Nuclear Plant

Accession Number (optional)

Work Order Number (optional)

Your Name

Donald W. Snodgrass

Date Submitted (YYYYMMDD)

20190905

Document Date (YYYYMMDD)

20190903

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: September 03, 2019

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: August 04 – 09, 2019
10. Average Flow on Days Sampled (MGD): 1727, 1729, 1733
11. Pertinent Site Conditions: Production / operation data will be provided upon request.
12. Test Dates: August 06 – 13, 2019
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: IC₂₅ = 42.8%
17. Test Results: Outfall 101: *Pimephales promelas*: IC₂₅ > 100%
Ceriodaphnia dubia: IC₂₅ > 100%



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: Donald W. Snodgrass Phone #: (256) 386-2787
22. Notes: Exposures to samples collected August 04 – 09, 2019 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 minnows and daphnids to intake samples resulted in no significant difference from the controls during this study period.



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	08-04-19 0700 to 08-05-19 0600	08-05-19 1420	2.3	<0.10	08-06-19 0932 08-07-19 0925
Intake	08-04-19 0700 to 08-05-19 0600	08-05-19 1420	1.0	<0.10	08-06-19 0932 08-07-19 0925
101	08-06-19 0700 to 08-07-19 0600	08-07-19 1345	1.5	<0.10	08-08-19 1007 08-09-19 0928
Intake	08-06-19 0700 to 08-07-19 0600	08-07-19 1345	1.1	<0.10	08-08-19 1007 08-09-19 0928
101	08-08-19 0700 to 08-09-19 0600	08-09-19 1315	1.7, 1.6 †	<0.10	08-10-19 1020 08-11-19 1017 08-12-19 0923
Intake	08-08-19 0700 to 08-09-19 0600	08-09-19 1315	4.0	<0.10	08-10-19 1020 08-11-19 1017 08-12-19 0923

*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.

	<i>Pimephales promelas</i>	<i>Ceriodaphnia dubia</i>
<u>Test Organisms:</u>		
1. Source:	<u>In-house Cultures</u>	<u>In-house Cultures</u>
2. Age:	<u>< 24-hours old</u>	<u>< 24-hours old</u>
<u>Test Method Summary:</u>		
1. Test Conditions:	<u>Static, Renewal</u>	<u>Static, Renewal</u>
2. Test Duration:	<u>7 days</u>	<u>Until at least 60% of control females have 3 broods</u>
3. Control / Dilution Water:	<u>Moderately Hard Synthetic</u>	<u>Moderately Hard Synthetic</u>
4. Number of Replicates:	<u>4</u>	<u>10</u>
5. Organisms per Replicate:	<u>10</u>	<u>1</u>
6. Test Initiation: (Date/Time):	<u>08-06-19 0834 ET</u>	<u>08-06-19 0932 ET</u>
7. Test Termination: (Date/Time):	<u>08-13-19 0740 ET</u>	<u>08-13-19 0913 ET</u>
8. Test Temperature: Outfall 101:	<u>Mean = 24.8°C</u> <u>(24.4 – 25.1°C)</u>	<u>Mean = 25.0°C</u> <u>(24.7 – 25.2°C)</u>
9. Physical / Chemical Measurements:	<u>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.</u>	
10. Statistics:	<u>Statistics were performed according to methods prescribed by EPA using ToxCalc version 5.0 statistical software (Tidepool Scientific Software, McKinneyville, CA).</u>	



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 August 06 – 13, 2019 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	0.734	0.755	0.702	0.756	0.737
10.7%	0.702	0.739	0.808	0.727	0.744
21.4%	0.817	0.812	0.857	0.754	0.810
42.8%	0.724	0.716	0.756	0.749	0.736
85.6%	0.807	0.756	0.820	0.710	0.773
100.0%	0.742	0.733	0.731	0.721	0.732
Intake	0.714	0.762	0.684	0.760	0.730
Control, Non-treated	0.788	0.737	0.713	0.818	0.764
IC ₂₅ Value: <u>> 100%</u> Permit Limit: <u>42.8%</u>			Calculated TU Estimates: <u>< 1.0 TUc*</u>		
95% Confidence Limits: Upper Limit: <u>NA</u> Lower Limit: <u>NA</u>			Permit Limit: <u>2.3 TUc</u>		

*TUa = 100/LC₅₀; TUc = 100/ IC₂₅



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 August 06 – 13, 2019 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	27	30	33	31	31	32	32	31	30	31	30.8
10.7%	33	29	28	33	33	32	32	33	32	30	31.5
21.4%	35	29	34	29	34	33	29	33	32	34	32.2
42.8%	30	34	36	36	34	35	33	34	33	37	34.2
85.6%	36	38	34	34	38	33	33	36	39	33	35.4
100.0%	39	36	38	35	35	37	35	37	38	41	37.1
IC ₂₅ Value: <u>> 100%</u> Permit Limit: <u>42.8%</u> 95% Confidence Limits: Upper Limit: <u>NA</u> Lower Limit: <u>NA</u>						Calculated TU Estimates: <u>< 1.0 TUc*</u> Permit Limit: <u>2.3 TUc</u>					

*TU_a = 100/LC₅₀; TU_c = 100/ IC₂₅



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 August 06 – 13, 2019 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	31	34	33	27	30	31	30	32	29	31	30.8
Intake	34	35	33	32	32	33	31	33	32	32	32.7
IC ₂₅ Value: <u>> 100%</u> Permit Limit: <u>N/A</u> 95% Confidence Limits: Upper Limit: <u>NA</u> Lower Limit: <u>NA</u>				Calculated TU Estimates: <u>≤ 1.0 TUc*</u> Permit Limit: <u>N/A</u>							

*TUa = 100/LC₅₀; TUc = 100/ IC₂₅

REFERENCE TOXICANT TEST RESULTS (see Appendix A and D)

Species	Date	Time	Duration	Toxicant	Results (IC ₂₅)
<i>Pimephales promelas</i>	August 06 – 13, 2019	0800	7 days	KCl	0.69 g/L
<i>Ceriodaphnia dubia</i>	August 06 – 13, 2019	0912	7-days	NaCl	1.10 g/L





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 August 06-13, 2019.

Test	Sample ID	Temperature (°C)		Dissolved Oxygen (mg/L)		pH (S.U.)		Conductance (µmhos/cm)	Alkalinity (mg/L CaCO ₃)	Hardness (mg/L CaCO ₃)	*Total Residual Chlorine (mg/L)
		Initial	Final	Initial	Final	Initial	Final				
<i>Pimephales promelas</i>	Control, Non-treated	24.8 24.7 - 25.0	24.7 24.6 - 24.9	7.8 7.7 - 7.9	7.7 7.5 - 7.9	7.89 7.83 - 7.99	7.77 7.62 - 7.95	320 306 - 326	59 58 - 60	85 84 - 88	- - -
	Control, UV-treated	24.8 24.7 - 24.9	24.7 24.6 - 25.1	8.1 7.9 - 8.4	7.7 7.0 - 8.0	7.98 7.93 - 8.06	7.75 7.53 - 7.97	324 307 - 340	59 58 - 61	85 84 - 86	- - -
	10.7%	24.9 24.8 - 24.9	24.7 24.5 - 24.9	8.1 7.8 - 8.4	7.6 7.0 - 8.0	7.99 7.95 - 8.06	7.72 7.52 - 7.95	309 292 - 326	- - -	- - -	- - -
	21.4%	24.9 24.8 - 24.9	24.7 24.5 - 24.9	8.1 7.8 - 8.4	7.6 7.0 - 8.0	7.99 7.96 - 8.06	7.71 7.49 - 7.91	292 276 - 304	- - -	- - -	- - -
	42.8%	24.9 24.9 - 25.0	24.7 24.5 - 25.1	8.1 7.9 - 8.4	7.5 6.9 - 8.0	7.99 7.96 - 8.06	7.69 7.50 - 7.90	258 244 - 263	- - -	- - -	- - -
	85.6%	25.0 24.9 - 25.0	24.7 24.4 - 24.8	8.2 8.0 - 8.4	7.5 7.0 - 8.0	7.98 7.92 - 8.06	7.67 7.50 - 7.88	187 182 - 190	- - -	- - -	- - -
	100%	25.0 24.9 - 25.0	24.7 24.6 - 24.8	8.2 8.0 - 8.4	7.5 6.9 - 8.1	7.97 7.93 - 8.06	7.67 7.47 - 7.88	162 157 - 170	63 61 - 65	72 64 - 76	< 0.10 < 0.10 - < 0.10
	Intake	25.0 24.9 - 25.0	24.7 24.4 - 25.0	8.2 8.0 - 8.4	7.6 6.9 - 8.1	7.97 7.93 - 8.06	7.70 7.57 - 7.87	159 154 - 166	62 61 - 63	72 68 - 80	< 0.10 < 0.10 - < 0.10
<i>Ceriodaphnia dubia</i>	Control, Non-treated	24.8 24.7 - 24.9	25.0 24.9 - 25.2	7.8 7.7 - 7.9	8.0 7.8 - 8.1	7.89 7.83 - 7.99	7.96 7.92 - 8.08	320 306 - 326	59 58 - 60	85 84 - 88	- - -
	10.7%	24.8 24.7 - 24.9	25.1 25.0 - 25.2	7.9 7.7 - 8.3	8.0 7.9 - 8.1	7.99 7.88 - 8.17	7.97 7.91 - 8.09	309 291 - 317	- - -	- - -	- - -
	21.4%	24.8 24.7 - 25.0	25.0 24.9 - 25.2	7.9 7.8 - 8.3	8.0 7.9 - 8.2	7.99 7.91 - 8.14	7.97 7.91 - 8.09	293 277 - 309	- - -	- - -	- - -
	42.8%	24.9 24.8 - 25.0	25.1 24.9 - 25.2	7.9 7.8 - 8.3	8.1 8.0 - 8.2	7.98 7.91 - 8.11	7.96 7.91 - 8.07	259 245 - 269	- - -	- - -	- - -
	85.6%	24.9 24.8 - 25.0	25.0 24.8 - 25.2	8.0 7.8 - 8.3	8.1 8.0 - 8.2	7.96 7.87 - 8.07	7.95 7.91 - 8.03	187 178 - 195	- - -	- - -	- - -
	100%	25.0 24.8 - 25.1	25.0 24.8 - 25.2	8.0 7.8 - 8.3	8.1 8.0 - 8.2	7.95 7.88 - 8.06	7.93 7.88 - 8.02	161 158 - 165	63 61 - 65	75 64 - 80	< 0.10 < 0.10 - < 0.10
	Intake	24.9 24.7 - 25.1	25.0 24.9 - 25.1	8.1 7.9 - 8.3	8.1 7.9 - 8.2	7.97 7.91 - 8.06	7.95 7.90 - 8.04	159 152 - 169	62 61 - 65	73 68 - 80	< 0.10 < 0.10 - < 0.10

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

Overall temperature (°C)	Average	Minimum	Maximum
<i>Pimephales promelas</i>	24.8	24.4	25.1
<i>Ceriodaphnia dubia</i>	25.0	24.7	25.2

SUMMARY / CONCLUSIONS

Exposures to samples collected August 04 – 09, 2019 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 minnows and daphnids to intake samples resulted in no significant difference from the controls during this study period.



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



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 IC₂₅ 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.



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).



**Sequoyah Nuclear Plant Biomonitoring
August 06 – 13, 2019**

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 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	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 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	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

**Sequoyah Nuclear Plant Biomonitoring
August 06 – 13, 2019**

Appendix C

**Chain of Custody Records and
Toxicity Test Bench Sheets**

BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA	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 CTI \$ _____
Project Name: Sequoyah NP Toxicity		Other (specify): <u>SONIC</u>
P.O. Number: N/A		General Comments:
Facility Sampled: Sequoyah NP		
NPDES Number: TN0026450		
Collected By: <u>Kelly Bonnette</u> <u>Chloe Moore</u>		

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Container Number & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)				Laboratory Use				
		Start	End			Yes	If Yes, Inches	No	Trace	ETS Log Number	Arrival Temp. (°C)	By	Time ET	Appearance
SQN-101-TOX	Comp	8-4-14 070700 ET	8-5-14 070000 ET	1(2.5gal)	1727.26	✓	0.03			190805.05	2.3°C	Y	1420	*
SQN-INT-TOX	Comp	8-4-14 070700 ET	8-5-14 070000 ET	1(2.5 gal)	1727.26	✓	0.03			190805.00	1.0°C	Y	1420	*

Sample Custody – Fill In From Top Down

* CUSTODY SEALS INTACT. SAMPLES RECEIVED

Relinquished By (Signature):	Date/Time	Received By (Signature):	IN GOOD CONDITION. Date/Time
<u>Kelly Bonnette</u> TVA	8-5-14 070705	<u>BR Skiles</u> SONIC DELIVERY	08-05-15 0705 ET
<u>BR Skiles</u> SONIC DELIVERY	08-05-15 1420	<u>ETS</u>	08-05-15 1420 ET

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them between 0°C and 6°C and shipping them in ice (samples should never be frozen). The hold time for each sample is 36 hours from the time of collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that timeframe. Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday.



Whole Effluent Sample Receipt Log

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*Sample temperature performed using Sample Receiving Thermometer: SN 160928622

Date Received	Time Received	Received by	Received from	*Sample Temp. (°C)	Project number	Sample number	Sample name and description	State	Comments
08-05-19	1030	J. Sumner	L. Keenan	0.8	14388	190805 .01	Waynesville WWTP	NC	
08-05-19	1231	K. Keenan	K. Deaver	1.5	14389	190805 .02	Marion WWTP	NC	
08-05-19	1313	J. Sumner	A. Coates	1.0/1.0	14390	190805 .03	Eastman - Blend	TN	
08-05-19	1313	J. Sumner	A. Coates	0.6/1.1	14390	190805 .04	Eastman - Riverwater	TN	
08-05-19	1420	J. Sumner	TVA Courier	2.3	14391	190805 .05	TVA - Sequoyah NP - 101	TN	
08-05-19	1420	J. Sumner	TVA Courier	1.0	14391	190805 .06	TVA - Sequoyah NP - Intake	TN	

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BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA	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 CTI \$ _____
Project Name: Sequoyah NP Toxicity		Other (specify): <u>Sonic</u>
P.O. Number: N/A		General Comments:
Facility Sampled: Sequoyah NP		
APDES Number: TN0026450		
Collected By: <u>Kelly Robinette, Obie Moore</u> <u>Kelly Robinette</u> <u>Obie Moore</u>		

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Container Number & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)				Project # 14391 Laboratory Use				
		Start	End			Yes	If Yes, Inches	No	Trace	ETS Log Number	Arrival Temp. (°C)	By	Time	Appear- ance
SQN-101-TOX	Comp	8-6-19 @ 0700 ET	8-7-19 @ 0600 ET	1(2.5gal)	1728.76			✓		190807.28	1.5°C	JL	1345	*
SQN-INT-TOX	Comp	8-6-19 @ 0700 ET	8-7-19 @ 0600 ET	1(2.5 gal)	1728.76			✓		190807.29	1.1°C	JL	1345	*

Sample Custody - Fill In From Top Down

* CUSTODY SEALS INTACT. SAMPLES RECEIVED

Relinquished By (Signature):	Date/Time	Received By (Signature):	IN GOOD CONDITION: <u>JL</u> Date/Time
<u>Kelly Robinette</u> TVA	8-7-19 @ 0900 ET	<u>BR Spive</u> SONIC DELIVERY	08-07-19 09:00 ET
<u>BR Spive</u> SONIC DELIVERY	08-07-19 13:45 ET	<u>JL</u> ETS	08-07-19 1345 ET

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them between 0°C and 6°C and shipping them in ice (samples should never be frozen). The hold time for each sample is 36 hours from the time of collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that timeframe. Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday.



Whole Effluent Sample Receipt Log

*Sample temperature performed using Sample Receiving Thermometer: SN 160928622

Date Received	Time Received	Received by	Received from	*Sample Temp. (°C)	Project number	Sample number	Sample name and description	State	Comments
08-07-19	0841	K. Keenan	Fed - Ex	1.4	14412	190807 .01	ALCOA - 005	NC	
08-07-19	0841	K. Keenan	Fed - Ex	1.4	14412	190807 .02	ALCOA - 013	NC	
08-07-19	0948	K. Keenan	Fed - Ex	2.2	14414	190807 .03	Allen SS - 006	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.6	14415	190807 .04	Apex WRF	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14416	190807 .05	Belews Creek SS - 006	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14417	190807 .06	Daikin	NC	
08-07-19	0948	K. Keenan	Fed - Ex	1.8	14418	190807 .07	Dallas WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14419	190807 .08	Durham County	NC	
08-07-19	0948	K. Keenan	Fed - Ex	5.8	14420	190807 .09	Enfield WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	1.6	14421	190807 .10	James Loughlin WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.3	14422	190807 .11	McGuire NS - 005	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14423	190807 .12	North Cary WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	5.8	14424	190807 .13	Scotland Neck WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.3	14425	190807 .14	Western Wake WRF	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.5	14426	190807 .15	Wilson WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	5.7	14427	190807 .16	Mayo Steam Electric Plant	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.9	14428	190807 .17	Washington WWTP	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.3	14429	190807 .18	Woodlake Yacht Club	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14430	190807 .19	McGuire NS - 001	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14431	190807 .20	McGuire NS - 002	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.4	14432	190807 .21	PCS Phosphate, Inc.	NC	
08-07-19	0948	K. Keenan	Fed - Ex	0.6	14433	190807 .22	Morehead City WWTP	NC	
08-07-19	1020	J. Sumner	UPS	1.3	14434	190807 .23	South Cary WWTP	NC	
08-07-19	1056	K. Keenan	M. Cihelka	0.1	14435	190807 .24	Waynesville WWTP	NC	
08-07-19	1237	J. Sumner	Dash Courier	1.2	14436	190807 .25	OWASA	NC	
08-07-19	1312	J. Sumner	A. Coates	0.4/0.6	14390	190807 .26	Eastman - Blend	TN	
08-07-19	1312	J. Sumner	A. Coates	0.9/0.8	14390	190807 .27	Eastman - Riverwater	TN	
08-07-19	1345	J. Sumner	TVA Courier	1.5	14391	190807 .28	TVA - Sequoyah Nuclear Plant - 101	TN	
08-07-19	1345	J. Sumner	TVA Courier	1.1	14391	190807 .29	TVA - Sequoyah Nuclear Plant - Intake	TN	
08-07-19	1051	K. Keenan	K. Deaver	3.1	14389	190807 .30	Marion WWTP	NC	

BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA	Environmental Testing Solution, Inc. 351 Depot Street. Asheville, NC 28801 Phone: 828-350-9364 Fax: 828-350-9368	Delivered By (Circle One):
Project Name: Sequoyah NP Toxicity		FedEx UPS Bus Client CTI \$ _____
P.O. Number: N/A		Other (specify): <u>Sonic</u>
Facility Sampled: Sequoyah NP		General Comments:
NPDES Number: TN0026450		
Collected By: <u>KELLY ROBINETTE, DEE MOORE</u> <i>Kelly Robinette</i> <i>Dee Moore</i>		

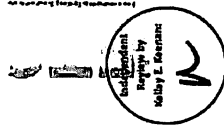
Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Container Number & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)				Laboratory Use				
		Start	End			Yes	If Yes, Inches	No	Trace	ETS Log Number	Arrival Temp. (°C)	By	Time ET	Appearance
SQN-101-TOX	Comp	8-8-19 0700 ET	8-9-19 0600 ET	21(2.5gal) KR	1733.25			✓		190809.12	1.7, 1.6°C	JL	1315	*
SQN-INT-TOX	Comp	8-8-19 0700 ET	8-9-19 0600 ET	1(2.5 gal)	1733.25			✓		190809.12	4.0°C	JL	1315	*

Sample Custody – Fill In From Top Down

* CUSTOMER SEALS INTACT. SAMPLES WERE

Relinquished By (Signature):	Date/Time	Received By (Signature):	IN GOOD CONDITION. Date/Time
<i>Kelly Robinette</i> TVA	8-9-19 0915 ET	<i>BR Shuler</i> SONIC DELIVERY	08-09-19 0915 ET
<i>BR Shuler</i> SONIC DELIVERY	08-09-19 1315 ET	<i>J</i> ETS	08-09-19 1315 ET

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them between 0°C and 6°C and shipping them in ice (samples should never be frozen). The hold time for each sample is 36 hours from the time of collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that timeframe. Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday.



