



Tennessee Valley Authority, Post Office Box 2000, Soddy Daisy, Tennessee 37384-2000

June 12, 2012

Ms: Christina Morgan  
Tennessee Department of Environment  
and Conservation  
Division of Water Pollution Control  
Enforcement & Compliance Section  
6<sup>th</sup> Floor, L & C Annex  
401 Church Street  
Nashville, Tennessee 37243-1534

Dear Ms. Morgan:

TENNESSEE VALLEY AUTHORITY (TVA) - SEQUOYAH NUCLEAR PLANT (SQN) -  
NPDES PERMIT NO. TN0026450 - DISCHARGE MONITORING REPORT (DMR) FOR  
MAY 2012

Enclosed is the May 2012 Discharge Monitoring Report for Sequoyah Nuclear Plant. On May 24, 2012 at 15:27 Eastern Daylight Time (EDT) visible oil sheen was identified on the east side of the essential raw cooling water (ERCW) intake building at SQN on the Tennessee River.

The source of the oil sheen was caused by an oil drip pan overflow located at the C-B ERCW traveling water screen gear drive. The leak was immediately secured and the discharge was stopped. The estimated quantity of oil discharged is less than one gallon. Because the oil spill occurred in a low flow area and favorable meteorological conditions (light winds, no rain), the water was fairly stagnant and the sheen was easily contained and cleaned up utilizing absorbent booms and pads. TVA provided oral notification to Angela Young of the Chattanooga Environmental Field Office via voice message on May 24, 2012. Documentation of the incident and corrective actions are being performed in accordance with the site's Corrective Action Program.

In a letter to Mr. Vojin Janjić dated December 21, 2011 SQN notified TDEC of upcoming activities for the installation of pilings into bedrock to support a crane during the Unit 2 Cycle 18 refueling outage scheduled to begin in October 2012. This project is complete and resulted in the discharge of approximately 700 gallons of water through the SQN low volume waste treatment process. The results for the pH and TSS collected during discharge have been incorporated into the DMR. The results for the TRC have been included as an attachment to the Outfall 103 reporting form.

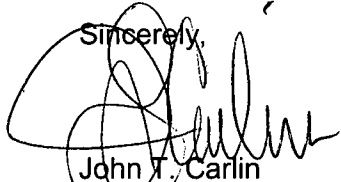
If you have any questions or need additional information, please contact Brad Love by email at [bmlove@tva.gov](mailto:bmlove@tva.gov) or by phone at (423) 843-6714.

IE25  
NR2

Ms. Christina Morgan  
Page 2  
June 12, 2012

*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.*

Sincerely,



John T. Carlin  
Site Vice President  
Sequoyah Nuclear Plant

Enclosures

cc (Enclosures):

Chattanooga Environmental Field Office  
Division of Water Pollution Control  
State Office Building, Suite 550  
540 McCallie Avenue  
Chattanooga, Tennessee 37402-2013

U.S. Nuclear Regulatory Commission  
Attn: Document Control Desk  
Washington, DC 20555

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  
 From YEAR 12 MO 05 DAY 01 To YEAR 12 MO 05 DAY 31

\*\*\* NO DISCHARGE ☐ \*\*\*

NOTE: Read instructions before completing this form.

ATTN: Brad Love

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	*****	*****	**	*****	*****	39.4	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	*****	*****	**	*****	*****	28.3	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	*****	*****	**	*****	*****	1	04	0	31 / 31	CALCTD
00016 1 S	PERMIT REQUIREMENT	*****	*****	****	*****	*****	3 DAILY MX	DEG. C.		CONTI NUOUS	CALCTD
EFFLUENT GROSS											
FLOW, IN CONDUIT OR THRU TREATMENT PLANT	SAMPLE MEASUREMENT	*****	1719	03	*****	*****	*****	**	0	31 / 31	RCORDR
50050 1 0	PERMIT REQUIREMENT	*****	Req. Mon. DAILY MAX	MGD	*****	*****	*****	****		CONTI NUOUS	RCORDR
EFFLUENT GROSS											
CHLORINE, TOTAL RESIDUAL	SAMPLE MEASUREMENT	*****	*****	**	*****	0.019	0.034	19	0	18 / 31	GRAB
50060 1 0	PERMIT REQUIREMENT	*****	*****	****	*****	0.1 MO AVG	0.1 DAILY MAX	MG/L		FIVE PER WEEK	CALCTD
EFFLUENT GROSS											
TEMPERATURE - C, RATE OF CHANGE	SAMPLE MEASUREMENT	*****	0	62	*****	*****		**	0	31 / 31	CALCTD
82234 1 0	PERMIT REQUIREMENT	*****	2 DAILY MX	DEG C/HR	*****	*****	*****	****		CONTI NUOUS	CALCTD
EFFLUENT GROSS											
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER	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.	TELEPHONE		DATE		
		AREA CODE	NUMBER	YEAR	MO	DAY
John T. Carlin		423	843-7001	12	06	12
Sequoyah Site Vice President						
TYPED OR PRINTED						

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

No closed mode operation. Veliger monitoring data is included as an attachment. B/CTP chemical injections during this period included 1. Floguard MS6236 (max. calc. conc. was 0.032mg/L--limit 0.2mg/L) 2. Biodetergent 73551 (max. calc. conc. was 0.032mg/L--limit 2.0mg/L) 3. Spectrus CT1300 (max. calc. conc. was 0.041mg/L--limit 0.050mg/L).

Sample Date	Mean # of ZM/m3	% Settlers	Water Temp. (°C)	Sample Date	Mean# of Asiatic Clams/m3	Water Temp. (°C)	LOCATION	SUB LOCATION	NOTES: % Gravid Asiatic Clam	COLLECTED BY
01/03/2012	14	100	26	01/03/2012	0	26	RCW	1-25-545		PKS
01/10/2012	0	0	9	01/10/2011	0	9				WBE
01/17/2011	0	0	10	01/17/2011	0	10		1-ISV-24-1234		PB
01/24/2012	0	0	13	01/24/2012	0	13		1-25-545		WDT
01/31/2012	0	0	17.6	01/31/2012	0	17.6		1-25-545		CR
02/07/2012	0	0	12	02/07/2012	0	12		1-25-545		BB
02/14/2012	0	0	8.3	02/14/2012	0	8.3		1-24-1234		WE
02/21/2012	0	0	26.5	02/21/2012	0	26.5		1-25-545		CR
02/28/2012	0	0	11.1	02/28/2011	0	11.1	1-ISV-24-1234		WBE	
03/06/2012	0	0	11.7	03/06/2012	0	11.7	1-ISV-24-1234		WBE	
03/13/2012	0	0	13	03/13/2012	0	13	1-ISV-24-1234		WBE	
03/20/2012	0	0	14.6	03/20/2012	0	14.6	1-ISV-24-1234		WBE	
03/27/2012	1623	1.3	17.2	03/27/2012	0	17.2	1-ISV-24-1234		WBE	
04/03/2012	229	0	18	04/03/2012	0	18	1-ISV-24-1234		PB	
04/10/2012	79	20	22	04/10/2012	0	22	1-ISV-24-1234		PB	
04/18/2012	326	5	18.8	04/18/2012	0	18.8	1-ISV-24-1234		MJW	
May 2012										No Samples Collected

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

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**(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)

Form Approved.  
 OMB No. 2040-0004

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

F - FINAL  
 BIOMONITORING FOR OUTFALL 101  
 EFFLUENT

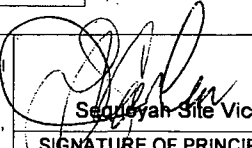
MONITORING PERIOD  
 From **12 05 01** To **12 05 31**

\*\*\* NO DISCHARGE ☐ \*\*\*

NOTE: Read instructions before completing this form.

ATTN: Brad Love

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 TRP3B 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	>100.0	*****	*****	23	0	1 / 180	COMPOS
	PERMIT REQUIREMENT	*****	*****	****	43.2 MINIMUM	*****	*****	PERCENT		SEMI ANNUAL	COMPOS
IC25 STATRE 7DAY CHR PIMEPHALES TRP6C 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	>100.0	*****	*****	23	0	1 / 180	COMPOS
	PERMIT REQUIREMENT	*****	*****	****	43.2 MINIMUM	*****	*****	PERCENT		SEMI ANNUAL	COMPOS
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER  John T. Carlin  Sequoyah 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.	 Sequoyah Site Vice President SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT	TELEPHONE		DATE		
			423	843-7001	12	06	12
			AREA CODE	NUMBER	YEAR	MO	DAY

COMMENTS AND EXPLANATION OF ANY VIOLATIONS (Reference all attachments here)  
 Toxicity sampling was performed May 6 - 11. The report is included as an attachment.

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)

Form Approved.  
 OMB No. 2040-0004

**TN0026450** **103 G**  
**PERMIT NUMBER** **DISCHARGE NUMBER**

F - FINAL  
 LOW VOL. WASTE TREATMENT POND  
 EFFLUENT

MONITORING PERIOD  

YEAR	MO	DAY	YEAR	MO	DAY
12	05	01	12	05	31

From

To

\*\*\* 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	*****	*****	**	7	*****	9	12	0	15 / 31	GRAB
	PERMIT REQUIREMENT	*****	*****	**	6 MINIMUM	*****	9 MAXIMUM	SU		THREE/ WEEK	GRAB
SOLIDS, TOTAL SUSPENDED 00530 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	*****	7	9	19	0	2 / 31	GRAB
	PERMIT REQUIREMENT	*****	*****	**	*****	30 MO AVG	100 DAILY MX	MG/L		TWICE/ MONTH	GRAB
OIL AND GREASE 00556 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	*****	*****	**	*****	<5	<6	19	0	2 / 31	GRAB
	PERMIT REQUIREMENT	*****	*****	**	*****	15 MO AVG	20 DAILY MX	MG/L		TWICE/ MONTH	GRAB
FLOW, IN CONDUIT OR THRU TREATMENT PLANT 50050 1 0 EFFLUENT GROSS	SAMPLE MEASUREMENT	1.038	1.216	03	*****	*****	*****	**	0	31 / 31	RCORDR
	PERMIT REQUIREMENT	Req. Mon. MO AVG	Req. Mon DAILY MX	MGD	*****	*****	*****	**		SEE PERMIT	RCORDR
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER

John T. Carlin

Sequoyah Site Vice President

TYPED OR PRINTED

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Sequoyah Site Vice President

SIGNATURE OF PRINCIPAL EXECUTIVE  
 OFFICER OR AUTHORIZED AGENT

TELEPHONE

423 843-7001

AREA  
 CODE

NUMBER

DATE

12 06 12

YEAR MO DAY

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

Outfall 103 TRC analysis collected during discharge of Steam Generator Process Water from drilling core pilings is included as an attachment.

DMR Attachment

**Outfall 103:** TRC Sample collected during discharge of Steam Generator Project discharge of process water from drilling core pilings. Reference DMR Cover Letter.

<b>Outfall 103</b>			
<b>Date/Time Collected</b>	<b>TRC</b>	<b>Analysis Date/Time</b>	<b>Analyst</b>
<b>5/15/2012 / 11:10</b>	<b>0.03 mg/L</b>	<b>5/15/2012 / 11:24</b>	<b>MLW</b>

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

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

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  

YEAR	MO	DAY	YEAR	MO	DAY
12	05	01	12	05	31

From

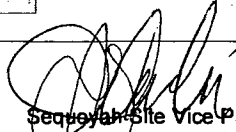
To

\*\*\* NO DISCHARGE ☒ \*\*\*

NOTE: Read instructions before completing this form.

ATTN: Brad Love

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	CALCULATED
EFFLUENT GROSS VALUE											
TEMPERATURE, WATER DEG. CENTIGRADE	SAMPLE MEASUREMENT	*****	*****	**	*****	*****		04			
00010 Z 0	PERMIT REQUIREMENT	*****	*****	**	*****	*****	30.5 DAILY MX	DEG C		CONTINUOUS	CALCULATED
INSTREAM MONITORING											
TEMP. DIFF. BETWEEN SAMP. & UPSTRM DEG.C	SAMPLE MEASUREMENT	*****	*****	**	*****	*****		04			
00016 1 0	PERMIT REQUIREMENT	*****	*****	**	*****	*****	5 DAILY MX	DEG C		CONTINUOUS	CALCULATED
EFFLUENT GROSS VALUE											
FLOW, IN CONDUIT OR THRU TREATMENT PLANT	SAMPLE MEASUREMENT	*****		03	*****	*****	*****	**			
50050 1 0	PERMIT REQUIREMENT	*****	Req. Mon. DAILY MX	MGD	*****	*****	*****	**		CONTINUOUS	RECORD
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	CALCULATED
EFFLUENT GROSS VALUE											
TEMPERATURE - C, RATE OF CHANGE	SAMPLE MEASUREMENT	*****		04	*****	*****	*****	**			
82234 1 0	PERMIT REQUIREMENT	*****	2 DAILY MX	DEG C	*****	*****	*****	**		CONTINUOUS	CALCULATED
EFFLUENT GROSS VALUE											
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER  John T. Carlin  Sequoyah 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.	 Sequoyah Site Vice President SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT	TELEPHONE		DATE		
			423	843-7001	12	06	12
			AREA CODE	NUMBER	YEAR	MO	DAY

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

No Discharge this Period



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

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 Location **HAMILTON COUNTY**

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

MAJOR  
 (SUBR 01)

Form Approved.  
 OMB No. 2040-0004

**TN0026450** **110 T**  
**PERMIT NUMBER** **DISCHARGE NUMBER**

F - FINAL  
 RECYCLED COOLING WATER  
 EFFLUENT

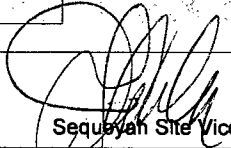
MONITORING PERIOD  
 YEAR MO DAY YEAR MO DAY  
 From **12 05 01** To **12 05 31**

\*\*\* NO DISCHARGE ☒ \*\*\*

ATTN: Brad Love

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PARAMETER		QUANTITY OR LOADING			QUALITY OR CONCENTRATION				NO. EX	FREQUENCY OF ANALYSIS	SAMPLE TYPE
		AVERAGE	MAXIMUM	UNITS	MINIMUM	AVERAGE	MAXIMUM	UNITS			
IC25 STATRE 7DAY CHR CERIODAPHNIA	SAMPLE MEASUREMENT	*****	*****	**		*****	*****	23			
TRP3B 1 0 0 EFFLUENT GROSS VALUE	PERMIT REQUIREMENT	*****	*****	****	43.2 MINIMUM	*****	*****	PERCENT		SEMI ANNUAL	COMPOS
IC25 STATRE 7DAY CHR PIMEPHALES	SAMPLE MEASUREMENT	*****	*****	**		*****	*****	23			
TRP6C 1 0 0 EFFLUENT GROSS VALUE	PERMIT REQUIREMENT	*****	*****	****	43.2 MINIMUM	*****	*****	PERCENT		SEMI ANNUAL	COMPOS
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
	PERMIT REQUIREMENT										
	SAMPLE MEASUREMENT										
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	PERMIT REQUIREMENT										

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			423	843-7001	12	06	12
			AREA CODE	NUMBER	YEAR	MO	DAY

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

No Discharge this Period

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

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

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  
 YEAR MO DAY YEAR MO DAY  
 From **12 05 01** To **12 05 31**

\*\*\* NO DISCHARGE ☒ \*\*\*

ATTN: Brad Love

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

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER

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.

Sequoyah Site Vice President

SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT

TELEPHONE

DATE

423 843-7001

12 06 12

TYPED OR PRINTED

AREA CODE NUMBER YEAR MO DAY

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

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

June 11, 2012

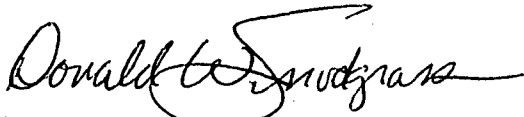
Bradley M. Love, OPS 5N-SQN

SEQUOYAH NUCLEAR PLANT (SQN) TOXICITY BIOMONITORING, NPDES PERMIT  
NO. TN0026450, COMPLIANCE TOXICITY TESTS, MAY, 2012

Per your request, I have also submitted an electronic copy of the subject report. Outfall 101 samples collected May 06-11, 2012, showed no toxic effects to fathead minnows or daphnids. The resulting  $IC_{25}$  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.

Fathead minnows were also exposed to UV treated Outfall 101 and intake samples since fish pathogens present in intake water have been the suspected cause of interference (anomalous dose response and high variability among replicates) in previous toxicity testing at Sequoyah. At the time this study was conducted, insignificant mortality occurred in minnows exposed to non-treated and UV-treated samples.

Please call me at your convenience if you have any questions or comments following your review of the report.



Donald W. Snodgrass  
Manager,  
Environmental Engineering Field Team-West  
CTR 2L - M

Attachment

Cc (Attachment):

R.M. Sherrard, PSC 1X - C (Electronic copy)  
EDMS, MPB 1E - M (Electronic copy)

**TENNESSEE VALLEY AUTHORITY  
TOXICITY TEST REPORT**

**INTRODUCTION / EXECUTIVE SUMMARY**

Report Date: June 11, 2012

1. Facility / Discharger: Sequoyah Nuclear Plant / TVA
2. County / State: Hamilton / Tennessee
3. NPDES Permit #: TN0026450
4. Type of Facility: Nuclear-Fueled Electric Generating Plant
5. Design Flow (MGD): 1,579
6. Receiving Stream: Tennessee River (TRM 483.6)
7. 1Q10: 3,491
8. Outfall Tested: 101
9. Dates Sampled: May 06 – 11, 2012
10. Average Flow on Days Sampled (MGD): 1715.29, 1713.71, 1700.72
11. Pertinent Site Conditions: Production / operation data will be provided upon request.
12. Test Dates: May 08 – 15, 2012
13. Test Type: Short-term Chronic Definitive
14. Test Species: Fathead Minnows (*Pimephales promelas*)  
Daphnids (*Ceriodaphnia dubia*)
15. Concentrations Tested (%): Outfall 101: 10.8, 21.6, 43.2, 86.4, 100  
Intake: 100  
*Pimephales promelas*: UV treated Outfall 101: 10.8, 21.6, 43.2, 86.4, 100  
UV treated Intake: 100
16. Permit Limit Endpoint (%): Outfall 101:  $IC_{25} = 43.2\%$
17. Test Results: Outfall 101: *Pimephales promelas*:  $IC_{25} > 100\%$   
*Ceriodaphnia dubia*:  $IC_{25} > 100\%$   
UV treated Outfall 101: *Pimephales promelas*:  $IC_{25} > 100\%$

18. Facility Contact: Brad Love 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: Outfall 101 samples collected May 06 – 11, 2012, showed no toxic effects to fathead minnows or daphnids. The resulting  $IC_{25}$  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.

Fathead minnows were also exposed to UV treated Outfall 101 and intake samples since fish pathogens present in intake water have been the suspected cause of interference (anomalous dose response and high variability among replicates) in previous toxicity testing at Sequoyah. At the time this study was conducted, insignificant mortality occurred in minnows exposed to non-treated and UV treated samples.

## METHODS SUMMARY

### Samples:

1. Sampling Point: Outfall 101, Intake
2. Sample Type: Composite
3. Sample Information:

Sample ID	Date (MM-DD-YY) Time (ET) Collected	Date (MM-DD-YY) Time (ET) Received	Arrival Temp. (°C)	Initial TRC* (mg/L)	Date (MM-DD-YY) Time (ET) Last Used By
101	05-06-12 0855 to 05-07-12 0755	05-07-12 1429	1.8, 1.8 <sup>†</sup>	<0.10	05-08-12 1130 05-09-12 1030
Intake	05-06-12 0910 to 05-07-12 0810	05-07-12 1429	1.0	<0.10	05-08-12 1130 05-09-12 1030
101	05-08-12 0655 to 05-09-12 0555	05-09-12 1300	1.6, 1.7 <sup>†</sup>	<0.10	05-10-12 1053 05-11-12 1030
Intake	05-08-12 0710 to 05-09-12 0610	05-09-12 1300	1.6	<0.10	05-10-12 1053 05-11-12 1030
101	05-10-12 0655 to 05-11-12 0555	05-11-12 1225	0.5, 0.6 <sup>†</sup>	<0.10	05-12-12 1030 05-13-12 1032 05-14-12 1030
Intake	05-10-12 0710 to 05-11-12 0610	05-11-12 1225	2.1	<0.10	05-12-12 1030 05-13-12 1032 05-14-12 1030

\*TRC = Total Residual Chlorine

<sup>†</sup>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.

*Pimephales promelas*

*Ceriodaphnia dubia*

Test Organisms:

- |            |                              |                          |
|------------|------------------------------|--------------------------|
| 1. Source: | <u>Aquatox, Inc.</u>         | <u>In-house Cultures</u> |
| 2. Age:    | <u>19.2 – 19.5 hours old</u> | <u>&lt; 24-hours old</u> |

Test Method Summary:

- |                                   |  |  |
|-----------------------------------|--|--|
| 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)   |  |  |
| Outfall 101                       | <u>05-08-12 1130 ET</u>                        | <u>05-08-12 0935 ET</u>                                    |
| UV Treated Outfall 101            | <u>05-08-12 1112 ET</u>                        |  |
| 7. Test Termination: (Date/Time)  |  |  |
| Outfall 101                       | <u>05-15-12 1030 ET</u>                        | <u>05-15-12 0842 ET</u>                                    |
| UV Treated Outfall 101            | <u>05-15-12 1015 ET</u>                        |  |
| 8. Test Temperature: Outfall 101: | <u>Mean = 24.8°C</u><br><u>(24.5 – 25.1°C)</u> | <u>Mean = 25.0°C</u><br><u>(24.7 – 25.3°C)</u>             |

Test Temperature: UV-Treated Outfall 101: Mean = 24.8°C  
(24.3 – 25.2°C)

9. Physical / Chemical Measurements: Alkalinity, hardness, total residual chlorine, and conductivity were measured at the laboratory in each 100% sample. Daily temperatures were measured in one replicate for each test concentration. Pre- and post-exposure test solutions were analyzed daily for pH and dissolved oxygen.
10. Statistics: Statistics were performed according to methods prescribed by EPA using ToxCalc version 5.0 statistical software (Tidepool Scientific Software, McKinneyville, CA).

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

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

Conducted May 08 – 15, 2012 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
10.8%	100	100	100	100	100	100	100
21.6%	100	100	100	100	98	98	98
43.2%	100	100	100	100	100	100	100
86.4%	100	100	100	100	100	100	100
100.0%	100	100	100	100	100	100	100
Intake	100	100	100	100	100	100	100

Test Solutions (% Effluent)	Mean Dry Weight (mg) (replicate number)				
	1	2	3	4	Mean
Control	0.658	0.686	0.707	0.778	0.707
10.8%	0.624	0.648	0.699	0.647	0.655
21.6%	0.629	0.717	0.594	0.716	0.664
43.2%	0.678	0.674	0.642	0.674	0.667
86.4%	0.660	0.604	0.657	0.648	0.642
100.0%	0.621	0.634	0.586	0.682	0.631
Intake	0.734	0.634	0.693	0.716	0.694
IC <sub>25</sub> Value: <u>&gt; 100%</u> Permit Limit: <u>43.2%</u>  95% Confidence Limits: Upper Limit: <u>NA</u> Lower Limit: <u>NA</u>			Calculated TU Estimates: <u>&lt; 1.0 TUc*</u>  Permit Limit: <u>2.3 TUc</u>		

\*TUa = 100/LC<sub>50</sub>; TUc = 100/ IC<sub>25</sub>



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

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

Conducted May 08 – 15, 2012 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
10.8%	100	100	100	100	100	100	100
21.6%	100	100	100	100	100	100	100
43.2%	100	100	100	100	100	100	100
86.4%	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	33	32	30	31	36	30	33	34	31	29	31.9
10.8%	33	33	30	33	31	32	28	35	35	32	32.2
21.6%	36	32	36	33	31	32	35	32	35	33	33.5
43.2%	35	31	33	39	33	30	35	37	33	33	33.9
86.4%	34	36	32	35	37	35	34	34	33	35	34.5
100.0%	37	36	35	35	37	38	33	36	35	36	35.8
IC <sub>25</sub> Value: <u>&gt; 100%</u> Permit Limit: <u>43.2%</u>  95% Confidence Limits: Upper Limit: <u>NA</u> Lower Limit: <u>NA</u>						Calculated TU Estimates: <u>&lt; 1.0 TUc*</u>  Permit Limit: <u>2.3 TUc</u>					

\*TUa = 100/LC<sub>50</sub>; TUc = 100/ IC<sub>25</sub>

TOXICITY TEST RESULTS (see Appendix C for Bench Sheets)

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

Conducted May 08 – 15, 2012 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	30	33	33	30	31	29	32	31	31	26	30.6
Intake	33	35	29	32	31	36	32	31	33	31	32.3
IC <sub>25</sub> Value: <u>&gt; 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>&lt; 1.0 TUc*</u>  Permit Limit: <u>N/A</u>							

\*TUa = 100/LC<sub>50</sub>; TUc = 100/ IC<sub>25</sub>

**TOXICITY TEST RESULTS, UV-TREATED** (see Appendix C for Bench Sheets)

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

Conducted May 08 – 15, 2012 using effluent from UV Treated 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
10.8%	100	100	100	100	100	100	100
21.6%	100	100	100	100	100	100	100
43.2%	100	100	100	100	100	100	100
86.4%	100	100	100	100	100	100	100
100.0%	100	100	100	100	100	100	100
Intake	100	100	98	98	98	98	98

Test Solutions (% Effluent)	Mean Dry Weight (mg) (replicate number)				
	1	2	3	4	Mean
Control	0.591	0.647	0.507	0.599	0.586
10.8%	0.646	0.621	0.616	0.698	0.645
21.6%	0.553	0.622	0.659	0.663	0.624
43.2%	0.565	0.609	0.634	0.606	0.604
86.4%	0.537	0.573	0.610	0.560	0.570
100.0%	0.673	0.680	0.676	0.653	0.671
Intake	0.597	0.592	0.561	0.704	0.614
IC <sub>25</sub> Value: <u>&gt; 100%</u>			Calculated TU Estimates: <u>&lt; 1.0 TUc*</u>		
95% Confidence Limits:					
Upper Limit: <u>NA</u>					
Lower Limit: <u>NA</u>					

\*TUa = 100/LC<sub>50</sub>; TUc = 100/ IC<sub>25</sub>

**REFERENCE TOXICANT TEST RESULTS** (see Appendix A and D)

Species	Date	Time	Duration	Toxicant	Results (IC <sub>25</sub> )
<i>Pimephales promelas</i>	May 08 – 15, 2012	1236	7 days	KCl	0.77 g/L
<i>Ceriodaphnia dubia</i>	May 08 – 15, 2012	0908	7 days	NaCl	1.06 g/L

**PHYSICAL/CHEMICAL SUMMARY**

Water Chemistry Mean Values and Ranges for *Pimephales promelas* and *Ceriodaphnia dubia* Tests, Non-treated Sequoyah Nuclear Plant (SQN) Outfall 101 performed May 08-15, 2012.

Test	Sample ID	Temperature (°C)		Dissolved Oxygen (mg/L)		pH (S.U.)		Conductance (µmhos/cm)	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Total Residual Chlorine (mg/L)
		Initial	Final	Initial	Final	Initial	Final				
<i>Pimephales promelas</i>	Control	24.8	24.7	7.6	7.2	7.96	7.72	310	63	88	
		24.8 - 24.9	24.6 - 25.0	7.4 - 7.8	6.4 - 8.0	7.85 - 8.06	7.49 - 7.90	294 - 327	62 - 63	87 - 89	
	10.8%	24.9	24.7	7.8	7.2	7.97	7.68	293			
		24.8 - 25.0	24.5 - 24.9	7.6 - 8.0	6.4 - 7.9	7.89 - 8.10	7.49 - 7.86	283 - 306			
	21.6%	24.9	24.7	7.8	7.1	7.98	7.68	283			
		24.8 - 25.0	24.5 - 24.9	7.6 - 8.0	6.5 - 7.9	7.89 - 8.10	7.49 - 7.86	276 - 291			
	43.2%	24.9	24.7	7.8	7.2	7.96	7.71	260			
		24.8 - 25.0	24.6 - 25.1	7.6 - 8.0	6.5 - 8.0	7.86 - 8.11	7.47 - 7.90	253 - 265			
	86.4%	24.9	24.7	7.9	7.2	7.95	7.72	213			
		24.8 - 25.0	24.6 - 25.0	7.6 - 8.0	6.5 - 8.0	7.81 - 8.12	7.46 - 7.90	210 - 218			
	100.0%	25.0	24.7	7.9	7.2	7.94	7.73	195	80	81	< 0.10
		24.8 - 25.0	24.5 - 25.0	7.6 - 8.0	6.2 - 8.0	7.76 - 8.16	7.42 - 7.92	188 - 199	79 - 81	81 - 81	< 0.10 - < 0.10
	Intake	25.0	24.8	7.9	7.3	8.06	7.75	190	78	80	< 0.10
		24.9 - 25.1	24.7 - 25.1	7.7 - 8.1	6.7 - 8.0	7.95 - 8.16	7.53 - 7.93	182 - 195	77 - 79	79 - 81	< 0.10 - < 0.10
<i>Ceriodaphnia dubia</i>	Control	24.8	25.0	7.6	7.8	7.96	7.95	310	63	88	
		24.7 - 25.0	24.9 - 25.2	7.4 - 7.8	7.6 - 8.0	7.85 - 8.06	7.87 - 8.02	294 - 327	62 - 63	87 - 89	
	10.8%	24.9	25.0	7.8	7.8	7.97	7.95	293			
		24.7 - 25.2	24.8 - 25.3	7.6 - 8.0	7.6 - 8.0	7.89 - 8.10	7.86 - 8.03	283 - 306			
	21.6%	24.9	25.0	7.8	7.8	7.98	7.95	283			
		24.7 - 25.2	24.8 - 25.2	7.6 - 8.0	7.6 - 8.0	7.89 - 8.10	7.86 - 8.02	276 - 291			
	43.2%	24.9	25.0	7.8	7.8	7.96	7.95	260			
		24.8 - 25.2	24.7 - 25.2	7.6 - 8.0	7.6 - 8.1	7.86 - 8.11	7.85 - 8.03	253 - 265			
	86.4%	24.9	25.0	7.9	7.8	7.95	7.98	213			
		24.8 - 25.2	25.0 - 25.2	7.6 - 8.0	7.6 - 8.0	7.81 - 8.12	7.86 - 8.07	210 - 218			
	100.0%	25.0	25.0	7.9	7.8	7.94	8.00	195	80	81	< 0.10
		24.8 - 25.2	24.8 - 25.2	7.6 - 8.0	7.6 - 8.1	7.76 - 8.16	7.87 - 8.06	188 - 199	79 - 81	81 - 81	< 0.10 - < 0.10
	Intake	25.0	25.0	7.9	7.9	8.06	7.98	190	78	80	< 0.10
		24.8 - 25.2	24.8 - 25.2	7.7 - 8.1	7.8 - 8.1	7.95 - 8.16	7.87 - 8.03	182 - 195	77 - 79	79 - 81	< 0.10 - < 0.10

Overall temperature (°C)	Average	Minimum	Maximum
<i>Pimephales promelas</i>	24.8	24.5	25.1
<i>Ceriodaphnia dubia</i>	25.0	24.7	25.3

**PHYSICAL/CHEMICAL SUMMARY**

Water Chemistry Mean Values and Ranges for *Pimephales promelas* Test, UV-treated Sequoyah Nuclear Plant (SQN) Outfall 101  
performed May 08-15, 2012.

Test	Sample ID	Temperature (°C)		Dissolved Oxygen (mg/L)		pH (S.U.)		Conductance (µmhos/cm)	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Total Residual Chlorine (mg/L)
		Initial	Final	Initial	Final	Initial	Final				
<i>Pimephales promelas</i>	Control	24.8	24.7	7.9	7.3	7.97	7.72	307	63	88	
		24.7 - 24.9	24.5 - 24.8	7.6 - 8.0	6.5 - 8.0	7.88 - 8.07	7.51 - 7.91	300 - 316	62 - 64	87 - 89	
	10.8%	24.9	24.6	7.9	7.2	7.97	7.70	296			
		24.8 - 25.0	24.4 - 24.9	7.6 - 8.0	6.6 - 7.9	7.88 - 8.09	7.50 - 7.85	283 - 305			
	21.6%	24.9	24.7	7.9	7.2	7.97	7.71	283			
		24.8 - 25.0	24.5 - 24.9	7.6 - 8.0	6.5 - 8.0	7.89 - 8.10	7.50 - 7.90	269 - 291			
	43.2%	24.9	24.6	7.9	7.2	7.96	7.71	260			
		24.8 - 25.1	24.5 - 24.8	7.6 - 8.0	6.5 - 7.9	7.86 - 8.11	7.50 - 7.89	248 - 266			
	86.4%	24.9	24.7	7.9	7.2	7.94	7.74	216			
		24.8 - 25.1	24.6 - 24.9	7.5 - 8.0	6.5 - 7.9	7.79 - 8.13	7.50 - 7.90	207 - 221			
	100.0%	25.0	24.6	7.9	7.2	7.94	7.74	198	79	81	< 0.10
		24.8 - 25.2	24.3 - 24.8	7.6 - 8.1	6.5 - 7.9	7.76 - 8.15	7.50 - 7.91	189 - 201	79 - 79	81 - 81	< 0.10 - < 0.10
	Intake	25.0	24.7	8.0	7.2	8.02	7.77	192	78	80	< 0.10
		24.8 - 25.2	24.5 - 24.9	7.6 - 8.2	6.6 - 8.0	7.94 - 8.16	7.52 - 7.95	184 - 196	77 - 79	79 - 81	< 0.10 - < 0.10

Overall temperature (°C)	Average	Minimum	Maximum
<i>Pimephales promelas</i>	24.8	24.3	25.2

## **SUMMARY / CONCLUSIONS**

Outfall 101 samples collected May 06 – 11, 2012, showed no toxic effects to fathead minnows or daphnids. The resulting  $IC_{25}$  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.

Fathead minnows were also exposed to UV treated Outfall 101 and intake samples since fish pathogens present in intake water have been the suspected cause of interference (anomalous dose response and high variability among replicates) in previous toxicity testing at Sequoyah. At the time this study was conducted, insignificant mortality occurred in minnows exposed to non-treated and UV treated samples.

## 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*

None

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.
4. Dissolved oxygen was measured by SM 4500 O G.
5. The pH was measured by SM 4500 H+ B.
6. Conductance was measured by SM 2510 B.
7. Alkalinity was measured by SM 2320 B.
8. Total hardness was measured by SM 2340 C.
9. Total residual chlorine was measured by ORION Electrode Method 97-70.

## **QUALITY ASSURANCE**

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

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

1. Test Type: 7-day chronic tests with results expressed as IC<sub>25</sub> 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, 21<sup>st</sup> Edition, 2005.
4. Quality Assurance Program: Standard Operating Procedures, Environmental Testing Solutions, Inc (most current version).

**Sequoyah Nuclear Plant Biomonitoring  
May 08 – 15, 2012**

**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**

Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge  
Concentrations of Chemicals Used to Control Microbiologically Induced Corrosion  
Mollusks, During Toxicity Test Sampling,  
March 12, 1998 – May 11, 2012

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
03/12/1998	0.016	-	-	-	-	-	-
03/13/1998	0.015	-	-	-	-	-	-
03/14/1998	0.013	-	-	-	-	-	-
03/15/1998	0.030	-	-	-	-	-	-
03/16/1998	0.013	-	-	-	-	-	-
03/17/1998	0.020	-	-	-	-	-	-
03/18/1998	0.018	-	-	-	-	-	-
09/08/1998	0.015	-	0.014	0.005	-	-	0.021
09/09/1998	0.003	-	0.031	0.011	-	-	-
09/10/1998	0.014	-	0.060	0.021	-	-	-
09/11/1998	0.013	-	0.055	0.019	-	-	-
09/12/1998	< 0.001	-	0.044	0.015	-	-	-
09/13/1998	< 0.001	-	0.044	0.015	-	-	-
09/14/1998	0.008	-	0.044	0.015	-	-	-
02/22/1999	< 0.001	-	-	-	-	-	-
02/23/1999	0.005	-	-	-	-	-	-
02/24/1999	0.009	-	-	-	-	-	-
02/25/1999	0.012	-	-	-	-	-	-
02/26/1999	0.008	-	-	-	-	-	-
02/27/1999	< 0.001	-	-	-	-	-	-
02/28/1999	< 0.001	-	-	-	-	-	-
08/18/1999	-	0.015	0.069	0.024	0.006	-	-
08/19/1999	-	0.012	0.068	0.024	-	-	-
08/20/1999	-	0.023	0.070	0.024	-	0.120	-
08/21/1999	-	0.022	0.068	0.024	-	-	-
08/22/1999	-	0.022	0.068	0.024	-	-	-
08/23/1999	-	0.025	0.068	0.024	0.006	-	-
08/24/1999	-	0.016	0.067	0.023	0.020	-	-

**Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of  
Chemicals Used to Control Microbiologically Induced Corrosion Mollusks, During  
Toxicity Test Sampling,  
March 12, 1998 – May 11, 2012**

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
01/31/2000	-	< 0.002	0.026	0.009	-	-	-
02/01/2000	-	0.011	0.026	0.028	-	-	-
02/02/2000	-	0.028	0.026	0.009	0.006	-	-
02/03/2000	-	0.008	0.027	0.009	-	-	-
02/04/2000	-	0.006	0.027	0.009	0.005	0.109	-
02/05/2000	-	< 0.002	0.027	0.009	-	-	-
02/06/2000	-	< 0.002	0.027	0.009	-	-	-
07/26/2000	-	< 0.0057	0.055	0.019	-	-	-
07/27/2000	-	0.019	0.055	0.019	-	-	-
07/28/2000	-	0.0088	0.053	0.018	0.004	0.108	-
07/29/2000	-	< 0.0088	0.055	0.019	-	-	-
07/30/2000	-	< 0.0076	0.055	0.019	-	-	-
07/31/2000	-	< 0.0152	0.055	0.019	0.006	-	-
08/01/2000	-	< 0.0141	0.055	0.019	0.005	-	-
12/11/2000	-	0.0143	0.025	0.020	0.005	-	-
12/12/2000	-	0.0092	0.025	0.020	0.005	-	-
12/13/2000	-	< 0.0120	0.025	0.020	-	-	-
12/14/2000	-	< 0.0087	0.025	0.020	-	-	-
12/15/2000	-	0.0120	0.025	0.020	0.005	-	-
12/16/2000	-	< 0.0036	0.025	0.020	-	-	-
12/17/2000	-	< 0.0036	0.025	0.020	-	-	-
08/26/2001	-	0.017	0.06	0.021	0.006	-	-
08/27/2001	-	<0.0096	0.06	0.021	0.005	-	0.021
08/28/2001	-	<0.0085	0.06	0.021	-	-	-
08/29/2001	-	<0.0094	0.059	0.020	0.005	-	0.021
08/30/2001	-	<0.0123	0.06	0.021	0.005	-	-
08/31/2001	-	<0.005	0.059	0.020	-	-	-
11/25/2001	-	<0.0044	-	-	-	-	-
11/26/2001	-	<0.0119	0.024	0.02	0.005	-	-
11/27/2001	-	0.0137	0.023	0.019	0.007	-	-
11/28/2001	-	<0.0089	0.022	0.019	0.006	-	-
11/29/2001	-	0.0132	0.024	0.02	0.007	-	-
11/30/2001	-	< 0.0043	0.024	0.02	-	-	-
12/09/2001	-	<0.0042	-	-	-	-	-
12/10/2001	-	<0.0042	-	-	-	-	-
12/11/2001	-	<0.0104	-	-	-	-	-
12/12/2001	-	0.0128	0.024	0.02	0.008	-	-
12/13/2001	-	<0.0088	0.024	0.02	-	-	-
12/14/2001	-	0.0134	0.024	0.02	0.007	-	-

Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of Chemicals Used to Control Microbiologically Induced Corrosion Mollusks, During Toxicity Test Sampling, March 12, 1998 – May 11, 2012

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
01/02/2002	-	< 0.0079	0.023	0.02	0.006	-	-
01/03/2002	-	< 0.0042	0.023	0.014	-	-	-
01/04/2002	-	0.0124	0.024	0.014	0.009	-	-
01/05/2002	-	< 0.0042	-	-	-	-	-
01/06/2002	-	< 0.0042	-	-	-	-	-
01/07/2002	-	< 0.0089	0.024	0.014	0.006	-	-
02/24/2002	-	< 0.004	-	-	-	-	-
02/25/2002	-	< 0.004	0.023	0.023	-	-	-
02/26/2002	-	0.0143	0.023	0.023	0.007	-	-
02/27/2002	-	< 0.0041	0.023	0.023	-	-	-
02/28/2002	-	< 0.0041	0.024	0.008	-	-	-
03/01/2002	-	< 0.0041	0.024	0.008	-	-	-
05/05/2002	-	-	-	-	-	-	-
05/06/2002	-	-	0.058	0.02	0.014	-	-
05/07/2002	-	-	0.058	0.02	0.015	-	-
05/08/2002	-	-	0.056	0.019	-	-	-
05/09/2002	-	-	0.057	0.02	0.014	-	-
05/10/2002	-	-	0.056	0.019	-	-	-
08/04/2002	-	<0.0058	-	-	-	-	-
08/05/2002	-	<0.0058	0.053	0.018	-	-	0.025
08/06/2002	-	0.0092	0.053	0.018	-	-	-
08/07/2002	-	<0.0107	0.055	0.019	0.007	-	-
08/08/2002	-	<0.0061	0.055	0.019	-	-	-
08/09/2002	-	0.0152	0.054	0.018	0.008	-	-
10/06/2002	-	<0.00497	-	-	-	-	-
10/07/2002	-	0.0153	0.054	0.018	0.009	-	-
10/08/2002	-	<0.0092	0.054	0.018	0.007	-	-
10/09/2002	-	0.0124	0.053	0.018	0.009	-	-
10/10/2002	-	0.0134	0.054	0.018	0.009	-	-
10/11/2002	-	<0.0042	0.054	0.018	-	-	-
01/12/2003	-	<0.0035	-	-	-	-	-
01/13/2003	-	<0.006	0.025	0.019	0.009	-	-
01/14/2003	-	<0.0118	0.026	0.020	-	-	-
01/15/2003	-	<0.0063	0.026	0.020	0.009	-	-
01/16/2003	-	<0.0034	0.026	0.020	-	-	-
01/17/2003	-	<0.0034	0.026	0.009	-	-	-
04/06/2003	-	<0.0073	-	-	-	-	-
04/07/2003	-	<0.0189	-	0.021	-	-	-
04/08/2003	-	<0.0117	-	0.021	-	-	-
04/09/2003	-	<0.0139	-	0.021	0.016	-	-
04/10/2003	-	<0.0113	-	0.021	0.018	-	-
04/11/2003	-	<0.0073	-	0.022	-	-	-

Table B-1 (continued). Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of Chemicals Used to Control Growth of Microbiologically Induced Bacteria and Mollusks, During Toxicity Test Sampling, March 12, 1998 – May 11, 2012

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
06/15/2003	-	< 0.0045	-	-	-	-	-
06/16/2003	-	< 0.0037	0.057	0.020	-	-	0.022
06/17/2003	-	< 0.0048	0.041	0.014	-	-	0.024
06/18/2003	-	< 0.0048	0.041	0.014	-	-	0.024
06/19/2003	-	< 0.0085	0.058	0.020	-	-	0.025
06/20/2003	-	< 0.0048	0.058	0.020	-	-	0.025
08/03/2003	-	<0.0050	-	-	-	-	-
08/04/2003	-	<0.0050	0.058	0.020	-	-	-
08/05/2003	-	<0.0051	0.057	0.020	-	-	0.025
08/06/2003	-	<0.0084	0.057	0.020	-	-	0.025
08/07/2003	-	0.0129	0.057	0.020	-	-	0.024
08/08/2003	-	0.0153	0.057	0.020	0.009	-	-
10/05/2003	-	<0.0043	0.057	0.020	-	-	-
10/06/2003	-	<0.0043	0.057	0.020	-	-	0.025
10/07/2003	-	<0.0090	0.057	0.020	-	-	0.025
10/08/2003	-	<0.0106	0.057	0.020	-	-	0.025
10/09/2003	-	0.0181	0.026	0.022	-	-	0.025
10/10/2003	-	0.0183	0.026	0.024	0.009	-	-
02/01/2004	-	0.0093	0.027	0.009	-	-	-
02/02/2004	-	<0.0034	0.026	0.009	-	-	-
02/03/2004	-	<0.0034	0.026	0.009	-	-	-
02/04/2004	-	0.0124	0.026	0.009	0.009	-	-
02/05/2004	-	<0.0034	0.026	0.009	-	-	-
02/06/2004	-	0.0105	0.026	0.009	0.010	-	-
05/04/2004	-	<0.0123	0.026	0.019	-	-	0.025
05/05/2004	-	<0.0144	0.026	0.014	0.009	-	0.025
05/06/2004	-	<0.0146	0.037	0.013	-	-	0.025
05/07/2004	-	0.0227	0.058	0.020	0.009	-	0.025
05/08/2004	-	0.016	0.060	0.021	-	-	-
05/09/2004	-	<0.0104	0.058	0.020	-	-	-
07/04/2004	-	0.0217	0.057	0.019	-	-	-
07/05/2004	-	<0.0085	0.057	0.020	0.009	-	-
07/06/2004	-	<0.0077	0.058	0.020	-	-	0.031
07/07/2004	-	0.0252	0.056	0.019	-	-	0.031
07/08/2004	-	0.0223	0.057	0.019	0.009	-	-
07/09/2004	-	0.0182	0.057	0.020	0.009	-	-

**Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of  
Chemicals Used to Control Microbiologically Induced Corrosion Mollusks, During  
Toxicity Test Sampling,  
March 12, 1998 – May 11, 2012**

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
11/07/2004	-	<0.0187	0.000	0.014	-	-	-	-	-
11/08/2004	-	<0.0192	0.047	0.030	-	-	-	-	-
11/09/2004	-	<0.0233	0.048	0.016	-	-	0.041	-	-
11/10/2004	-	<0.0149	0.047	0.016	-	-	0.041	-	-
11/11/2004	-	<0.0149	0.049	0.017	-	-	0.043	-	-
11/12/2004	-	<0.0253	0.048	0.017	-	-	0.042	-	-
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	-	-	-	-	-	-	-

Table B-1 (continued). Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge  
Concentrations of Chemicals Used to Control Growth of Microbiologically Induced  
Bacteria and Mollusks, During Toxicity Test Sampling,  
March 12, 1998 – May 11, 2012

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



**Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of  
Chemicals Used to Control Microbiologically Induced Corrosion Mollusks, During Toxicity Test  
Sampling,  
March 12, 1998 – May 11, 2012**

Date	Sodium Hypochlorite mg/L TRC	Towerbrom mg/L TRC	PCL- 222 mg/L Phosph ate	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	-	-

**Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of  
Chemicals Used to Control Microbiologically Induced Corrosion Mollusks, During Toxicity Test  
Sampling,  
March 12, 1998 – May 11, 2012**

Date	Sodium Hypochlorite mg/L TRC	Towerbrom mg/L TRC	PCL- 222 mg/L Phosph ate	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
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	-	-	-	-	-	-	-	-	-

**Sequoyah Nuclear Plant Biomonitoring  
May 08 – 15, 2012**

**Appendix C**

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

Page 25 of 110

# BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA

Project Name: Sequoyah NP Toxicity

P.O. Number: N/A

Facility Sampled: Sequoyah NP

NPDES Number: TN0026450

Collected By: *Dustin Binegar*  
*Lyndell Williams*

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 Courier

Other (specify):

General Comments: *Arrived at outfall 0755,  
and arrived at intake at 0830 outfall.  
Sample B did not collect bottles 19-23.*

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Container Number & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)				Laboratory Use				
		Date	Time			Yes	If Yes, Inches	No	Trace	ETS Log Number	Arrival Temp. (°C)	By	Time ET	Appear- ance
SQN-101-TOX	Comp	05/07/12	Start 0753 ET Stop 0755 ET	2(2.5gal)	4466-ET 1215.29		6-1-8 dura	X		120507.01	1.8°C	JL	1429	*
SQN-JNT-TOX	Comp	05/07/12	Start 0710 ET Stop 0710 ET	1(2.5 gal)	N/A					120507.02	1.0°C	JL	1429	*

Sample Custody - Fill In From Top Down

\* CUSTODY SEALS INTACT. SAMPLES RECEIVED

Relinquished By (Signature):	Date/Time	Received By (Signature):	SONIC	ETS	IN GOOD CONDITION. TSC ABSENT IN ALL SAMPLES
<i>Dustin Binegar</i> TVA	5-7-12 1030 ET	<i>BR Skiles</i>	SONIC		05-07-12 10:30 ET
<i>BR Skiles</i> SONIC	05-07-12 1429 ET	<i>Jim</i>	ETS		05-07-12 1429 ET

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 6°C and shipping them in ice. 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 time frame. 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 Toxicity Sample Receipt Log

\*Sample temperature performed using Sample Receiving Thermometer: SN 6338.

Date received	Time received	Received by	Received from	Sample temperature (°C)*	Project number	Sample	Sample name and description	State	Comments
05-07-12	1429	J. Sumner	TVA Courier	1.8 / 1.8	7875	120507.01	TVA - Sequoyah Nuclear Plant - Outfall 101	TN	
05-07-12	1429	J. Sumner	TVA Courier	1.0	7875	120507.02	TVA - Sequoyah Nuclear Plant - Intake	TN	
05-07-12	1451	J. Sumner	TVA Courier	1.7	7876	120507.03	TVA - Watts Bar Nuclear Plant - Outfall 101	TN	
05-07-12	1451	J. Sumner	TVA Courier	3.7	7876	120507.04	TVA - Watts Bar Nuclear Plant - Intake 101	TN	
05-07-12	1451	J. Sumner	TVA Courier	2.6	7876	120507.05	TVA - Watts Bar Nuclear Plant - Outfall 113	TN	
05-07-12	1451	J. Sumner	TVA Courier	3.5	7876	120507.06	TVA - Watts Bar Nuclear Plant - Intake 113	TN	

## BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA

Project Name: Sequoyah NP Toxicity

P.O. Number: N/A

Facility Sampled: Sequoyah NP

NPDES Number: TN0026450

Collected By: Dustin Binegar

Lyndell Williams

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

Courier

Other (specify):

General Comments: Arrived at outfall at 0655 and at intake at 0625.