Whole Effluent Sample Receipt Log

*Sample temperature performed using Sample Receiving Thermometer: SN 160928622

Date	Received Time	Received by	Received from	*Sample Temp. (°C)	Project number	Sample number	Sample name and description	State	Comments
08-09-19	0936	K. Keenan	UPS	0.8	14434	190809 .01	South Cary WWTp	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.8	14414	190809 .02	Allen SS - 006	NC	
08-09-19	0940	K. Keenan	Fed - Ex	2.3	14415	190809 .03	Apex WRF	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.9	14417	190809 .04	Dakin	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.7	14418	190809 .05	Dallas WWTp	NC	
08-09-19	0940	K. Keenan	Fed - Ex	5.8	14420	190809 .06	Enfield WWTp	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.3	14421	190809 .07	James Loughlin WWTp	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.8	14422	190809 .08	McGuire NS - 005	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.9	14423	190809 .09	North Cary WWTp	NC	
08-09-19	0940	K. Keenan	Fed - Ex	5.8	14424	190809 .10	Scotland Neck WWTp	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.6	14425	190809 .11	Western Wake WRF	NC	
08-09-19	0940	K. Keenan	Fed - Ex	0.3	14426	190809 .12	Wilson WWTp	NC	
08-09-19	0955	K. Keenan	M. Stone	1.3	14435	190809 .13	Waynesville WWTp	NC	
08-09-19	1230	K. Keenan	The Lewis Co.	3.4	14416	190809 .14	Belews Creek SS - 006	NC	
08-09-19	1240	J. Sumner	A. Coates	0.9/1	14390	190809 .15	Eastman - Blend	TN	
08-09-19	1240	J. Sumner	A. Coates	0.6/0.5	14390	190809 .16	Eastman - River Water	TN	
08-09-19	1315	J. Sumner	TVA Courier	1.7/1.6	14391	190809 .17	TVA - Sequoyah Nuclear Plant - 101	TN	
08-09-19	1315	J. Sumner	TVA Courier	4.0	14391	190809 .18	TVA - Sequoyah Nuclear Plant - Intake	TN	
08-09-19	1316	K. Keenan	K. Deaver	2.5	14389	190809 .19	Marion WWTp	NC	
08-09-19	1345	J. Sumner	Dash Courier	2.7	14436	190809 .20	OWASA	NC	

Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013 Method 1000.0)

Species: Pimephales promelas

Client: Tennessee Valley Authority

County: Hamilton

Facility: Sequoyah Nuclear Plant

Outfall: 101

NPDES #: TN0026450

Project #: 14251

Dilution preparation information:						Comments:
Dilution prep (%)	10.7	21.4	42.8	85.6	100	Each concentration was UV-treated for 2 minutes to remove pathogenic Interferences.
Effluent volume (mL)	267.5	535	1070	2140	2500	
Diluent volume (mL)	2232.5	1965	1430	360	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism information:				Test information:	
Organism source:	In-house culture			Randomizing template:	GREEN
Age:	< 24-hours old			Incubator number and shelf location:	7E
Spawn date:	07-31-19			Artemia CHM number:	CHM1048
Hatch dates and times:	08-05-19 1630 TO 08-06-19 0550			Drying information for weight determination:	
Transfer vessel information:	pH = 8.22 S.U. Temperature = 24.4 °C			Date / Time in oven:	08-13-19 0810
Average transfer volume:	< 0.25 mL			Initial oven temperature:	60 °C
				Date / Time out of oven:	08-14-19 0810
				Final oven temperature:	60 °C
				Total drying time:	24 HOURS

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	08-06-19	0600	H	1200	H	0834	H	190805.05	190805.06	08-01-19B
1	08-07-19	0600	H	1200	H	0831	H	190805.05	190805.06	08-01-19B
2	08-08-19	0630	H	1230	H	0902	H	190807.28	190807.29	08-05-19A
3	08-09-19	0600	H	1200	H	0832.1	H	190807.28	190807.29	08-05-19A
4	08-10-19	0700	H	1300	H	0930	H	190809.17	190809.18	08-07-19
5	08-11-19	0700	H	1300	H	0930	H	190809.17	190809.18	08-07-19
6	08-12-19	0600	H	1200	H	0830	H	190809.17	190809.18	08-07-19
7	08-13-19					0940	H			

Control information: UV-TREATED		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC50	> 1007.
Average weight per initial larvae:	0.737		NOEC	1007.
Average weight per surviving larvae:	0.737	≥ 0.25mg/larvae	LOEC	> 1007.
			ChV	> 1007.
			IC25	> 1007.



Species: *Pimephales promelas*Client: TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treatedDate: 08-06-19Survival 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) Tray color code: <u>magenta</u> Analyst: <u>TS</u> Date: <u>07.31.19</u>	14.59	14.39	14.17	15.56	13.11	15.76	15.32	13.57	16.13	14.55	14.14	14.79
B = Pan + Larvae weight (mg) Analyst: <u>TS</u> Date: <u>08.15.19</u>	21.93	21.94	21.19	23.12	20.13	23.15	23.40	20.84	24.30	22.67	22.71	22.33
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>JA</u>	7.34	7.55	7.02	7.56	7.02	7.39	8.08	7.27	8.17	8.12	8.57	7.54
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>JA</u>	0.734	0.755	0.702	0.756	0.702	0.739	0.808	0.727	0.817	0.812	0.857	0.754
Average weight per initial number of larvae (mg)	0.737				0.744				0.810			
Percent reduction from control (%)					-1.07				-9.97			

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:



Species: *Pimephales promelas*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treated

Date: 08-06-19

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) Tray color code: <u>magenta</u> Analyst: <u>TS</u> Date: <u>07.31.19</u>	16.09	15.67	14.26	15.37	15.33	15.47	16.00	14.57	15.87	14.70	15.83	14.01
B = Pan + Larvae weight (mg) Analyst: <u>HS</u> Date: <u>08.15.19</u>	23.33	22.83	21.82	22.86	23.40	23.03	24.20	21.67	23.29	22.03	23.74	21.22
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>HS</u>	7.24	7.16	7.56	7.49	8.07	7.56	8.20	7.10	7.42	7.33	7.31	7.21
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>HS</u>	0.724	0.716	0.756	0.749	0.807	0.756	0.820	0.710	0.742	0.733	0.731	0.721
Average weight per initial number of larvae (mg)	0.736				0.773				0.737			
Percent reduction from control (%)	0.17.				-5.07.				0.77.			

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:



Species: *Pimephales promelas*Client: TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treatedDate: 08-06-19Survival and Growth Data

Day	100% Intake				Control - Non-treated							
	Y	Z	AA	BB	CC	DD	EE	FF	GG	HH	II	JJ
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) Tray color code:: <u>magenta</u> Analyst: <u>TS</u> Date: <u>07.31.19</u>	14.48	15.32	15.59	12.98	16.09	15.43	15.98	14.66				
B = Pan + Larvae weight (mg) Analyst: <u>HA</u> Date: <u>08.15.19</u>	21.62	22.94	22.43	20.58	23.97	22.80	23.11	22.84				
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>HA</u>	7.14	7.62	6.84	7.60	7.88	7.37	7.13	8.18				
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>HA</u>	0.714	0.762	0.684	0.760	0.788	0.737	0.713	0.818				
Average weight per initial number of larvae (mg)	0.730				0.764				NA			
Percent reduction from control (%)	0.97.											

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:





Environmental Testing Solutions, Inc.

TVA / Sequoyah Nuclear Plant, Outfall 101

August 06-13, 2019

Pimephales promelas Chronic Whole Effluent Toxicity Test
EPA-821-R-02-013, Method 1000.0Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Project number: 14391

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 / Initial 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 (%)
							Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)								
Control, Non-treated	CC	10	10	16.09	23.97	7.88	0.788					0.788				
	DD	10	10	15.43	22.80	7.37	0.737					0.737				
	EE	10	10	15.98	23.11	7.13	0.713		0.764	6.2		0.713	100.0	0.764	6.2	Not applicable
	FF	10	10	14.66	22.84	8.18	0.818					0.818				
Control, UV-treated	A	10	10	14.59	21.93	7.34	0.734					0.734				
	B	10	10	14.39	21.94	7.55	0.755					0.755				
	C	10	10	14.17	21.19	7.02	0.702		0.737	3.4		0.702	100.0	0.737	3.4	Not applicable
	D	10	10	15.56	23.12	7.56	0.756					0.756				
10.7%	E	10	10	13.11	20.13	7.02	0.702					0.702				
	F	10	10	15.76	23.15	7.39	0.739					0.739				
	G	10	10	15.32	23.40	8.08	0.808		0.744	6.1		0.808	100.0	0.744	6.1	-1.0
	H	10	10	13.57	20.84	7.27	0.727					0.727				
21.4%	I	10	10	16.13	24.30	8.17	0.817					0.817				
	J	10	10	14.55	22.67	8.12	0.812					0.812				
	K	10	10	14.14	22.71	8.57	0.857		0.810	5.2		0.857	100.0	0.810	5.2	-9.9
	L	10	10	14.79	22.33	7.54	0.754					0.754				
42.8%	M	10	10	16.09	23.33	7.24	0.724					0.724				
	N	10	10	15.67	22.83	7.16	0.716					0.716				
	O	10	10	14.26	21.82	7.56	0.756		0.736	2.6		0.756	100.0	0.736	2.6	0.1
	P	10	10	15.37	22.86	7.49	0.749					0.749				
85.6%	Q	10	10	15.33	23.40	8.07	0.807					0.807				
	R	10	10	15.47	23.03	7.56	0.756					0.756				
	S	10	10	16.00	24.20	8.20	0.820		0.773	6.5		0.820	100.0	0.773	6.5	-5.0
	T	10	10	14.57	21.67	7.10	0.710					0.710				
100%	U	10	10	15.87	23.39	7.42	0.742					0.742				
	V	10	10	14.70	22.03	7.33	0.733					0.733				
	W	10	10	15.83	23.14	7.31	0.731		0.732	1.2		0.731	100.0	0.732	1.2	0.7
	X	10	10	14.01	21.22	7.21	0.721					0.721				
100% Intake	Y	10	10	14.48	21.62	7.14	0.714					0.714				
	Z	10	10	15.32	22.94	7.62	0.762					0.762				
	AA	10	10	15.59	22.43	6.84	0.684		0.730	5.2		0.684	100.0	0.730	5.2	0.9
	BB	10	10	12.98	20.58	7.60	0.760					0.760				

Outfall 101:

Dunnett's MSD value: 0.0602
PMSD: 8.2MSD =
PMSD =

Minimum Significant Difference

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.

Intake:

Dunnett's MSD value: 0.0442
PMSD: 6.0

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



TVA / Sequoyah Nuclear Plant, Outfall 101
August 06-13, 2019

Statistical Analyses

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 8/6/2019	Test ID: PpFRCR	Sample ID: TVA / SQN Outfall 101
End Date: 8/13/2019	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report
Sample Date: August 2019	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas
Comments: UV-treated		

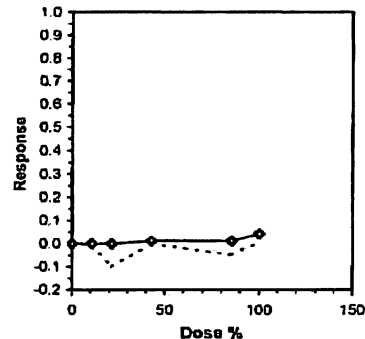
Conc-%	1	2	3	4
Non-Control	0.7880	0.7370	0.7130	0.8180
UV-Control	0.7340	0.7550	0.7020	0.7560
10.7	0.7020	0.7390	0.8080	0.7270
21.4	0.8170	0.8120	0.8570	0.7540
42.8	0.7240	0.7160	0.7560	0.7490
85.6	0.8070	0.7560	0.8200	0.7100
100	0.7420	0.7330	0.7310	0.7210
Intake	0.7140	0.7620	0.6840	0.7600

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Non-Control	0.7640	1.0370	0.7640	0.7130	0.8180	6.242	4				0.7636	1.0000
UV-Control	0.7368	1.0000	0.7368	0.7020	0.7560	3.433	4	*			0.7636	1.0000
10.7	0.7440	1.0098	0.7440	0.7020	0.8080	6.097	4	-0.290	2.410	0.0602	0.7636	1.0000
21.4	0.8100	1.0994	0.8100	0.7540	0.8570	5.237	4	-2.933	2.410	0.0602	0.7636	1.0000
42.8	0.7363	0.9993	0.7363	0.7160	0.7560	2.616	4	0.020	2.410	0.0602	0.7548	0.9884
85.6	0.7733	1.0495	0.7733	0.7100	0.8200	6.519	4	-1.462	2.410	0.0602	0.7548	0.9884
100	0.7318	0.9932	0.7318	0.7210	0.7420	1.178	4	0.200	2.410	0.0602	0.7318	0.9583
Intake	0.7300	0.9908	0.7300	0.6840	0.7620	5.184	4					

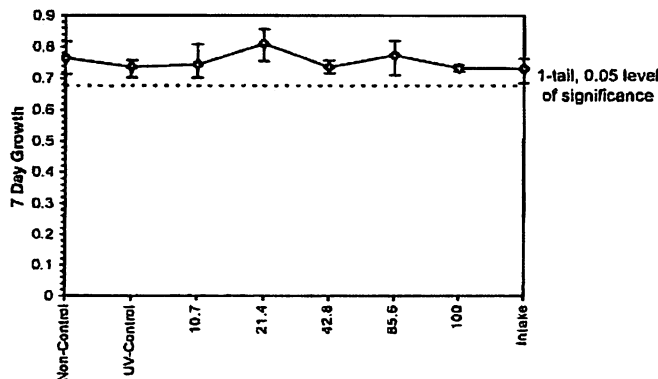
Auxiliary Tests	Statistic	Critical	Skow	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98227	0.884	-0.0549	0.05938						
Bartlett's Test indicates equal variances (p = 0.14)	8.29262	15.0863								
The control means are not significantly different (p = 0.35)	1.00968	2.44691								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.06018	0.08169	0.00376	0.00125	0.03768	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



Entered and
Reviewed by
[Signature]



TVA / Sequoyah Nuclear Plant, Outfall 101 - Intake
August 06-13, 2019

Statistical Analyses

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

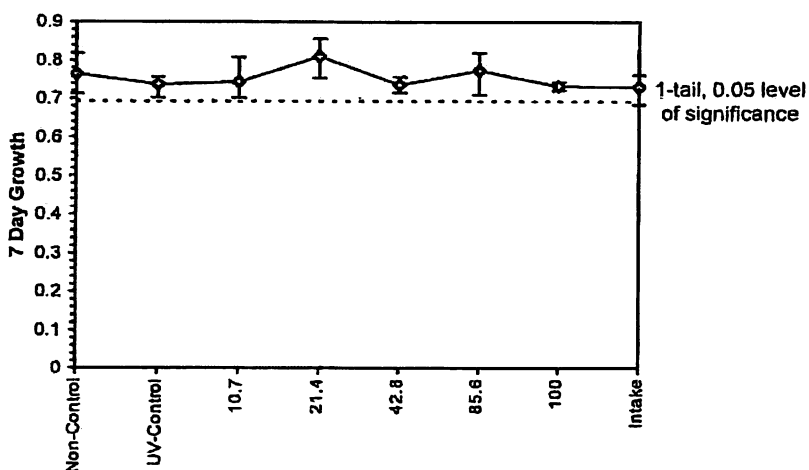
Start Date: 8/6/2019	Test ID: PpFRCR	Sample ID: TVA / SQN Outfall 101, Intake
End Date: 8/13/2019	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report
Sample Date: August 2019	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas
Comments: UV-treated		

Conc-%	1	2	3	4
Non-Control	0.7880	0.7370	0.7130	0.8180
UV-Control	0.7340	0.7550	0.7020	0.7560
10.7	0.7020	0.7390	0.8080	0.7270
21.4	0.8170	0.8120	0.8570	0.7540
42.8	0.7240	0.7160	0.7560	0.7490
85.6	0.8070	0.7560	0.8200	0.7100
100	0.7420	0.7330	0.7310	0.7210
Intake	0.7140	0.7620	0.6840	0.7600

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed	
			Mean	Min	Max	CV%			Critical	MSD
Non-Control	0.7640	1.0370	0.7640	0.7130	0.8180	6.242	4			
UV-Control	0.7368	1.0000	0.7368	0.7020	0.7560	3.433	4	*		
10.7	0.7440	1.0098	0.7440	0.7020	0.8080	6.097	4			
21.4	0.8100	1.0994	0.8100	0.7540	0.8570	5.237	4			
42.8	0.7363	0.9993	0.7363	0.7160	0.7560	2.616	4			
85.6	0.7733	1.0495	0.7733	0.7100	0.8200	6.519	4			
100	0.7318	0.9932	0.7318	0.7210	0.7420	1.178	4			
Intake	0.7300	0.9908	0.7300	0.6840	0.7620	5.184	4	0.297	1.943	0.0442

Auxiliary Tests	Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90546	0.749	-0.5072	-1.3732		
F-Test indicates equal variances (p = 0.53)	2.23896	47.4683				
The control means are not significantly different (p = 0.35)	1.00968	2.44691				
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs UV-Control	0.04422	0.06002	9.1E-05	0.00104	0.77677	1, 6

Dose-Response Plot



Reviewed and
Reviewed by
Jim Sumner
JS



Species: *Pimephales promelas*

Date: 08-06-19

Client: TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treated

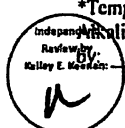
Daily Chemistry:

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		IL	MS	MS	MS	MS	IL
Concentration	Parameter						
CONTROL UV-treated	pH (S.U.)	8.05	7.97	8.06	7.72	7.96	7.72
	DO (mg/L)	8.1	8.0	8.0	7.7	8.1	7.4
	Conductivity (µmhos/cm)	307		318		328	
	*Alkalinity (mg CaCO ₃ /L)	58				58	
	*Hardness (mg CaCO ₃ /L)	84				86	
	*Temperature (°C)	24.9	24.6	24.8	24.6	24.8	24.6
10.7%	pH (S.U.)	8.00	7.95	8.05	7.74	7.97	7.68
	DO (mg/L)	8.1	8.0	8.0	7.7	8.2	7.3
	Conductivity (µmhos/cm)	292		304		314	
	*Temperature (°C)	24.9	24.5	24.8	24.5	24.9	24.6
21.4%	pH (S.U.)	8.04	7.91	8.05	7.72	7.97	7.66
	DO (mg/L)	8.1	8.0	8.1	7.7	8.2	7.2
	Conductivity (µmhos/cm)	274		291		295	
	*Temperature (°C)	24.9	24.6	24.8	24.5	24.9	24.7
42.8%	pH (S.U.)	8.06	7.89	8.04	7.68	7.97	7.65
	DO (mg/L)	8.2	8.0	8.1	7.8	8.2	7.2
	Conductivity (µmhos/cm)	244		262		259	
	*Temperature (°C)	25.0	24.6	24.9	24.5	25.0	24.6
85.6%	pH (S.U.)	8.06	7.87	8.03	7.68	7.94	7.59
	DO (mg/L)	8.2	8.0	8.1	7.8	8.2	7.1
	Conductivity (µmhos/cm)	182		189		186	
	*Temperature (°C)	25.0	24.6	24.1	24.7	25.0	24.7
100%	pH (S.U.)	8.06	7.88	8.02	7.68	7.94	7.57
	DO (mg/L)	8.2	8.1	8.2	7.8	8.2	7.3
	Conductivity (µmhos/cm)	162		155 (70)		161	
	*Alkalinity (mg CaCO ₃ /L)	63				65	
	*Hardness (mg CaCO ₃ /L)	76				64	
	*TR chlorine (mg/L)	<0.10				<0.10	
	*Temperature (°C)	25.0	24.6	24.9	24.7	25.0	24.7
		Initial	Final	Initial	Final	Initial	Final
100% Intake	pH (S.U.)	8.06	7.86	8.00	7.71	7.94	7.58
	DO (mg/L)	8.3	8.1	8.2	7.9	8.2	7.2
	Conductivity (µmhos/cm)	157		166		158	
	*Alkalinity (mg CaCO ₃ /L)	63				61	
	*Hardness (mg CaCO ₃ /L)	80				68	
	*TR chlorine (mg/L)	<0.10				<0.10	
	*Temperature (°C)	25.0	24.4	25.0	24.6	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final

*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 total residual chlorine performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

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

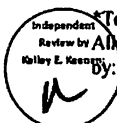


Species: *Pimephales promelas*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treated

Date: 08-06-19

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		N	N	N	BSL	BSL	MS	MS	MS
Concentration	Parameter								
CONTROL UV-treated	pH (S.U.)	7.96	7.53	7.93	7.63	7.96	7.79	7.95	7.90
	DO (mg/L)	7.9	7.0	7.9	7.8	7.9 (6.9)	8.0	8.0	7.8
	Conductivity (µmhos/cm)	326		320		330		340	
	*Alkalinity (mg CaCO ₃ /L)			61					
	*Hardness (mg CaCO ₃ /L)			84					
	*Temperature (°C)	24.7	24.6	24.8	24.8	24.8	25.1	24.8	24.7
10.7%	pH (S.U.)	7.97	7.52	7.95	7.56	7.97	7.73	7.76	7.89
	DO (mg/L)	7.8	7.0	7.9	7.5	8.4	7.8	8.1	7.8
	Conductivity (µmhos/cm)	308		309		313		326	
	*Temperature (°C)	24.8	24.5	24.8	24.9	24.9	24.9	24.9	24.9
21.4%	pH (S.U.)	7.98	7.49	7.96	7.56	7.97	7.71	7.76	7.90
	DO (mg/L)	7.8	7.0	7.9	7.5	8.4	7.8	8.1	7.8
	Conductivity (µmhos/cm)	293		291		294		304	
	*Temperature (°C)	24.8	24.5	24.9	24.7	24.9	24.9	24.9	24.8
42.8%	pH (S.U.)	7.98	7.50	7.96	7.56	7.98	7.67	7.76	7.90
	DO (mg/L)	7.9	6.9	7.9	7.5	8.4	7.6	8.2	7.8
	Conductivity (µmhos/cm)	260		250		259		263	
	*Temperature (°C)	24.9	24.7	24.9	24.7	24.9	25.1	24.9	25.0
85.6%	pH (S.U.)	7.98	7.50	7.96	7.53	7.97	7.63	7.92	7.88
	DO (mg/L)	8.0	7.0	8.0	7.4	8.4	7.6	8.2	7.8
	Conductivity (µmhos/cm)	189		184		188		190	
	*Temperature (°C)	24.9	24.4	25.0	24.7	24.9	24.8	25.0	24.7
100%	pH (S.U.)	7.97	7.47	7.94	7.52	7.95	7.69	7.93	7.86
	DO (mg/L)	8.0	6.9	8.0	7.3	8.4	7.6	8.2	7.8
	Conductivity (µmhos/cm)	143		157		159		161	
	*Alkalinity (mg CaCO ₃ /L)			61					
	*Hardness (mg CaCO ₃ /L)			76					
	*TR chlorine (mg/L)			<0.10					
	*Temperature (°C)	25.0	24.7	25.0	24.8	24.9	24.8	25.0	24.7
100% Intake	pH (S.U.)	7.98	7.57	7.94	7.58	7.96	7.71	7.98	7.87
	DO (mg/L)	8.0	6.9	8.0	7.8	8.4	7.6	8.3	7.8
	Conductivity (µmhos/cm)	157		154		166		158	
	*Alkalinity (mg CaCO ₃ /L)			61					
	*Hardness (mg CaCO ₃ /L)			68					
	*TR chlorine (mg/L)			<0.10					
	*Temperature (°C)	25.0	24.8	25.0	24.6	24.9	25.0	25.0	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final



*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 total residual chlorine performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.
 Total residual chlorine was performed on non-treated Outfall 101 and Intake samples.


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

Date: 08-06-19

Daily Chemistry:

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
Analyst		N	MS	MS	MS	MS	N
Concentration	Parameter						
Control Non-treated	pH (S.U.)	7.99	7.95	7.85	7.76	7.83	7.70
	DO (mg/L)	7.7	7.8	7.8	7.7	7.7	7.7
	Conductivity (µmhos/cm)	300		317		319	
	*Alkalinity (mg CaCO ₃ /L)	59				60	
	*Hardness (mg CaCO ₃ /L)	84				88	
	*Temperature (°C)	24.8	24.7	24.9	24.6	24.7	24.6
		Initial	Final	Initial	Final	Initial	Final

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Analyst		N	N	N	BSU	BSU	MS	MS	MS
Concentration	Parameter								
Control Non-treated	pH (S.U.)	7.88	7.62	7.90	7.68	7.87	7.99	7.89	7.89
	DO (mg/L)	7.8	7.6	7.7	7.9	7.9	7.5	7.9	7.8
	Conductivity (µmhos/cm)	324		320		325		322	
	*Alkalinity (mg CaCO ₃ /L)			58					
	*Hardness (mg CaCO ₃ /L)			84					
	*Temperature (°C)	24.7	24.7	24.7	24.9	24.9	24.8	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

*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 total residual chlorine performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet by: 





TVA / Sequoyah Nuclear Plant, Outfall 101, UV-treated

August 06-13, 2019

Pimephales promelas Chronic Whole Effluent Toxicity Test

EPA-821-R-02-013, Method 1000.0

Daily Chemical Analyses

Environmental Testing Solutions, Inc.

Project number:

14391

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)	7.99	7.95	7.85	7.76	7.83	7.70	7.88	7.62	7.90	7.68	7.87	7.79	7.89	7.89
	DO (mg/L)	7.7	7.8	7.8	7.7	7.7	7.7	7.8	7.6	7.7	7.9	7.9	7.5	7.9	7.8
	Conductivity (µmhos/cm)	306		317		319		324		326		325		322	
	Alkalinity (mg/L CaCO ₃)	59				60				58					
	Hardness (mg/L CaCO ₃)	84				88				84					
	Temperature (°C)	24.8	24.7	24.9	24.6	24.7	24.6	24.7	24.7	24.7	24.9	24.9	24.8	25.0	24.9
Control, UV-treated	pH (SU)	8.05	7.97	8.06	7.72	7.96	7.72	7.96	7.53	7.93	7.63	7.96	7.79	7.95	7.90
	DO (mg/L)	8.1	8.0	8.0	7.7	8.1	7.4	7.9	7.0	7.9	7.8	8.4	8.0	8.0	7.8
	Conductivity (µmhos/cm)	307		318		328		326		320		330		340	
	Alkalinity (mg/L CaCO ₃)	58				58				61					
	Hardness (mg/L CaCO ₃)	84				86				84					
	Temperature (°C)	24.9	24.6	24.8	24.6	24.8	24.6	24.7	24.6	24.8	24.8	24.8	25.1	24.8	24.7
10.7%	pH (SU)	8.06	7.95	8.05	7.74	7.97	7.68	7.97	7.52	7.95	7.56	7.97	7.73	7.96	7.89
	DO (mg/L)	8.1	8.0	8.0	7.7	8.2	7.3	7.8	7.0	7.9	7.5	8.4	7.8	8.1	7.8
	Conductivity (µmhos/cm)	292		304		314		308		309		313		326	
	Temperature (°C)	24.9	24.5	24.8	24.5	24.9	24.6	24.8	24.5	24.8	24.9	24.9	24.9	24.9	24.9
21.4%	pH (SU)	8.06	7.91	8.05	7.72	7.97	7.66	7.98	7.49	7.96	7.56	7.97	7.71	7.96	7.90
	DO (mg/L)	8.1	8.0	8.1	7.7	8.2	7.2	7.8	7.0	7.9	7.5	8.4	7.8	8.1	7.8
	Conductivity (µmhos/cm)	276		291		295		293		291		294		304	
	Temperature (°C)	24.9	24.6	24.8	24.5	24.9	24.7	24.8	24.5	24.9	24.7	24.9	24.9	24.9	24.8
42.8%	pH (SU)	8.06	7.89	8.04	7.68	7.97	7.65	7.98	7.50	7.96	7.56	7.98	7.67	7.96	7.90
	DO (mg/L)	8.2	8.0	8.1	7.8	8.2	7.2	7.9	6.9	7.9	7.5	8.4	7.6	8.2	7.8
	Conductivity (µmhos/cm)	244		262		259		260		256		259		263	
	Temperature (°C)	25.0	24.6	24.9	24.5	25.0	24.6	24.9	24.7	24.9	24.7	24.9	25.1	24.9	25.0
85.6%	pH (SU)	8.06	7.87	8.03	7.68	7.94	7.59	7.98	7.50	7.96	7.53	7.97	7.63	7.92	7.88
	DO (mg/L)	8.2	8.0	8.1	7.8	8.2	7.1	8.0	7.0	8.0	7.4	8.4	7.6	8.2	7.8
	Conductivity (µmhos/cm)	182		189		186		189		184		188		190	
	Temperature (°C)	25.0	24.6	24.9	24.7	25.0	24.7	24.9	24.4	25.0	24.7	24.9	24.8	25.0	24.7
100%	pH (SU)	8.06	7.88	8.02	7.68	7.94	7.57	7.97	7.47	7.94	7.52	7.95	7.69	7.93	7.86
	DO (mg/L)	8.2	8.1	8.2	7.8	8.2	7.3	8.0	6.9	8.0	7.3	8.4	7.6	8.2	7.8
	Conductivity (µmhos/cm)	162		170		161		163		157		159		161	
	Alkalinity (mg/L CaCO ₃)	63				65				61					
	Hardness (mg/L CaCO ₃)	76				64				76					
	*Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.0	24.6	24.9	24.7	25.0	24.7	25.0	24.7	25.0	24.8	24.9	24.8	25.0	24.7
100% Intake	pH (SU)	8.06	7.86	8.00	7.71	7.94	7.58	7.98	7.57	7.94	7.58	7.96	7.71	7.93	7.87
	DO (mg/L)	8.3	8.1	8.2	7.9	8.2	7.2	8.0	6.9	8.0	7.8	8.4	7.6	8.3	7.8
	Conductivity (µmhos/cm)	157		166		158		157		154		166		158	
	Alkalinity (mg/L CaCO ₃)	63				61				61					
	Hardness (mg/L CaCO ₃)	80				68				68					
	*Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.0	24.4	25.0	24.6	25.0	24.8	25.0	24.8	25.0	24.6	24.9	25.0	25.0	24.7

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

File: sqn101_080619chem

Entered by: T. Sleeper

Reviewed by:

Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013 Method 1002.0)Species: Ceriodaphnia dubiaClient: Tennessee Valley AuthorityCounty: HamiltonFacility: Sequoyah Nuclear PlantOutfall: 101NPDES #: TN0026450Project #: 14391

Dilution preparation information:						Comments:
Dilution prep (%)	10.7	21.4	42.8	85.6	100	
Effluent volume (mL)	267.5	535	1070	2140	2500	
Diluent volume (mL)	2232.5	1965	1430	360	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism source information:												Test information:		
Organism age:				< 24-hours old								Randomizing template color:		Bwe
Date and times organisms were born between:				08-06-19 0615 TO 0900								Incubator number and shelf location:		2C1
Culture board:		08-30-19 B										YWT batch:		08-08-19
Replicate number:		1	2	3	4	5	6	7	8	9	10			
Culture board cup number:		6	7	8	11	12	14	18	19	20	24	Selenastrum batch:		08-08-19
Transfer vessel information:		pH = 7.98 S.U. Temperature = 75.1 °C												
Average transfer volume (mL):		< 0.25 mL												

Daily renewal information:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	MHSW batch used	Sample numbers used		Analyst
				Outfall 101	Intake	
0	08-06-19	0932	08-01-19B	190805.05	190805.06	J
1	08-07-19	0925	08-01-19B	190805.05	190805.06	J
2	08-08-19	1007	08-05-19A	190807.28	190807.29	J
3	08-09-19	0928	08-05-19A	190807.28	190807.29	J
4	08-10-19	1020	08-07-19	190809.17	190809.18	J
5	08-11-19	1017	08-07-19	190809.17	190809.18	J
6	08-12-19	0923	08-07-19	190809.17	190809.18	J
7	08-13-19	0913				J

Control information:				Summary of test endpoints:	
	Control-1	Control-2	Acceptance criteria		
% of Male Adults:	07.	07.	≤ 20%	7-day LC ₅₀	> 1007.
% Adults having 3 rd Broods:	1007.	1007.	≥ 60% surviving adults	NOEC	1007.
% Mortality:	07.	07.	≤ 20%	LOEC	> 1007.
Mean Offspring/Female:	30.8	30.8	≥ 15.0 offspring/female	ChV	> 1007.
% CV:	5.37.	6.57.	< 42.0 %	IC ₂₅	> 1007.



Species: Ceriodaphnia dubiaClient: TVA / Sequoyah Nuclear Plant, Outfall 101Date: 08-06-19**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	5	5	4	5	4	4	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	11	13	10	13	11	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	13	14	17	14	16	15	17	15	16	16
Total young produced		27	30	33	31	31	32	32	31	30	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd 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:

07.

Mean Offspring/Female:

30.6

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	3	5	4	4	5	4	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	10	13	11	12	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	14	14	16	17	16	15	18	16	15
Total young produced		33	29	28	33	33	32	32	33	32	30
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:

07.

Mean Offspring/Female:

31.5

% Reduction from Control-1:

-2.37.



Species: *Ceriodaphnia dubia*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 08-06-19

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	4	4	5	4	6	4	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	13	12	10	10	10	12	11	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	14	16	13	18	19	15	17	17	18
Total young produced		35	29	34	29	34	33	29	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:	07.
Mean Offspring/Female:	32.2
% Reduction from Control-1:	-4.57.

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	5	4	4	4	5	4	5	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	13	12	13	10	11	11	11	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	15	19	19	20	17	20	18	18	17	19
Total young produced		30	34	36	36	34	35	33	34	33	37
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:	07.
Mean Offspring/Female:	34.2
% Reduction from Control-1:	-11.07.



Species: Ceriodaphnia dubiaClient: TVA / Sequoyah Nuclear Plant, Outfall 101Date: 08-06-19CONC: 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	6	6	4	5	4	4	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	14	12	10	13	10	10	11	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	19	18	18	19	21	19	19	20	22	18
Total young produced		36	38	34	34	38	33	33	36	39	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:	07.
Mean Offspring/Female:	35.4
% Reduction from Control-1:	-14.97.

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	7	5	4	6	6	4	5	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	11	14	10	12	12	11	12	13
	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	21	18	22	17	19	19	19	21	20	22
Total young produced		39	36	38	35	35	37	35	37	38	41
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:	07.
Mean Offspring/Female:	37.1
% Reduction from Control-1:	-20.57.



Species: Ceriodaphnia dubia

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 08-06-19

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	3	4	5	4	5	5	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	14	11	11	10	10	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	16	16	17	12	15	16	14	16	15	17
Total young produced		31	34	33	27	30	31	30	32	29	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd 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: 07.

Mean Offspring/Female: 30.8

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	5	5	7	5	5	5	5	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	14	12	12	13	10	13	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	16	16	16	13	14	16	13	16	14	16
Total young produced		34	35	33	32	32	33	31	33	32	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: 07.

Mean Offspring/Female: 32.7

% Reduction from Control-2: -6.22.





TVA / Sequoyah Nuclear Plant, Outfall 101
August 06-13, 2019



Verification of *Ceriodaphnia* Reproduction Totals

Control-1

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	5	4	5	4	4	4	4	5	44
5	10	11	11	13	10	13	11	12	10	10	111
6	0	0	0	0	0	0	0	0	0	0	0
7	13	14	17	14	16	15	17	15	16	16	153
Total	27	30	33	31	31	32	32	31	30	31	308

85.6%

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	6	6	4	5	4	4	4	5	5	5	48
5	11	14	12	10	13	10	10	11	12	10	113
6	0	0	0	0	0	0	0	0	0	0	0
7	19	18	18	19	21	19	19	20	22	18	193
Total	36	38	34	34	38	33	33	36	39	33	354

10.7%

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	4	5	4	5	5	5	4	44
5	13	10	10	13	11	12	12	10	11	11	113
6	0	0	0	0	0	0	0	0	0	0	0
7	17	14	14	16	17	16	15	18	16	15	158
Total	33	29	28	33	33	32	32	33	32	30	315

100%

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	7	5	4	6	6	4	5	6	6	54
5	13	11	11	14	10	12	12	11	12	13	119
6	0	0	0	0	0	0	0	0	0	0	0
7	21	18	22	17	19	19	19	21	20	22	198
Total	39	36	38	35	35	37	35	37	38	41	371

21.4%

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	4	5	4	6	4	4	4	4	4	43
5	13	11	13	12	10	10	10	12	11	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	18	14	16	13	18	19	15	17	17	18	165
Total	35	29	34	29	34	33	29	33	32	34	322

Control-2

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	4	5	4	5	5	4	4	4	4	42
5	12	14	11	11	10	10	12	12	10	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	17	12	15	16	14	16	15	17	154
Total	31	34	33	27	30	31	30	32	29	31	308

42.8%

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	5	4	4	4	5	4	5	5	6	47
5	10	10	13	12	13	10	11	11	11	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	19	19	20	17	20	18	18	17	19	182
Total	30	34	36	36	34	35	33	34	33	37	342

100% Intake

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	6	5	5	7	5	5	5	5	6	4	53
5	12	14	12	12	13	10	13	12	12	12	122
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	16	13	14	18	13	16	14	16	152
Total	34	35	33	32	32	33	31	33	32	32	327

Reviewed and
Approved by
the Engineer



Environmental Testing Solutions, Inc.

TVA / Sequoyah Nuclear Plant, Outfall 101

August 06-13, 2019

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: 14391

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	27	30	33	31	31	32	32	31	30	31	100	30.8	5.3	Not applicable
10.7%	33	29	28	33	33	32	32	33	32	30	100	31.5	5.8	-2.3
21.4%	35	29	34	29	34	33	29	33	32	34	100	32.2	7.3	-4.5
42.8%	30	34	36	36	34	35	33	34	33	37	100	34.2	5.8	-11.0
85.6%	36	38	34	34	38	33	33	36	39	33	100	35.4	6.6	-14.9
100%	39	36	38	35	35	37	35	37	38	41	100	37.1	5.3	-20.5
Control - 2	31	34	33	27	30	31	30	32	29	31	100	30.8	6.5	Not applicable
100% Intake	34	35	33	32	32	33	31	33	32	32	100	32.7	3.5	-6.2

Outfall 101:

Dunnett's MSD value: 2.076

PMSD: 6.7

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.

Intake:

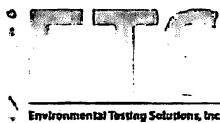
Dunnett's MSD value: 1.262

PMSD: 4.1

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).