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Container Number & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)				Laboratory Use				
		Date	Time			Yes	If Yes, Inches	No	Trace	ETS Log Number	Arrival Temp (°C)	By	Time ET	Appearance
SQN-101-TOX	Comp	05/09/12	start 0635 stop 0535	2(2.5gal)	1713.31 4425.40			X		120509.25	1.6°C/1.7°C	JL	1300	*
SQN-INT-TOX	Comp	05/09/12	start 0710 stop 0610	1(2.5 gal)	N/A			X		120609.20	1.6°C	JL	1300	*

Sample Custody - Fill In From Top Down

\* CUSTODY SEALS INTACT. SAMPLES RECEIVED

Relinquished By (Signature):	Date/Time	Received By (Signature):	Date/Time
Dustin Binegar	5/9/12 0815 ET	B R Skiles	05-09-12 0815 ET
B R Skiles	05-09-12 1300 ET	JL	05-09-12 1300 ET

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 6°C and shipping them in ice. 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 time frame. 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 Toxicity Sample Receipt Log

\*Sample temperature performed using Sample Receiving Thermometer: SN 6338.

Date received	Time received	Received by	Received from	Sample temperature (°C)*	Project number	Sample	Sample name and description	State	Comments
05-09-12	1002	K. Keenan	Fed - Ex	1.4	7898	120509.01	CORR ERI - AAP McQuay International	NC	
05-09-12	1002	K. Keenan	Fed - Ex	2.0	7899	120509.02	Bladenboro WWTP	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.7	7900	120509.03	Duke Energy - Belows Creek Steam Station	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.5	7901	120509.04	Progress Energy - Cape Fear S.E.	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.5	7901	120509.05	Progress Energy - Cape Fear S.E. - Upstream/Intake	NC	
05-09-12	1002	K. Keenan	Fed - Ex	0.7	7902	120509.06	City of Gastonia - Dallas WWTP	NC	
05-09-12	1002	K. Keenan	Fed - Ex	5.8	7903	120509.07	United Water - Enfield WWTP	NC	
05-09-12	1002	K. Keenan	Fed - Ex	2.3	7904	120509.08	Duke Energy - Marshall Steam Station	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.6	7905	120509.09	Ammons Resources - Raleigh CC	NC	
05-09-12	1002	K. Keenan	Fed - Ex	2.6	7906	120509.10	Bell Enterprises - Scarlett Acres MHP	NC	
05-09-12	1002	K. Keenan	Fed - Ex	5.8	7907	120509.11	United Water - Scotland Neck WWTP	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.7	7908	120509.12	Duke Energy - McGuire NS - Outfall 005	NC	
05-09-12	1002	K. Keenan	Fed - Ex	2.4	7909	120509.13	Duke Energy - McGuire NS - Outfall 001	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.7	7910	120509.14	Duke Energy - McGuire NS - Outfall 002	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.5	7911	120509.15	Elements	NC	
05-09-12	1002	K. Keenan	Fed - Ex	1.1	7912	120509.16	Carolina Beach WWTP	NC	
05-09-12	1020	K. Keenan	UPS	0.8	7913	120509.17	South Cary WWTP	NC	
05-09-12	1020	K. Keenan	UPS	5.8	7914	120509.18	Enviro Chemists - Manteo WWTP	NC	
05-09-12	1020	K. Keenan	UPS	2.0	7915	120509.19	Microbac Environmental - Rockingham WWTP	NC	
05-09-12	1020	K. Keenan	UPS	2.0	7916	120509.20	Microbac Environmental - Roseboro WWTP	NC	
05-09-12	1020	K. Keenan	UPS	2.0	7917	120509.21	Microbac Environmental - JP Stevens - Wagram WWTP	NC	
05-09-12	1020	K. Keenan	UPS	2.0	7918	120509.22	Microbac Environmental - TransMontaigne - Outfall 002	NC	
05-09-12	1020	K. Keenan	UPS	2.0	7918	120509.23	Microbac Environmental - TransMontaigne - Outfall 003	NC	
05-09-12	1125	K. Keenan	Dash Courier	1.2	7919	120509.24	OWASA	NC	
05-09-12	1300	J. Sumner	TVA Courier	1.6/1.7	7875	120509.25	TVA - Sequoyah Nuclear Plant - Outfall 101	TN	
05-09-12	1300	J. Sumner	TVA Courier	1.6	7875	120509.26	TVA - Sequoyah Nuclear Plant - Intake	TN	
05-09-12	1522	J. Sumner	TVA Courier	2.2	7876	120509.27	TVA - Watts Bar Nuclear Plant - Outfall 101	TN	
05-09-12	1522	J. Sumner	TVA Courier	1.9	7876	120509.28	TVA - Watts Bar Nuclear Plant - Intake 101	TN	
05-09-12	1522	J. Sumner	TVA Courier	2.5	7876	120509.29	TVA - Watts Bar Nuclear Plant - Outfall 113	TN	
05-09-12	1522	J. Sumner	TVA Courier	1.8	7876	120509.30	TVA - Watts Bar Nuclear Plant - Intake 113	TN	

## BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA

Project Name: Sequoyah NP Toxicity

P.O. Number: N/A

Facility Sampled: Sequoyah NP

NPDES Number: TN0026450

Collected By: Dustin Binegar  
Lyndell Williams

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 Courier


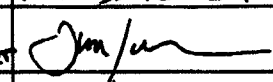
Other (specify): \_\_\_\_\_

General Comments: Arrived at outfall at 0555  
and at intake at 0630.

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Container Number & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)				Laboratory Use				
		Date	Time			Yes	If Yes, Inches	No	Trace	ETS Log Number	Arrival Temp (°C)	By	Time ET	Appearance
SQN-101-TOX	Comp	05/10/12 - 05/11/12	Start 0630 ET Stop 0635 ET	2(2.5gal)	1.00-1.2 4.68-1.2		6-1.2 Inches	X		120511-20	05°C / 40°C	JL	1225	*
SQN-INT-TOX	Comp	05/10/12 - 05/11/12	Start 0710 ET Stop 0610 ET	1(2.5 gal) 20-05/11/12				X		120511-21	21°C	JL	1225	*

Sample Custody - Fill In From Top Down

\* CUSTODY SEALS INTACT. SAMPLES RECEIVED

Relinquished By (Signature):	Date/Time	Received By (Signature):	IN GOOD CONDITION. TILL ABSENT IN ALL SAMPLES
 TVA	05/11/2012 0830 ET	BR Skalen	SONIC 05-11-12 0830 ET
BR Skalen	05-11-12 1225 ET		ETS 05-11-12 1225 ET

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 6°C and shipping them in ice. 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 time frame. 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 Toxicity Sample Receipt Log

\*Sample temperature performed using Sample Receiving Thermometer: SN 6338.

Date received	Time received	Received by	Received from	Sample temperature (°C)*	Project number	Sample	Sample name and description	State	Comments
05-11-12	1014	K. Keenan	Fed - Ex	0.7	7898	120511.01	CORR ERI - AAP McQuay International	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.0	7899	120511.02	Bladenboro WWTP	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.6	7900	120511.03	Duke Energy - Belevs Creek Stream Station	NC	
05-11-12	1014	K. Keenan	Fed - Ex	0.4	7901	120511.04	Progress Energy - Cape Fear S.E.	NC	
05-11-12	1014	K. Keenan	Fed - Ex	0.4	7901	120511.05	Progress Energy - Cape Fear S.E. - Upstream/Intake	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.1	7902	120511.06	City of Gastonia - Dallas WWTP	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.5	7903	120511.07	United Water - Enfield WWTP	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.4	7904	120511.08	Duke Energy - Marshall Steam Station	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.2	7905	120511.09	Ammons Resources - Raleigh CC	NC	
05-11-12	1014	K. Keenan	Fed - Ex	2.2	7906	120511.10	Bell Enterprises - Scarlett Acres MHP	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.5	7907	120511.11	United Water - Scotland Neck WWTP	NC	
05-11-12	1014	K. Keenan	Fed - Ex	0.9	7908	120511.12	Duke Energy - McGuire NS - Outfall 005	NC	
05-11-12	1014	K. Keenan	Fed - Ex	1.0	7911	120511.13	Elementis	NC	
05-11-12	1014	K. Keenan	UPS	0.8	7913	120511.14	South Cary WWTP	NC	
05-11-12	1014	K. Keenan	UPS	0.8	7915	120511.15	Microbac Environmental - Rockingham WWTP	NC	
05-11-12	1014	K. Keenan	UPS	0.8	7916	120511.16	Microbac Environmental - Roseboro WWTP	NC	
05-11-12	1014	K. Keenan	UPS	0.8	7917	120511.17	Microbac Environmental - JP Stevens - Wagram WWTP	NC	
05-11-12	1014	K. Keenan	UPS	0.8	7921	120511.18	Microbac Environmental - Motiva Enterprises, LLC	NC	
05-11-12	1123	K. Keenan	Dash Courier	0.9	7919	120511.19	OWASA	NC	
05-11-12	1225	J. Sumner	TVA Courier	0.5/0.6	7875	120511.20	TVA - Sequoyah Nuclear Plant - Outfall 101	TN	
05-11-12	1225	J. Sumner	TVA Courier	2.1	7875	120511.21	TVA - Sequoyah Nuclear Plant - Intake	TN	
05-11-12	1437	J. Sumner	TVA Courier	3.6	7876	120511.22	TVA - Watts Bar Nuclear Plant - Outfall 101	TN	
05-11-12	1437	J. Sumner	TVA Courier	4.5	7876	120511.23	TVA - Watts Bar Nuclear Plant - Intake 101	TN	
05-11-12	1437	J. Sumner	TVA Courier	2.4	7876	120511.24	TVA - Watts Bar Nuclear Plant - Outfall 113	TN	
05-11-12	1437	J. Sumner	TVA Courier	5.8	7876	120511.25	TVA - Watts Bar Nuclear Plant - Intake 113	TN	

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

Species: *Pimephales promelas*

Client: Tennessee Valley Authority

County: Rhea

Facility: Sequoyah Nuclear Plant

Outfall: 101

NPDES #: TN0020168

Project #: 7875

Dilution preparation information:						Comments:
Dilution prep (%)	10.8	21.6	43.2	86.4	100	
Effluent volume (mL)	270	540	1080	2160	2500	
Diluent volume (mL)	2230	1960	1420	340	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism information:		Test information:	
Organism age:	19.5 <sup>19.5 HOURS OLD</sup>	Randomizing template:	Yellow
Date and times organisms were born between:	05-07-12 1600	Incubator number and shelf location:	3C
Organism source:	ACTOX BATCH Pp 05-07-12	Artemia CHM number:	CHM652
Transfer bowl information:		Drying information for weight determination:	
pH = 7.79 S.U. Temperature = 24.8 °C		Date / Time in oven:	05-15-12 1140
Average transfer volume:		Initial oven temperature:	60 °C
0.1209 mL		Date / Time out of oven:	05-16-12 1140
		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	05-06-12			1545	JL	1130	JL	120507.01	120507.02	05-03-12A
1	05-09-12	0800	JL	1400	JL	1030	JL	120507.01	120507.02	05-03-12B
2	05-10-12	0830	JL	1430	JL	1040	JL	120509.25	120509.26	05-07-12
3	05-11-12	0800	JL	1400	JL	1030	JL	120509.25	120509.26	05-09-12A
4	05-12-12	0800	JL	1400	JL	1030	JL	120511.20	120511.21	05-10-12A
5	05-13-12	0800	JL	1400	JL	1032	JL	120511.20	120511.21	05-10-12B
6	05-14-12	0800	JL	1400	JL	1030	JL	120511.20	120511.21	05-10-12B
7	05-15-12					1030	JL			

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC <sub>50</sub>	>1007.
Average weight per initial larvae:	0.707		NOEC	86.47.
Average weight per surviving larvae:	0.707	≥ 0.25mg/larvae	LOEC	1007.
			ChV	93.07.
			IC <sub>25</sub>	>1007.



Species: *Pimephales promelas*

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

Date: 05-08-12

**Survival and Growth Data**

Day	CONTROL				10.8%				21.6%			
	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	9 <sup>d</sup>	10
6	10	10	10	10	10	10	10	10	10	10	9	10
7	10	10	10	10	10	10	10	10	10	10	9	10
A = Pan weight (mg) Tray color code: <u>Light Blue</u> Analyst: <u>MJE</u> Date: <u>05-02-12</u>												
B = Pan + Larvae weight (mg) Analyst: <u>CDJ</u> Date: <u>05-15-12</u>												
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>[Signature]</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>[Signature]</u>												
Average weight per initial number of larvae (mg)	0.707				0.655				0.664			
Percent reduction from control (%)					7.57				6.17			

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, Non-treated

Date: 05-08-12

### Survival and Growth Data

Day	43.2%				86.4%				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>Light Blue</u> Analyst: <u>MHC</u> Date: <u>05-02-12</u>												
	14.30	14.69	14.77	12.48	13.72	13.67	13.07	13.24	13.23	14.98	14.65	13.58
B = Pan + Larvae weight (mg) Analyst: <u>GDJ</u> Date: <u>05-13-12</u>												
	21.08	21.43	21.19	19.72	20.32	19.71	19.64	19.92	19.44	21.32	20.51	20.40
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>JLH</u>												
	6.78	6.74	6.42	6.74	6.60	6.04	6.57	6.48	6.21	6.34	5.86	6.82
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>JLH</u>												
	0.678	0.674	0.642	0.674	0.660	0.604	0.657	0.648	0.621	0.634	0.586	0.682
Average weight per initial number of larvae (mg)      Percent reduction from control (%)												
	0.667			5.77	0.642			9.27	0.631			10.87

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:


Species: *Pimephales promelas*

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

Date: 05-08-12

**Survival and Growth Data**

Day	100% Intake							
	Y	Z	AA	BB				
0	10	10	10	10				
1	10	10	10	10				
2	10	10	10	10				
3	10	10	10	10				
4	10	10	10	10				
5	10	10	10	10				
6	10	10	10	10				
7	10	10	10	10				
A = Pan weight (mg) Tray color code: <u>Light Blue</u> Analyst: <u>MLP</u> Date: <u>05-02-12</u>					14.01	13.43	13.70	13.51
B = Pan + Larvae weight (mg) Analyst: <u>CDJ</u> Date: <u>05-11-12</u>					21.35	19.77	20.43	20.67
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>[Signature]</u>					7.34	6.34	6.73	7.16
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>[Signature]</u>					0.734	0.634	0.673	0.716
Average weight per initial number of larvae (mg)		Percent reduction from control (%)			0.694	1.87.		

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:**

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TVA / Sequoyah Nuclear Plant, Outfall 101  
Non-treated  
May 08-15, 2012

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

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

ETS

Environmental Testing Solutions, Inc.

Project number: 7875

Concentration (%)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = A - B	Not for Compliance Assessment, Internal Laboratory QC			Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (Observed weight per initial number of larvae) (%)	Percent reduction from control (%)
							Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Observed weight per surviving number of larvae) (%)					
Control	A	10	10	13.69	20.27	6.58	0.658	0.707	7.2	0.658	100.0	0.707	7.2	Not applicable
	B	10	10	12.78	19.64	6.86	0.686			0.686				
	C	10	10	14.17	21.24	7.07	0.707			0.707				
	D	10	10	13.67	21.45	7.78	0.778			0.778				
10.8%	E	10	10	14.65	20.89	6.24	0.624	0.655	4.8	0.624	100.0	0.655	4.8	7.5
	F	10	10	15.54	22.02	6.48	0.648			0.648				
	G	10	10	14.07	21.06	6.99	0.699			0.699				
	H	10	10	13.39	19.86	6.47	0.647			0.647				
21.6%	I	10	10	12.86	19.15	6.29	0.629	0.681	6.4	0.629	97.5	0.664	9.4	6.1
	J	10	10	14.60	21.77	7.17	0.717			0.717				
	K	10	9	14.08	20.02	5.94	0.660			0.594				
	L	10	10	14.11	21.27	7.16	0.716			0.716				
43.2%	M	10	10	14.30	21.08	6.78	0.678	0.667	2.5	0.678	100.0	0.667	2.5	5.7
	N	10	10	14.69	21.43	6.74	0.674			0.674				
	O	10	10	14.77	21.19	6.42	0.642			0.642				
	P	10	10	12.48	19.22	6.74	0.674			0.674				
86.4%	Q	10	10	13.72	20.32	6.60	0.660	0.642	4.0	0.660	100.0	0.642	4.0	9.2
	R	10	10	13.67	19.71	6.04	0.604			0.604				
	S	10	10	13.07	19.64	6.57	0.657			0.657				
	T	10	10	13.24	19.72	6.48	0.648			0.648				
100%	U	10	10	13.23	19.44	6.21	0.621	0.631	6.3	0.621	100.0	0.631	6.3	10.8
	V	10	10	14.98	21.32	6.34	0.634			0.634				
	W	10	10	14.65	20.51	5.86	0.586			0.586				
	X	10	10	13.58	20.40	6.82	0.682			0.682				
100% Intake	Y	10	10	14.01	21.35	7.34	0.734	0.694	6.3	0.734	100.0	0.694	6.3	1.8
	Z	10	10	13.43	19.77	6.34	0.634			0.634				
	AA	10	10	13.70	20.63	6.93	0.693			0.693				
	BB	10	10	13.51	20.67	7.16	0.716			0.716				

Outfall 101:

Dunnett's MSD value: 0.0697  
PMSD: 9.9

MSD =  
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: 0.0653  
PMSD: 9.2

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

Non-treated

May 08-15, 2012



## Statistical Analyses

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

Start Date: 5/8/2012 Test ID: PpFRCR Sample ID: TVA / SQN, Outfall 101  
 End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report  
 Sample Date: May 2012 Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas  
 Comments: Non-treated

Conc-%	1	2	3	4
D-Control	0.8580	0.8860	0.7070	0.7780
10.8	0.8240	0.8480	0.6990	0.6470
21.6	0.8290	0.7170	0.5940	0.7160
43.2	0.6780	0.8740	0.6420	0.8740
86.4	0.8600	0.8040	0.8570	0.8480
100	0.8210	0.8340	0.6860	0.6820
Intake	0.7340	0.8340	0.6930	0.7160

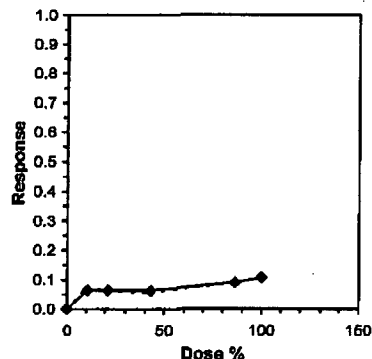
Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.7073	1.0000	0.7073	0.6580	0.7780	7.248	4				0.7073	1.0000
10.8	0.8545	0.9254	0.8545	0.6240	0.6990	4.839	4	1.823	2.410	0.0697	0.6818	0.9358
21.6	0.6640	0.9388	0.6640	0.5940	0.7170	9.380	4	1.495	2.410	0.0697	0.6618	0.9358
43.2	0.6670	0.9431	0.6670	0.8420	0.6780	2.515	4	1.391	2.410	0.0697	0.6818	0.9358
86.4	0.8423	0.9081	0.8423	0.8040	0.6600	4.049	4	2.247	2.410	0.0697	0.8423	0.9081
*100	0.6308	0.8918	0.6308	0.5860	0.6820	6.298	4	2.644	2.410	0.0697	0.6308	0.8918
Intake	0.6943	0.9816	0.6943	0.6340	0.7340	6.270	4					

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )					0.97188	0.884	0.17128	-0.4665						
Bartlett's Test indicates equal variances ( $p = 0.39$ )					5.23369	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test					86.4	100	92.9518	1.15741	0.06973	0.09859	0.00279	0.00167	0.19306	5, 18
Treatments vs D-Control														

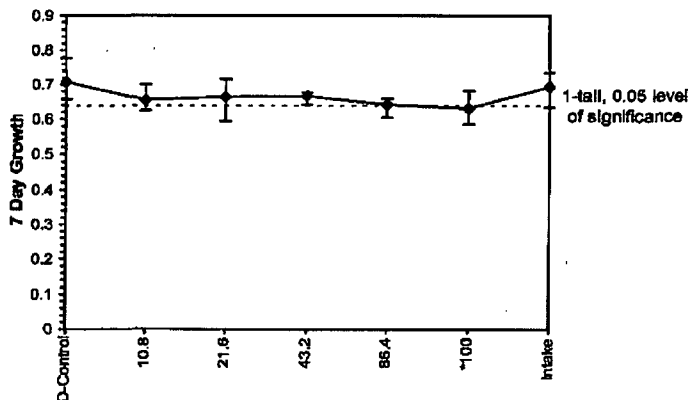
## Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	8.409			
IC10	93.170			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

\* Indicates IC estimate less than the lowest concentration



Dose-Response Plot



**TVA / Sequoyah Nuclear Plant, Intake**  
**Non-treated**  
**May 08-15, 2012**



Statistical Analyses

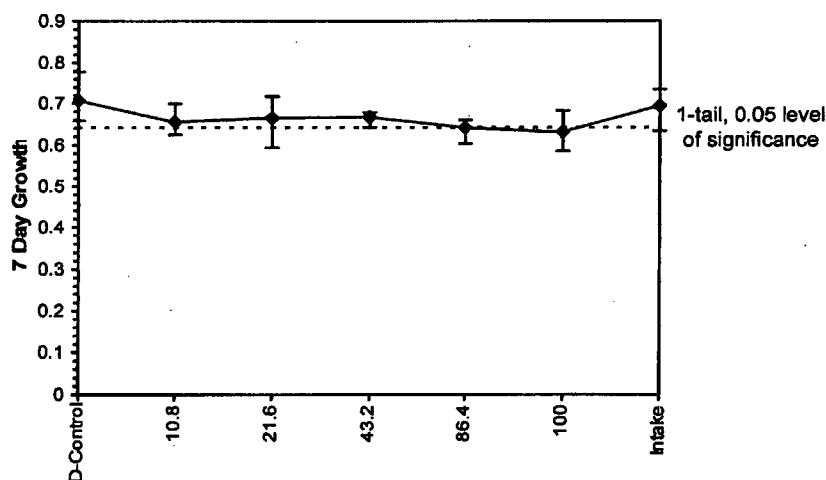
Larval Fish Growth and Survival Test-7 Day Growth			
Start Date: 5/8/2012	Test ID: PpFRCR	Sample ID: TVA / SQN, Intake	
End Date: 5/15/2012	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date: May 2012	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas	
Comments: Non-treated			

Conc-%	1	2	3	4
D-Control	0.6580	0.6860	0.7070	0.7780
10.8	0.6240	0.6480	0.6990	0.6470
21.6	0.6290	0.7170	0.5940	0.7160
43.2	0.6780	0.6740	0.6420	0.6740
86.4	0.6600	0.6040	0.6570	0.6480
100	0.6210	0.6340	0.5860	0.6820
Intake	0.7340	0.6340	0.6930	0.7160

Conc-%	Transform: Untransformed						N	1-Tailed		
	Mean	N-Mean	Mean	Min	Max	CV%		t-Stat	Critical	MSD
D-Control	0.7073	1.0000	0.7073	0.6580	0.7780	7.248	4			
10.8	0.6545	0.9254	0.6545	0.6240	0.6990	4.839	4			
21.6	0.6640	0.9388	0.6640	0.5940	0.7170	9.380	4			
43.2	0.6670	0.9431	0.6670	0.6420	0.6780	2.515	4			
86.4	0.6423	0.9081	0.6423	0.6040	0.6600	4.049	4			
100	0.6308	0.8918	0.6308	0.5860	0.6820	6.298	4			
Intake	0.6943	0.9816	0.6943	0.6340	0.7340	6.270	4	0.387	1.943	0.0653

Auxiliary Tests	Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97462	0.749	0.1774	-0.6068		
F-Test indicates equal variances (p = 0.79)	1.38665	47.4683				
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	0.06534	0.09238	0.00034	0.00226	0.71238	1, 6
Treatments vs D-Control						

Dose-Response Plot





Species: Pimephales promelas

Date: 05-08-12

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

Daily Chemistry:

Analyst		Day					
		0		1		2	
Concentration	Parameter	CDT	CDT	CDT	CDT	CDT	MNF
CONTROL Non-treated	pH (S.U.)	7.85	7.90	7.92	7.79	7.95	7.85
	DO (mg/L)	7.6	8.0	7.8	7.5	7.7	7.7
	Conductivity (µmhos/cm)	327		310		303	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		63	
	Hardness (mg CaCO <sub>3</sub> /L)	88		88		89	
	Temperature (°C)	24.9	24.7	24.9	24.7	24.8	24.6
10.8%	pH (S.U.)	7.89	7.86	7.96	7.38	7.90	7.85
	DO (mg/L)	7.9	7.9	7.8	7.4	7.8	7.7
	Conductivity (µmhos/cm)	306		297		292	
	Temperature (°C)	24.9	24.9	25.0	24.7	24.8	24.6
21.6%	pH (S.U.)	7.89	7.86	7.98	7.38	7.92	7.79
	DO (mg/L)	7.9	7.9	7.8	7.6	7.8	7.7
	Conductivity (µmhos/cm)	291		284		280	
	Temperature (°C)	24.9	24.6	25.0	24.7	24.8	24.5
43.2%	pH (S.U.)	7.89	7.90	7.99	7.82	7.91	7.85
	DO (mg/L)	7.9	8.0	7.8	7.6	7.8	7.7
	Conductivity (µmhos/cm)	265		259		257	
	Temperature (°C)	24.9	24.6	25.0	24.8	24.8	24.8
86.4%	pH (S.U.)	7.81	7.90	8.01	7.82	7.88	7.87
	DO (mg/L)	7.9	8.0	7.9	7.6	7.9	7.7
	Conductivity (µmhos/cm)	212		210		211	
	Temperature (°C)	25.0	24.6	25.0	24.6	24.8	24.8
100%	pH (S.U.)	7.92	7.90	8.02	7.86	7.89	7.92
	DO (mg/L)	7.9	8.0	7.9	7.6	7.9	7.8
	Conductivity (µmhos/cm)	197		193		197	
	Alkalinity (mg CaCO <sub>3</sub> /L)	79				81	
	Hardness (mg CaCO <sub>3</sub> /L)	81				81	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	25.0	24.8	25.0	24.6	25.0	24.7
100% Intake	pH (S.U.)	7.95	7.93	8.04	7.84	8.04	7.90
	DO (mg/L)	7.9	7.9	8.1	7.5	8.1	8.0
	Conductivity (µmhos/cm)	195		190		192	
	Alkalinity (mg CaCO <sub>3</sub> /L)	77				79	
	Hardness (mg CaCO <sub>3</sub> /L)	79				81	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	25.1	24.8	25.1	24.7	25.0	24.7
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

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

Date: 05-08-12

Analyst		Day							
		3		4		5		6	
		MW	MW	MW	MW	MW	MW	MW	MW
Concentration	Parameter								
CONTROL Non-treated	pH (S.U.)	7.97	7.70	8.02	7.07	7.97	7.62	8.06	7.49
	DO (mg/L)	7.4	7.2	7.8	6.9	7.6	7.0	7.6	6.4
	Conductivity (µmhos/cm)	294		309		313		312	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		62			
	Hardness (mg CaCO <sub>3</sub> /L)	87		89		89			
	Temperature (°C)	24.8	24.7	24.8	24.8	24.8	25.0	24.8	24.6
10.8%	pH (S.U.)	8.07	7.59	7.95	7.60	7.94	7.62	8.10	7.49
	DO (mg/L)	7.3	7.1	8.0	6.8	8.0	7.0	7.6	6.4
	Conductivity (µmhos/cm)	294		283		291		291	
	Temperature (°C)	24.9	24.5	24.8	24.7	24.8	24.8	24.8	24.7
21.6%	pH (S.U.)	8.10	7.62	7.92	7.50	7.93	7.64	8.10	7.49
	DO (mg/L)	7.3	6.8	8.0	6.5	8.0	6.9	7.6	6.6
	Conductivity (µmhos/cm)	280		270		280		281	
	Temperature (°C)	24.9	24.8	24.8	24.9	24.8	24.8	24.8	24.7
43.2%	pH (S.U.)	8.09	7.73	7.80	7.50	7.87	7.63	8.11	7.47
	DO (mg/L)	7.3	6.8	8.0	6.6	8.0	7.0	7.6	6.5
	Conductivity (µmhos/cm)	258		253		263		263	
	Temperature (°C)	24.9	24.6	24.8	24.6	24.8	25.1	24.8	24.7
86.4%	pH (S.U.)	8.10	7.69	7.81	7.63	7.83	7.65	8.12	7.46
	DO (mg/L)	7.3	7.0	8.0	6.9	8.0	6.9	7.6	6.5
	Conductivity (µmhos/cm)	212		216		215		218	
	Temperature (°C)	24.9	24.6	24.9	24.8	24.8	25.0	24.8	24.8
100%	pH (S.U.)	8.05	7.75	7.76	7.61	7.90	7.65	8.16	7.42
	DO (mg/L)	7.3	6.9	8.0	6.9	8.0	6.9	7.6	6.2
	Conductivity (µmhos/cm)	196		189		196		199	
	Alkalinity (mg CaCO <sub>3</sub> /L)			79					
	Hardness (mg CaCO <sub>3</sub> /L)			81					
	TR Chlorine (mg/L)			20.10					
100% Intake	Temperature (°C)	24.9	24.7	25.0	24.9	24.8	25.0	25.0	24.5
	pH (S.U.)	8.12	7.76	8.06	7.65	8.02	7.68	8.16	7.53
	DO (mg/L)	7.3	7.0	8.0	6.7	8.1	7.0	7.7	6.7
	Conductivity (µmhos/cm)	194		182		190		190	
	Alkalinity (mg CaCO <sub>3</sub> /L)			79					
	Hardness (mg CaCO <sub>3</sub> /L)			79					
	TR chlorine (mg/L)			20.10					
	Temperature (°C)	24.9	24.7	25.1	24.9	24.9	25.1	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Reviewed by  
Kathy E. Keenan

## TVA / Sequoyah Nuclear Plant, Outfall 101 - Non-treated

May 08-15, 2012

*Pimephales promelas* Chronic Whole Effluent Toxicity Test

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


## Daily Chemical Analyses

Project number: 7875

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)	7.85	7.90	7.92	7.79	7.95	7.85	7.98	7.70	8.02	7.67	7.97	7.62	8.06	7.49
	DO (mg/L)	7.6	8.0	7.8	7.5	7.7	7.7	7.4	7.2	7.8	6.9	7.6	7.0	7.6	6.4
	Conductivity (µmhos/cm)	327		310		303		294		309		313		312	
	Alkalinity (mg/L CaCO <sub>3</sub> )	63		63		63		63		63		62			
	Hardness (mg/L CaCO <sub>3</sub> )	88		88		89		87		89		89			
	Temperature (°C)	24.9	24.7	24.9	24.7	24.8	24.6	24.8	24.7	24.8	24.8	24.8	25.0	24.8	24.6
10.8%	pH (SU)	7.89	7.86	7.96	7.78	7.90	7.85	8.07	7.59	7.95	7.60	7.94	7.62	8.10	7.49
	DO (mg/L)	7.9	7.9	7.8	7.4	7.8	7.7	7.7	7.1	8.0	6.8	8.0	7.0	7.6	6.4
	Conductivity (µmhos/cm)	306		297		292		294		283		291		291	
	Temperature (°C)	24.9	24.9	25.0	24.7	24.8	24.6	24.9	24.5	24.8	24.7	24.8	24.8	24.8	24.7
21.6%	pH (SU)	7.89	7.86	7.98	7.78	7.92	7.79	8.10	7.62	7.92	7.56	7.93	7.64	8.10	7.49
	DO (mg/L)	7.9	7.9	7.8	7.6	7.8	7.7	7.7	6.8	8.0	6.5	8.0	6.9	7.6	6.6
	Conductivity (µmhos/cm)	291		284		280		280		276		286		281	
	Temperature (°C)	24.9	24.6	25.0	24.7	24.8	24.5	24.9	24.8	24.8	24.9	24.8	24.8	24.8	24.7
43.2%	pH (SU)	7.89	7.90	7.99	7.82	7.91	7.85	8.09	7.73	7.86	7.56	7.87	7.63	8.11	7.47
	DO (mg/L)	7.9	8.0	7.8	7.6	7.8	7.7	7.7	6.8	8.0	6.6	8.0	7.0	7.6	6.5
	Conductivity (µmhos/cm)	265		259		257		258		253		263		263	
	Temperature (°C)	24.9	24.6	25.0	24.8	24.8	24.8	24.9	24.6	24.8	24.6	24.8	25.1	24.8	24.7
86.4%	pH (SU)	7.91	7.90	8.01	7.82	7.88	7.87	8.10	7.69	7.81	7.63	7.83	7.65	8.12	7.46
	DO (mg/L)	7.9	8.0	7.9	7.6	7.9	7.7	7.7	7.0	8.0	6.9	8.0	6.9	7.6	6.5
	Conductivity (µmhos/cm)	212		210		211		212		216		215		218	
	Temperature (°C)	25.0	24.6	25.0	24.6	24.8	24.8	24.9	24.6	24.9	24.8	24.8	25.0	24.8	24.8
100%	pH (SU)	7.92	7.90	8.02	7.86	7.89	7.92	8.05	7.75	7.76	7.61	7.78	7.65	8.16	7.42
	DO (mg/L)	7.9	8.0	7.9	7.6	7.9	7.8	7.7	6.9	8.0	6.9	8.0	6.9	7.6	6.2
	Conductivity (µmhos/cm)	197		193		197		196		188		196		199	
	Alkalinity (mg/L CaCO <sub>3</sub> )	79				81				79					
	Hardness (mg/L CaCO <sub>3</sub> )	81				81				81					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.0	24.8	25.0	24.6	25.0	24.7	24.9	24.7	25.0	24.9	24.8	25.0	25.0	24.5
100% Intake	pH (SU)	7.95	7.93	8.04	7.84	8.04	7.90	8.12	7.75	8.06	7.65	8.02	7.68	8.16	7.53
	DO (mg/L)	7.9	7.9	8.1	7.5	8.1	8.0	7.7	7.0	8.0	6.7	8.1	7.0	7.7	6.7
	Conductivity (µmhos/cm)	195		190		192		194		182		190		190	
	Alkalinity (mg/L CaCO <sub>3</sub> )	77				79				79					
	Hardness (mg/L CaCO <sub>3</sub> )	79				81				79					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.1	24.8	25.1	24.7	25.0	24.7	24.9	24.7	25.1	24.9	24.9	25.1	24.9	24.7

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Entered by: C. Johnson

Reviewed by: 

ETS

Environmental Testing Solutions, Inc.

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

Species: Ceriodaphnia dubia

Client: Tennessee Valley Authority  
Facility: Sequoyah Nuclear Plant  
NPDES #: TN0026450  
Project #: 7875

County: Hamilton  
Outfall: 101

Dilution preparation information:						Comments:
Dilution prep (%)	10.8	21.6	43.2	86.4	100	
Effluent volume (mL)	270	540	1080	2160	2500	
Diluent volume (mL)	2230	1960	1420	340	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism source information:													Test information:			
Organism age:				< 24-hours old									Randomizing template color:		GOLD	
Date and times organisms were born between:				05-08-12 0640 TO 0700									Incubator number and shelf location:		2C1	
Culture board:				05-01-12 A									YWT batch:		05-01-12	
Replicate number:				1	2	3	4	5	6	7	8	9	10	Selenastrum batch:		04-30-12
Culture board cup number:				21	25	26	27	32	33	34	35	36	40			
Transfer vessel information:				pH = 7.85 S.U. Temperature = 25.1 °C												
Average transfer volume (mL):				0.0327 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	05-08-12	0935	05-03-12A	120507.01	120507.02	JL
1	05-09-12	0836	05-03-12B	120507.01	120507.02	JL
2	05-10-12	0835	05-07-12	120509.25	120509.26	JL
3	05-11-12	0837	05-09-12A	120509.25	120509.26	JL
4	05-12-12	0836	05-11-12A	120511.20	120511.21	JL
5	05-13-12	0838	05-11-12B	120511.20	120511.21	JL
6	05-14-12	0840	05-11-12B	120511.20	120511.21	JL
7	05-15-12	0842				JL

Control information:				Summary of test endpoints:	
	Control-1	Control-2	Acceptance criteria		
% of Male Adults:	07.	07.	≤ 20%	7-day LC <sub>50</sub>	> 1007.
% Adults having 3 <sup>rd</sup> Broods:	1007. 31.9	1007. 30.6	≥ 80%	NOEC	1007.
% Mortality:	07. 6.77	07. 6.87	≤ 20%	LOEC	> 1007.
Mean Offspring/Female:	31.9	30.6	≥ 15.0 offspring/female	ChV	> 1007.
% CV:	6.77.	6.87.	< 40.0 %	IC <sub>25</sub>	> 1007.

Species: Ceriodaphnia dubia

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 05.08.12

**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	4	5	4	6	4	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	12	14	12	13	12	13	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	17	15	15	15	16	14	15	17	14	14
Total young produced		33	32	30	31	36	36	33	34	31	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 <sup>rd</sup> 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).

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	31.9

CONC: 10.8%

**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	4	4	5	5	3	4	4	6	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	12	12	13	12	10	12	13	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	17	16	13	16	15	16	14	17	17	15
Total young produced		33	33	30	33	31	32	28	35	35	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).

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	32.2
% Reduction from Control-1:	-0.17.

Species: Ceriodaphnia dubia

Client: TVA/Sequoyah Nuclear Plant, Outfall 101

Date: 05-08-12

CONC: 21.6%

**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	4	4	6	5	5	4	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	14	13	11	13	12	12	15	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	19	16	16	15	15	15	18	15	16	16
Total young produced		36	32	36	33	31	32	35	32	35	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).

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	33.5
% Reduction from Control-1:	-5.07.

CONC: 43.2%

**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	4	4	4	6	4	4	6	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	12	14	14	12	12	11	13	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	17	15	15	14	17	14	18	19	16	15
Total young produced		35	31	33	31	33	36	35	37	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).

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	33.9
% Reduction from Control-1:	-6.3%

Species: *Ceriodaphnia dubia*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 05-08-17

CONC: 86.4%

**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	5	5	4	5	5	6	4	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	13	15	13	13	11	13	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	17	18	15	15	19	16	19	17	17	17
Total young produced		34	36	32	35	37	35	34	34	33	35
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:	34.5
% Reduction from Control-1:	-8.27.

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	6	5	6	4	4	5	5	6	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	13	12	13	15	13	12	14	14
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	19	16	19	20	18	15	18	17	17
Total young produced		37	36	35	35	37	38	33	36	35	36
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

**Concentration:**

% Mortality:	07.
Mean Offspring/Female:	36.8
% Reduction from Control-1:	-12.27.

Species: Ceriodaphnia dubia

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 05-08-12

**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	4	6	5	4	4	5	4	4	4	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	12	12	13	11	11	11	11	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	14	16	14	14	13	17	16	16	13
Total young produced		30	33	33	30	31	29	32	31	31	26
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 <sup>rd</sup> 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).

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	30.6

**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	4	4	5	4	5	6	6	6	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	14	10	14	13	12	13	10	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	17	14	14	13	18	13	15	17	15
Total young produced		33	35	29	32	31	36	32	31	33	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).

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	32.3
% Reduction from Control-2:	-5.67.



**TVA / Sequoyah Nuclear Plant, Outfall 101 - Non-treated**  
**May 08-15, 2012**



**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	4	5	4	6	4	5	5	4	5	46
5	12	13	10	12	14	12	13	12	13	10	121
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	15	15	16	14	15	17	14	14	152
Total	33	32	30	31	36	30	33	34	31	29	319

**86.4%**

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	5	5	4	5	5	6	4	4	4	6	48
5	12	13	13	15	13	13	11	13	12	12	127
6	0	0	0	0	0	0	0	0	0	0	0
7	17	18	15	15	19	16	19	17	17	17	170
Total	34	36	32	35	37	35	34	34	33	35	345

**10.8%**

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	4	4	5	5	3	4	4	6	5	5	45
5	12	13	12	12	13	12	10	12	13	12	121
6	0	0	0	0	0	0	0	0	0	0	0
7	17	16	13	16	15	16	14	17	17	15	156
Total	33	33	30	33	31	32	28	35	35	32	322

**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	6	5	6	4	4	5	5	6	4	5	50
5	13	12	13	12	13	15	13	12	14	14	131
6	0	0	0	0	0	0	0	0	0	0	0
7	18	19	16	19	20	18	15	18	17	17	177
Total	37	36	35	35	37	38	33	36	35	36	358

**21.6%**

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	4	4	6	5	5	4	5	5	4	5	47
5	13	12	14	13	11	13	12	12	15	12	127
6	0	0	0	0	0	0	0	0	0	0	0
7	19	16	16	15	15	15	18	15	16	16	161
Total	36	32	36	33	31	32	35	32	35	33	335

**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	4	6	5	4	4	5	4	4	4	3	43
5	10	13	12	12	13	11	11	11	11	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	16	14	14	13	17	16	16	13	149
Total	30	33	33	30	31	29	32	31	31	26	306

**43.2%**

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	4	4	4	6	4	4	6	5	5	5	47
5	14	12	14	14	12	12	11	13	12	13	127
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	15	19	17	14	18	19	16	15	165
Total	35	31	33	39	33	30	35	37	33	33	339

**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	4	4	5	4	5	6	6	6	4	5	49
5	13	14	10	14	13	12	13	10	12	11	122
6	0	0	0	0	0	0	0	0	0	0	0
7	16	17	14	14	13	18	13	13	17	15	152
Total	33	35	29	32	31	36	32	31	33	31	323

**TVA / Sequoyah Nuclear Plant, Outfall 101**  
**Non-treated**  
**May 08-15, 2012**



***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: 7875

Concentration (%)	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 - 1	33	32	30	31	36	30	33	34	31	29	100	31.9	6.7	Not applicable
10.8%	33	33	30	33	31	32	28	35	35	32	100	32.2	6.7	-0.9
21.6%	36	32	36	33	31	32	35	32	35	33	100	33.5	5.5	-5.0
43.2%	35	31	33	39	33	30	35	37	33	33	100	33.9	7.9	-6.3
86.4%	34	36	32	35	37	35	34	34	33	35	100	34.5	4.2	-8.2
100%	37	36	35	35	37	38	33	36	35	36	100	35.8	3.9	-12.2
Control - 2	30	33	33	30	31	29	32	31	31	26	100	30.6	6.8	Not applicable
100% Intake	33	35	29	32	31	36	32	31	33	31	100	32.3	6.4	-5.6

**Outfall 101:**

Dunnett's MSD value: 2.036  
 PMSD: 6.4

**Intake:**

Dunnett's MSD value: 1.599  
 PMSD: 5.2

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 (10<sup>th</sup> percentile) = 13%.

Upper PMSD bound determined by USEPA (90<sup>th</sup> percentile) = 47%.

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

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

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.

File: sqn101\_050812data.xlsx  
 Table populated from associated "Verification of *Ceriodaphnia* Reproduction Totals" spreadsheet.  
 Spreadsheet entered by: J. Sumner  
 Reviewed by:

## TVA / Sequoyah Nuclear Plant, Outfall 101

Non-treated  
May 08-15, 2012



## Statistical Analyses

## Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 5/8/2012 Test ID: CdFRCR Sample ID: TVA / SQN 101  
End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report  
Sample Date: May 2012 Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia

## Comments:

Conc-%	1	2	3	4	5	6	7	8	9	10
Control-1	33.000	32.000	30.000	31.000	36.000	30.000	33.000	34.000	31.000	29.000
Control-2	30.000	33.000	33.000	30.000	31.000	29.000	32.000	31.000	31.000	28.000
10.8	33.000	33.000	30.000	33.000	31.000	32.000	28.000	35.000	35.000	32.000
21.6	36.000	32.000	36.000	33.000	31.000	32.000	35.000	32.000	35.000	33.000
43.2	35.000	31.000	33.000	39.000	33.000	30.000	35.000	37.000	33.000	33.000
86.4	34.000	36.000	32.000	36.000	37.000	35.000	34.000	34.000	33.000	35.000
100	37.000	36.000	35.000	35.000	37.000	38.000	33.000	36.000	35.000	36.000
Intake	33.000	35.000	29.000	32.000	31.000	36.000	32.000	31.000	33.000	31.000

Conc-%	Transform: Untransformed							1-Tailed		Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
Control-1	31.900	1.0425	31.900	29.000	36.000	6.683	10				33.633
Control-2	30.600	1.0000	30.600	28.000	33.000	6.750	10				1.0000
10.8	32.200	1.0523	32.200	28.000	35.000	6.677	10	-0.337	2.287	2.036	33.633
21.6	33.500	1.0948	33.500	31.000	36.000	6.496	10	-1.797	2.287	2.036	33.633
43.2	33.900	1.1078	33.900	30.000	39.000	7.921	10	-2.246	2.287	2.036	33.633
86.4	34.500	1.1275	34.500	32.000	37.000	4.158	10	-2.920	2.287	2.036	33.633
100	35.800	1.1699	35.800	33.000	38.000	3.908	10	-4.381	2.287	2.036	33.633
Intake	32.300	1.0558	32.300	29.000	36.000	6.370	10				

## Auxiliary Tests

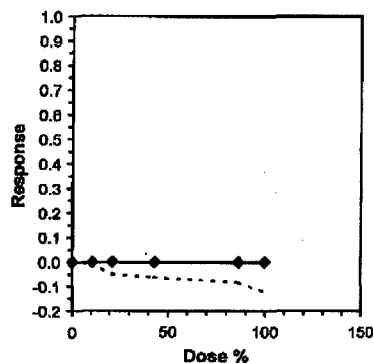
Kolmogorov D Test indicates normal distribution ( $p > 0.01$ )  
Bartlett's Test indicates equal variances ( $p = 0.37$ )  
The control means are not significantly different ( $p = 0.18$ )

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	2.03577	0.06382	21.1867	3.98296	4.6E-04	5, 64

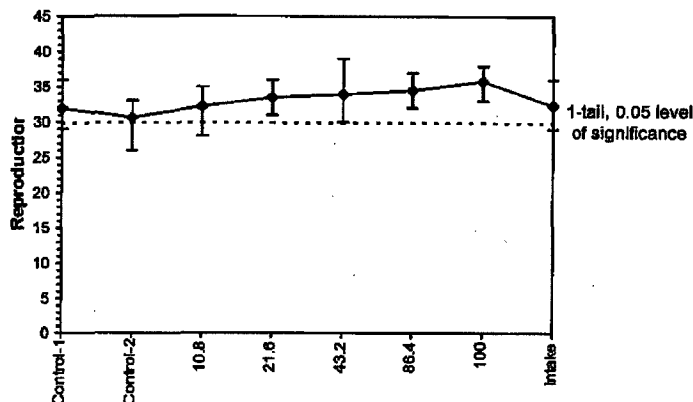
Treatments vs Control-1

## Linear Interpolation (200 Resamples)

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



Dose-Response Plot



Independent  
Review by  
Kerley E. Kaufman  
Initials:

## TVA / Sequoyah Nuclear Plant, Intake

Non-treated

May 08-15, 2012



## Statistical Analyses

## Ceriodaphnia Survival and Reproduction Test-Reproduction

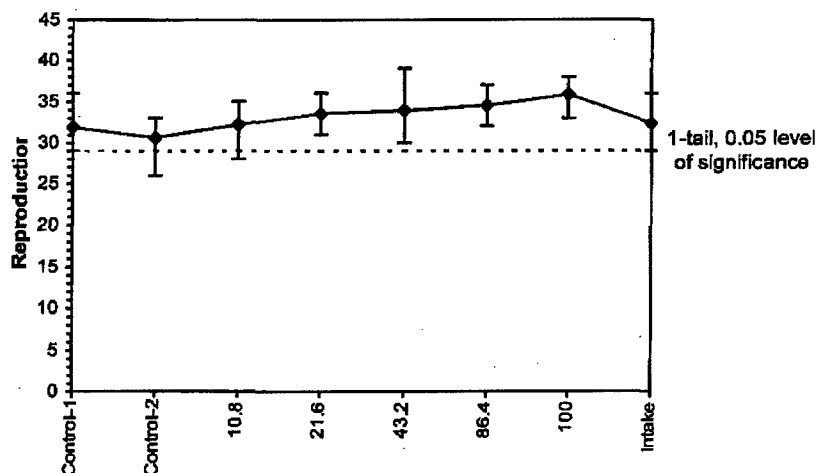
Start Date: 5/8/2012 Test ID: CdFRCR Sample ID: TVA / SQN 101 - Intake  
 End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report  
 Sample Date: May 2012 Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia  
 Comments:

Conc-%	1	2	3	4	5	6	7	8	9	10
Control-1	33.000	32.000	30.000	31.000	36.000	30.000	33.000	34.000	31.000	29.000
Control-2	30.000	33.000	33.000	30.000	31.000	29.000	32.000	31.000	31.000	26.000
10.8	33.000	33.000	30.000	33.000	31.000	32.000	28.000	35.000	35.000	32.000
21.6	36.000	32.000	36.000	33.000	31.000	32.000	35.000	32.000	35.000	33.000
43.2	35.000	31.000	33.000	39.000	33.000	30.000	35.000	37.000	33.000	33.000
86.4	34.000	36.000	32.000	35.000	37.000	35.000	34.000	34.000	33.000	35.000
100	37.000	36.000	35.000	35.000	37.000	38.000	33.000	36.000	35.000	36.000
Intake	33.000	35.000	29.000	32.000	31.000	36.000	32.000	31.000	33.000	31.000