TVA / Sequoyah Nuclear Plant, Outfall 101 August 06-13, 2019



Statistical Analyses

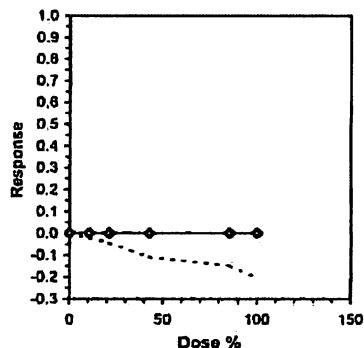
Ceriodaphnia Survival and Reproduction Test-Reproduction			
Start Date: 8/6/2019	Test ID: CdFRCR	Sample ID: TVA / SQN Outfall 101	
End Date: 8/13/2019	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date: August 2019	Protocol: FWCHR-EPA-821-R-02-013	Test Species: CD-Ceriodaphnia dubia	
Comments: Non-treated			

Conc-%	1	2	3	4	5	6	7	8	9	10
Control-1	27.000	30.000	33.000	31.000	31.000	32.000	32.000	31.000	30.000	31.000
Control-2	31.000	34.000	33.000	27.000	30.000	31.000	30.000	32.000	29.000	31.000
10.7	33.000	29.000	28.000	33.000	33.000	32.000	32.000	33.000	32.000	30.000
21.4	35.000	29.000	34.000	29.000	34.000	33.000	29.000	33.000	32.000	34.000
42.8	30.000	34.000	36.000	36.000	34.000	35.000	33.000	34.000	33.000	37.000
85.6	36.000	38.000	34.000	34.000	38.000	33.000	33.000	36.000	39.000	33.000
100	39.000	36.000	38.000	35.000	35.000	37.000	35.000	37.000	38.000	41.000
Intake	34.000	35.000	33.000	32.000	32.000	33.000	31.000	33.000	32.000	32.000

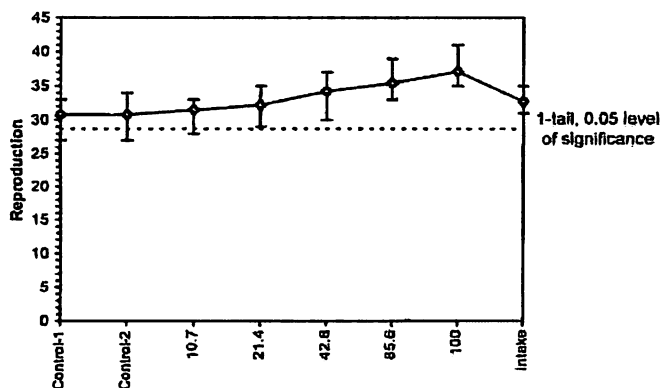
Transform: Untransformed										
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	Isotonic Mean
Control-1	30.800	1.0000	30.800	27.000	33.000	5.258	10			33.533
Control-2	30.800	1.0000	30.800	27.000	34.000	6.457	10			1.0000
10.7	31.500	1.0227	31.500	28.000	33.000	5.844	10	-0.771	2.287	33.533
21.4	32.200	1.0455	32.200	29.000	35.000	7.291	10	-1.542	2.287	33.533
42.8	34.200	1.1104	34.200	30.000	37.000	5.815	10	-3.745	2.287	33.533
85.6	35.400	1.1494	35.400	33.000	39.000	6.551	10	-5.066	2.287	33.533
100	37.100	1.2045	37.100	35.000	41.000	5.308	10	-6.938	2.287	33.533
Intake	32.700	1.0617	32.700	31.000	35.000	3.546	10			1.0000

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ($p > 0.01$)				0.85514	1.035	-0.2805	-0.6141
Bartlett's Test indicates equal variances ($p = 0.89$)				1.67718	15.0883		
The control means are not significantly different ($p = 1.00$)				0	2.10092		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnett's Test				100	>100		1
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			



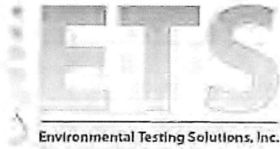
Dose-Response Plot



Reviewed and
Initialed by
JL



TVA / Sequoyah Nuclear Plant, Outfall 101 - Intake
August 06-13, 2019



Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

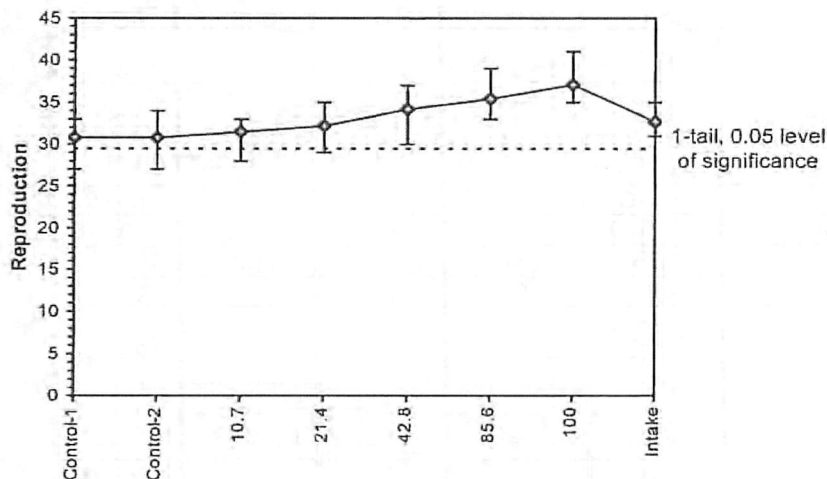
Start Date: 8/6/2019	Test ID: CdFRCR	Sample ID: TVA / SQN Outfall 101 - Intake
End Date: 8/13/2019	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report
Sample Date: August 2019	Protocol: FWCHR-EPA-821-R-02-013	Test Species: CD-Ceriodaphnia dubia
Comments: Non-treated		

Conc-%	1	2	3	4	5	6	7	8	9	10
Control-1	27.000	30.000	33.000	31.000	31.000	32.000	32.000	31.000	30.000	31.000
Control-2	31.000	34.000	33.000	27.000	30.000	31.000	30.000	32.000	29.000	31.000
10.7	33.000	29.000	28.000	33.000	33.000	32.000	32.000	33.000	32.000	30.000
21.4	35.000	29.000	34.000	29.000	34.000	33.000	29.000	33.000	32.000	34.000
42.8	30.000	34.000	36.000	29.000	34.000	35.000	33.000	34.000	33.000	37.000
85.6	36.000	38.000	34.000	34.000	38.000	33.000	33.000	36.000	39.000	33.000
100	39.000	36.000	38.000	35.000	35.000	37.000	35.000	37.000	38.000	41.000
Intake	34.000	35.000	33.000	32.000	32.000	33.000	31.000	33.000	32.000	32.000

Conc-%	Transform: Untransformed						N	1-Tailed		
	Mean	N-Mean	Mean	Min	Max	CV%		t-Stat	Critical	MSD
Control-1	30.800	1.0000	30.800	27.000	33.000	5.258	10			
Control-2	30.800	1.0000	30.800	27.000	34.000	6.457	10			
10.7	31.500	1.0227	31.500	28.000	33.000	5.844	10			
21.4	32.200	1.0455	32.200	29.000	35.000	7.291	10			
42.8	34.200	1.1104	34.200	30.000	37.000	5.815	10			
85.6	35.400	1.1494	35.400	33.000	39.000	6.551	10			
100	37.100	1.2045	37.100	35.000	41.000	5.308	10			
Intake	32.700	1.0617	32.700	31.000	35.000	3.546	10	-2.610	1.734	1.262

Auxiliary Tests	Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95504	0.868	-0.1235	0.93503		
F-Test indicates equal variances ($p = 0.12$)	2.94215	6.54109				
The control means are not significantly different ($p = 1.00$)	0	2.10092				
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	1.26242	0.04099	18.05	2.65	0.01773	1, 18
Treatments vs Control-2						

Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS



Species: Ceriodaphnia dubia

Date: 08-06-19

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Daily Chemistry:

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		N	MS	MC	MS	MC	N
Concentration	Parameter						
CONTROL	pH (S.U.)	7.99	8.08	7.85	7.92	7.83	7.92
	DO (mg/L)	7.7	7.9	7.8	7.9	7.7	7.8
	Conductivity (µmhos/cm)	306		317		319	
	*Alkalinity (mg CaCO ₃ /L)	59		64		60	
	*Hardness (mg CaCO ₃ /L)	84		88		88	
	*Temperature (°C)	24.8	25.2	24.7	25.2	24.8	24.9
10.7%	pH (S.U.)	8.10	8.09	8.17	7.93	7.95	7.91
	DO (mg/L)	8.0	8.0	7.9	7.9	7.8	7.9
	Conductivity (µmhos/cm)	291		310		317	
	*Temperature (°C)	24.8	25.0	24.8	25.2	24.8	25.1
21.4%	pH (S.U.)	8.07	8.09	8.14	7.93	7.95	7.91
	DO (mg/L)	8.0	8.0	7.9	7.9	7.8	7.9
	Conductivity (µmhos/cm)	277		294		298	
	*Temperature (°C)	24.8	25.0	24.8	25.2	24.9	25.0
42.8%	pH (S.U.)	8.06	8.07	8.11	7.94	7.95	7.91
	DO (mg/L)	8.0	8.1	7.9	8.0	7.8	8.0
	Conductivity (µmhos/cm)	245		263		266	
	*Temperature (°C)	24.9	25.0	24.9	25.2	24.9	25.2
85.6%	pH (S.U.)	8.05	8.03	8.07	7.94	7.93	7.91
	DO (mg/L)	8.1	8.1	7.9	8.0	7.8	8.0
	Conductivity (µmhos/cm)	178		193		190	
	*Temperature (°C)	24.9	24.8	25.0	24.9	25.0	25.2
100%	pH (S.U.)	8.05	8.02	8.06	7.94	7.94	7.90
	DO (mg/L)	8.1	8.2	8.0	8.2	7.8	8.0
	Conductivity (µmhos/cm)	159		165		160	
	*Alkalinity (mg CaCO ₃ /L)	65				63	
	*Hardness (mg CaCO ₃ /L)	84 80				64	
	*TR chlorine (mg/L)	<0.10				<0.10	
	*Temperature (°C)	25.0	25.0	25.0	25.1	25.0	25.2
100% Intake	pH (S.U.)	8.05	8.04	8.06	7.95	7.94	7.90
	DO (mg/L)	8.2	8.2	8.1	8.2	7.9	7.9
	Conductivity (µmhos/cm)	155		159		152	
	*Alkalinity (mg CaCO ₃ /L)	65				61	
	*Hardness (mg CaCO ₃ /L)	80				72	
	*TR chlorine (mg/L)	<0.10				<0.10	
	*Temperature (°C)	24.8	24.9	25.0	24.9	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final

*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 total residual chlorine performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet by:



Species: *Ceriodaphnia dubia*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 08-06-19

Concentration		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Analyst		N	N	N	BSC	BSC	MS	MS	MS
Concentration	Parameter								
CONTROL	pH (S.U.)	7.03	7.97	7.90	7.97	7.87	7.93	7.89	7.76
	DO (mg/L)	7.0	8.0	7.7	8.1	8.0	8.0	7.9	8.1
	Conductivity (µmhos/cm)	324		326		325		322	
	*Alkalinity (mg CaCO ₃ /L)			58					
	*Hardness (mg CaCO ₃ /L)			84					
	*Temperature (°C)	24.9	25.0	24.8	24.9	24.8	25.0	24.7	25.0
10.7%	pH (S.U.)	7.03	7.97	7.97	7.97	7.99	7.93	7.90	7.97
	DO (mg/L)	7.7	8.0	7.0	8.1	8.3	8.1	7.9	8.1
	Conductivity (µmhos/cm)	308		303		305		317	
	*Temperature (°C)	24.9	25.2	24.8	25.1	24.9	25.2	24.7	25.0
21.4%	pH (S.U.)	7.92	7.96	7.97	7.97	7.98	7.93	7.91	7.97
	DO (mg/L)	7.0	8.0	7.0	8.1	8.3	8.1	7.9	8.2
	Conductivity (µmhos/cm)	294		207		295		309	
	*Temperature (°C)	25.0	24.9	24.8	25.0	24.9	25.0	24.7	24.9
42.8%	pH (S.U.)	7.92	7.96	7.91	7.98	7.98	7.93	7.92	7.96
	DO (mg/L)	7.0	8.0	7.0	8.2	8.3	8.1	7.9	8.2
	Conductivity (µmhos/cm)	260		253		257		269	
	*Temperature (°C)	25.0	24.9	24.8	25.0	24.9	25.0	24.8	25.1
85.6%	pH (S.U.)	7.93	7.96	7.07	7.95	7.97	7.93	7.92	7.92
	DO (mg/L)	7.0	8.0	7.0	8.1	8.3	8.1	8.0	8.2
	Conductivity (µmhos/cm)	189		103		182		195	
	*Temperature (°C)	25.0	24.9	24.9	25.0	25.0	24.9	24.8	25.1
100%	pH (S.U.)	7.92	7.96	7.08	7.92	7.92	7.88	7.90	7.92
	DO (mg/L)	7.9	8.0	7.9	8.1	8.3	8.2	8.0	8.2
	Conductivity (µmhos/cm)	102		150		160		165	
	*Alkalinity (mg CaCO ₃ /L)			61					
	*Hardness (mg CaCO ₃ /L)			80					
	*TR chlorine (mg/L)			<0.10					
	*Temperature (°C)	25.0	24.9	25.1	24.9	25.0	24.8	24.8	25.2
100% Intake	pH (S.U.)	7.93	7.95	7.93	7.95	7.94	7.91	7.91	7.92
	DO (mg/L)	8.0	8.1	7.9	8.1	8.3	8.2	8.0	8.2
	Conductivity (µmhos/cm)	161		154		161		169	
	*Alkalinity (mg CaCO ₃ /L)			61					
	*Hardness (mg CaCO ₃ /L)			68					
	*TR chlorine (mg/L)			<0.10					
	*Temperature (°C)	24.9	25.0	24.7	24.9	25.1	25.1	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

*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 total residual chlorine performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet by:

SOP AT11 - Exhibit AT11.2, revision 11-01-14



TVA / Sequoyah Nuclear Plant, Outfall 101, Non-treated
August 06-13, 2019



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

Daily Chemical Analyses

Project number: 14391

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)	7.99	8.08	7.85	7.92	7.83	7.92	7.88	7.97	7.90	7.97	7.87	7.93	7.89	7.96
	DO (mg/L)	7.7	7.9	7.8	7.9	7.7	7.8	7.8	8.0	7.7	8.1	7.7	8.0	7.9	8.1
	Conductivity (µmhos/cm)	306		317		319		324		326		325		322	
	Alkalinity (mg/L CaCO ₃)	59				60				58					
	Hardness (mg/L CaCO ₃)	84				88				84					
	Temperature (°C)	24.8	25.2	24.7	25.2	24.8	24.9	24.9	25.0	24.8	24.9	24.8	25.0	24.7	25.0
10.7%	pH (SU)	8.10	8.09	8.17	7.93	7.95	7.91	7.88	7.97	7.97	7.97	7.99	7.93	7.90	7.97
	DO (mg/L)	8.0	8.0	7.9	7.9	7.8	7.9	7.7	8.0	7.8	8.1	8.3	8.1	7.9	8.1
	Conductivity (µmhos/cm)	291		310		317		308		303		315		317	
	Temperature (°C)	24.8	25.0	24.8	25.2	24.8	25.1	24.9	25.2	24.8	25.1	24.9	25.2	24.7	25.0
21.4%	pH (SU)	8.07	8.09	8.14	7.93	7.95	7.91	7.92	7.96	7.97	7.97	7.98	7.93	7.91	7.97
	DO (mg/L)	8.0	8.0	7.9	7.9	7.8	7.9	7.8	8.0	7.8	8.1	8.3	8.1	7.9	8.2
	Conductivity (µmhos/cm)	277		294		298		294		287		295		309	
	Temperature (°C)	24.8	25.0	24.8	25.2	24.9	25.0	25.0	24.9	24.8	25.0	24.9	25.0	24.7	24.9
42.8%	pH (SU)	8.06	8.07	8.11	7.94	7.95	7.91	7.92	7.96	7.91	7.98	7.98	7.93	7.92	7.96
	DO (mg/L)	8.0	8.1	7.9	8.0	7.8	8.0	7.8	8.0	7.8	8.2	8.3	8.1	7.9	8.2
	Conductivity (µmhos/cm)	245		263		266		260		253		257		269	
	Temperature (°C)	24.9	25.0	24.9	25.2	24.9	25.2	25.0	24.9	24.8	25.0	24.9	25.0	24.8	25.1
85.6%	pH (SU)	8.05	8.03	8.07	7.94	7.93	7.91	7.93	7.96	7.87	7.95	7.97	7.93	7.92	7.92
	DO (mg/L)	8.1	8.1	7.9	8.0	7.8	8.0	7.8	8.0	7.8	8.1	8.3	8.1	8.0	8.2
	Conductivity (µmhos/cm)	178		193		190		189		183		182		195	
	Temperature (°C)	24.9	24.8	25.0	24.9	25.0	25.2	25.0	24.9	24.9	25.0	25.0	24.9	24.8	25.1
100%	pH (SU)	8.05	8.02	8.06	7.94	7.94	7.90	7.92	7.96	7.88	7.92	7.92	7.88	7.90	7.92
	DO (mg/L)	8.1	8.2	8.0	8.2	7.8	8.0	7.9	8.0	7.9	8.1	8.3	8.2	8.0	8.2
	Conductivity (µmhos/cm)	159		165		160		162		158		160		165	
	Alkalinity (mg/L CaCO ₃)	65				63				61					
	Hardness (mg/L CaCO ₃)	80				64				80					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.0	25.0	25.0	25.1	25.0	25.2	25.0	24.9	25.1	24.9	25.0	24.8	24.8	25.2
100% Intake	pH (SU)	8.05	8.04	8.06	7.95	7.94	7.90	7.93	7.95	7.93	7.95	7.94	7.91	7.91	7.92
	DO (mg/L)	8.2	8.2	8.1	8.2	7.9	7.9	8.0	8.1	7.9	8.1	8.3	8.2	8.0	8.2
	Conductivity (µmhos/cm)	155		159		152		161		154		161		169	
	Alkalinity (mg/L CaCO ₃)	65				61				61					
	Hardness (mg/L CaCO ₃)	80				72				68					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	24.8	24.9	25.0	24.9	25.0	25.0	24.9	25.0	24.7	24.9	25.1	25.1	25.0	24.9

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

Matrix: Water, RL = 0.10 mg/L

Analyst TS
Date analyzed 08.06.19DPD: 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
190805.03	EASTMAN - RIVER	no color, clear		✓
190805.04	↓ EFFLUENT	no color, clear		✓
190805.05	TVA / SQN 101	no color, clear, particles		✓
190805.06	↓ INTAKE	no color, clear		✓
190805.02	HARION	no color, clear, particles		✓
190805.01	WAYNESVILLE	no color, clear, particles		✓
190806.08	N. CARY	light tan, clear		✓
190806.01	S. CARY	no color, clear		✓
190806.02	CHATTANOOGA	orange, clear		✓
190806.03	DAIKIN	no color, clear, particles		✓
190806.11	TVA / CUF 002	light tan, clear		✓
190806.12	↓ INTAKE	light tan, clear		✓
190806.13	↓ UASTREAM	light tan, clear		✓
190806.04	BOGUE BANKS	light yellow, clear		✓
190806.05	CAROLINA BEACH	light yellow, clear		✓
190806.06	ENGELHART	yellow, clear, sulfur smell		✓
190806.07	PASQUOTANK	no color, clear		✓
190806.09	MANTEO	no color, clear		✓
190806.10	APEX OIL	no color, clear		✓
	TS 08.06.19			

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 81
Date reviewed 08.06.19

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

Matrix: Water, RL = 0.10 mg/L, **Meter:** Accumet Model AB250 pH/mV/Ion Meter

Analyst	TS
Date analyzed	08.06.19

Iodide reagent:	INR 928
Acid reagent:	INR 896

Calibration:

	0.10 mg/L	1.00 mg/L	Slope (mV) (suggested range = 26 to 30 mV)
Reference standard #	INSS 176	INSS 176	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 1761	0.50	0.513	102.6%

Duplicate sample precision:

Sample number	Sample ID	Total residual chlorine (mg/L)	%RPD = $\{(S - D) / [(S+D)/2]\} \times 100$ (acceptable range = $\pm 10\%$)
190805.05	TVA/SQJ101	S 0.035	
↓	Duplicate	D 0.033	<u>7.08.06.19</u>

Sample measurements:

Sample number	Sample ID	Total residual chlorine (mg/L)
TV < 0.05 mg/L	Method Blank (MB)	< 0.020
TV = 0.30 mg/L	Method Detection Limit spike sample (MDLs)	0.332
190805.06	TVA / SQN INTAKE	< 0.017
190806.11	TVA / CUF 002	< 0.005
190806.12	↓ INTAKE	< 0.003
190806.13	↓ UPSTREAM	< 0.003
TS 08.06.19		