Conc-%	Mean	N-Mean	Transform: Untransformed				N	1-Tailed		
			Mean	Min	Max	CV%		t-Stat	Critical	MSD
Control-1	31.900	1.0425	31.900	29.000	36.000	6.683	10			
Control-2	30.600	1.0000	30.600	26.000	33.000	6.750	10			
10.8	32.200	1.0523	32.200	28.000	35.000	6.677	10			
21.6	33.500	1.0948	33.500	31.000	36.000	5.495	10			
43.2	33.900	1.1078	33.900	30.000	39.000	7.921	10			
86.4	34.500	1.1275	34.500	32.000	37.000	4.156	10			
100	35.800	1.1699	35.800	33.000	38.000	3.906	10			
Intake	32.300	1.0556	32.300	29.000	36.000	6.370	10	-1.844	1.734	1.599

Auxiliary Tests	Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )	0.97072	0.688	-0.3101	0.44033		
F-Test indicates equal variances ( $p = 0.99$ )	1.00787	6.54109				
The control means are not significantly different ( $p = 0.18$ )	1.38493	2.10092				
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	1.59873	0.05225	14.45	4.25	0.08172	1, 18
Treatments vs Control-2						

Dose-Response Plot



Species: Ceriodaphnia dubia

Date: 05-08-12

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

**Daily Chemistry:**

Concentration		Day					
		0		1		2	
		CDT	ODT	CDT	ODT	CDT	Min
Analyst	Parameter						
CONTROL	pH (S.U.)	7.05	7.94	7.92	7.96	7.95	8.01
	DO (mg/L)	7.6	8.0	7.8	7.7	7.7	7.8
	Conductivity (µmhos/cm)	327		310		303	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		63	
	Hardness (mg CaCO <sub>3</sub> /L)	88		88		89	
	Temperature (°C)	24.9	25.2	25.0	25.1	24.9	24.9
10.8%	pH (S.U.)	7.89	7.94	7.96	7.97	7.90	8.01
	DO (mg/L)	7.9	8.0	7.8	7.6	7.8	7.8
	Conductivity (µmhos/cm)	306		297		292	
	Temperature (°C)	25.0	25.2	25.2	24.8	25.0	24.9
21.6%	pH (S.U.)	7.89	7.95	7.98	7.97	7.92	8.01
	DO (mg/L)	7.9	8.0	7.8	7.6	7.8	7.8
	Conductivity (µmhos/cm)	291		284		280	
	Temperature (°C)	25.0	25.2	25.2	25.1	25.0	24.9
43.2%	pH (S.U.)	7.89	7.95	7.99	7.97	7.91	8.02
	DO (mg/L)	7.9	8.1	7.8	7.6	7.8	7.8
	Conductivity (µmhos/cm)	265		259		257	
	Temperature (°C)	25.0	25.2	25.2	25.2	25.0	25.0
86.4%	pH (S.U.)	7.91	7.99	8.01	8.01	7.88	8.03
	DO (mg/L)	7.9	8.0	7.9	7.6	7.9	7.8
	Conductivity (µmhos/cm)	212		210		211	
	Temperature (°C)	25.0	25.1	25.2	25.2	25.0	25.0
100%	pH (S.U.)	7.92	8.00	8.02	8.04	7.99	8.06
	DO (mg/L)	7.9	8.1	7.9	7.6	7.9	7.8
	Conductivity (µmhos/cm)	197		193		197	
	Alkalinity (mg CaCO <sub>3</sub> /L)	79				81	
	Hardness (mg CaCO <sub>3</sub> /L)	81				81	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	25.2	25.1	25.2	25.0	25.0	24.8
100% Intake	pH (S.U.)	7.95	8.01	8.04	8.02	8.04	8.03
	DO (mg/L)	7.9	8.1	8.1	7.9	8.1	7.9
	Conductivity (µmhos/cm)	195		190		192	
	Alkalinity (mg CaCO <sub>3</sub> /L)	77				79	
	Hardness (mg CaCO <sub>3</sub> /L)	79				81	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	25.0	25.1	25.2	25.0	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final

Species: *Ceriodaphnia dubia*

Client: TVA / Sequoyah Nuclear Plant, Outfall 101

Date: 05-08-12

Analyst		Day							
		3		4		5		6	
Concentration	Parameter	Mnt	Mxn	Mnt	Mxn	Mnt	Mxn	Mnt	Mxn
CONTROL	pH (S.U.)	8.07	8.02	8.02	7.92	7.97	7.91	8.06	7.87
	DO (mg/L)	7.4	7.6	7.8	7.6	7.6	7.6	7.6	8.0
	Conductivity (µmhos/cm)	194		309		313		312	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		62			
	Hardness (mg CaCO <sub>3</sub> /L)	87		89		89			
	Temperature (°C)	24.8	24.9	24.9	24.9	24.7	25.2	24.7	24.9
10.8%	pH (S.U.)	8.07	8.03	7.95	7.91	7.94	7.93	8.10	7.86
	DO (mg/L)	7.7	7.9	8.0	7.8	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	294		283		291		291	
	Temperature (°C)	24.8	25.3	24.9	25.1	24.7	24.8	24.8	25.1
21.6%	pH (S.U.)	8.10	8.02	7.92	7.92	7.93	7.93	8.10	7.86
	DO (mg/L)	7.7	7.9	8.0	7.7	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	280		270		286		281	
	Temperature (°C)	24.8	25.2	24.9	25.1	24.7	24.8	24.8	24.9
43.2%	pH (S.U.)	8.09	8.03	7.86	7.92	7.87	7.94	8.11	7.85
	DO (mg/L)	7.7	7.9	8.0	7.7	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	258		253		263		263	
	Temperature (°C)	24.8	25.0	24.9	25.1	24.8	24.7	24.8	25.0
86.4%	pH (S.U.)	8.10	8.07	7.81	7.94	7.83	7.99	8.12	7.86
	DO (mg/L)	7.7	7.9	8.0	7.8	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	212		216		215		218	
	Temperature (°C)	24.8	25.0	24.9	25.0	24.8	25.0	24.8	25.0
100%	pH (S.U.)	8.05	8.06	7.76	7.98	7.78	8.01	8.16	7.87
	DO (mg/L)	7.7	7.8	8.0	7.8	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	196		188		196		199	
	Alkalinity (mg CaCO <sub>3</sub> /L)			79					
	Hardness (mg CaCO <sub>3</sub> /L)			81					
	TR Chlorine (mg/L)			20.10					
	Temperature (°C)	25.0	25.0	25.1	24.8	24.8	25.0	24.8	25.2
100% Intake	pH (S.U.)	8.12	8.02	8.06	7.95	8.02	7.93	8.16	7.87
	DO (mg/L)	7.7	7.8	8.0	7.9	8.1	7.8	7.7	8.1
	Conductivity (µmhos/cm)	194		182		190		190	
	Alkalinity (mg CaCO <sub>3</sub> /L)			79					
	Hardness (mg CaCO <sub>3</sub> /L)			79					
	TR chlorine (mg/L)			20.10					
	Temperature (°C)	24.9	24.9	24.8	24.9	25.0	25.1	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

**TVA / Sequoyah Nuclear Plant, Outfall 101 - Non-treated**  
**May 08-15, 2012**

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

**Daily Chemical Analyses**

Project number: 7875

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)	7.85	7.94	7.92	7.96	7.95	8.01	7.98	8.02	8.02	7.92	7.97	7.91	8.06	7.87
	DO (mg/L)	7.6	8.0	7.8	7.7	7.7	7.8	7.4	7.6	7.8	7.6	7.6	7.6	7.6	8.0
	Conductivity (µmhos/cm)	327		310		303		294		309		313		312	
	Alkalinity (mg/L CaCO <sub>3</sub> )	63		63		63		63		63		62			
	Hardness (mg/L CaCO <sub>3</sub> )	88		88		89		87		89		89			
	Temperature (°C)	24.9	25.2	25.0	25.1	24.9	24.9	24.8	24.9	24.9	24.9	24.7	25.2	24.7	24.9
16.8%	pH (SU)	7.89	7.94	7.96	7.97	7.90	8.01	8.07	8.03	7.95	7.91	7.94	7.93	8.10	7.86
	DO (mg/L)	7.9	8.0	7.8	7.6	7.8	7.8	7.7	7.9	8.0	7.8	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	306		297		292		294		283		291		291	
	Temperature (°C)	25.0	25.2	25.2	24.8	25.0	24.9	24.8	25.3	24.9	25.1	24.7	24.8	24.8	25.1
21.6%	pH (SU)	7.89	7.95	7.98	7.97	7.92	8.01	8.10	8.02	7.92	7.92	7.93	7.93	8.10	7.86
	DO (mg/L)	7.9	8.0	7.8	7.6	7.8	7.8	7.7	7.9	8.0	7.7	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	291		284		280		280		276		286		281	
	Temperature (°C)	25.0	25.2	25.2	25.1	25.0	24.9	24.8	25.2	24.9	25.1	24.7	24.8	24.8	24.9
43.2%	pH (SU)	7.89	7.95	7.99	7.97	7.91	8.02	8.09	8.03	7.86	7.92	7.87	7.94	8.11	7.85
	DO (mg/L)	7.9	8.1	7.8	7.6	7.8	7.8	7.7	7.9	8.0	7.7	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	265		259		257		258		253		263		263	
	Temperature (°C)	25.0	25.2	25.2	25.2	25.0	25.0	24.8	25.0	24.9	25.1	24.8	24.7	24.8	25.0
86.4%	pH (SU)	7.91	7.99	8.01	8.01	7.88	8.03	8.10	8.07	7.81	7.94	7.83	7.99	8.12	7.86
	DO (mg/L)	7.9	8.0	7.9	7.6	7.9	7.8	7.7	7.9	8.0	7.8	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	212		210		211		212		216		215		218	
	Temperature (°C)	25.0	25.1	25.2	25.2	25.0	25.0	24.8	25.0	24.9	25.0	24.8	25.0	24.8	25.0
100%	pH (SU)	7.92	8.00	8.02	8.04	7.89	8.06	8.05	8.06	7.76	7.98	7.78	8.01	8.16	7.87
	DO (mg/L)	7.9	8.1	7.9	7.6	7.9	7.8	7.7	7.8	8.0	7.8	8.0	7.6	7.6	8.0
	Conductivity (µmhos/cm)	197		193		197		196		188		196		199	
	Alkalinity (mg/L CaCO <sub>3</sub> )	79				81				79					
	Hardness (mg/L CaCO <sub>3</sub> )	81				81				81					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
100% Intake	Temperature (°C)	25.2	25.1	25.2	25.0	25.0	24.8	25.0	25.0	25.1	24.8	24.8	25.0	24.8	25.2
	pH (SU)	7.95	8.01	8.04	8.02	8.04	8.03	8.12	8.02	8.06	7.95	8.02	7.93	8.16	7.87
	DO (mg/L)	7.9	8.1	8.1	7.9	8.1	7.9	7.7	7.8	8.0	7.9	8.1	7.8	7.7	8.1
	Conductivity (µmhos/cm)	195		190		192		194		182		190		190	
	Alkalinity (mg/L CaCO <sub>3</sub> )	77				79				79					
	Hardness (mg/L CaCO <sub>3</sub> )	79				81				79					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
100% Intake	Temperature (°C)	25.0	25.1	25.2	25.0	25.0	24.8	24.9	24.9	24.8	24.9	25.0	25.1	24.9	25.2

File: sqn101\_050812chem.xls

Entered by: C. Johnson

Reviewed by: *X*

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

Species: Pimephales promelas

Client: Tennessee Valley Authority

County: Rhea

Facility: Sequoyah Nuclear Plant

Outfall: 101

NPDES #: TN0020168

Project #: 7875

Dilution preparation information:						Comments:
Dilution prep (%)	10.8	21.6	43.2	86.4	100	Each concentration was UV-treated for 2 minutes to remove pathogenic Interferences.
Effluent volume (mL)	270	540	1080	2160	2500	
Diluent volume (mL)	2230	1960	1420	340	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism information:		Test information:	
Organism age:	19.2 HOURS OLD	Randomizing template:	BLUE
Date and times organisms were born between:	05-07-12 1600	Incubator number and shelf location:	3B
Organism source:	ATOX BATCH Pp 05-07-12	Artemia CHM number:	CHM652
Transfer bowl information: pH = 7.79 S.U. Temperature = 24.8 °C		Drying information for weight determination:	
		Date / Time in oven:	05-15-12 1140
Average transfer volume: 0.1209 mL		Initial oven temperature:	60 °C
		Date / Time out of oven:	05-16-12 1140
		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	05-08-12		X	1545	JL	1112	JL	120507.01	120507.02	05-03-12A
1	05-09-12	0800	JL	1400	JL	1015	JL	120507.01	120507.02	05-03-12B
2	05-10-12	0830	JL	1430	JL	1053	JL	120509.25	120509.26	05-07-12
3	05-11-12	0800	JL	1400	JL	1015	JL	120509.25	120509.26	05-09-12A
4	05-12-12	0800	JL	1400	JL	1013	JL	120511.20	120511.21	05-10-12A
5	05-13-12	0800	JL	1400	JL	1014	JL	120511.20	120511.21	05-10-12B
6	05-14-12	0800	JL	1400	JL	1012	JL	120511.20	120511.21	05-10-12B
7	05-15-12					1015	JL			

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC <sub>50</sub>	> 1007.
Average weight per initial larvae:	0.586		NOEC	1007.
Average weight per surviving larvae:	0.586	≥ 0.25mg/larvae	LOEC	> 1007.
			ChV	> 1007.
			IC <sub>25</sub>	> 1007.



Species: *Pimephales promelas*

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

Date: 05-08-12

**Survival and Growth Data**

Day	CONTROL				10.8%				21.6%			
	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
<b>A = Pan weight (mg)</b> Tray color code: <u>Magenta</u> Analyst: <u>MLP</u> Date: <u>05-02-12</u>												
13.77 13.56 14.13 13.25 14.39 13.44 13.89 13.94 14.12 13.54 12.70 14.06												
<b>B = Pan + Larvae weight (mg)</b> Analyst: <u>COJ</u> Date: <u>05-12-12</u>												
19.68 20.03 19.20 19.24 20.85 20.15 20.03 20.92 19.65 19.76 19.29 20.69												
<b>C = Larvae weight (mg) = B - A</b> Hand calculated. Analyst: <u>[Signature]</u>												
5.91 6.47 5.07 5.99 6.46 6.21 6.16 6.98 5.53 6.22 6.59 6.63												
<b>Weight per initial number of larvae (mg)</b> = C / Initial number of larvae Hand calculated. Analyst: <u>[Signature]</u>												
0.591 0.647 0.507 0.599 0.646 0.621 0.616 0.698 0.553 0.622 0.659 0.663												
<b>Average weight per initial number of larvae (mg)</b> <b>Percent reduction from control (%)</b> 0.586      0.645      -10.17      0.624      -6.57												

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: 05-08-12

**Survival and Growth Data**

Day	43.2%				86.4%				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>MKT</u> Date: <u>05.02.12</u>												
13.93 13.52 14.95 13.26 14.40 14.46 13.36 14.21 13.58 14.42 14.09 13.12												
B = Pan + Larvae weight (mg) Analyst: <u>MSJ</u> Date: <u>05.13.12</u>												
19.58 19.41 21.29 19.32 19.77 20.19 19.46 19.51 20.31 21.22 20.83 19.65												
C = Larvae weight (mg) = B - A												
Hand calculated. <u>[Signature]</u> Analyst:												
5.65 6.09 6.34 6.06 5.37 5.73 6.10 5.60 6.73 6.80 6.76 6.53												
Weight per initial number of larvae (mg) = C / Initial number of larvae												
Hand calculated. <u>[Signature]</u> Analyst:												
0.565 0.609 0.634 0.606 0.537 0.573 0.610 0.560 0.673 0.680 0.676 0.653												
Average weight per initial number of larvae (mg)	0.604			-3.07		0.570		2.77		0.671		-14.47
Percent reduction from control (%)												

**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.

<b>Comments:</b>

Species: *Pimephales promelas*

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

Date: 05-08-12

**Survival and Growth Data**

Day	100% Intake				
	Y	Z	AA	BB	
0	10	10	10	10	
1	10	10	10	10	
2	10	10	10	10	
3	10	9 <sup>nd</sup>	10	10	
4	10	9	10	10	
5	10	9	10	10	
6	10	9	10	10	
7	10	9	10	10 <sup>th</sup>	
<b>A = Pan weight (mg)</b>					
Tray color code: <u>Magenta</u>					
Analyst: <u>MKD</u>					
Date: <u>05-02-12</u>					
	13.71	13.83	15.29	13.20	
<b>B = Pan + Larvae weight (mg)</b>					
Analyst: <u>CDJ</u>					
Date: <u>05-13-12</u>					
	19.68	19.75	20.90	20.24	
<b>C = Larvae weight (mg) = B - A</b>					
Hand calculated. <u>[Signature]</u>					
	5.97	5.92	5.61	7.04	
<b>Weight per initial number of larvae (mg) = C / Initial number of larvae</b>					
Hand calculated. <u>[Signature]</u>					
	0.597	0.592	0.561	0.704	
<b>Average weight per initial number of larvae (mg)</b>					
<b>Percent reduction from control (%)</b>					
	0.614	-4.77			

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:**

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TVA / Sequoyah Nuclear Plant, Outfall 101  
UV-treated  
May 08-15, 2012

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

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

Environmental Testing Solutions, Inc.

Project number: 7875

Concentration (%)	Replicate	Initial number of larvae	Final number of larvae	A = Pns weight (mg)	B = Pns + Larvae weight (mg)	Larvae weight (mg) = A - B	Not for Compliance Assessment, Internal Laboratory QC			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)	Coefficient of variation (Mean weight per surviving number of larvae) (%)					
Control	A	10	10	13.77	19.68	5.91	0.591	0.586	9.9	0.591	100.0	0.586	9.9	Not applicable
	B	10	10	13.56	20.03	6.47	0.647			0.647				
	C	10	10	14.13	19.20	5.07	0.507			0.507				
	D	10	10	13.25	19.24	5.99	0.599			0.599				
10.8%	E	10	10	14.39	20.85	6.46	0.646	0.645	5.8	0.646	100.0	0.645	5.8	-10.1
	F	10	10	13.94	20.15	6.21	0.621			0.621				
	G	10	10	13.89	20.05	6.16	0.616			0.616				
	H	10	10	13.94	20.92	6.98	0.698			0.698				
21.6%	I	10	10	14.12	19.65	5.53	0.553	0.624	8.2	0.553	100.0	0.624	8.2	-6.5
	J	10	10	13.54	19.76	6.22	0.622			0.622				
	K	10	10	12.70	19.29	6.59	0.659			0.659				
	L	10	10	14.06	20.69	6.63	0.663			0.663				
43.2%	M	10	10	13.93	19.58	5.65	0.565	0.604	4.7	0.565	100.0	0.604	4.7	-3.0
	N	10	10	13.52	19.61	6.09	0.609			0.609				
	O	10	10	14.95	21.29	6.34	0.634			0.634				
	P	10	10	13.26	19.32	6.06	0.606			0.606				
86.4%	Q	10	10	14.40	19.77	5.37	0.537	0.570	5.4	0.537	100.0	0.570	5.4	2.7
	R	10	10	14.46	20.19	5.73	0.573			0.573				
	S	10	10	13.36	19.46	6.10	0.610			0.610				
	T	10	10	14.21	19.81	5.60	0.560			0.560				
100%	U	10	10	13.58	20.31	6.73	0.673	0.671	1.8	0.673	100.0	0.671	1.8	-14.4
	V	10	10	14.42	21.22	6.80	0.680			0.680				
	W	10	10	14.07	20.83	6.76	0.676			0.676				
	X	10	10	13.12	19.65	6.53	0.653			0.653				
100% Intake	Y	10	10	13.71	19.68	5.97	0.597	0.630	10.1	0.597	97.5	0.614	10.2	-4.7
	Z	10	9	13.83	19.75	5.92	0.658			0.592				
	AA	10	10	15.29	20.90	5.61	0.561			0.561				
	BB	10	10	13.20	20.24	7.04	0.704			0.704				

Outfall 101:

Dunnnett's MSD value: 0.0670  
PMDS: 11.4

MSD =  
PMDS =

Minimum Significant Difference  
Percent Minimum Significant Difference

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

Intake:

Dunnnett's MSD value: 0.0829  
PMDS: 14.1

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

Lower and upper PMDS bounds were determined from the 10th and 90th percentile, respectively, of PMDS 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

UV-treated

May 08-15, 2012

## Statistical Analyses



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

Start Date: 5/8/2012 Test ID: PpFRCR Sample ID: TVA / SQN, Outfall 101  
End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report  
Sample Date: May 2012 Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas  
Comments: UV-treated

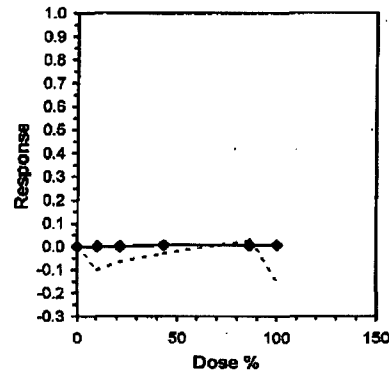
Conc-%	1	2	3	4
D-Control	0.5910	0.6470	0.5070	0.5990
10.8	0.6460	0.6210	0.6160	0.6980
21.6	0.5530	0.6220	0.6590	0.6630
43.2	0.5650	0.6090	0.6340	0.6060
86.4	0.5370	0.5730	0.6100	0.5600
100	0.6730	0.6800	0.6760	0.6530
Intake	0.5970	0.5920	0.5810	0.7040

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.5860	1.0000	0.5860	0.5070	0.6470	9.929	4				0.6185	1.0000
10.8	0.6453	1.1011	0.6453	0.6160	0.6980	5.817	4	-2.130	2.410	0.0670	0.6185	1.0000
21.6	0.6243	1.0853	0.6243	0.5530	0.6630	8.163	4	-1.375	2.410	0.0670	0.6185	1.0000
43.2	0.6035	1.0299	0.6035	0.5650	0.6340	4.734	4	-0.629	2.410	0.0670	0.6147	0.9938
86.4	0.5700	0.9727	0.5700	0.5370	0.6100	5.358	4	0.575	2.410	0.0670	0.6147	0.9938
100	0.6705	1.1442	0.6705	0.6530	0.6800	1.792	4	-3.038	2.410	0.0670	0.6147	0.9938
Intake	0.6135	1.0489	0.6135	0.5810	0.7040	10.171	4					

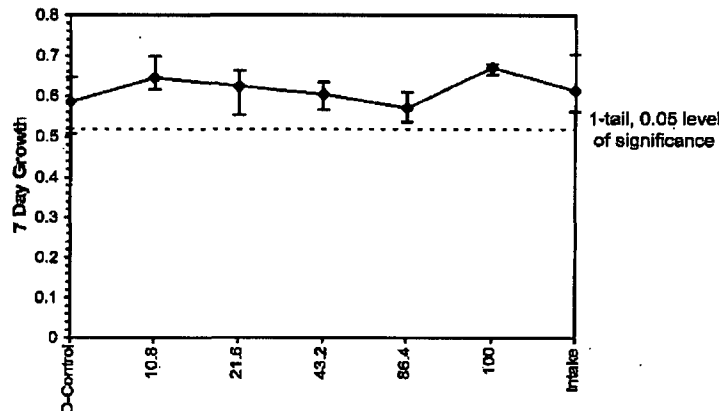
Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )					0.9571	0.884	-0.4767	0.34009						
Bartlett's Test indicates equal variances ( $p = 0.29$ )					6.13792	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					100	>100		1	0.06704	0.1144	0.00565	0.00155	0.01863	5, 18
Treatments vs D-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



File: sqn101\_050812data-uv.xlsx  
Entered by: J. Sumner  
Reviewed by:



# TVA / Sequoyah Nuclear Plant, Intake

UV-treated

May 08-15, 2012



## Statistical Analyses

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

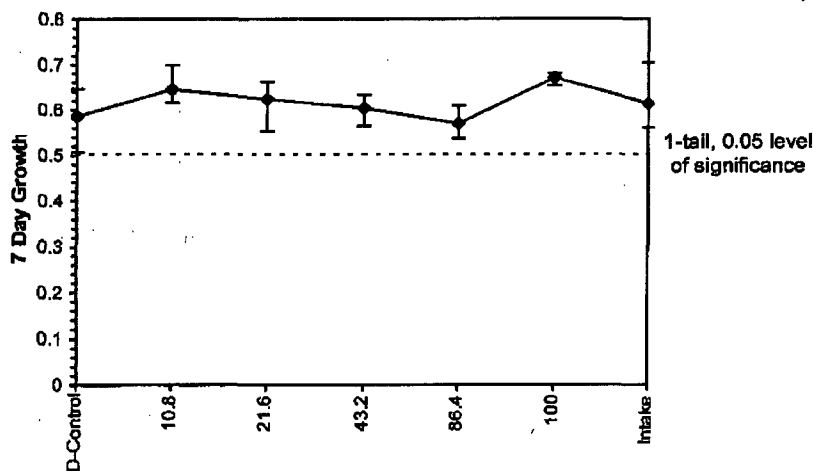
Start Date: 5/8/2012 Test ID: PpFRCR Sample ID: TVA / SQN, Intake  
 End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report  
 Sample Date: May 2012 Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas  
 Comments: UV-treated

Conc-%	1	2	3	4
D-Control	0.5910	0.6470	0.5070	0.5990
10.8	0.6460	0.6210	0.6160	0.6980
21.6	0.5530	0.6220	0.6590	0.6630
43.2	0.5650	0.6090	0.6340	0.6060
86.4	0.5370	0.5730	0.6100	0.5600
100	0.6730	0.6800	0.6760	0.6530
Intake	0.5970	0.5920	0.5610	0.7040

Conc-%	Mean	N-Mean	Transform: Untransformed				N	1-Tailed		
			Mean	Min	Max	CV%		t-Stat	Critical	MSD
D-Control	0.5660	1.0000	0.5660	0.5070	0.6470	9.929	4			
10.8	0.6453	1.1011	0.6453	0.6160	0.6980	5.817	4			
21.6	0.6243	1.0653	0.6243	0.5530	0.6630	8.163	4			
43.2	0.6035	1.0299	0.6035	0.5650	0.6340	4.734	4			
86.4	0.5700	0.9727	0.5700	0.5370	0.6100	5.358	4			
100	0.6705	1.1442	0.6705	0.6530	0.6800	1.792	4			
Intake	0.6135	1.0469	0.6135	0.5610	0.7040	10.171	4	-0.645	1.943	0.0829

Auxiliary Tests	Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )	0.9723	0.749	0.34802	-0.3978		
F-Test indicates equal variances ( $p = 0.91$ )	1.15016	47.4683				
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	0.08289	0.14146	0.00151	0.00364	0.543	1, 6
Treatments vs D-Control						

### Dose-Response Plot



Species: Pimephales promelas

Date: 05-08-12

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

Daily Chemistry:

		Day					
		0		1		2	
Analyst		CDT	CDT	CDT	CDT	CDT	MAF
Concentration	Parameter						
CONTROL UV-treated	pH (S.U.)	7.90	7.91	7.90	7.91	7.90	7.84
	DO (mg/L)	7.9	8.0	8.0	7.8	7.8	7.8
	Conductivity (µmhos/cm)	316		313		300	
	Alkalinity (mg CaCO <sub>3</sub> /L)	62		63		64	
	Hardness (mg CaCO <sub>3</sub> /L)	87		87		89	
	Temperature (°C)	24.9	24.8	24.9	24.8	24.8	24.5
10.8%	pH (S.U.)	7.88	7.84	7.78	7.82	7.98	7.85
	DO (mg/L)	7.9	7.7	8.0	7.7	7.9	7.8
	Conductivity (µmhos/cm)	305		301		290	
	Temperature (°C)	25.0	24.8	25.0	24.9	24.8	24.5
21.6%	pH (S.U.)	7.89	7.90	7.99	7.82	7.98	7.85
	DO (mg/L)	8.0	8.0	8.0	7.6	7.9	7.8
	Conductivity (µmhos/cm)	291		289		280	
	Temperature (°C)	25.0	24.8	25.0	24.9	24.8	24.5
43.2%	pH (S.U.)	7.89	7.89	7.99	7.83	7.97	7.84
	DO (mg/L)	8.0	7.9	8.0	7.6	8.0	7.8
	Conductivity (µmhos/cm)	266		263		259	
	Temperature (°C)	25.0	24.5	25.1	24.6	24.8	24.6
86.4%	pH (S.U.)	7.90	7.90	8.00	7.82	7.94	7.88
	DO (mg/L)	7.9	7.9	8.0	7.6	8.0	7.8
	Conductivity (µmhos/cm)	220		215		213	
	Temperature (°C)	25.0	24.7	25.1	24.6	24.8	24.6
100%	pH (S.U.)	7.92	7.91	8.02	7.84	7.94	7.89
	DO (mg/L)	7.9	7.9	8.0	7.7	8.0	7.8
	Conductivity (µmhos/cm)	199		198		197	
	Alkalinity (mg CaCO <sub>3</sub> /L)	79				79	
	Hardness (mg CaCO <sub>3</sub> /L)	81				81	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	25.0	24.6	25.1	24.5	25.0	24.7
100% Intake	pH (S.U.)	7.94	7.95	8.05	7.85	8.01	7.91
	DO (mg/L)	8.0	7.9	8.0	7.6	8.2	8.0
	Conductivity (µmhos/cm)	194		195		189	
	Alkalinity (mg CaCO <sub>3</sub> /L)	79				77	
	Hardness (mg CaCO <sub>3</sub> /L)	81				79	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	25.1	24.6	25.2	24.5	25.1	24.7
		Initial	Final	Initial	Final	Initial	Final

Species: Pimephales promelas

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

Date: 05-08-12

Analyst		Day							
		3		4		5		6	
		Min	Max	Min	Max	Min	Max	Min	Max
Concentration	Parameter								
CONTROL UV-treated	pH (S.U.)	7.97	7.69	7.97	7.62	7.92	7.69	8.07	7.57
	DO (mg/L)	8.0	7.1	8.0	6.9	7.6	6.5	7.0	6.8
	Conductivity (µmhos/cm)	304		300		309		304	
	Alkalinity (mg CaCO <sub>3</sub> /L)	64		63		62			
	Hardness (mg CaCO <sub>3</sub> /L)	89		89		89			
	Temperature (°C)	24.8	24.6	24.9	24.8	24.7	24.7	24.9	24.7
10.8%	pH (S.U.)	7.98	7.67	7.94	7.60	7.91	7.63	8.09	7.50
	DO (mg/L)	8.0	6.9	8.0	6.9	7.6	6.6	7.0	6.8
	Conductivity (µmhos/cm)	295		283		289		290	
	Temperature (°C)	24.8	24.4	25.0	24.5	24.8	24.6	25.0	24.5
21.6%	pH (S.U.)	7.98	7.68	7.93	7.61	7.89	7.61	8.10	7.50
	DO (mg/L)	8.0	6.9	8.0	6.8	7.6	6.5	7.8	6.8
	Conductivity (µmhos/cm)	283		269		284		287	
	Temperature (°C)	24.8	24.7	25.0	24.7	24.8	24.6	25.0	24.5
43.2%	pH (S.U.)	7.99	7.68	7.88	7.63	7.86	7.60	8.11	7.50
	DO (mg/L)	8.0	6.9	7.9	6.8	7.6	6.5	7.8	6.8
	Conductivity (µmhos/cm)	260		248		259		266	
	Temperature (°C)	24.8	24.8	25.0	24.7	24.8	24.6	25.0	24.5
86.4%	pH (S.U.)	8.00	7.68	7.81	7.73	7.79	7.65	8.13	7.50
	DO (mg/L)	8.0	6.9	8.0	6.8	7.5	6.5	7.8	6.7
	Conductivity (µmhos/cm)	215		207		221		220	
	Temperature (°C)	24.9	24.6	25.0	24.8	24.8	24.9	25.0	24.6
100%	pH (S.U.)	8.02	7.69	7.78	7.69	7.76	7.68	8.15	7.50
	DO (mg/L)	8.1	6.8	8.0	7.0	7.6	6.5	7.9	6.7
	Conductivity (µmhos/cm)	201		189		199		200	
	Alkalinity (mg CaCO <sub>3</sub> /L)			79					
	Hardness (mg CaCO <sub>3</sub> /L)			81					
	TR Chlorine (mg/L)			20.10					
	Temperature (°C)	25.0	24.6	25.1	24.8	24.8	24.8	25.2	24.3
100% Intake	pH (S.U.)	8.04	7.76	7.99	7.71	7.94	7.70	8.16	7.52
	DO (mg/L)	8.2	6.8	8.2	6.8	7.6	6.7	7.9	6.6
	Conductivity (µmhos/cm)	196		184		191		193	
	Alkalinity (mg CaCO <sub>3</sub> /L)			79					
	Hardness (mg CaCO <sub>3</sub> /L)			81					
	TR chlorine (mg/L)			20.10					
	Temperature (°C)	24.8	24.6	25.0	24.7	24.8	24.9	25.1	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Independent review by  
Kathy E. Keenan  
Initials



**TVA / Sequoyah Nuclear Plant, Outfall 101 - UV-treated**  
**May 08-15, 2012**

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

**Daily Chemical Analyses**

Project number: 7875

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)	7.88	7.91	7.98	7.81	7.98	7.84	7.97	7.69	7.97	7.62	7.92	7.69	8.07	7.51
	DO (mg/L)	7.9	8.0	8.0	7.8	7.8	7.8	8.0	7.1	8.0	6.9	7.6	6.5	7.8	6.8
	Conductivity (µmhos/cm)	316		313		300		304		306		309		304	
	Alkalinity (mg/L CaCO <sub>3</sub> )	62		63		64		64		63		62			
	Hardness (mg/L CaCO <sub>3</sub> )	87		87		89		89		89		89			
	Temperature (°C)	24.9	24.8	24.9	24.8	24.8	24.5	24.8	24.6	24.9	24.8	24.7	24.7	24.9	24.7
10.8%	pH (SU)	7.88	7.84	7.98	7.82	7.98	7.85	7.98	7.67	7.94	7.60	7.91	7.63	8.09	7.50
	DO (mg/L)	7.9	7.9	8.0	7.7	7.9	7.8	8.0	6.9	8.0	6.9	7.6	6.6	7.8	6.8
	Conductivity (µmhos/cm)	305		301		290		295		283		299		298	
	Temperature (°C)	25.0	24.8	25.0	24.9	24.8	24.5	24.8	24.4	25.0	24.5	24.8	24.6	25.0	24.5
21.6%	pH (SU)	7.89	7.90	7.99	7.82	7.98	7.85	7.98	7.68	7.93	7.61	7.89	7.61	8.10	7.50
	DO (mg/L)	8.0	8.0	8.0	7.6	7.9	7.8	8.0	6.9	8.0	6.8	7.6	6.5	7.8	6.8
	Conductivity (µmhos/cm)	291		289		280		283		269		284		287	
	Temperature (°C)	25.0	24.8	25.0	24.9	24.8	24.5	24.8	24.7	25.0	24.7	24.8	24.6	25.0	24.5
43.2%	pH (SU)	7.89	7.89	7.99	7.83	7.97	7.84	7.99	7.68	7.88	7.63	7.86	7.60	8.11	7.50
	DO (mg/L)	8.0	7.9	8.0	7.6	8.0	7.8	8.0	6.9	7.9	6.8	7.6	6.5	7.8	6.8
	Conductivity (µmhos/cm)	266		263		259		260		248		259		266	
	Temperature (°C)	25.0	24.5	25.1	24.6	24.8	24.6	24.8	24.8	25.0	24.7	24.8	24.6	25.0	24.5
86.4%	pH (SU)	7.90	7.90	8.00	7.82	7.94	7.88	8.00	7.68	7.81	7.73	7.79	7.65	8.13	7.50
	DO (mg/L)	7.9	7.9	8.0	7.6	8.0	7.8	8.0	6.9	8.0	6.8	7.5	6.5	7.8	6.7
	Conductivity (µmhos/cm)	220		215		213		215		207		221		220	
	Temperature (°C)	25.0	24.7	25.1	24.6	24.8	24.6	24.9	24.6	25.0	24.8	24.8	24.9	25.0	24.6
100%	pH (SU)	7.92	7.91	8.02	7.84	7.94	7.89	8.02	7.69	7.78	7.69	7.76	7.68	8.15	7.50
	DO (mg/L)	7.9	7.9	8.0	7.7	8.0	7.8	8.1	6.8	8.0	7.0	7.6	6.5	7.9	6.7
	Conductivity (µmhos/cm)	199		198		197		201		189		199		200	
	Alkalinity (mg/L CaCO <sub>3</sub> )	79				79				79					
	Hardness (mg/L CaCO <sub>3</sub> )	81				81				81					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.0	24.6	25.1	24.5	25.0	24.7	25.0	24.6	25.1	24.8	24.8	24.8	25.2	24.3
100% Intake	pH (SU)	7.94	7.95	8.05	7.85	8.01	7.91	8.04	7.76	7.99	7.71	7.94	7.70	8.16	7.52
	DO (mg/L)	8.0	7.9	8.0	7.6	8.2	8.0	8.2	6.8	8.2	6.8	7.6	6.7	7.9	6.6
	Conductivity (µmhos/cm)	194		195		189		196		184		191		193	
	Alkalinity (mg/L CaCO <sub>3</sub> )	79				77				79					
	Hardness (mg/L CaCO <sub>3</sub> )	81				79				81					
	Total Residual Chlorine (mg/L)	<0.10				<0.10				<0.10					
	Temperature (°C)	25.1	24.6	25.2	24.5	25.1	24.7	24.8	24.6	25.0	24.7	24.8	24.9	25.1	24.6



**Total Residual Chlorine**  
**(Orion Electrode Method, Orion 97-70)**

Matrix: Water, RL = 0.10 mg/L

Meter: Accumet Model AR25 pH/Ion Meter

Analyst MHC  
Date analyzed 05-08-12

Iodide reagent: INRS16  
Acid reagent: INRA12

**Calibration:**

	0.10 mg/L	1.00 mg/L	Slope
Reference standard number	<u>INSS1064</u>	<u>INSS1064</u>	<u>-49.1%</u>

Note: For samples with a residual chlorine of > 1.0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples.

**Laboratory control standard:**

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS1064</u>	<u>0.50</u>	<u>0.528</u>	<u>105.6%</u>

**Duplicate sample precision:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\{(S - D) / [(S + D) / 2]\} \times 100$ (acceptable range = $\pm 10\%$ )
<u>120508-03</u>	<u>CARR</u>	<u>clear, particles, pale tan</u>	<u>S &lt; 0.00505</u>	
<u>↓</u>	<u>Duplicate</u>		<u>D &lt; 0.00335</u>	<u>05-08-12</u> <u>MHC</u>

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	<b>Reagent Blank</b>		<u>&lt; 0.0195</u>
<u>120508-01</u>	<u>FOXWOOD</u>	<u>clear, particles, pale tan</u>	<u>&lt; 0.00190</u>
<u>120508-02</u>	<u>HOLCOM</u>	<u>clear, particles, pale tan</u>	<u>&lt; 0.00149</u>
<u>120508-07</u>	<u>FARMVILLE</u>	<u>clear, particles, no color</u>	<u>&lt; 0.00302</u>
<u>120508-10</u>	<u>SOUTH CARY</u>	<u>clear, particles, no color</u>	<u>&lt; 0.00155</u>
<u>120508-05</u>	<u>ENGELHARD</u>	<u>clear, particles, tan</u>	<u>&lt; <math>8.28 \times 10^{-10}</math></u>
<u>120508-06</u>	<u>PASQUOTANK</u>	<u>clear, particles, no color</u>	<u>&lt; <math>4.39 \times 10^{-9}</math></u>
<u>120507-01</u>	<u>TVA/SQN101</u>	<u>clear, particles, no color</u>	<u>&lt; 0.00235</u>
<u>120507-02</u>	<u>↓ INTAKE</u>	<u>clear, particles, no color</u>	<u>&lt; 0.00131</u>
<u>120507-03</u>	<u>TVA/WBN101</u>	<u>clear, particles, no color</u>	<u>&lt; 0.000802</u>

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

**Laboratory control standard:**

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS1064</u>	<u>0.50</u>	<u>0.516</u>	<u>103.2%</u>

Reviewed by JP  
Date reviewed 05-08-12

**Meter:** Accumet Model AR25 pH/Ion Meter

Iodide reagent: 05-08-12  
Acid reagent: me

	0.10 mg/L	1.00 mg/L	Slope
Reference standard number			25-08-12

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = $MV / TV \times 100$ (acceptable range = 90 to 110%)
	0.50		08.08.12 <i>ml</i>

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\{[(S-D)/(S+D)/2]\} \times 100$ (acceptable range = $\pm 10\%$ )
170507.04	TVA/WORKING INTAKE	clear, particles, color	S < 0.0025	
↓	Duplicate		D < 0.0053	05.08.12 JK

[illegible]

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INS1064	0.50	0.458	91.6%

**SOP C8 – Exhibit C8.1, revision 06-01-11**



**Total Residual Chlorine**  
**(Orion Electrode Method, Orion 97-70)**

Matrix: Water, RL = 0.10 mg/L

Meter: Accumet Model AR25 pH/Ion Meter

Analyst JTB  
Date analyzed 05-10-12

Iodide reagent: INR 516  
Acid reagent: INR 472

**Calibration:**

	0.10 mg/L	1.00 mg/L	Slope
Reference standard number	<u>INSS 1064</u>	<u>INSS 1064</u>	<u>-45.9%</u>

Note: For samples with a residual chlorine of > 1.0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples.

**Laboratory control standard:**

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1064</u>	<u>0.50</u>	<u>0.510</u>	<u>102.0%</u>

**Duplicate sample precision:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\frac{(S - D)}{((S + D)/2)} \times 100$ (acceptable range = $\pm 10\%$ )
<u>120510.04</u>	<u>ARCELOR</u>	<u>pale grey, clear particles</u>	<u>S 0.00549</u>	
<u>1</u>	<u>Duplicate</u>		<u>D 0.00506</u>	<u>30 05-10-12</u>

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	<b>Reagent Blank</b>		<u>0.00484</u>
<u>120510.05</u>	<u>ENGELHARD</u>	<u>yellow, clear, no particles</u>	<u>0.00392</u>
<u>120510.06</u>	<u>PASQUOTANK</u>	<u>no color, clear, no particles</u>	<u>0.000867</u>
<u>120509.25</u>	<u>TVA / SQN 101</u>	<u>no color, clear, particles</u>	<u>0.00486</u>
<u>120509.26</u>	<u>↓ INTAKE</u>	<u>no color, clear, no particles</u>	<u>0.00113</u>
<u>120509.27</u>	<u>TVA / WBN 101</u>	<u>no color, clear, no particles</u>	<u>0.00122</u>
<u>120509.28</u>	<u>↓ INTAKE</u>	<u>no color, clear, no particles</u>	<u>0.00431</u>
<u>120509.29</u>	<u>TVA / WBN 113</u>	<u>no color, cloudy, particles</u>	<u>0.00255</u>
<u>120509.30</u>	<u>↓ INTAKE</u>	<u>no color, cloudy, no particles</u>	<u>0.00213</u>
<u>120510.08</u>	<u>CFPU - LOUGHLIN</u>	<u>pale yellow, clear, no particles</u>	<u>0.00567</u>

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

**Laboratory control standard:**

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
	<u>0.50</u>		<u>105 05-10-12</u>

Reviewed by K  
Date reviewed 051012



**Total Residual Chlorine**  
**(Orion Electrode Method, Orion 97-70)**

Matrix: Water, RL = 0.10 mg/L  
Meter: Accumet Model AR25 pH/Ion Meter

Analyst JSB  
Date analyzed 05.10.12

Iodide reagent: JSB 05.10.12  
Acid reagent: JSB 05.10.12

**Calibration:**

	0.10 mg/L	1.00 mg/L	Slope
Reference standard number			<u>JSB 05.10.12</u>

Note: For samples with a residual chlorine of > 1.0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples.

**Laboratory control standard:**

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1064</u>	<u>0.50</u>	<u>0.460</u>	<u>92.0%</u>

**Duplicate sample precision:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\frac{(S - D)}{[(S + D)/2]} \times 100$ (acceptable range = $\pm 10\%$ )
<u>120510.03</u>	<u>CORR</u>	<u>no color, cloudy, particles</u>	<u>S 0.000798</u>	
<u>1</u>	<u>Duplicate</u>		<u>D 0.000776</u>	<u>JSB 05.10.12</u>

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	<u>Reagent Blank</u>		<u>JSB 05.10.12</u>
<u>120510.01</u>	<u>FOXWOOD</u>	<u>pale yellow, clear, no particles</u>	<u>0.00668</u>
<u>120510.02</u>	<u>HOLCIM</u>	<u>no color, cloudy, no particles</u>	<u>0.00642</u>
<u>120510.07</u>	<u>FARMVILLE</u>	<u>no color, clear, no particles</u>	<u>0.00102</u>
<u>120510.09</u>	<u>S. CARY</u>	<u>no color, clear, no particles</u>	<u>0.00218</u>
			<u>JSB 05.10.12</u>

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

**Laboratory control standard:**

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1064</u>	<u>0.50</u>	<u>0.516</u>	<u>103.6%</u>

Reviewed by W  
Date reviewed 05.10.12



### Total Residual Chlorine (Orion Electrode Method, Orion 97-70)

Matrix: Water, RL = 0.10 mg/L

Meter: Accumet Model AR25 pH/Ion Meter

Analyst: PLW  
Date analyzed: 05.12.12

Iodide reagent: IN 2516  
Acid reagent: IN 2517

#### Calibration:

	0.10 mg/L	1.00 mg/L	Slope
Reference standard number	<u>IN 251064</u>	<u>IN 251064</u>	<u>-52.0%</u>

Note: For samples with a residual chlorine of &gt; 1.0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples.

#### Laboratory control standard:

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>IN 251064</u>	<u>0.50</u>	<u>0.520</u>	<u>105.6%</u>

#### Duplicate sample precision:

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\frac{(S - D)}{[(S + D)/2]} \times 100$ (acceptable range = $\pm 10\%$ )
<u>120512.03</u>	<u>CORR</u>	<u>particulate</u>	<u>S 0.00213</u>	
<u>↓</u>	<u>Duplicate</u>		<u>D 0.00206</u>	<u>4.4%</u>

#### Sample measurements:

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	Reagent Blank		<u>0.00921</u>
<u>120512.01</u>	<u>FOXWOOD</u>	<u>no color, clear</u>	<u>0.00650</u>
<u>120512.02</u>	<u>HOLCIM</u>	<u>no color, clear</u>	<u>0.00746</u>
<u>120512.07</u>	<u>FARMVILLE</u>	<u>pale tan, clear</u>	<u>0.00503</u>
<u>120512.08</u>	<u>SOUTH CARY</u>	<u>pale tan, clear</u>	<u>0.00280</u>
<u>120512.05</u>	<u>ENGELHARD</u>	<u>yellow, clear, sulfur smell</u>	<u>0.0000569</u>
<u>120512.06</u>	<u>PASQUOTANK</u>	<u>no color, clear</u>	<u>0.000310</u>
<u>120512.04</u>	<u>ARCELOR</u>	<u>pale grey, <sup>bill</sup>particulate, clear</u>	<u>0.0000500</u>
<u>120512.09</u>	<u>CAMP LEBLANC</u>	<u>no color, clear</u>	<u>0.0000183</u>
<u>120512.10</u>	<u>PINOVA</u>	<u>no color, clear, particulate</u>	<u>0.0000310</u>

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

#### Laboratory control standard:

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
	<u>0.50</u>		<u>0.5212</u>

Reviewed by: PL  
Date reviewed: 05.12.12

**Matrix:** Water, RL = 0.10 mg/L

**Meter:** Accumet Model AR25 pH/Ion Meter

Iodide reagent: 051212  
Acid reagent: 12

	0.10 mg/L	1.00 mg/L	Slope
Reference standard number	<u>                    </u> <i>n osnn</i>		

**Note:** For samples with a residual chlorine of  $> 1.0 \text{ mg/L}$ , the calibration range must be adjusted to bracket the chlorine levels of the samples.

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INS51064	0.50	0.486	97.2%

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\{(S - D) / [(S + D) / 2]\} \times 100$ (acceptable range = $\pm 10\%$ )
120511-20	TVASDN101	pale tan, clear	S 0.00122	
↓	Duplicate		D 0.00109	-2.42%

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	Reagent Blank		→ 0.0122
120511.21	TVA SQN INT	pale tan, clear, particles	10.00170
120511.22	TVA WBN 101	no color, clear	10.00094
120511.23	101, INT	no color, clear	10.00075
120511.24	113	no color, clear	10.00175
120511.25	113 INT	no color, clear	10.00317
			0.0134

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

Reference standard number	True value (TV) (mg/L)	Measured value (MV) (mg/L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
1N551064	0.50	0.489	97.87

Reviewed by	<i>JS</i>
Date reviewed	05.12.12

### Alkalinity (SM 2320 B)

**Matrix:** Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst	JJS
Date analyzed	05-05-12

Time initiated	1015
Time completed	1045

**Titrate samples to  
pH = 4.5 S.U.**

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

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
4.6	INR 484	INSS 1018	0.0	12.0	12.0	0.0208	10.4

**RIK CON. 0.0-00:00**  
**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1037	100	100	12.0	21.6	9.6	10.4	100	100.0%

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = $\frac{ (S - D) }{((S+D)/2)} \times 100$ (acceptable range = $\pm 10\%$ )
06-03-12	SSW	100	24.9	24.9	3.3	10.4	S 34	
1	Duplicate (B)	1	24.9	28.2	3.3	1	D 34	50% 06-05-12

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
INSS 1037	50	100	24.9	39.1	8.0	10.4	83

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
34	47	98.0%

**Sample measurements:**

[illegible]

Reviewed by:

Date reviewed: 05.07.12



**Alkalinity (SM 2320 B)**

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JSB  
Date analyzed 05.12.12

Time initiated 0910  
Time completed 1419

Titrate samples to  
pH = 4.5 S.U.