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 1761	0.50	0.474	94.8 %

Reviewed by X Date reviewed 08-06-19

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

Matrix: Water, RL = 0.10 mg/L

Analyst TS
Date analyzed 08.08.19DPD: 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
190807.26	EASTMAN - RIVER	no color, clear		✓
190807.27	↓ EFFLUENT	no color, clear		✓
190807.28	TVA/SQN 101	no color, clear, particles		✓
190807.29	↓ INTAKE	light tan, clear, particles		✓
190807.30	MARION	light tan, clear, particles		✓
190807.24	WAYNESVILLE	light tan, clear, particles		✓
190808.08	N. CARY	light yellow, clear		✓
190808.01	S. CARY	light tan, clear		✓
190808.02	CHATTANOOGA	yellow, slightly cloudy		✓
190808.03	DAIKIN	no color, clear, particles		✓
190808.12	TVA/CVF 002	light tan, clear		✓
190808.13	↓ INTAKE	light tan, clear, particles		✓
190808.14	↓ UPSTREAM	light tan, clear		✓
190808.04	BOGUE BANKS	light yellow, clear, particles		✓
190808.05	CAROLINA BEACH	no color, clear		✓
190808.06	ENGELHART	light yellow, clear, sulfur smell		✓
190808.07	PASQUOTANK	no color, clear		✓
190808.09	SUTTON 001	no color, clear		✓
190808.10	↓ 008	light yellow, clear		✓
190808.11	CHEMTRADE	light tan, clear		✓

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 TS
Date reviewed 08.08.19

Matrix: Water, RL = 0.10 mg/L, Meter: Accumet Model AB250 pH/mV/Ion Meter

Iodide reagent:	INR 928
Acid reagent:	INR 896

	0.10 mg/L	1.00 mg/L	Slope (mV) (suggested range = 25 to 30 mV)
Reference standard #	INSS 1761	INSS 1761	30

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1761	0.50	0.533	106.6%

Sample number	Sample ID	Total residual chlorine (mg/L)	%RPD = $\{ (S - D) / [(S+D)/2] \} \times 100$ (acceptable range = $\pm 10\%$)
190807.26	TJA/SO101	S 0.029	
↓	Duplicate	D 0.024	TS 08.08/19

Sample number	Sample ID	Total residual chlorine (mg/L)
TV < 0.05 mg/L	Method Blank (MB)	40.012
TV = 0.30 mg/L	Method Detection Limit spike sample (MDL _s)	0.346
190807.29	TVA / SQN INTAKE	< 0.009
190808.12	TVA / CUF 002	< 0.002
190808.13	↓ INTAKE	< 0.003
190808.14	↓ UPSTREAM	< 0.003
TS 08.08.19		

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1701	0.50	0.533	106.6%

SOP C8-Revision 4-Exhibit C8.2

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

Matrix: Water, RL = 0.10 mg/L

 Analyst TS
 Date analyzed 08.10.19
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
190809.15	EASTMAN- RIVER	no color, clear, particles		✓
190809.16	↓ EFFLUENT	no color, clear		✓
190809.17	TVA / SQN 101	no color, clear, particles		✓
190809.18	↓ INTAKE	no color, clear, particles		✓
190809.19	MARION	light tan, clear, particles		✓
190809.13	WAYNESVILLE	light tan, clear, particles		✓
190810.07	N. CARY	light tan, clear		✓
190810.11	S. CARY	light tan, clear, particles		✓
190810.01	CHATTANOOGA	light brown, clear		✓
190810.02	DAIKIN	light tan, clear, particles		✓
190810.08	TVA / CUF 002	light tan, clear		✓
190810.09	↓ INTAKE	light tan, clear		✓
190810.10	↓ UPSTREAM	light tan, clear		✓
190810.03	BOGUE BANKS	light yellow, clear		✓
190810.04	CAROLINA BEACH	light yellow, clear		✓
190810.05	ENGELHART	yellow, clear, sulfur smell		✓
190810.06	PASQUOTANK	no color, clear		✓
TS 08.10.19				

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
 Date reviewed

TS
08.10.19

Analyst AL
Date analyzed 08.10.19

Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg CaCO₃/L, Samples are titrated to pH = 4.5 S.U.

Titrant normality and multiplier determination:

Titrant reference number	Normality check standard number	Begin mL	End mL	Total mL (E)	Normality (N) of H ₂ SO ₄ = (5 mL Na ₂ CO 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 911	INSS 1735	0.2	12.6	12.4	0.0202	10.1

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 1742	100	100	12.6	22.2	9.6	10.1	97	97.0%

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%)
08-07-19	SSW	100	22.2	25.4	3.2	10.1	32	
1	Duplicate (D)	1	25.4	28.4	3.2	1	32	~0.0019

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 = 7.66 S.U.	100	0.0	0.2	0.2	10.1	2.0
08-01-19A	SSW		28.4	31.6	3.2		32
08-01-19B	↓		31.6	34.9	3.1		31
08-07-19	MHSW		34.9	40.6	5.7		58
08-09-19A			40.6	46.5	5.9		60
08-09-19B			0.0	5.8	5.8		59
07-25-19A			5.8	11.4	5.6		59
07-25-19B			11.4	17.5	5.9		60
08-01-19A			17.5	23.4	5.9		60
08-01-19B			23.4	29.2	5.8		59
08-05-19A			29.2	35.1	5.9		60
08-05-19B			35.1	41.1	6.0		61
08-01-19B	MHSW DU 1		41.1	46.8	5.7		58
08-05-19A	↓ 2		0.0	5.7	5.7		58
08-05-19	↓ 3		5.7	11.7	6.0		61
08-01-19B	MHSW Anti 1		11.7	17.4	5.7		58
08-05-19A	↓ 2		17.4	23.2	5.8		59
08-05-19	↓ 3		23.2	29.1	5.9		60
190805.03	Eastman 1	50	29.1	33.6	4.5 (2)		91
190807.26	↓ 2	↓	33.6	38.5	4.9	↓	99

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					10.1	

Reviewed by:

AL

Date reviewed:

08-10-19

Analyst N
Date analyzed 08.10.19

Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg CaCO₃/L, Samples are titrated to pH = 4.5 S.U.

Titrant normality and multiplier determination:

Titrant reference number	Normality check standard number	Begin mL	End mL	Total mL (E)	Normality (N) of H ₂ SO ₄ = (5 mL Na ₂ CO 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					<u>0.01019</u>

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 <u>1742</u>	100	100	<u>30.5</u>	<u>47.9</u>	<u>9.4</u>	<u>10.1</u>	<u>95</u>	<u>95.0%</u>

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%)
<u>190809.15</u>	<u>Eastman 3</u>	<u>50</u>	<u>0.0</u>	<u>4.0</u>	<u>4.0</u>	<u>(2) 10.1</u>	<u>93</u>	
<u>↓</u>	Duplicate (D)	<u>↓</u>	<u>4.4</u>	<u>9.3</u>	<u>4.7</u>	<u>↓ ↓</u>	<u>95</u>	<u>2.1%</u>

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 = <u>8.0</u>	<u>100</u>					<u>0.01019</u>
<u>190805.03</u>	<u>Eastman Anti 1</u>	<u>50</u>	<u>9.3</u>	<u>13.0</u>	<u>4.5</u>	<u>(2) 10.1</u>	<u>91</u>
<u>190807.26</u>	<u>↓ 2</u>		<u>13.0</u>	<u>16.6</u>	<u>4.0</u>		<u>97</u>
<u>190809.15</u>	<u>↓ 3</u>		<u>16.6</u>	<u>23.2</u>	<u>4.6</u>		<u>93</u>
<u>190805.04</u>	<u>Eastman RW 1</u>		<u>23.2</u>	<u>27.5</u>	<u>4.3</u>		<u>97</u>
<u>190807.27</u>	<u>↓ 2</u>		<u>27.5</u>	<u>32.2</u>	<u>4.7</u>		<u>96</u>
<u>190809.16</u>	<u>↓ 3</u>		<u>32.2</u>	<u>36.8</u>	<u>4.6</u>		<u>93</u>
<u>190805.04</u>	<u>Eastman RW Anti 1</u>		<u>36.8</u>	<u>41.1</u>	<u>4.3</u>		<u>97</u>
<u>190807.27</u>	<u>↓ 2</u>		<u>41.1</u>	<u>45.8</u>	<u>4.7</u>		<u>95</u>
<u>190809.16</u>	<u>↓ 3</u>		<u>0.0</u>	<u>4.0</u>	<u>4.0</u>		<u>93</u>
<u>190805.05</u>	<u>TVA SON 101 1</u>		<u>4.0</u>	<u>7.0</u>	<u>3.2</u>		<u>65</u>
<u>190807.28</u>	<u>↓ 2</u>		<u>7.9</u>	<u>11.0</u>	<u>3.1</u>		<u>63</u>
<u>190809.17</u>	<u>↓ 3</u>		<u>11.0</u>	<u>14.0</u>	<u>3.0</u>		<u>61</u>
<u>190805.05</u>	<u>TVA SON 101 UV 1</u>		<u>14.0</u>	<u>17.1</u>	<u>3.1</u>		<u>63</u>
<u>190807.28</u>	<u>↓ 2</u>		<u>17.1</u>	<u>20.3</u>	<u>3.2</u>		<u>65</u>
<u>190809.17</u>	<u>↓ 3</u>		<u>20.3</u>	<u>23.3</u>	<u>3.0</u>		<u>61</u>
<u>190805.06</u>	<u>TVA SON INT 1</u>		<u>23.3</u>	<u>26.5</u>	<u>3.2</u>		<u>65</u>
<u>190807.29</u>	<u>↓ 2</u>		<u>26.5</u>	<u>29.5</u>	<u>3.0</u>		<u>61</u>
<u>190809.18</u>	<u>↓ 3</u>		<u>29.5</u>	<u>32.5</u>	<u>3.0</u>		<u>61</u>
<u>190805.06</u>	<u>TVA SON INT UV 1</u>		<u>32.5</u>	<u>35.6</u>	<u>3.1</u>		<u>63</u>

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					<u>0.01019</u>	

Reviewed by:

N

Date reviewed:

08.10.19

Analyst W
Date analyzed 08.10.19

Alkalinity (SM 2320 B-2011)

Matrix: Water, RL = 5.0 mg CaCO₃/L, Samples are titrated to pH = 4.5 S.U.

Titrant normality and multiplier determination:

Titrant reference number	Normality check standard number	Begin mL	End mL	Total mL (E)	Normality (N) of H ₂ SO ₄ = (5 mL Na ₂ CO 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					0.01019

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 1702	100	100	35.0	45.3	9.7	10.1	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%)
190801.29	TVA SAN INTUV 2	50	0.0	3.0	3.0	(2) 10.1	61	
↓	Duplicate (D)	↓	3.0	6.0	3.0	↓	61	-0.0019

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		9.0	9.0		0.01019
190801.18	TVA SAN INTUV 3	50	6.0	3.0	3.0	(2) 10.1	61
190806.11	TVA CUF 002 1		9.0	12.7	3.7		75
190808.12	↓ 2		12.7	16.5	3.8		77
190810.08	↓ 3		16.5	20.5	4.0		81
190806.11	TVA CUF 002VU 1		20.5	24.2	3.7		75
190808.12	↓ 2		24.2	28.0	3.8		77
190810.08	↓ 3		28.0	32.0	4.0		81
190806.12	TVA CUF INT 1		32.0	35.8	3.8		77
190808.13	↓ 2		35.8	39.9	4.0		81
190810.09	↓ 3		39.9	43.9	4.0		81
190806.12	TVA CUF INTUV 1		43.9	47.4	3.7		75
190808.13	↓ 2		47.4	50.3	3.8		77
190810.09	↓ 3		50.3	54.2	4.1		83
190806.13	TVA CUF UP 1		54.2	58.3	4.1		83
190808.14	↓ 2		58.3	62.1	3.8		77
190810.10	↓ 3		62.1	66.1	4.0		81
190806.13	TVA CUF UP W 1		66.1	70.1	4.0		81
190808.14	↓ 2		70.1	74.0	3.9		79
190810.10	↓ 3		74.0	78.1	4.1		83

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 1702	100	100	38.0	47.5	9.5	10.1	96	96.0%

Reviewed by:

W

Date reviewed:

08.10.19

Analyst AL
Date analyzed 08.10.19

Hardness (SM 2340 C-2011)
Matrix: Water, RL = 5.0 mg CaCO₃/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 907	INSS 1701	0.0	10.0	10.0	0.0200	20.0

Laboratory control standard (LCS): 081019

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 1744	40	50	10.0	12.2	2.2	20.0	44	110.0%

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%)
	F city eff	10	12.2	16.8	4.6	20.0	460	
	Duplicate (D)	10	16.8	21.4	4.6	1	460	100.0%

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	20.0	ND
	F city op	50	21.4	22.1	0.7		14
	K-T Pm 01	25	22.1	25.8	3.7	62	150
	Pm 02	25	25.8	29.2	3.4	1	140
	Pm 03	50	29.2	34.4	5.2		100
080719	SSW		34.4	36.5	2.1		42
08.07.19	MHSW		36.5	40.7	4.2		84
080919A			40.7	44.9	4.2		84
080919B			44.9	49.0	4.1		82
08.01.19A	SSW		0.0	2.3	2.3		46
08.01.19B			2.3	4.5	2.2		44
07.25.19A	MHSW		4.5	9.2	4.7		94
07.25.19B			9.2	13.6	4.4		88
08.01.19A			13.6	17.9	4.3		86
08.01.19B			17.9	22.1	4.2		84
08.05.19A			22.1	26.5	4.4		88
08.05.19B			26.5	31.0	4.5		90
08.01.19B	MHSW VV 1		31.0	35.2	4.2		84
08.05.19A		2	35.2	39.5	4.3		86
08.07.19		3	39.5	43.7	4.2		84

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					100.0%	

Reviewed by:

AL

Date reviewed:

08-10-19

Analyst W
Date analyzed 08.10.19

Hardness (SM 2340 C-2011)
Matrix: Water, RL = 5.0 mg CaCO₃/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					<u>0.01019</u>

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 1744	40	50	<u>43.7</u>	<u>45.7</u>	<u>2.0</u>	<u>20.0</u>	<u>40</u>	<u>100.0</u>

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%)
<u>190805.02</u>	<u>Marum 1</u>	<u>50</u>	<u>0.0</u>	<u>2.5</u>	<u>2.5</u>	<u>20</u>	<u>50</u>	
<u>↓</u>	Duplicate (D)	<u>↓</u>	<u>2.5</u>	<u>5.0</u>	<u>2.5</u>	<u>↓</u>	<u>50</u>	<u>-48.1019</u>

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	<u>50</u>					<u>0.01019</u>
<u>190801.30</u>	<u>Marum 2</u>	<u>50</u>	<u>5.0</u>	<u>7.5</u>	<u>2.5</u>	<u>20.0</u>	<u>50</u>
<u>190809.19</u>	<u>↓ 3</u>	<u>↓</u>	<u>7.5</u>	<u>9.9</u>	<u>2.4</u>		<u>40</u>
<u>190805.01</u>	<u>Wayneville 1</u>	<u>2.5</u>	<u>9.9</u>	<u>13.0</u>	<u>3.9</u>	<u>(2)</u>	<u>100</u>
<u>190801.24</u>	<u>↓ 2</u>	<u>↓</u>	<u>13.0</u>	<u>20.2</u>	<u>6.4</u>		<u>260</u>
<u>190809.13</u>	<u>↓ 3</u>	<u>↓</u>	<u>20.2</u>	<u>32.2</u>	<u>12.0</u>		<u>400</u>
<u>190806.08</u>	<u>N Cary 1</u>	<u>50</u>	<u>32.2</u>	<u>38.35</u>	<u>6.15</u>		<u>64</u>
<u>190808.08</u>	<u>↓ 2</u>	<u>↓</u>	<u>35.4</u>	<u>38.7</u>	<u>3.3</u>		<u>66</u>
<u>190810.01</u>	<u>↓ 3</u>	<u>↓</u>	<u>0.0</u>	<u>3.0</u>	<u>3.0</u>		<u>60</u>
<u>190806.01</u>	<u>S Cary 1</u>		<u>38.7</u>	<u>41.6</u>	<u>2.9</u>		<u>50</u>
<u>190808.01</u>	<u>↓ 2</u>	<u>↓</u>	<u>41.6</u>	<u>44.2</u>	<u>2.6</u>		<u>52</u>
<u>190810.11</u>	<u>↓ 3</u>	<u>↓</u>	<u>3.0</u>	<u>5.7</u>	<u>2.7</u>		<u>64</u>
<u>190806.02</u>	<u>Chattanooga 1</u>	<u>2.5</u>	<u>41.2</u>	<u>44.1</u>	<u>4.9</u>	<u>(2)</u>	<u>200</u>
<u>190808.02</u>	<u>↓ 2</u>	<u>↓</u>	<u>0.0</u>	<u>4.6</u>	<u>4.6</u>		<u>180</u>
<u>190810.01</u>	<u>↓ 3</u>	<u>↓</u>	<u>5.7</u>	<u>10.4</u>	<u>4.7</u>		<u>190</u>
<u>190806.03</u>	<u>Daikin AL 1</u>		<u>4.6</u>	<u>9.1</u>	<u>4.5</u>		<u>100</u>
<u>190808.03</u>	<u>↓ 2</u>	<u>↓</u>	<u>9.1</u>	<u>14.0</u>	<u>4.9</u>		<u>200</u>
<u>190810.02</u>	<u>↓ 3</u>	<u>↓</u>	<u>10.4</u>	<u>15.2</u>	<u>4.8</u>		<u>190</u>
<u>190808.11</u>	<u>Chemtrade</u>	<u>50</u>	<u>14.0</u>	<u>19.7</u>	<u>5.7</u>		<u>110</u>
<u>190805.03</u>	<u>Epstein 1</u>	<u>↓</u>	<u>19.7</u>	<u>25.0</u>	<u>5.3</u>		<u>110</u>

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					<u>0.01019</u>	

Reviewed by:

W

Date reviewed:

08.10.19

Analyst ✓
 Date analyzed 08.10.19

Hardness (SM 2340 C-2011)
 Matrix: Water, RL = 5.0 mg CaCO₃/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				<u>0.01019</u>	

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 1744	40	50	25.0	27.0	2.0	20.0	<u>0.01019</u> <u>20.40</u>	<u>100.0</u>

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%)
190801.26	Eastman 2	50	27.0	32.6	5.6	20.0	^S 110	
↓	Duplicate (D)	↓	32.6	38.1	5.5	↓	^D 110	→ <u>0.01019</u>

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					<u>0.01019</u>
190809.15	Eastman 3	50	28.1	32.5	5.4	20.0	110
190805.03	Eastman Anti 1			43.5	40.9	5.4	110
190807.26	↓ 2			0.0	5.5	5.5	110
190809.15	↓ 3			5.5	11.3	5.8	120
190805.04	Eastman RW 1			11.3	16.4	5.1	100
190807.27	↓ 2			16.4	21.5	5.1	100
190809.15	↓ 3			21.5	26.6	5.1	100
190805.04	Eastman RW Anti 1			26.6	31.6	5.0	100
190807.27	↓ 2			31.6	36.7	5.1	100
190809.16	↓ 3			36.7	41.7	5.0	100
08-01-19B	MHSW Anti 1			41.7	45.8	4.1	82
08-05-19A	↓ 2			0.0	4.5	4.5	90
08-07-19	↓ 3			4.5	9.0	4.5	90
190805.05	TVA SQN 101 1	25	9.0	11.0	2.0 (2)		80
190807.28	↓ 2			11.0	12.0	<u>2.1.0</u>	64
190809.17	↓ 3			12.0	14.6	<u>2.0</u>	80
190805.05	TVA SQN 10100 1			14.6	16.5	1.9	74
190807.28	↓ 2			16.5	18.1	1.6	64
190809.17	↓ 3			18.1	20.0	1.9	76

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					<u>0.01019</u>	

Reviewed by:

✓

Date reviewed:

08-10-19

Analyst W
Date analyzed 08/10/19

Hardness (SM 2340 C-2011)
Matrix: Water, RL = 5.0 mg CaCO₃/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				<u>0.021015</u>	

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 1744	40	50	20.0	22.0	2.0	20.0	40	100.0

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%)
190805.06	SON INT 1	25	22.0	24.0	2.0	20.0	90	
↓	Duplicate (D)	↓	24.0	26.0	2.0	↓	90	<u>0.01019</u>

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					<u>0.01019</u>
190807.29	TJA SON INT 2	25	26.0	27.8	1.8	20.0	72
190808.12	↓	3	27.8	29.5	1.7		68
190805.06	TJA SON INT UV 1		29.5	31.5	2.0		90
190807.29	↓	2	31.5	33.2	1.7		68
190809.18	↓	3	33.2	34.9	1.7		68
190806.11	TJA CUF 002 1		34.9	38.1	3.2		128-130
190808.12	↓	2	38.1	41.1	3.0		120
190810.08	↓	3	41.1	44.3	3.2		132-130
190806.11	TJA CUF 002 UV 1		44.3	47.3	3.0		128-130
190808.12	↓	2	47.3	50.5	3.2		120
190810.08	↓	3	50.5	53.9	3.4		136-140
190806.12	TJA CUF INT 1		53.9	57.1	3.2		112-110
190808.13	↓	2	57.1	60.5	3.4		100
190810.09	↓	3	60.5	64.5	4.0		104-100
190806.12	TJA CUF INT UV 1		64.5	68.5	4.0		112-110
190808.13	↓	2	68.5	72.9	4.4		96
190810.09	↓	3	72.9	77.1	4.2		104-100
190806.13	TJA CUF UP 1		77.1	81.0	3.9		108-110
190808.14	↓	2	81.0	85.2	4.2		104-100

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					<u>0.01019</u>	

Reviewed by:

J

Date reviewed:

08-11-19

Analyst	u
Date analyzed	08.10.19

Hardness (SM 2340 C-2011)
Matrix: Water, RL = 5.0 mg CaCO₃/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					0.018

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 1744	40	50	26.2	28.2	2.0	20.0	40	100.0.