**Titrant normality and multiplier determination:**

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
5.1	INR 484	INSS 1018	0.1	12.2	12.1	0.0207	10.4

pk corr 0.0 - 0.10, 1

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1037	100	100	12.2	21.8	9.6	10.4	100	100.0%

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100 (acceptable range = ± 10%)
05.10.12	SSW	100	21.6	25.1	3.3	10.4	S 34	
↓	Duplicate (B)	↓	25.1	28.4	3.3	↓	D 34	JSB 05.12.12

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
INSS 1037	50	100	25.1	33.2	8.1	10.4	84

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
34	50	100.0%

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
05.09.12B	MHSW	100	33.2	39.4	6.2	10.4	64
05.11.12A			39.4	45.5	6.1		63
05.11.12B			40	6.0	60		62
05.07.12A			60	12.1	6.1		63
05.09.12A			12.1	18.2	6.1		63
93322	Calcium Chloride	50	18.2	22.5	4.3	(2)	89
120321.01	OdACDMC	100	22.5	28.6	6.1		63
↓	CaCl <sub>2</sub> DMC	↓	28.6	34.7	6.1		63
↓	PPACDMC	↓	34.7	40.9	6.2	↓	64

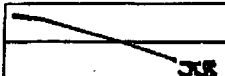
Reviewed by: JSB

Date reviewed: 05.12.12

**Alkalinity (SM 2320 B)**

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst **JAS**  
Date analyzed **05-12-12**

Time initiated   
Time completed **05-12-12**

Titrate samples to  
pH = 4.5 S.U.

**Titrant normality and multiplier determination:**

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
							<b>05-12-12</b>

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<b>JN55 1037</b>	<b>100</b>	<b>100</b>	<b>0.0</b>	<b>9.6</b>	<b>9.6</b>	<b>10.4</b>	<b>100</b>	<b>100.0%</b>

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100 (acceptable range = ± 10%)
<b>05-05-12A</b>	<b>MHSUV 1</b>	<b>100</b>	<b>9.6</b>	<b>15.6</b>	<b>6.0</b>	<b>10.4</b>	<b>S 62</b>	
	Duplicate (B)		<b>15.6</b>	<b>21.7</b>	<b>6.1</b>		<b>D 63</b>	<b>1.6%</b>

**Matrix spike recovery:**


Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
<b>JN65 1037</b>	<b>50</b>	<b>100</b>	<b>15.6</b>	<b>26.4</b>	<b>10.8</b>	<b>10.4</b>	<b>110</b>

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<b>63</b>	<b>47</b>	<b>94.6%</b>

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
<b>05-05-12B</b>	<b>MHSUV 2</b>	<b>100</b>	<b>26.4</b>	<b>32.5</b>	<b>6.1</b>	<b>10.4</b>	<b>63</b>
<b>05-07-12</b>	<b>3</b>		<b>32.5</b>	<b>38.7</b>	<b>6.2</b>		<b>64</b>
<b>05-09-12A</b>	<b>4</b>		<b>38.7</b>	<b>44.9</b>	<b>6.2</b>		<b>64</b>
<b>05-11-12A</b>	<b>5</b>		<b>0.0</b>	<b>6.1</b>	<b>6.1</b>		<b>63</b>
<b>120521.01</b>	<b>ABALDMR</b>		<b>6.1</b>	<b>16.0</b>	<b>9.9</b>		<b>100</b>
	<b>ABCRDMR</b>		<b>16.0</b>	<b>25.8</b>	<b>9.8</b>		<b>100</b>
	<b>MBALDMR</b>		<b>25.8</b>	<b>35.1</b>	<b>9.3</b>		<b>97</b>
	<b>MBCRDMR</b>		<b>35.1</b>	<b>44.2</b>	<b>9.1</b>		<b>95</b>
<b>05-11-12</b>	<b>SCUTSW</b>		<b>0.0</b>	<b>8.6</b>	<b>8.6</b>		<b>89</b>

Reviewed by:



Date reviewed:

**05-12-12**

**Alkalinity (SM 2320 B)**

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst **JTB**  
Date analyzed **05.12.12**

Titrate samples to  
**pH = 4.5 S.U.**

Time initiated  
Time completed **JTB 05.12.12**

**Titrant normality and multiplier determination:**

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
							<b>JTB 05.12.12</b>

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<b>INSS 1037</b>	<b>100</b>	<b>100</b>	<b>8.6</b>	<b>18.3</b>	<b>9.7</b>	<b>10.4</b>	<b>101</b>	<b>101.0%</b>

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = {(S - D) / [(S+D)/2]} x 100 (acceptable range = ± 10%)
<b>05-03-12A</b>	<b>Saltsw</b>	<b>100</b>	<b>18.3</b>	<b>28.3</b>	<b>10.0</b>	<b>10.4</b>	<b>S 100</b>	
<b>↓</b>	<b>Duplicate (B)</b>	<b>↓</b>	<b>28.3</b>	<b>38.3</b>	<b>10.0</b>	<b>↓</b>	<b>D 100</b>	<b>— JTB 05.12.12</b>

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
<b>INSS 1037</b>	<b>50</b>	<b>100</b>	<b>28.3</b>	<b>43.0</b>	<b>14.7</b>	<b>10.4</b>	<b>150</b>

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<b>100</b>	<b>50</b>	<b>100.0%</b>

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
<b>05-03-12B</b>	<b>Saltsw</b>	<b>100</b>	<b>0.0</b>	<b>9.9</b>	<b>9.9</b>	<b>10.4</b>	<b>100</b>
<b>05-10-12</b>	<b>↓</b>	<b>↓</b>	<b>9.9</b>	<b>19.3</b>	<b>9.4</b>	<b>↓</b>	<b>98</b>
<b>05-08-12</b>	<b>↓</b>	<b>↓</b>	<b>19.3</b>	<b>29.1</b>	<b>9.8</b>	<b>↓</b>	<b>100</b>
<b>120507.01</b>	<b>TVASQN 101 1</b>	<b>50</b>	<b>29.1</b>	<b>32.9</b>	<b>3.8</b>	<b>(12)</b>	<b>79</b>
<b>120509.25</b>	<b>↓</b>	<b>↓</b>	<b>32.9</b>	<b>36.8</b>	<b>3.9</b>	<b>↓</b>	<b>81</b>
<b>120511.21</b>	<b>↓</b>	<b>↓</b>	<b>36.8</b>	<b>40.6</b>	<b>3.8</b>	<b>↓</b>	<b>79</b>
<b>120507.02</b>	<b>TVASQN 101 INT 1</b>	<b>↓</b>	<b>40.6</b>	<b>44.3</b>	<b>3.7</b>	<b>↓</b>	<b>77</b>
<b>120509.26</b>	<b>↓</b>	<b>↓</b>	<b>44.3</b>	<b>48.1</b>	<b>3.8</b>	<b>↓</b>	<b>79</b>
<b>120511.21</b>	<b>↓</b>	<b>↓</b>	<b>0.0</b>	<b>3.8</b>	<b>3.8</b>	<b>↓</b>	<b>79</b>

Reviewed by: **JTB**

Date reviewed: **05.12.12**

**Alkalinity (SM 2320 B)**

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JTB  
Date analyzed 05-12-12

Time initiated                       
Time completed JTB 05-12-12

**Titrate samples to  
pH = 4.5 S.U.**

**Titrant normality and multiplier determination:**

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
							<u>JTB 05-12-12</u>

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1037</u>	<u>100</u>	<u>100</u>	<u>3.8</u>	<u>13.4</u>	<u>9.6</u>	<u>10.4</u>	<u>100</u>	<u>100.0%</u>

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100 (acceptable range = ± 10%)
<u>120507.01</u>	<u>TVASQNI01UV1</u>	<u>50</u>	<u>13.4</u>	<u>17.2</u>	<u>3.8</u>	<u>(2) 10.4</u>	<u>S 79</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>17.2</u>	<u>20.9</u>	<u>3.7</u>	<u>↓ ↓</u>	<u>D 77</u>	<u>2.6%</u>

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
<u>INSS 1037</u>	<u>100</u>	<u>50</u>	<u>17.2</u>	<u>25.6</u>	<u>8.4</u>	<u>(2) 10.4</u>	<u>051212 170 → 180</u>

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>77</u>	<u>051212 83 → 103</u>	<u>051212 93.0% → 103.0%</u>

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
<u>120509.25</u>	<u>TVASQNI01UV2</u>	<u>50</u>	<u>25.6</u>	<u>29.4</u>	<u>3.8</u>	<u>(2) 10.4</u>	<u>79</u>
<u>120511.20</u>	<u>↓ 3</u>		<u>29.4</u>	<u>33.2</u>	<u>3.8</u>		<u>79</u>
<u>120507.02</u>	<u>TVASQNI01UV1</u>		<u>33.2</u>	<u>37.0</u>	<u>3.8</u>		<u>79</u>
<u>120509.26</u>	<u>↓ 2</u>		<u>37.0</u>	<u>40.7</u>	<u>3.7</u>		<u>77</u>
<u>120511.21</u>	<u>↓ 3</u>		<u>40.7</u>	<u>44.5</u>	<u>3.8</u>		<u>79</u>
<u>120507.03</u>	<u>TVASQNI01UV1</u>		<u>0.0</u>	<u>4.1</u>	<u>4.1</u>		<u>85</u>
<u>120509.27</u>	<u>↓ 2</u>		<u>4.1</u>	<u>8.3</u>	<u>4.2</u>		<u>87</u>
<u>120511.22</u>	<u>↓ 3</u>		<u>8.3</u>	<u>12.4</u>	<u>4.1</u>		<u>85</u>
<u>120507.04</u>	<u>TVASQNI01UV1</u>	<u>↓</u>	<u>12.4</u>	<u>16.3</u>	<u>3.9</u>	<u>↓ ↓</u>	<u>81</u>

Reviewed by: JTB

Date reviewed: 05-12-12

**Alkalinity (SM 2320 B)**

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JCS  
Date analyzed 05-12-12

Time initiated                       
Time completed                       
JCS 05-12-12

Titrate samples to  
pH = 4.5 S.U.

**Titrant normality and multiplier determination:**

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
							<u>JCS 05-12-12</u>

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>JN55 1037</u>	<u>100</u>	<u>100</u>	<u>16.3</u>	<u>26.0</u>	<u>9.7</u>	<u>10.4</u>	<u>101</u>	<u>101.0%</u>

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = [(S - D) / ((S+D)/2)] x 100 (acceptable range = ± 10%)
<u>120504.28</u>	<u>TVA WBN 101 INT 2</u>	<u>50</u>	<u>26.0</u>	<u>29.9</u>	<u>3.9</u>	<u>(2) 10.4</u>	<u>S 81</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>29.9</u>	<u>33.9</u>	<u>4.0</u>	<u>↓ ↓</u>	<u>D 83</u>	<u>2.4%</u>

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
<u>JN55 1037</u>	<u>100</u>	<u>50</u>	<u>29.9</u>	<u>38.6</u>	<u>8.7</u>	<u>(2) 10.4</u>	<u>180</u>

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>83</u>	<u>97</u>	<u>97.0%</u>

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
<u>120511.25</u>	<u>TVA WBN 101 INT 3</u>	<u>50</u>	<u>38.6</u>	<u>42.6</u>	<u>4.0</u>	<u>(2) 10.4</u>	<u>83</u>
<u>120507.05</u>	<u>TVA WBN 113 1</u>		<u>42.6</u>	<u>46.8</u>	<u>4.2</u>		<u>87</u>
<u>120509.29</u>	<u>↓ 2</u>		<u>0.0</u>	<u>4.3</u>	<u>4.3</u>		<u>89</u>
<u>120511.24</u>	<u>↓ 3</u>		<u>4.3</u>	<u>8.4</u>	<u>4.1</u>		<u>85</u>
<u>120507.06</u>	<u>TVA WBN 113 INT 1</u>		<u>8.4</u>	<u>12.1</u>	<u>3.7</u>		<u>77</u>
<u>120509.30</u>	<u>↓ 2</u>		<u>12.1</u>	<u>15.9</u>	<u>3.8</u>		<u>79</u>
<u>120511.25</u>	<u>↓ 3</u>		<u>15.9</u>	<u>19.9</u>	<u>4.0</u>	<u>↓</u>	<u>83</u>
<u>120508.01</u>	<u>Farmville 1</u>	<u>100</u>	<u>21.5</u>	<u>22.4</u>	<u>0.9</u>		<u>9.4</u>
<u>120510.01</u>	<u>↓ 2</u>	<u>↓</u>	<u>22.4</u>	<u>25.2</u>	<u>2.8</u>	<u>↓</u>	<u>29</u>

Reviewed by:

Date reviewed:

05-12-12

**Alkalinity (SM 2320 B)**

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JLB  
Date analyzed 05.12.12

Time initiated                       
Time completed                       
JLB  
05.12.12

**Titrate samples to  
pH = 4.5 S.U.**

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

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
							<u>JLB 05.12.12</u>

***Laboratory control standard:***

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INAS 1037</u>	<u>100</u>	<u>100</u>	<u>35.2</u>	<u>44.7</u>	<u>9.5</u>	<u>10.4</u>	<u>99</u>	<u>99.0%</u>

***Duplicate sample precision:***

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = [(S - D) / ((S+D)/2)] x 100 (acceptable range = ± 10%)
<u>120512.01</u>	<u>Farmville 3</u>	<u>100</u>	<u>0.0</u>	<u>2.8</u>	<u>2.8</u>	<u>10.4</u>	<u>S 29</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>2.8</u>	<u>5.6</u>	<u>2.8</u>	<u>↓</u>	<u>D 29</u>	<u>JLB 05.12.12</u>

***Matrix spike recovery:***

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
<u>INAS 1037</u>	<u>50</u>	<u>100</u>	<u>2.8</u>	<u>10.3</u>	<u>7.5</u>	<u>10.4</u>	<u>78</u>

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>29</u>	<u>49</u>	<u>98.0%</u>

***Sample measurements:***

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
<u>120508.10</u>	<u>Savtr Cary 1</u>	<u>100</u>	<u>10.3</u>	<u>15.2</u>	<u>4.9</u>	<u>10.4</u>	<u>51</u>
<u>120510.09</u>	<u>↓ 2</u>	<u>↓</u>	<u>15.2</u>	<u>20.0</u>	<u>4.8</u>	<u>↓</u>	<u>50</u>
<u>120512.08</u>	<u>↓ 3</u>	<u>↓</u>	<u>20.0</u>	<u>25.1</u>	<u>5.1</u>	<u>↓</u>	<u>53</u>
<u>120508.03</u>	<u>COLE AL 1</u>	<u>25</u>	<u>25.1</u>	<u>29.2</u>	<u>4.1</u>	<u>(H)</u>	<u>170</u>
<u>120510.03</u>	<u>↓ 2</u>	<u>↓</u>	<u>29.2</u>	<u>34.5</u>	<u>5.3</u>	<u>↓</u>	<u>220</u>
<u>120512.03</u>	<u>↓ 3</u>	<u>↓</u>	<u>34.5</u>	<u>39.0</u>	<u>4.5</u>	<u>↓</u>	<u>190</u>
<u>120508.01</u>	<u>Foxwood 1</u>	<u>100</u>	<u>39.0</u>	<u>42.6</u>	<u>3.6</u>	<u>↓</u>	<u>37</u>
<u>120510.01</u>	<u>↓ 2</u>	<u>↓</u>	<u>42.6</u>	<u>46.9</u>	<u>4.3</u>	<u>↓</u>	<u>45</u>
<u>120512.01</u>	<u>↓ 3</u>	<u>↓</u>	<u>0.0</u>	<u>6.4</u>	<u>6.4</u>	<u>↓</u>	<u>66 + 67</u>

Reviewed by: JH

Date reviewed: 05.12.12

## Alkalinity (SM 2320 B)

Matrix: Water, RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JBS  
Date analyzed 05-12-12

Time initiated                       
Time completed                       
05-12-12

Titrate samples to  
pH = 4.5 S.U.

### Titrant normality and multiplier determination:

pH of Deionized water = 4.5 S.U.	Titrant reference number	Normality check standard number	Begin ml	End ml	Total ml (E)	Normality (N) of H <sub>2</sub> SO <sub>4</sub> = (5 ml Na <sub>2</sub> CO <sub>3</sub> 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
							<u>JBS 05-12-12</u>

### Laboratory control standard:

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1037</u>	<u>100</u>	<u>100</u>	<u>6.4</u>	<u>16.0</u>	<u>9.6</u>	<u>10.4</u>	<u>100</u>	<u>100.0%</u>

### Duplicate sample precision:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = {(S - D) / [(S+D)/2]} x 100 (acceptable range = ± 10%)
<u>120508-02</u>	<u>Holcim 1</u>	<u>25</u>	<u>16.0</u>	<u>18.8</u>	<u>2.8</u>	<u>(14) 10.4</u>	<u>S 120</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>18.8</u>	<u>21.4</u>	<u>2.6</u>	<u>↓ ↓</u>	<u>D 110</u>	<u>8.7%</u>

### Matrix spike recovery:

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)
<u>INSS 1037</u>	<u>200</u>	<u>25</u>	<u>24.1</u>	<u>27.9</u>	<u>3.8</u>	<u>(14) 10.4</u>	<u>310</u>

31.5

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>110</u>	<u>200</u>	<u>100.0%</u>

### Sample measurements:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
<u>120510-02</u>	<u>Holcim 2</u>	<u>25</u>	<u>21.4</u>	<u>24.1</u>	<u>2.7</u>	<u>(14) 10.4</u>	<u>110</u>
<u>120512-02</u>	<u>↓ 3</u>	<u>↓</u>	<u>31.5</u>	<u>34.4</u>	<u>2.9</u>	<u>↓</u>	<u>120</u>
<u>120508-05</u>	<u>Engelhard 1</u>	<u>2</u>	<u>34.4</u>	<u>40.3</u>	<u>5.9</u>	<u>(50)</u>	<u>3100</u>
<u>120510-05</u>	<u>↓ 2</u>	<u>↓</u>	<u>40.3</u>	<u>46.4</u>	<u>6.1</u>	<u>↓</u>	<u>3200</u>
<u>120512-05</u>	<u>↓ 3</u>	<u>↓</u>	<u>0.0</u>	<u>6.1</u>	<u>6.1</u>	<u>↓</u>	<u>3200</u>
<u>120508-06</u>	<u>Pasquottank 1</u>	<u>↓</u>	<u>6.1</u>	<u>10.1</u>	<u>4.0</u>	<u>↓</u>	<u>2100</u>
<u>120510-06</u>	<u>↓ 2</u>	<u>↓</u>	<u>10.1</u>	<u>14.1</u>	<u>4.0</u>	<u>↓</u>	<u>2100</u>
<u>120512-06</u>	<u>↓ 3</u>	<u>↓</u>	<u>14.1</u>	<u>17.9</u>	<u>3.8</u>	<u>↓</u>	<u>2000</u>
<u>120508-04</u>	<u>Arccelor 1</u>	<u>50</u>	<u>17.9</u>	<u>21.5</u>	<u>3.6</u>	<u>(7) ↓</u>	<u>75</u>

Reviewed by: JH

Date reviewed: 05-12-12

### Alkalinity (SM 2320 B)

**Matrix:** Water, RL = 1.0 mg CaCO<sub>3</sub>/L

**Analyst**

১১৩

Date analyzed

05.17.12

Time initiated

Time completed

941212

**Titrate samples to  
pH = 4.5 S.U.**

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

[illegible]

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1037	100	100	21.5	31.2	9.7	10.4	101	101.0%

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)	%RPD = $\frac{ (S - D) }{ (S + D)/2 } \times 100$ (acceptable range = ± 10%)
							S	
	Duplicate (B)						D	

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike alkalinity (A) (mg CaCO <sub>3</sub> /L)

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
120510-01	ARCCELW 2	50	31.2	34.6	3.4	(2) 10.4	71
120512-04	↓ 3	↓	34.6	38.2	3.6	↓	75
05-11-12B	MHSW 6	100	38.2	44.2	6.0	↓	62
38 05.12.12							

Reviewed by:

**Date reviewed:**

05-12-17



**Total Hardness (SM 2340 C)**
$$RL = 1.0 \text{ mg CaCO}_3/\text{L}$$

Analyst	JLB
Date analyzed	05-05-12

Time initiated	0134
Time completed	0158

***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
JNR 458	JNS 919	0.0	10.0	10.0	0.0200	20.0

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1033	40	50	10.0	12.0	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 <sub>3</sub> /L)	%RPD = $\{(S - D) / ((S+D)/2)\} \times 100$
050312	SSW	50	12.0	14.0	2.0	20.0	<sup>S</sup> 40	
↓	Duplicate (B)	↓	14.0	16.0	2.0	↓	<sup>D</sup> 40	— 0.0 0503-12

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
INSS 1033	40	50	14.0	16.0	4.0	20.0	80

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
40	40	100.0%

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)
TV = ND	Blank (should be = 0 mg CaCO <sub>3</sub> /L)	50	0.0	0.0	6.0	20.0	ND
05-DI-12	MHSW	↓	18.0	22.4	4.4	↓	88
05-03-12A	↓	↓	22.4	26.8	4.4	↓	88
05-03-12B	↓	↓	26.8	31.2	4.4	↓	88
04-30-12	↓	↓	31.2	35.8	4.6	↓	92
							JTB 03-05-12

## Total Hardness (SM 2340 C)

RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JS  
Date analyzed 05-13-12

Time initiated 0914  
Time completed 1223

### 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 488	INSS 911	0.0	10.1	10.1	0.0198	19.8

### Laboratory control standard:

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1033	40	50	10.1	12.1	2.0	19.8	40	100.0%

### Duplicate sample precision:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100
05-10-12	SSW	50	12.1	14.1	2.0	19.8	S 40	
↓	Duplicate (B)	↓	14.1	16.1	2.0	↓	D 40	0% 05-13-12

### Matrix spike recovery:

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
INSS 1033	40	50	14.1	18.1	4.0	19.8	79

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
40	39	97.5%

### Sample measurements:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)
TV = ND	Blank (should be = 0 mg CaCO <sub>3</sub> /L)	50	0.0	0.0	0.0	19.8	ND
05-11-12 A	MHSW		18.1	22.6	4.5		89
05-11-12 B			22.6	27.1	4.5		89
05-07-12			27.1	31.6	4.5		89
05-09-12 A			31.6	36.0	4.4		87
05-09-12 B	↓		36.0	40.5	4.5		89
93341	Waynesville		40.5	47.4	6.9		140
120321-01	CAACONE		0.0	4.5	4.5		89
↓	CLERDMR		4.5	9.0	4.5		89
↓	PDAE DMR	↓	9.0	13.5	4.5	↓	89

## Total Hardness (SM 2340 C)

RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JSB  
Date analyzed 05-13-12

Time initiated                       
Time completed                       
JSB 05-13-12

### 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
						<u>JSB 05-13-12</u>

### Laboratory control standard:

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1033</u>	<u>40</u>	<u>50</u>	<u>13.5</u>	<u>15.5</u>	<u>2.0</u>	<u>19.8</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 <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100
<u>05-03-12B</u>	<u>MHSUU 2</u>	<u>50</u>	<u>15.5</u>	<u>19.9</u>	<u>4.4</u>	<u>19.8</u>	<u>S 87</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>19.9</u>	<u>24.3</u>	<u>4.4</u>	<u>↓</u>	<u>D 87</u>	<u>JSB 05-13-12</u>

### Matrix spike recovery:

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
<u>INSS 1033</u>	<u>40</u>	<u>50</u>	<u>19.9</u>	<u>26.3</u>	<u>6.4</u>	<u>19.8</u>	<u>130</u>

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>87</u>	<u>43</u>	<u>107.5%</u>

### Sample measurements:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)
<u>TV = ND</u>	<u>Blank</u> (should be = 0 mg CaCO <sub>3</sub> /L)					<u>19.8</u>	<u>JSB 05-13-12</u>
<u>05-03-12A</u>	<u>MHSUU 1</u>	<u>50</u>	<u>26.3</u>	<u>30.7</u>	<u>4.4</u>	<u>19.8</u>	<u>87</u>
<u>05-07-12</u>	<u>3</u>		<u>30.7</u>	<u>35.2</u>	<u>4.5</u>		<u>89</u>
<u>05-09-12A</u>	<u>4</u>		<u>35.2</u>	<u>39.7</u>	<u>4.5</u>		<u>89</u>
<u>05-11-12A</u>	<u>5</u>		<u>39.7</u>	<u>44.2</u>	<u>4.5</u>		<u>89</u>
<u>05-11-12B</u>	<u>↓ 6</u>		<u>44.2</u>	<u>48.7</u>	<u>4.5</u>		<u>89</u>
<u>120507.01</u>	<u>TVA SANIOL 1</u>		<u>0.0</u>	<u>4.1</u>	<u>4.1</u>		<u>81</u>
<u>120509.25</u>	<u>↓ 2</u>		<u>4.1</u>	<u>8.2</u>	<u>4.1</u>		<u>81</u>
<u>120511.20</u>	<u>↓ 3</u>		<u>8.2</u>	<u>12.3</u>	<u>4.1</u>		<u>81</u>
<u>120507.02</u>	<u>TVA SANIOL 1</u>	<u>↓</u>	<u>12.3</u>	<u>16.3</u>	<u>4.0</u>	<u>↓</u>	<u>79</u>