Duplicate sample precision:

Sample number	Sample ID	Sample volume (mL)	Begin mL	End mL	Total mL	Multiplier	Hardness (mg CaCO ₃ /L)	%RPD = $\{(S - D) / [(S + D) / 2]\} \times 100$ (acceptable range = $\pm 10\%$)
190610-10	CUF up 3	25	27.1	30.0	2.9	(2) 20.0	^S 116-120	
↓	Duplicate (D)	1	30.0	32.8	2.8	↓	^D 112-110	8.7%

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					
190806.13	TVA CUF up UV oblong 1st #1	25	28.2	31.5	3.3*	(2) 200	132-130 n REACH
190806.14	↓ 2		31.5	34.1	2.6		104-100
190806.10	↓ 3		32.0	35.6	2.6		112-110
190806.13	TVA CUF up 1 recheck		34.1	36.9	2.8		112-110 ←
190806.13	TVA CUF up UV 1 recheck	✓ -	36.9	39.5	2.6*		104-100 n

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 1744	40	50	36.0	37.0	2.0	200	40	100.152

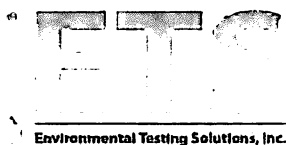
Reviewed by:

Date reviewed: 08-10-19

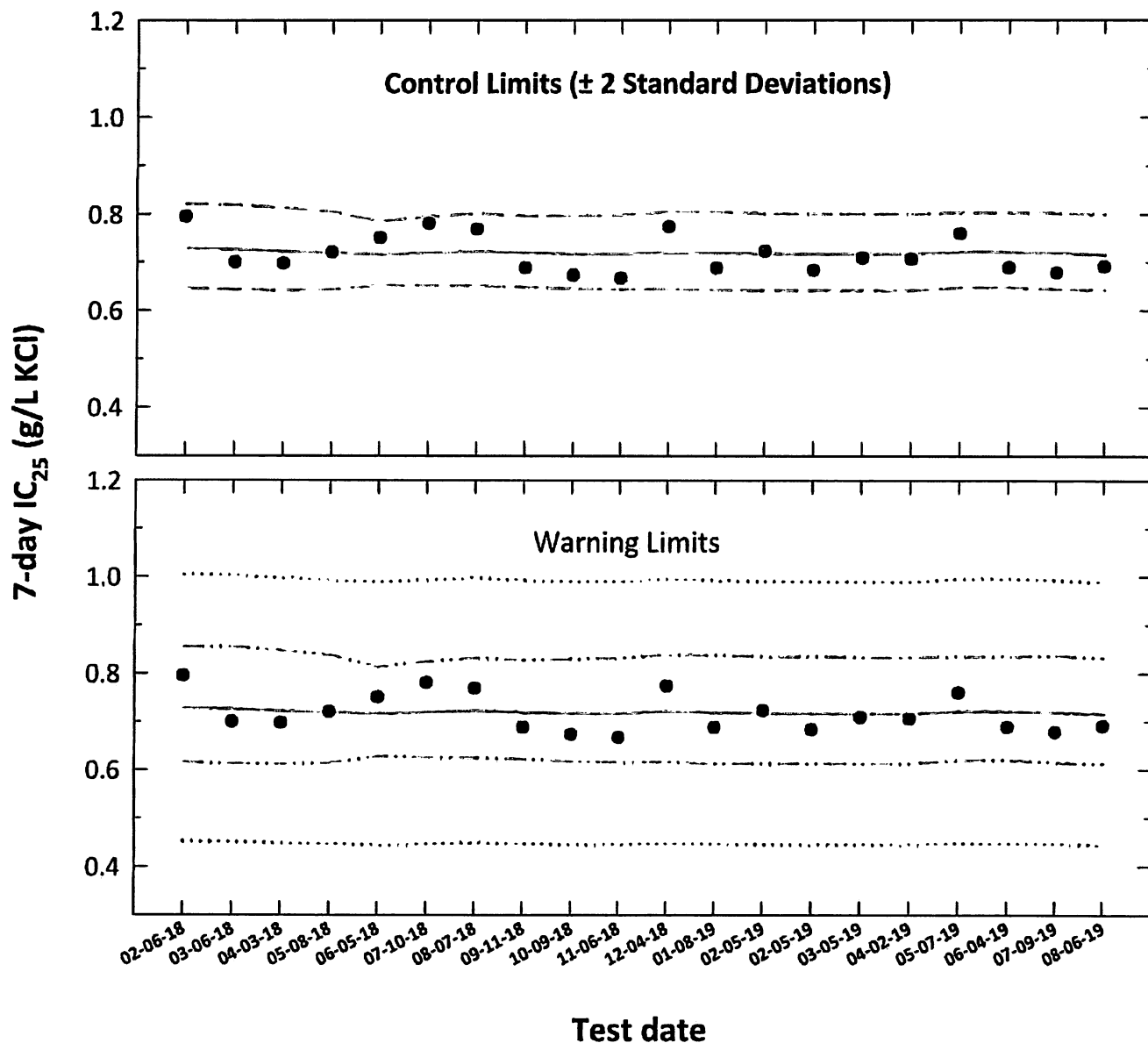
**Sequoyah Nuclear Plant Biomonitoring
August 06 – 13, 2019**

Appendix D

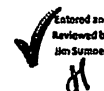
**Reference Toxicant Test and
Control Chart**



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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . Laboratory Warning Limits (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- USEPA Warning Limits (mean logarithmic $IC_{25} \pm S_{A.75}$ converted to anti-logarithmic values,
 $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)



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

Test number	Test date	7-day IC ₂₅ ToxCat 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	02-06-18	0.7951	-0.0996	-0.1377	0.0260	0.7283	0.6461	0.8211	0.6154	0.8557	0.4516	1.0051
2	03-06-18	0.7002	-0.1548	-0.1387	0.0263	0.7266	0.6437	0.8201	0.6126	0.8552	0.4505	1.0027
3	04-03-18	0.6973	-0.1566	-0.1411	0.0256	0.7225	0.6423	0.8128	0.6115	0.8474	0.4480	0.9971
4	05-08-18	0.7204	-0.1424	-0.1429	0.0243	0.7196	0.6435	0.8048	0.6138	0.8380	0.4462	0.9931
5	06-05-18	0.7512	-0.1243	-0.1451	0.0200	0.7160	0.6530	0.7852	0.6280	0.8126	0.4440	0.9881
6	07-10-18	0.7808	-0.1075	-0.1429	0.0216	0.7196	0.6514	0.7950	0.6248	0.8244	0.4462	0.9931
7	08-07-18	0.7690	-0.1141	-0.1410	0.0225	0.7228	0.6518	0.8015	0.6245	0.8317	0.4481	0.9974
8	09-11-18	0.6884	-0.1621	-0.1432	0.0222	0.7191	0.6491	0.7967	0.6217	0.8270	0.4458	0.9924
9	10-09-18	0.6735	-0.1717	-0.1442	0.0231	0.7175	0.6452	0.7979	0.6167	0.8296	0.4448	0.9901
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCat).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

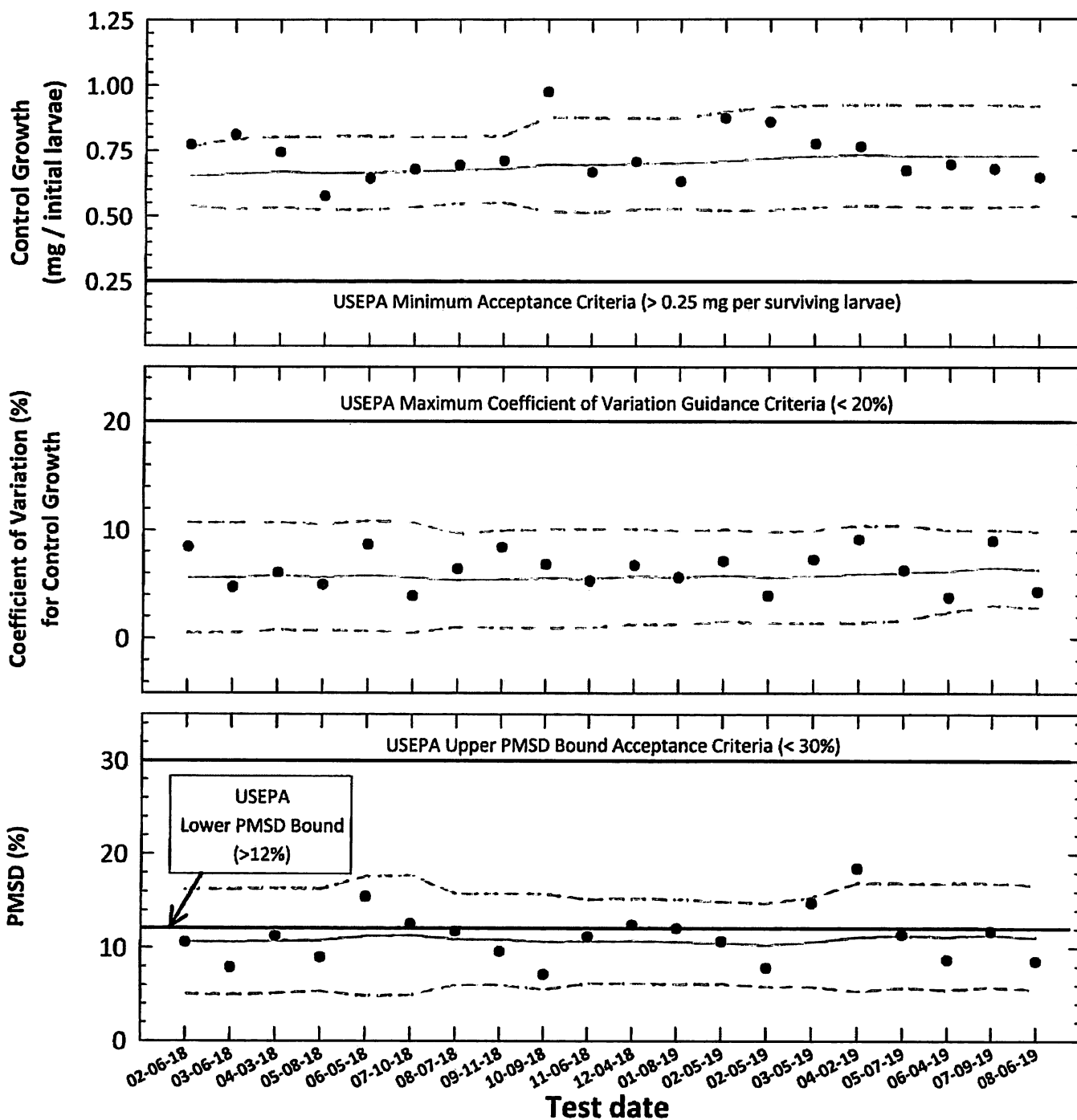
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A.75} converted to anti-logarithmic values.

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
 JTH/SUMMER


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 \pm 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		(mg/initial larvae)			(%)			(%)		
			Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
								CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	02-06-18	100	0.772	8.4	0.0815	10.6	0.651	0.538	0.765	5.6	0.5	10.7	10.6	5.0	16.2
2	03-06-18	100	0.810	4.7	0.0640	7.9	0.660	0.526	0.794	5.6	0.5	10.7	10.6	4.9	16.3
3	04-03-18	100	0.743	6.1	0.0836	11.3	0.668	0.534	0.802	5.8	0.8	10.7	10.7	5.1	16.3
4	05-08-18	100	0.576	4.9	0.0516	9.0	0.663	0.523	0.803	5.6	0.7	10.5	10.8	5.3	16.3
5	06-05-18	100	0.643	8.7	0.0992	15.4	0.664	0.525	0.803	5.8	0.7	10.9	11.2	4.8	17.6
6	07-10-18	100	0.678	3.9	0.0850	12.5	0.669	0.535	0.803	5.6	0.5	10.7	11.3	4.9	17.7
7	08-07-18	100	0.692	6.4	0.0813	11.7	0.675	0.547	0.802	5.3	1.0	9.7	10.9	6.0	15.8
8	09-11-18	100	0.709	8.4	0.0680	9.6	0.678	0.551	0.805	5.5	0.9	10.0	10.9	6.0	15.8
9	10-09-18	100	0.973	6.8	0.0693	7.1	0.696	0.516	0.876	5.5	1.0	10.1	10.6	5.5	15.7
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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.

Intercept and
Treatment by
Date Reported
OK

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: 55

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>1811</u>				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L Milli-Q 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:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>07-31-19</u>	Artemia CHM number:	<u>CHM1048</u>
Hatch dates and times:	<u>08-05-19 1630 TO</u> <u>08-06-19 0550</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>8.22</u> S.U. Temperature = <u>24.4</u> °C	Date / Time in oven:	<u>08-13-19 0810</u>
Average transfer volume:	< 0.25 mL	Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>08-14-19 0810</u>
		Final oven temperature:	<u>60 °C</u>
		Total drying time:	<u>24-HOURS</u>

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	08-06-19	0600	<u>JL</u>	1200	<u>JL</u>	0800	<u>JL</u>	<u>08-01-19B</u>
1	08-07-19	0600	<u>JL</u>	1200	<u>JL</u>	0800	<u>JL</u>	<u>08-01-19B</u>
2	08-08-19	<u>0630</u>	<u>JL</u>	<u>1230</u>	<u>JL</u>	<u>0830</u>	<u>JL</u>	<u>08-05-19 A</u>
3	08-09-19	<u>0600</u>	<u>JL</u>	<u>1200</u>	<u>JL</u>	<u>0800</u>	<u>JL</u>	<u>08-05-19 A</u>
4	08-10-19	<u>0700</u>	<u>JL</u>	<u>1300</u>	<u>JL</u>	<u>0900</u>	<u>JL</u>	<u>08-07-19</u>
5	08-11-19	<u>0700</u>	<u>JL</u>	<u>1300</u>	<u>JL</u>	<u>0900</u>	<u>JL</u>	<u>08-07-19</u>
6	08-12-19	<u>0600</u>	<u>JL</u>	<u>1200</u>	<u>JL</u>	<u>0800</u>	<u>JL</u>	<u>08-07-19</u>
7	08-13-19					<u>0700</u>	<u>JL</u>	

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>0%</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>742.8</u>
Average weight per initial larvae:	<u>0.645</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.645</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>670.8</u>
			IC ₂₅ (mg/L KCl)	<u>691.2</u>



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*PpKCICR Test Number: 55**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) Tray color code:: <u>forest green</u> Analyst: <u>TS</u> Date: <u>07.31.19</u>												
B = Pan + Larvae weight (mg) Analyst: <u>JS</u> Date: <u>08.15.19</u>												
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>JS</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>JS</u>												
Average weight per initial number of larvae (mg)	0.645				0.816				0.727			
Percent reduction from control (%)					-26.67.				-12.87.			

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:



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*PpKCICR Test Number: 55

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}	9 ^{1d}	8 ^{2d}	9 ^{1d}
2	10	10	10	10	10	10	10	10	8	9	8	9
3	10	10	10	10	10	10	10	10	8	9	7 ^{1d}	9
4	10	10	10	10	10	10	10	10	8	8 ^{1d}	7	9
5	10	10	10	10	7 ^{3d}	7 ^{3d}	7 ^{3d}	7 ^{3d}	4 ^{4d}	6 ^{2d}	5 ^{2d}	5 ^{4d}
6	10	10	10	10	5 ^{2d}	6 ^{1d}	5 ^{2d}	5 ^{2d}	4	6	5	5
7	10	10	10	10	5	6	5	5 ^{10d}	3 ^{1d}	3 ^{1d}	2 ^{2d}	2 ^{2d}
A = Pan weight (mg) Tray color code: <u>forest green</u> Analyst: <u>TS</u> Date: <u>07.31.19</u>												
B = Pan + Larvae weight (mg) Analyst: <u>X</u> Date: <u>08-15-19</u>												
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>X</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>X</u>												
Average weight per initial number of larvae (mg)	0.738				0.431				0.186			
Percent reduction from control (%)	-14.57				33.27				71.27			

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:



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*PpKCICR Test Number: 55**Survival and Growth Data**

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	7 ^{sd}	6 ^{sd}	7 ^{sd}	7 ^{sd}
2	7	5 ^{sd}	7	7
3	7	5	7	5 ^{sd}
4	6 ^{sd}	4 ^{sd}	6 ^{sd}	5
5	3 ^{sd}	3 ^{sd}	3 ^{sd}	1 ^{sd}
6	2 ^{sd}	1 ^{sd}	1 ^{sd}	1
7	1 ^{sd}	0 ^{sd}	1	0 ^{sd}
A = Pan weight (mg) Tray color code: <u>forest green</u> Analyst: <u>TS</u> Date: <u>07.31.19</u>				
B = Pan + Larvae weight (mg) Analyst: <u>J</u> Date: <u>08.15.19</u>				
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>J</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>J</u>				
Average weight per initial number of larvae (mg)	Percent reduction from control (%)			
	0.034		94.87.	

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:



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: 55
Test dates: August 06-13, 2019

Concentration (mg/L KD)	Replicate	Initial number of larvae	Final number of larvae	A = Post weight (mg)	B = Pre + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Percent weight per 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	16.05	22.59	6.54	0.654			0.654				
	B	10	10	16.26	22.93	6.67	0.667			0.667				
	C	10	10	14.74	20.78	6.04	0.604	0.645	4.3	0.645	100.0	0.645	4.3	Not applicable
	D	10	10	14.70	21.24	6.54	0.654			0.654				
300	E	10	10	13.83	21.36	7.53	0.753			0.753				
	F	10	10	15.32	23.81	8.49	0.849			0.849				
	G	10	10	14.21	22.55	8.34	0.834	0.816	5.3	0.816	100.0	0.816	5.3	-26.6
	H	10	10	14.06	21.34	7.28	0.728			0.728				
450	I	10	10	14.48	21.65	7.17	0.717			0.717				
	J	10	10	15.83	23.25	7.42	0.742	0.727	5.2	0.727	100.0	0.727	5.2	-12.8
	K	10	10	13.87	20.67	6.80	0.680			0.680				
	L	10	10	14.32	21.01	6.69	0.669			0.669				
600	M	10	10	13.54	20.90	7.36	0.736			0.736				
	N	10	10	15.81	22.90	7.09	0.709	0.738	3.3	0.738	100.0	0.738	3.3	-14.5
	O	10	10	13.21	20.89	7.68	0.768			0.768				
	P	10	10	15.59	22.98	7.39	0.739			0.739				
750	Q	10	5	12.86	17.30	4.44	0.888			0.434				
	R	10	6	15.02	19.39	4.37	0.728	0.825	17.5	0.431	52.5	0.431	15.0	33.2
	S	10	5	15.19	18.66	3.47	0.694			0.347				
	T	10	5	16.02	21.07	5.05	1.010			0.505				
900	U	10	3	15.60	17.26	1.66	0.720			0.216				
	V	10	3	14.56	16.67	2.11	0.703	0.750	11.7	0.211	25.0	0.186	19.3	71.2
	W	10	2	13.73	15.12	1.39	0.695			0.139				
	X	10	2	16.62	18.38	1.76	0.880			0.176				
1050	Y	10	1	15.06	15.70	0.64	0.640			0.064				
	Z	10	0	0.00	0.00	0.00	0.000	0.675	7.3	0.000	5.0	0.034	115.8	94.8
	AA	10	1	13.93	14.64	0.71	0.710			0.071				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: PMSD = 0.0551
PMSD = 8.5

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



Statistical Analyses

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 8/6/2019	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 8/13/2019	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	1.0000
750	0.5000	0.6000	0.5000	0.5000
900	0.3000	0.3000	0.2000	0.2000
1050	0.1000	0.0000	0.1000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
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	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.5250	0.5250	0.8106	0.7854	0.8861	6.210	4	10.00	10.00	19	40
*900	0.2500	0.2500	0.5216	0.4636	0.5796	12.838	4	10.00	10.00	30	40
*1050	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84579	0.896	0.20611	0.43364

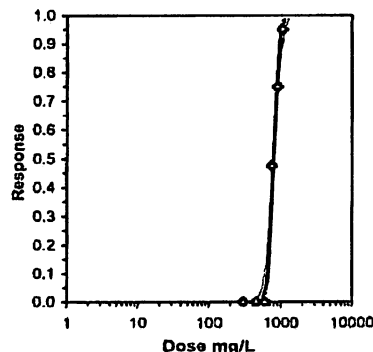
Equality of variance cannot be confirmed

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

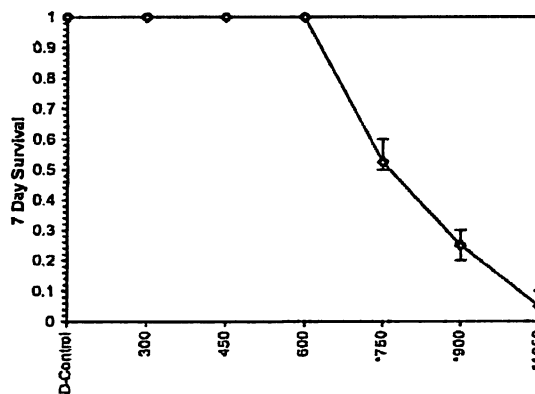
Treatments vs D-Control

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.4714	1.81435	10.9153 18.0275	0	4.28481	9.48773	0.36883	2.89916	0.0691	5
Intercept	-36.955	5.27632	-47.296 -26.613							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	547.521	478.464 596.007
EC05	3.355	610.229	550.897 652.032
EC10	3.718	646.544	593.377 684.613
EC15	3.964	672.258	623.507 707.925
EC20	4.158	693.423	648.221 727.377
EC25	4.326	712.11	669.888 744.84
EC40	4.747	781.463	725.693 792.97
EC50	5.000	792.785	759.384 825.681
EC60	5.253	825.396	792.59 861.963
EC75	5.674	882.6	846.38 930.927
EC80	5.842	906.386	867.417 961.249
EC85	6.036	934.921	891.911 998.611
EC90	6.282	972.105	922.802 1048.61
EC95	6.645	1029.95	969.643 1128.74
EC99	7.326	1147.92	1061.45 1298.81



Dose-Response Plot



Reviewed and
Approved by
(Signature)



Statistical Analyses

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 8/6/2019 Test ID: PpKICR Sample ID: REF-Ref Toxicant
End Date: 8/13/2019 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	0.6540	0.6670	0.8040	0.6540
300	0.7530	0.8490	0.8340	0.8280
450	0.7170	0.7420	0.6800	0.7690
600	0.7360	0.7090	0.7680	0.7390
750	0.4340	0.4370	0.3470	0.5050
900	0.2160	0.2110	0.1390	0.1760
1050	0.0640	0.0000	0.0710	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.6448	1.0000	0.6448	0.6040	0.6670	4.319	4				0.7314	1.0000
300	0.8160	1.2656	0.8160	0.7530	0.8490	5.260	4	-7.117	2.290	0.0551	0.7314	1.0000
450	0.7270	1.1276	0.7270	0.6800	0.7690	5.206	4	-3.418	2.290	0.0551	0.7314	1.0000
600	0.7380	1.1446	0.7380	0.7090	0.7680	3.269	4	-3.875	2.290	0.0551	0.7314	1.0000
750	0.4308	0.6681	0.4308	0.3470	0.5050	15.031	4				0.4308	0.5889
900	0.1855	0.2877	0.1855	0.1390	0.2160	19.269	4				0.1855	0.2536
1050	0.0338	0.0523	0.0338	0.0000	0.0710	115.780	4				0.0338	0.0461

Auxiliary Tests

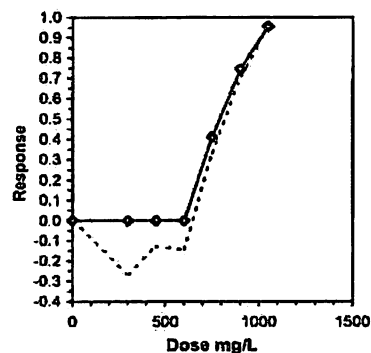
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$) 0.92853 0.844 -0.7738 -0.2669
Bartlett's Test indicates equal variances ($p = 0.78$) 1.08534 11.3449

Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU MSDu MSDp MSB MSE F-Prob df
Dunnett's Test 600 >600 0.0551 0.08547 0.01964 0.00118 1.3E-04 3, 12

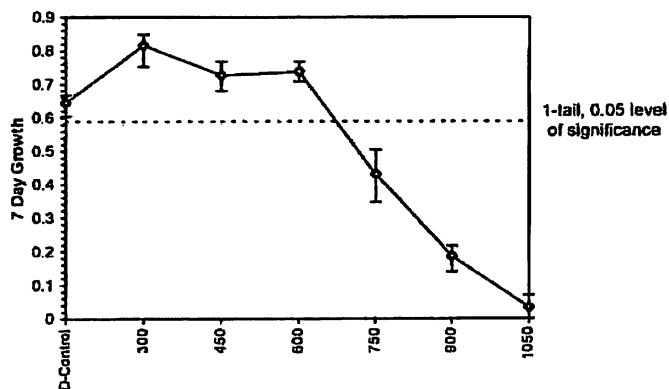
Treatments vs D-Control

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	618.24	18.11	597.45 623.37	-8.1870
IC10	636.49	4.84	615.76 646.73	-0.5699
IC15	654.73	5.83	631.77 670.10	-0.0862
IC20	672.98	7.09	647.45 693.46	0.1004
IC25	691.22	8.50	663.48 716.83	0.1633
IC40	745.95	12.71	704.59 784.07	0.0435
IC50	789.77	13.70	727.95 820.64	-0.7916



Dose-Response Plot



Reviewed and
Printed on
8/13/2019



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*PpKCICR Test Number: 55**Daily Chemistry:**

Concentration		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		Analyst					
Concentration	Parameter						
CONTROL	pH (S.U.)	7.99	7.84	7.85	7.79	7.83	7.67
	DO (mg/L)	7.7	7.7	7.8	7.7	7.7	7.2
	Conductivity (µmhos/cm)	306		317		319	
	*Alkalinity (mg CaCO ₃ /L)	59		59.4		60	
	*Hardness (mg CaCO ₃ /L)	84				88	
	*Temperature (°C)	24.8	24.6	24.7	24.7	24.8	24.8
300 mg KCl/L	pH (S.U.)	8.04	7.83	7.97	7.75	7.98	7.61
	DO (mg/L)	7.9	7.7	7.9	7.7	7.9	7.4
	Conductivity (µmhos/cm)	840		875		850	
	*Temperature (°C)	24.8	24.7	24.8	24.5	24.7	24.7
450 mg KCl/L	pH (S.U.)	8.03	7.77	7.97	7.72	7.96	7.66
	DO (mg/L)	7.8	7.7	8.0	7.7	8.0	7.4
	Conductivity (µmhos/cm)	1090		1120		1110	
	*Temperature (°C)	24.9	24.7	24.8	24.5	24.7	24.7
600 mg KCl/L	pH (S.U.)	8.03	7.80	7.96	7.73	7.97	7.60
	DO (mg/L)	7.9	7.7	8.0	7.7	8.0	7.4
	Conductivity (µmhos/cm)	1350		1410		1360	
	*Temperature (°C)	24.9	24.5	24.8	24.5	24.7	24.7
750 mg KCl/L	pH (S.U.)	8.02	7.80	7.95	7.71	7.97	7.59
	DO (mg/L)	8.0	7.7	8.1	7.8	8.0	7.5
	Conductivity (µmhos/cm)	1000		1660		1620	
	*Temperature (°C)	24.9	24.5	24.8	24.7	24.7	24.9
900 mg KCl/L	pH (S.U.)	8.01	7.80	7.95	7.69	7.97	7.57
	DO (mg/L)	8.0	7.8	8.1	7.8	8.1	7.5
	Conductivity (µmhos/cm)	1360		1830 (1900)	1880	1880	
	*Temperature (°C)	24.9	24.6	24.8	24.7	24.7	24.6
1050 mg KCl/L	pH (S.U.)	8.01	7.79	7.94	7.77	7.97	7.50
	DO (mg/L)	8.0	7.7	8.1	7.8	8.1	7.4
	Conductivity (µmhos/cm)	2190		2230		2160	
	*Temperature (°C)	24.9	24.4	24.7	24.7	24.7	24.9
		Initial	Final	Initial	Final	Initial	Final

*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 by: JL



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*PpKCICR Test Number: 55

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		Analyst							
Concentration	Parameter								
CONTROL	pH (S.U.)	7.88	7.63	7.90	7.65	7.87	7.63	7.89	7.82
	DO (mg/L)	7.0	7.3	7.7	7.6	7.9	7.5	7.9	7.5
	Conductivity (µmhos/cm)	324		326		325		322	
	*Alkalinity (mg CaCO ₃ /L)			58					
	*Hardness (mg CaCO ₃ /L)	84		84					
	*Temperature (°C)	24.7	24.8	24.8	24.7	24.7	25.0	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.95	7.50	7.96	7.52	7.98	7.61	7.98	7.88
	DO (mg/L)	7.9	7.3	7.6	7.6	7.9	7.5	8.0	7.5
	Conductivity (µmhos/cm)	860		864		883		875	
	*Temperature (°C)	24.6	24.7	24.9	24.8	24.7	24.8	24.9	24.6
450 mg KCl/L	pH (S.U.)	7.94	7.50	7.97	7.52	7.99	7.67	7.98	7.88
	DO (mg/L)	8.0	7.5	7.7	7.1	8.1	7.5	8.0	7.6
	Conductivity (µmhos/cm)	1120		1130		990		988	
	*Temperature (°C)	24.6	24.7	24.9	24.9	24.7	24.8	24.9	24.9
600 mg KCl/L	pH (S.U.)	7.95	7.50	7.97	7.64	8.00	7.73	7.96	7.88
	DO (mg/L)	8.1	7.5	7.7	7.2	8.1	7.6	8.1	7.6
	Conductivity (µmhos/cm)	1370		1390		1390		1390	
	*Temperature (°C)	24.6	24.5	25.0	24.9	24.7	24.9	25.0	24.9
750 mg KCl/L	pH (S.U.)	7.94	7.50	7.98	7.64	8.00	7.71	7.96	7.89
	DO (mg/L)	8.1	7.6	7.8	7.2	8.1	7.6	8.2	7.6
	Conductivity (µmhos/cm)	1630		1640		1640		1630	
	*Temperature (°C)	24.6	24.8	25.0	24.9	24.7	25.1	25.0	24.8
900 mg KCl/L	pH (S.U.)	7.94	7.55	7.98	7.64	8.00	7.75	7.96	7.87
	DO (mg/L)	8.1	7.6	7.8	7.2	8.1	7.6	8.2	7.6
	Conductivity (µmhos/cm)	1890		1910		1950		1920	
	*Temperature (°C)	24.7	24.6	25.0	24.7	24.8	25.1	25.0	24.8
1050 mg KCl/L	pH (S.U.)	7.95	7.64	7.99	7.55	7.99	7.70	7.96	7.86
	DO (mg/L)	8.1	7.7	7.8	7.3	8.1	7.5	8.2	7.6
	Conductivity (µmhos/cm)	2120		2120		2120		2180	
	*Temperature (°C)	24.7	24.7	25.0	24.9	24.8	24.8	25.0	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

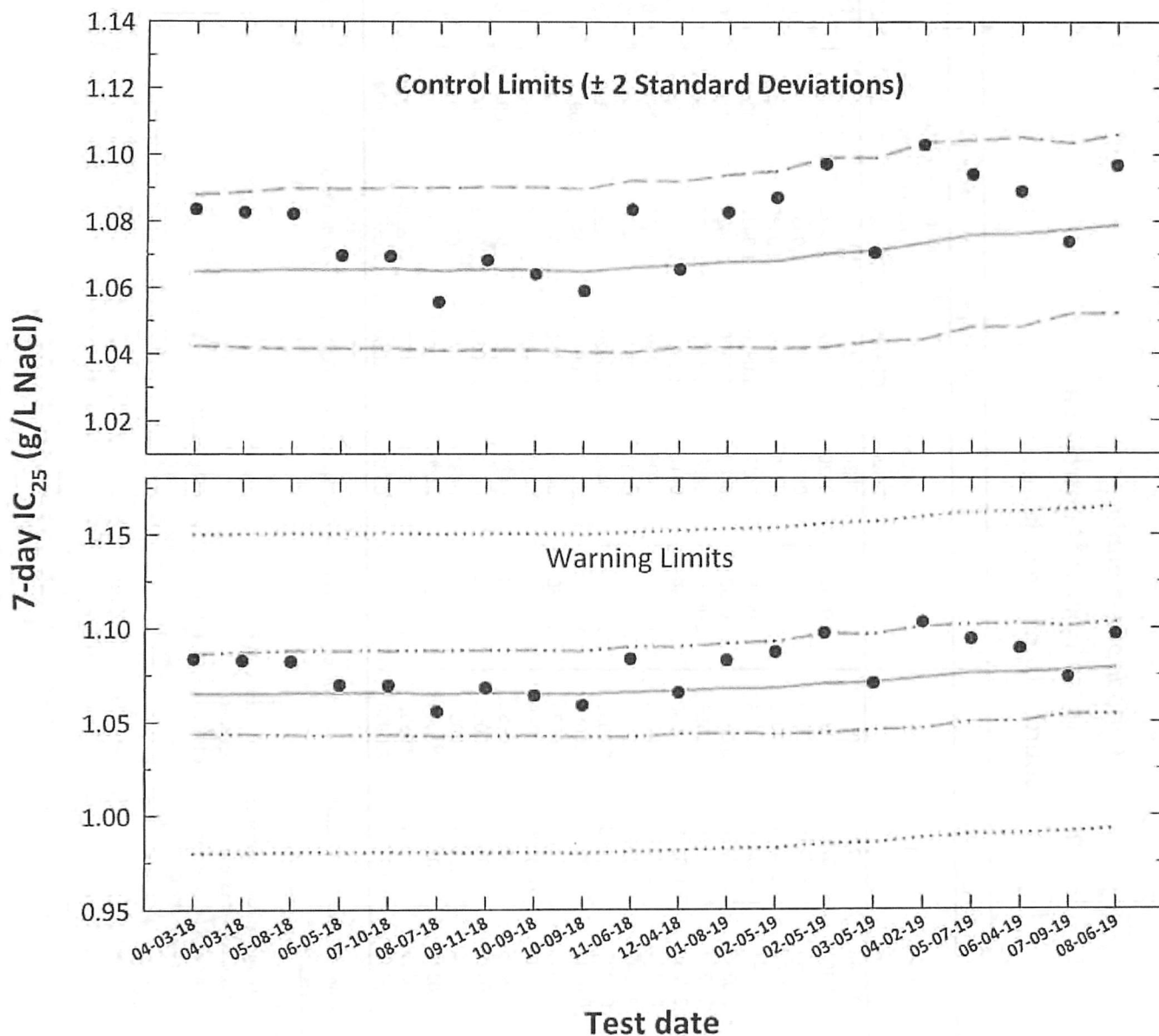


*Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1.
 Alkalinity and hardness performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet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

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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . - . **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient 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)



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	04-03-18	1.0835	0.0348	0.0273	0.0047	1.0649	1.0424	1.0880	1.0437	1.0861	0.9797	1.1501
2	04-03-18	1.0827	0.0345	0.0274	0.0048	1.0651	1.0420	1.0888	1.0434	1.0869	0.9799	1.1503
3	05-08-18	1.0822	0.0343	0.0275	0.0049	1.0655	1.0415	1.0899	1.0430	1.0879	0.9802	1.1507
4	06-05-18	1.0696	0.0292	0.0275	0.0049	1.0653	1.0415	1.0897	1.0430	1.0877	0.9801	1.1506
5	07-10-18	1.0694	0.0291	0.0276	0.0049	1.0656	1.0417	1.0900	1.0432	1.0880	0.9804	1.1508
6	08-07-18	1.0555	0.0235	0.0274	0.0050	1.0652	1.0409	1.0900	1.0424	1.0879	0.9800	1.1504
7	09-11-18	1.0682	0.0287	0.0276	0.0050	1.0655	1.0413	1.0903	1.0428	1.0883	0.9803	1.1508
8	10-09-18	1.0640	0.0269	0.0275	0.0050	1.0654	1.0412	1.0902	1.0427	1.0882	0.9802	1.1507
9	10-09-18	1.0589	0.0248	0.0273	0.0050	1.0649	1.0406	1.0898	1.0421	1.0878	0.9797	1.1501
10	11-06-18	1.0835	0.0348	0.0278	0.0053	1.0660	1.0404	1.0922	1.0420	1.0900	0.9807	1.1513
11	12-04-18	1.0655	0.0275	0.0280	0.0051	1.0667	1.0420	1.0920	1.0435	1.0899	0.9814	1.1521
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 7-day IC₂₅ = 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 IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A.10} converted to anti-logarithmic values.

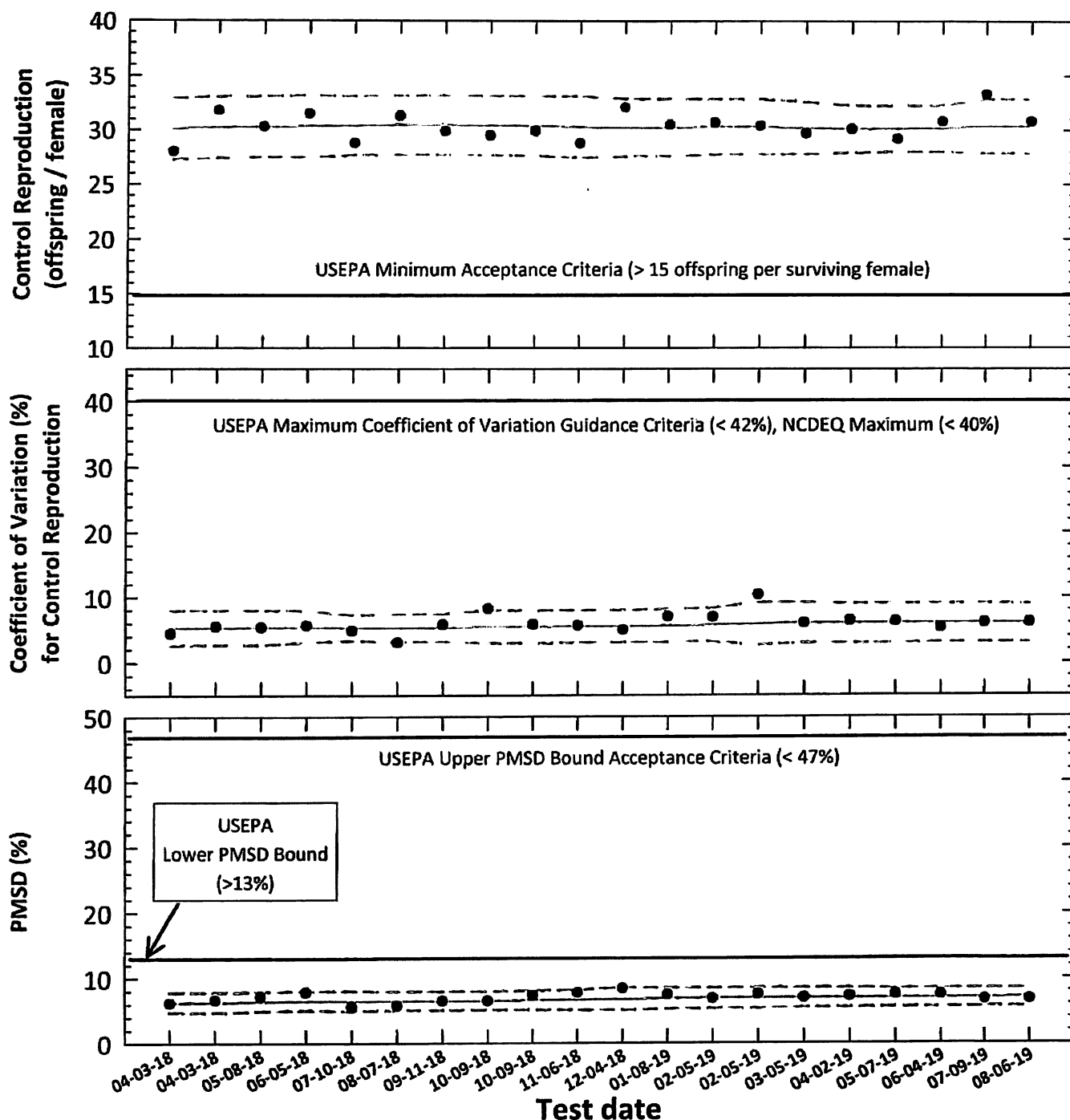
S_{A.10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A.10} = 0.08).

CV = Coefficient of variation.

Entered and
Reviewed by
Jen Sumner

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 \pm 2 Standard Deviations)

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

061 Test number	Test date	ToxCal Determination					Control Reproduction			Control Reproduction CV			Test PMSD		
		Control Survival (%)	Control Reproduction		Test		(offspring/female)			(%)			(%)		
			Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
								CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	04-03-18	100	28.0	4.5	1.729	6.2	30.1	27.3	32.9	5.3	2.6	7.9	6.2	4.7	7.8
2	04-03-18	100	31.8	5.5	2.108	6.6	30.2	27.4	33.1	5.3	2.7	7.9	6.3	4.7	7.8
3	05-08-18	100	30.3	5.4	2.172	7.2	30.3	27.5	33.1	5.3	2.7	8.0	6.4	4.9	7.8
4	06-05-18	100	31.5	5.6	2.469	7.8	30.3	27.5	33.2	5.5	3.0	7.9	6.5	5.0	8.0
5	07-10-18	100	28.8	4.9	1.598	5.5	30.4	27.7	33.1	5.3	3.3	7.2	6.4	4.9	8.0
6	08-07-18	100	31.3	3.0	1.806	5.8	30.5	27.7	33.2	5.2	3.1	7.4	6.5	5.0	7.9
7	09-11-18	100	29.9	5.8	1.943	6.5	30.5	27.7	33.2	5.3	3.1	7.4	6.5	5.0	7.9
8	10-09-18	100	29.5	8.2	1.912	6.5	30.4	27.7	33.2	5.4	2.9	7.9	6.5	5.1	7.9
9	10-09-18	100	29.9	5.8	2.182	7.3	30.4	27.6	33.1	5.4	2.9	7.9	6.5	5.1	8.0
10	11-06-18	100	28.8	5.6	2.231	7.7	30.3	27.4	33.1	5.4	3.0	7.9	6.6	5.1	8.2
11	12-04-18	100	32.1	5.0	2.687	8.4	30.2	27.6	32.9	5.5	3.0	7.9	6.7	5.0	8.5
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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.

Reviewed and
Approved by
Date: Summer
JL

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013 Method 1002.0)

Species: *Ceriodaphnia dubia*

CdNaCICR #: 232

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>1769</u>				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL Milli-Q 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 information:												Test information:			
Organism age:					< 24-hours old							Randomizing template color:		Yellow	
Date and times organisms were born between:					08-06-19 0615 to 0900							Incubator number and shelf location:		2B1	
Culture board:			07-30-19 A									YWT batch:		08-07-19	
Replicate number:			1	2	3	4	5	6	7	8	9				10
Culture board cup number:			1	2	3	9	10	11	17	18	21	25	Selenastrum batch:		08-07-19
Transfer vessel information:			pH = 7.98 S.U. Temperature = 25.1 °C												
Average transfer volume (mL):			< 0.25 mL												

Daily renewal information:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	MHSW batch used	Analyst
0	08-06-19	<u>0912</u>	<u>08-01-19 B</u>	<u>H</u>
1	08-07-19	<u>0902</u>	<u>08-01-19 B</u>	<u>H</u>
2	08-08-19	<u>0945</u>	<u>08-05-19 A</u>	<u>H</u>
3	08-09-19	<u>0906</u>	<u>08-05-19 A</u>	<u>H</u>
4	08-10-19	<u>1000</u>	<u>08-07-19</u>	<u>H</u>
5	08-11-19	<u>0958</u>	<u>08-07-19</u>	<u>H</u>
6	08-12-19	<u>0902</u>	<u>08-07-19</u>	<u>H</u>
7	08-13-19	<u>0842</u>		<u>11</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>21400</u>
% Adults having 3 rd Broods:	<u>1007.</u>	≥ 80%	NOEC (mg/L NaCl)	<u>1000</u>
% Mortality:	<u>07.</u>	≤ 20%	LOEC (mg/L NaCl)	<u>1200</u>
Mean Offspring/Female:	<u>30.8</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>6.17.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1096.7</u>



Environmental Testing Solutions, Inc.

Species: Ceriodaphnia dubiaCdNaClCR #: 232**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	4	5	5	5	4	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	10	13	13	10	12	10	10	10	13
	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	16	17	13	15	15	15	14	17	15
Total young produced		31	30	35	31	30	31	29	28	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd 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: 07.

Mean Offspring/Female: 30.8

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	4	6	4	5	5	5	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	12	13	13	12	13	10	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	16	15	13	17	15	18	15	17	15	15
Total young produced		31	31	29	35	33	35	33	31	31	31
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: 07.

Mean Offspring/Female: 32.0

% Reduction from Control: -3.97.



Environmental Testing Solutions, Inc.

Species: Ceriodaphnia dubiaCdNaClCR #: 232

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	5	4	5	5	6	4	6	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	13	12	10	11	10	10	13	11	13
	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	14	11	14	14	16	14	17	17	15
Total young produced		31	31	34	29	31	30	30	35	33	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:	07.
Mean Offspring/Female:	31.7
% Reduction from Control:	-2.97.

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	5	4	4	4	4	5	3	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	11	13	11	10	10	10	9	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	12	16	14	16	15	16	16	15	17	17
Total young produced		28	30	29	33	30	31	29	28	31	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:	07.
Mean Offspring/Female:	30.3
% Reduction from Control:	1.67.



Environmental Testing Solutions, Inc.

Species: Ceriodaphnia dubiaCdNaClCR #: 232

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	2	4	2	3	3	3	4	2	2	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	8	9	9	0	9	5	7	10	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	2	0	0	0	5	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	10	7	6	5	5	7	9	4	5	6
Total young produced		19	19	17	17	13	19	18	13	17	13
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:	07.
Mean Offspring/Female:	16.5
% Reduction from Control:	46.47.

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	2	1	1	3	1	2	1	1	1	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	3	0	0	2	0	0	0	0	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	1	0	0	0	2	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	1	0	0	0	2	0	0	1	0	0
Total young produced		6	2	1	5	3	4	1	2	2	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:	07.
Mean Offspring/Female:	2.9
% Reduction from Control:	90.47.



Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	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	5	5	4	4	4	4	4	44
5	9	10	13	13	10	12	10	10	10	13	110
6	0	0	0	0	0	0	0	0	0	0	0
7	17	16	17	13	15	15	15	14	17	15	154
Total	31	30	35	31	30	31	29	28	31	32	308

600 mg NaCl/L

Day	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	6	4	5	5	5	4	4	4	4	46
5	11	10	12	13	13	12	13	10	12	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	16	15	13	17	15	18	15	17	15	15	156
Total	31	31	29	35	33	35	33	31	31	31	320

800 mg NaCl/L

Day	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	5	6	4	6	5	5	5	50
5	13	13	12	10	11	10	10	13	11	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	13	14	17	14	14	16	14	17	17	15	151
Total	31	31	34	29	31	30	30	35	33	33	317

1000 mg NaCl/L

Day	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	4	4	4	5	3	3	5	5	42
5	11	10	11	13	11	10	10	10	9	12	107
6	0	0	0	0	0	0	0	0	0	0	0
7	12	16	14	16	15	16	16	15	17	17	154
Total	28	30	29	33	30	31	29	28	31	34	303

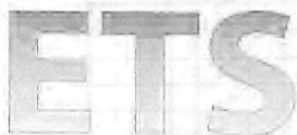
1200 mg NaCl/L

Day	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	2	4	2	3	3	3	4	2	2	1	26
5	0	8	9	9	0	9	5	7	10	6	63
6	7	0	0	0	5	0	0	0	0	0	12
7	10	7	6	5	5	7	9	4	5	6	64
Total	19	19	17	17	13	19	18	13	17	13	165

1400 mg NaCl/L

Day	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	2	1	1	3	1	2	1	1	1	1	14
5	3	0	0	2	0	0	0	0	1	2	8
6	0	1	0	0	0	2	0	0	0	0	3
7	1	0	0	0	2	0	0	1	0	0	4
Total	6	2	1	5	3	4	1	2	2	3	29

Unrevised and
Reviewed by
Program Manager



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 #232

Test dates: August 06-13, 2019

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	31	30	35	31	30	31	29	28	31	32	100	30.8	6.1	Not applicable
600	31	31	29	35	33	35	33	31	31	31	100	32.0	6.1	-3.9
800	31	31	34	29	31	30	30	35	33	33	100	31.7	6.1	-2.9
1000	28	30	29	33	30	31	29	28	31	34	100	30.3	6.6	1.6
1200	19	19	17	17	13	19	18	13	17	13	100	16.5	15.5	46.4
1400	6	2	1	5	3	4	1	2	2	3	100	2.9	57.4	90.6

Dunnett's MSD value: 2.060
PMSD: 6.7

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.

Reviewed and
Reviewed by
Jim Summers

Statistical Analyses

Environmental Testing Solutions, Inc.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 8/6/2019	Test ID: CdNaClCR	Sample ID: REF-Ref Toxicant
End Date: 8/13/2019	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	31.000	30.000	35.000	31.000	30.000	31.000	29.000	28.000	31.000	32.000
600	31.000	31.000	29.000	35.000	33.000	35.000	33.000	31.000	31.000	31.000
800	31.000	31.000	34.000	29.000	31.000	30.000	30.000	35.000	33.000	33.000
1000	28.000	30.000	29.000	33.000	30.000	31.000	29.000	28.000	31.000	34.000
1200	19.000	19.000	17.000	17.000	13.000	19.000	18.000	13.000	17.000	13.000
1400	6.000	2.000	1.000	5.000	3.000	4.000	1.000	2.000	2.000	3.000

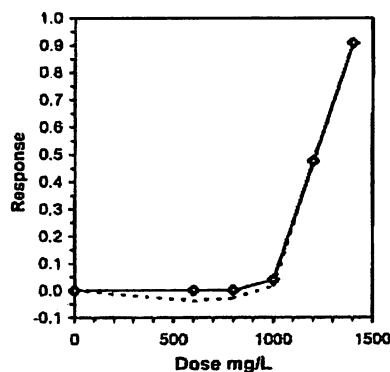
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean
D-Control	30.800	1.0000	30.800	28.000	35.000	6.084	10				31.500	1.0000
600	32.000	1.0390	32.000	29.000	35.000	6.074	10	-1.332	2.287	2.060	31.500	1.0000
800	31.700	1.0292	31.700	29.000	35.000	6.140	10	-0.999	2.287	2.060	31.500	1.0000
1000	30.300	0.9838	30.300	28.000	34.000	6.610	10	0.555	2.287	2.060	30.300	0.9619
*1200	16.500	0.5357	16.500	13.000	19.000	15.452	10	15.871	2.287	2.060	16.500	0.5238
*1400	2.900	0.0942	2.900	1.000	6.000	57.356	10	30.965	2.287	2.060	2.900	0.0921

Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Kolmogorov D Test indicates normal distribution ($p > 0.01$)					0.85068	1.035	0.20009	-0.6092			
Bartlett's Test indicates equal variances ($p = 0.87$)					1.81472	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		2.06035	0.06689	1421.35	4.05926	7.7E-40	5, 54
Treatments vs D-Control											

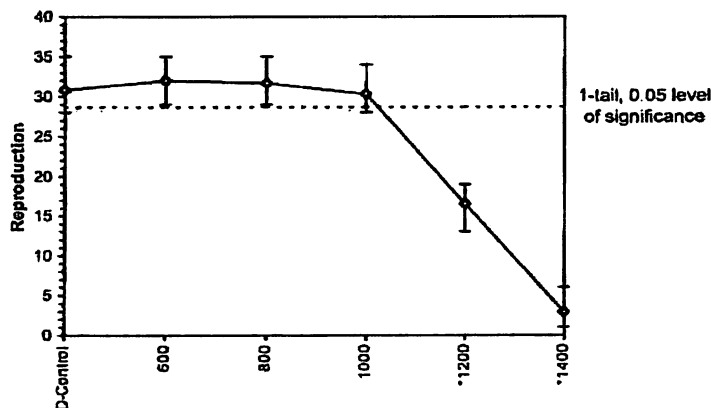
Treatments vs D-Control

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1005.43	28.3614	914.792	1019.97	-1.8546
IC10	1028.26	7.89191	1010.28	1041.48	-0.4520
IC15	1051.09	7.30185	1033.53	1062.52	-0.3950
IC20	1073.91	7.13193	1056.63	1086.42	-0.2653
IC25	1096.74	7.4111	1080.84	1110.32	-0.0917
IC40	1165.22	10.3044	1147.72	1186.7	0.2473
IC50	1211.03	11.3728	1188.69	1231.88	-0.1739



Dose-Response Plot



Reviewed and
Approved by
Date: 8/13/2019

Independent
Review by
Kathy E. Maanen

Species: Ceriodaphnia dubiaCdNaClCR #: 232**Daily Chemistry:**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		Analyst					
Concentration	Parameter						
CONTROL	pH (S.U.)	W	MS	MS	MS	MS	W
	DO (mg/L)	7.99	7.95	7.85	7.92	7.83	7.90
	Conductivity (µmhos/cm)	7.7	7.9	7.8	8.0	7.7	7.9
	*Alkalinity (mg CaCO ₃ /L)	300		317		319	
	*Hardness (mg CaCO ₃ /L)	59		11-14-9		60	
	*Temperature (°C)	84				88	
600 mg NaCl/L	pH (S.U.)	24.8	25.2	24.8	25.2	24.9	24.9
	DO (mg/L)	0.01	7.90	7.91	7.91	7.92	7.90
	Conductivity (µmhos/cm)	7.4	7.8	7.8	8.0	7.7	8.0
	*Temperature (°C)	1400		1430		1440	
800 mg NaCl/L	pH (S.U.)	24.9	25.0	24.9	25.3	25.0	25.2
	DO (mg/L)	0.01	7.91	7.93	7.90	7.93	7.88
	Conductivity (µmhos/cm)	7.7	7.8	7.8	8.1	7.8	8.0
	*Temperature (°C)	1770		1830		1810	
1000 mg NaCl/L	pH (S.U.)	24.9	25.0	24.9	25.0	25.0	25.3
	DO (mg/L)	0.01	7.91	7.93	7.90	7.93	7.88
	Conductivity (µmhos/cm)	7.8	7.9	7.8	8.1	7.9	8.0
	*Temperature (°C)	2140		2190		2180	
1200 mg NaCl/L	pH (S.U.)	25.0	24.9	24.9	25.0	25.0	25.1
	DO (mg/L)	0.00	7.91	7.91	7.90	7.92	7.87
	Conductivity (µmhos/cm)	7.8	7.9	7.9	8.1	7.9	8.0
	*Temperature (°C)	2510		2620		2580	
1400 mg NaCl/L	pH (S.U.)	25.0	24.9	25.0	25.2	25.0	25.3
	DO (mg/L)	7.99	7.90	7.91	7.89	7.94	7.85
	Conductivity (µmhos/cm)	7.9	7.9	7.9	8.1	8.0	8.0
	*Temperature (°C)	2970		3000		2950	
		Initial	Final	Initial	Final	Initial	Final

*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 by: W



Environmental Testing Solutions, Inc.

Species: Ceriodaphnia dubiaCdNaClCR #: 232

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		W	W	W	BSL	BSL	MS	MS	MP
Concentration	Parameter								
CONTROL	pH (S.U.)	7.03	7.96	7.90	8.02	7.87	7.95	7.89	7.91
	DO (mg/L)	7.8	7.9	7.7	8.1	7.93	8.0	7.9	8.0
	Conductivity (µmhos/cm)	324		326		325		322	
	*Alkalinity (mg CaCO ₃ /L)	7.4		58					
	*Hardness (mg CaCO ₃ /L)	7.4		84				7.4	
	*Temperature (°C)	24.8	25.2	24.9	25.0	24.8	25.1	24.7	25.2
600 mg NaCl/L	pH (S.U.)	7.89	7.93	7.90	7.96	7.93	7.94	7.95	7.91
	DO (mg/L)	7.8	7.9	7.6	8.1	8.3	7.9	7.8	8.0
	Conductivity (µmhos/cm)	1460		1410		1430		1460	
	*Temperature (°C)	24.9	25.2	25.0	25.3	24.9	24.8	24.8	25.2
800 mg NaCl/L	pH (S.U.)	7.89	7.93	7.90	7.95	7.95	7.94	7.96	7.92
	DO (mg/L)	7.8	7.9	7.7	8.1	8.4	8.0	7.8	8.0
	Conductivity (µmhos/cm)	1840		1790		1840		1890	
	*Temperature (°C)	24.9	25.0	24.9	25.2	24.8	25.1	24.8	25.2
1000 mg NaCl/L	pH (S.U.)	7.89	7.92	7.91	7.94	7.95	7.94	7.96	7.92
	DO (mg/L)	7.9	8.0	7.7	8.0	8.4	8.0	7.8	8.0
	Conductivity (µmhos/cm)	2200		2160		2210		2230	
	*Temperature (°C)	24.9	24.9	24.9	25.2	24.8	24.9	24.8	25.0
1200 mg NaCl/L	pH (S.U.)	7.89	7.92	7.90	7.93	7.95	7.93	7.95	7.93
	DO (mg/L)	8.0	8.0	7.8	8.0	8.4	8.0	7.8	8.0
	Conductivity (µmhos/cm)	2530		2490		2540		2620	
	*Temperature (°C)	25.0	25.1	24.9	25.2	24.8	24.9	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.90	7.91	7.90	7.92	7.95	7.93	7.95	7.92
	DO (mg/L)	8.0	8.1	7.9	8.0	8.4	8.1	7.8	8.2
	Conductivity (µmhos/cm)	2940		2880		2930		3000	
	*Temperature (°C)	25.0	25.1	24.9	25.0	24.8	25.0	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

*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 by: H