Page 80 of 110  
No. of titrant used, sample must be diluted. Reviewed by:

JSB

Date reviewed 05-13-12

## Total Hardness (SM 2340 C)

RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JSB  
Date analyzed 05-13-12

Time initiated                       
Time completed                       
05-13-12

### 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
						<u>JSB 05-13-12</u>

### Laboratory control standard:

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>INSS 1633</u>	<u>40</u>	<u>50</u>	<u>16.3</u>	<u>18.3</u>	<u>2.0</u>	<u>19.8</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 <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100
<u>120509.24</u>	<u>TVA SGNINT 2</u>	<u>50</u>	<u>18.3</u>	<u>22.4</u>	<u>4.1</u>	<u>19.8</u>	<u>S 81</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>22.4</u>	<u>26.5</u>	<u>4.1</u>	<u>↓</u>	<u>D 81</u>	<u>JSB 05-13-12</u>

### Matrix spike recovery:

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
<u>INSS 1633</u>	<u>40</u>	<u>50</u>	<u>22.4</u>	<u>28.4</u>	<u>6.0</u>	<u>19.8</u>	<u>120</u>

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>81</u>	<u>39</u>	<u>97.5%</u>

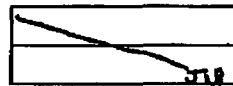
### Sample measurements:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)
<u>TV ND</u>	<u>Blank</u> (should be = 0 mg CaCO <sub>3</sub> /L)						<u>JSB 05-13-12</u>
<u>120511.21</u>	<u>TVA SGNINT 3</u>	<u>50</u>	<u>28.4</u>	<u>32.4</u>	<u>4.0</u>	<u>19.8</u>	<u>79</u>
<u>120507.01</u>	<u>TVA SGN 101 DU 1</u>		<u>32.4</u>	<u>36.5</u>	<u>4.1</u>		<u>81</u>
<u>120509.25</u>	<u>↓ 2</u>		<u>36.5</u>	<u>40.6</u>	<u>4.1</u>		<u>81</u>
<u>120511.20</u>	<u>↓ 3</u>		<u>40.6</u>	<u>44.7</u>	<u>4.1</u>		<u>81</u>
<u>120507.02</u>	<u>TVA SGN 101 INT DU 1</u>		<u>44.7</u>	<u>48.8</u>	<u>4.1</u>		<u>81</u>
<u>120509.26</u>	<u>↓ 2</u>		<u>0.0</u>	<u>4.0</u>	<u>4.0</u>		<u>79</u>
<u>120511.21</u>	<u>↓ 3</u>		<u>4.0</u>	<u>8.1</u>	<u>4.1</u>		<u>81</u>
<u>120507.03</u>	<u>TVA WBN 101 1</u>		<u>8.1</u>	<u>12.5</u>	<u>4.4</u>		<u>87</u>
<u>120509.27</u>	<u>↓ 2</u>	<u>↓</u>	<u>12.5</u>	<u>16.9</u>	<u>4.4</u>	<u>↓</u>	<u>87</u>

## Total Hardness (SM 2340 C)

RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst **JSB**  
Date analyzed **05-13-12**

Time initiated   
Time completed **05-13-12**

### 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
						<b>JSB 05-13-12</b>

### Laboratory control standard:

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<b>INSS 1033</b>	<b>40</b>	<b>50</b>	<b>16.9</b>	<b>18.9</b>	<b>2.0</b>	<b>19.8</b>	<b>40</b>	<b>100.0%</b>

### Duplicate sample precision:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100
<b>120511.22</b>	<b>TAWBNI101 3</b>	<b>50</b>	<b>18.9</b>	<b>23.4</b>	<b>4.5</b>	<b>19.8</b>	<b>S 89</b>	
<b>↓</b>	<b>Duplicate (B)</b>	<b>↓</b>	<b>23.4</b>	<b>27.9</b>	<b>4.5</b>	<b>↓</b>	<b>D 89</b>	<b>JSB 05-13-12</b>

### Matrix spike recovery:

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
<b>INSS 1033</b>	<b>40</b>	<b>50</b>	<b>23.4</b>	<b>29.9</b>	<b>6.4</b>	<b>19.8</b>	<b>130</b>

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<b>89</b>	<b>41</b>	<b>102.5%</b>

### Sample measurements:

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)
<b>TV ND</b>	<b>Blank</b> (should be = 0 mg CaCO <sub>3</sub> /L)						<b>JSB 05-13-12</b>
<b>120507.04</b>	<b>TAWBNI101 NT 1</b>	<b>50</b>	<b>29.8</b>	<b>34.1</b>	<b>4.3</b>	<b>19.8</b>	<b>85</b>
<b>120509.28</b>	<b>↓ 2</b>		<b>34.1</b>	<b>38.5</b>	<b>4.4</b>		<b>87</b>
<b>120511.23</b>	<b>↓ 3</b>		<b>38.5</b>	<b>42.8</b>	<b>4.3</b>		<b>85</b>
<b>120507.05</b>	<b>TAWBNI113 1</b>		<b>42.8</b>	<b>47.2</b>	<b>4.4</b>		<b>87</b>
<b>120509.29</b>	<b>↓ 2</b>		<b>0.0</b>	<b>4.5</b>	<b>4.5</b>		<b>89</b>
<b>120511.24</b>	<b>↓ 3</b>		<b>4.5</b>	<b>8.9</b>	<b>4.4</b>		<b>87</b>
<b>120507.06</b>	<b>TAWBNI131 NT 1</b>		<b>8.9</b>	<b>12.8</b>	<b>3.9</b>		<b>77</b>
<b>120509.30</b>	<b>↓ 2</b>		<b>12.8</b>	<b>16.9</b>	<b>4.1</b>		<b>81</b>
<b>120511.25</b>	<b>↓ 3</b>		<b>16.9</b>	<b>20.8</b>	<b>3.9</b>		<b>77</b>

**Total Hardness (SM 2340 C)**

RL = 1.0 mg CaCO<sub>3</sub>/L

Analyst JB  
Date analyzed 05.13.12

Time initiated                       
Time completed                       
JB  
05.13.12

**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
						<u>JB 05.13.12</u>

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
<u>IN05 1033</u>	<u>40</u>	<u>50</u>	<u>20.9</u>	<u>22.7</u>	<u>1.9</u>	<u>19.8</u>	<u>38</u>	<u>95.0%</u>

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)	%RPD = ((S - D) / ((S+D)/2)) x 100
<u>120508.07</u>	<u>Farmville 1</u>	<u>25</u>	<u>22.7</u>	<u>22.8</u>	<u>0.1</u>	<u>19.8</u>	<u>4.0</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>↓</u>	<u>22.8</u>	<u>22.9</u>	<u>0.1</u>	<u>↓</u>	<u>4.0</u>	<u>05.13.12</u>

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
<u>IN05 1033</u>	<u>80</u>	<u>25</u>	<u>22.8</u>	<u>24.9</u>	<u>2.1</u>	<u>19.8</u>	<u>83</u>

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<u>4.0</u>	<u>79</u>	<u>98.8%</u>

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)
<u>TV = ND</u>	<u>Blank</u> (should be = 0 mg CaCO <sub>3</sub> /L)						<u>JB 05.13.12</u>
<u>120510.07</u>	<u>Farmville 2</u>	<u>50</u>	<u>21.9</u>	<u>25.8</u>	<u>0.9</u>	<u>19.8</u>	<u>18</u>
<u>120512.07</u>	<u>↓ 3</u>	<u>↓</u>	<u>25.8</u>	<u>26.7</u>	<u>0.9</u>	<u>↓</u>	<u>18</u>
<u>120508.10</u>	<u>South Cary 1</u>	<u>↓</u>	<u>26.7</u>	<u>29.6</u>	<u>2.9</u>	<u>↓</u>	<u>57</u>
<u>120510.09</u>	<u>↓ 2</u>	<u>↓</u>	<u>29.6</u>	<u>32.3</u>	<u>2.7</u>	<u>↓</u>	<u>54</u>
<u>120512.08</u>	<u>↓ 3</u>	<u>↓</u>	<u>32.3</u>	<u>34.9</u>	<u>2.6</u>	<u>↓</u>	<u>52</u>
<u>120508.03</u>	<u>Corl. AL 1</u>	<u>10</u>	<u>34.9</u>	<u>36.7</u>	<u>1.8</u>	<u>6)</u>	<u>180</u>
<u>120510.03</u>	<u>↓ 2</u>	<u>↓</u>	<u>36.7</u>	<u>38.8</u>	<u>2.1</u>	<u>↓</u>	<u>210</u>
<u>120512.03</u>	<u>↓ 3</u>	<u>↓</u>	<u>38.8</u>	<u>40.7</u>	<u>1.9</u>	<u>↓</u>	<u>190</u>
<u>120508.01</u>	<u>Fuquoo 1</u>	<u>50</u>	<u>40.7</u>	<u>45.2</u>	<u>4.5</u>	<u>↓</u>	<u>89</u>

### Total Hardness (SM 2340 C)

$$RL = 1.0 \text{ mg CaCO}_3/\text{L}$$

Analyst	JS
Date analyzed	05.13.12

Time initiated \_\_\_\_\_  
Time completed \_\_\_\_\_ JJS  
05-13-12

***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
						0.0013-12

**Laboratory control standard:**

Reference standard number	True value (TV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (MV) (mg CaCO <sub>3</sub> /L)	% RS = MV / TV x 100 (acceptable range = 90 to 110%)
INSS 1033	40	50	0.0	1.1	1.1	14.8	38	95.0%

**Duplicate sample precision:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Hardness (mg CaCO <sub>3</sub> /L)	%RPD = $\{(S - D) / ((S+D)/2)\} \times 100$
120510-01	Flywood 2	56	1.9	6.5	4.6	12.8	<sup>S</sup> 91	
↓	Duplicate (B)	↓	6.5	11.1	4.6	↓	<sup>D</sup> 91	— 53 05-12-12

**Matrix spike recovery:**

Reference standard number	Spike value (SV) (mg CaCO <sub>3</sub> /L)	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Spike hardness (A) (mg CaCO <sub>3</sub> /L)
INSS 1033	40	50	6.5	13.1	6.6	19.8	130

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) MV = A - B (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
91	39	97.5%

**Sample measurements:**

[illegible]

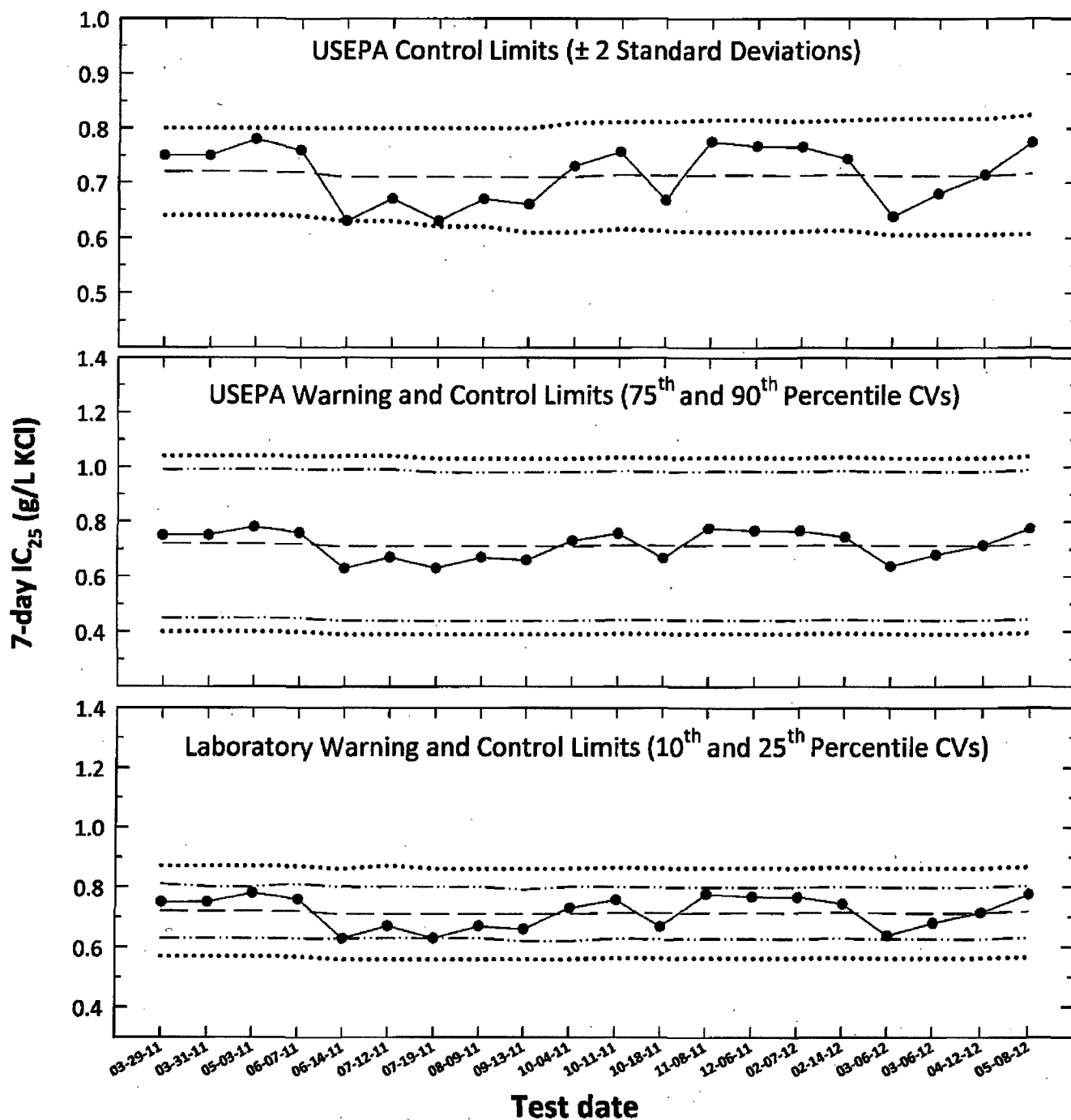
Sequoyah Nuclear Plant Biomonitoring  
May 08 – 15, 2012

Appendix D

Reference Toxicant Test and  
Control Chart



***Pimephales promelas***  
**Chronic Reference Toxicant Control Chart**  
**Organism Source: Aquatox, Inc.**



- **7-day  $IC_{25}$**  = 25% inhibition concentration. An estimation of the concentration of potassium chloride that would cause a 25% reduction in *Pimephales* growth for the test population.
- — **Central Tendency (mean  $IC_{25}$ )**
- **Warning Limits (mean  $IC_{25} \pm S_{A.10}$  or  $S_{A.75}$ )**
- **Control Limits (mean  $IC_{25} \pm S_{A.25}$ ,  $S_{A.90}$ , or 2 Standard Deviations)**

Graphs generated from associated excel spreadsheet.  
Excel spreadsheet entered by: J. Sumner  
Reviewed by: *[Signature]*





Environmental Testing Solutions, Inc.

## Pimephales promelas Chronic Reference Toxicant Control Chart

Test number	Test date	7-day $IC_{25}$ (g/L KCl)	CT (g/L KCl)	S	State and USEPA Control Limits		Laboratory Warning Limits		$S_{A,25}$	Laboratory Control Limits		$S_{A,75}$	USEPA Warning Limits		$S_{A,90}$	USEPA Control Limits		CV
					CT - 2S	CT + 2S	CT - $S_{A,10}$	CT + $S_{A,10}$		CT - $S_{A,25}$	CT + $S_{A,25}$		CT - $S_{A,75}$	CT + $S_{A,75}$		CT - $S_{A,90}$	CT + $S_{A,90}$	
1	03-29-11	0.75	0.72	0.04	0.64	0.80	0.63	0.81	0.15	0.57	0.87	0.27	0.45	0.99	0.32	0.40	1.04	0.05
2	03-31-11	0.75	0.72	0.04	0.64	0.80	0.63	0.80	0.15	0.57	0.87	0.27	0.45	0.99	0.32	0.40	1.04	0.05
3	05-03-11	0.78	0.72	0.04	0.64	0.80	0.63	0.80	0.15	0.57	0.87	0.27	0.45	0.99	0.32	0.40	1.04	0.05
4	06-07-11	0.76	0.72	0.04	0.64	0.80	0.63	0.81	0.15	0.57	0.87	0.27	0.45	0.99	0.32	0.40	1.04	0.05
5	06-14-11	0.63	0.71	0.04	0.63	0.80	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.99	0.32	0.39	1.04	0.06
6	07-12-11	0.67	0.71	0.04	0.63	0.80	0.63	0.80	0.15	0.56	0.87	0.27	0.44	0.99	0.32	0.39	1.04	0.06
7	07-19-11	0.63	0.71	0.05	0.62	0.80	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.06
8	08-09-11	0.67	0.71	0.05	0.62	0.80	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
9	09-13-11	0.66	0.71	0.05	0.61	0.80	0.62	0.79	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
10	10-04-11	0.73	0.71	0.05	0.61	0.81	0.62	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
11	10-11-11	0.76	0.71	0.05	0.62	0.81	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.99	0.32	0.39	1.03	0.07
12	10-18-11	0.67	0.71	0.05	0.61	0.81	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
13	11-08-11	0.78	0.71	0.05	0.61	0.82	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
14	12-06-11	0.77	0.71	0.05	0.61	0.82	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
15	02-07-12	0.77	0.71	0.05	0.61	0.81	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
16	02-14-12	0.74	0.71	0.05	0.61	0.82	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.99	0.32	0.39	1.04	0.07
17	03-06-12	0.64	0.71	0.05	0.61	0.82	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
18	03-06-12	0.68	0.71	0.05	0.61	0.82	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
19	04-12-12	0.71	0.71	0.05	0.61	0.82	0.63	0.80	0.15	0.56	0.86	0.27	0.44	0.98	0.32	0.39	1.03	0.07
20	05-08-12	0.77	0.72	0.05	0.61	0.82	0.63	0.80	0.15	0.57	0.87	0.27	0.44	0.99	0.32	0.39	1.04	0.08

Note: 7-d  $IC_{25}$  = 7-day 25% inhibition concentration. An estimation of the concentration of potassium chloride that would cause a 25% reduction in Pimephales growth for the test population.

CT = Central tendency (mean  $IC_{25}$ ).

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

#### Laboratory Control and Warning Limits

Laboratory control and warning limits were established using the standard deviation of the  $IC_{25}$  values corresponding to the 10th and 25th percentile CVs. These ranges are more stringent than the control and warning limits recommended by USEPA for the test method and endpoint.

$S_{A,10}$  = Standard deviation corresponding to the 10<sup>th</sup> percentile CV. ( $S_{A,10}$  = 0.12)

$S_{A,25}$  = Standard deviation corresponding to the 25<sup>th</sup> percentile CV. ( $S_{A,25}$  = 0.21)

#### USEPA Control and Warning Limits

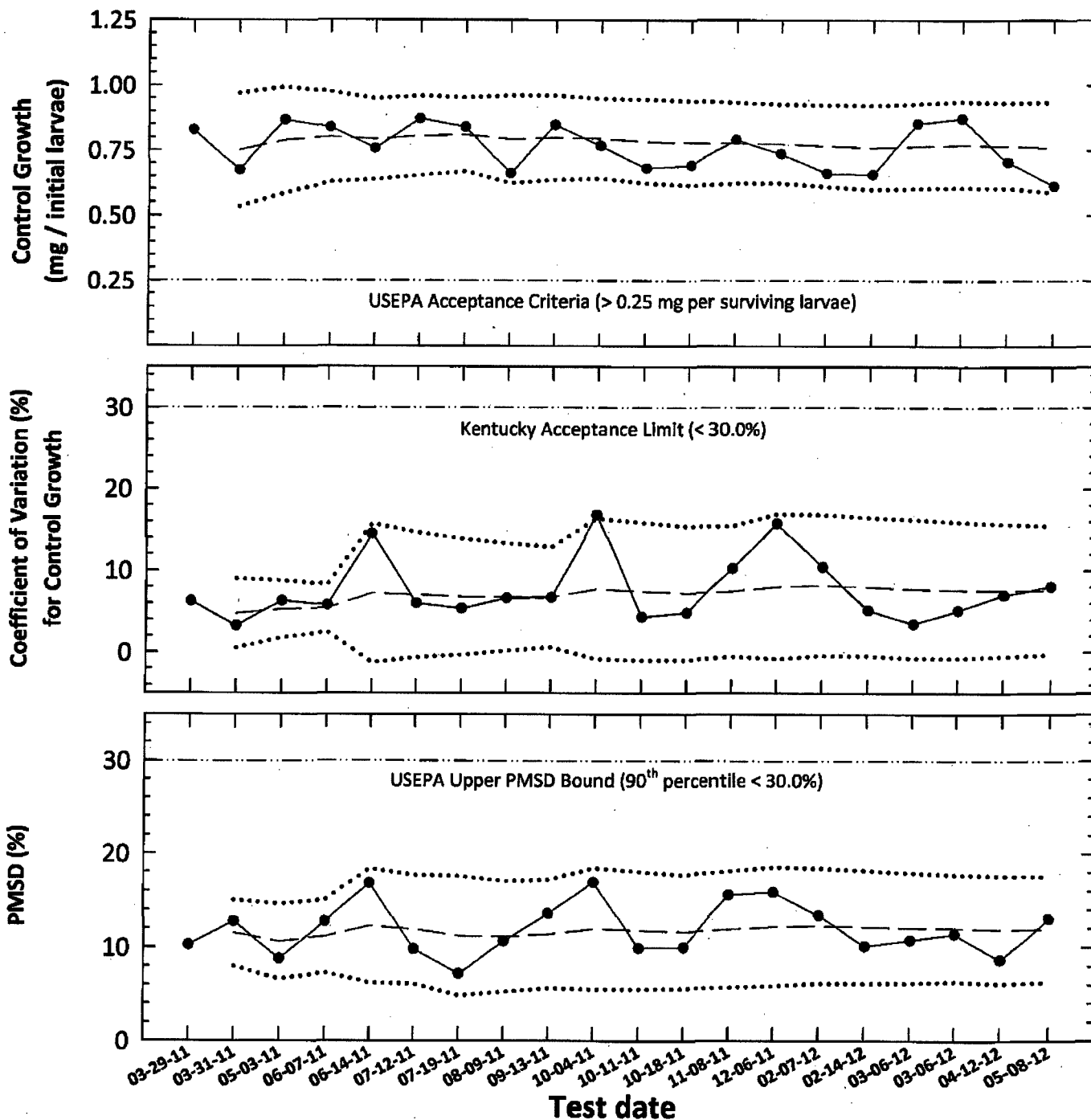
$S_{A,75}$  = Standard deviation corresponding to the 75<sup>th</sup> percentile CV. ( $S_{A,75}$  = 0.38)

$S_{A,90}$  = Standard deviation corresponding to the 90<sup>th</sup> percentile CV. ( $S_{A,90}$  = 0.45)

CV = Coefficient of variation of the  $IC_{25}$  values.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

*Pimephales promelas*  
**Chronic Reference Toxicant Control Chart**  
**Precision of Endpoint Measurements**  
**Organism Source: Aquatox, Inc.**



- Control Reproduction, Coefficient of Variation (CV), or Percent Minimum Significant Difference (PMSD) PMSD is the minimum significant difference between the control and treatment that can be declared statistically significant.
- Central Tendency (mean Control Growth, CV, or PMSD)
- Control Limits (mean Control Growth, CV, or PMSD  $\pm$  2 Standard Deviations)





## Precision of Endpoint Measurements

### *Pimephales promelas* Chronic Reference Toxicant Data

Test number	Test date	Control Survival	Control Mean Growth	CT	CV	CT	MSD	PMSD	CT
		(%)	(mg/larvae)	for Control Growth (mg/larvae)	(%)	for Control Growth CV (%)		(%)	for PMSD (%)
1	03-29-11	100	0.829		6.3		0.09	10.3	
2	03-31-11	100	0.675	0.752	3.2	4.8	0.09	12.8	11.5
3	05-03-11	100	0.867	0.790	6.3	5.3	0.08	8.8	10.6
4	06-07-11	97.5	0.839	0.802	5.8	5.4	0.11	12.7	11.1
5	06-14-11	100	0.758	0.793	14.5	7.2	0.13	16.8	12.3
6	07-12-11	97.5	0.872	0.806	6.0	7.0	0.09	9.8	11.9
7	07-19-11	100	0.839	0.811	5.4	6.8	0.06	7.2	11.2
8	08-09-11	100	0.662	0.792	6.6	6.7	0.07	10.6	11.1
9	09-13-11	100	0.848	0.799	6.7	6.7	0.12	13.6	11.4
10	10-04-11	100	0.768	0.796	16.8	7.7	0.13	16.9	11.9
11	10-11-11	100	0.681	0.785	4.3	7.4	0.07	9.9	11.8
12	10-18-11	100	0.693	0.777	4.8	7.2	0.07	10.0	11.6
13	11-08-11	100	0.792	0.779	10.3	7.5	0.12	15.6	11.9
14	12-06-11	100	0.738	0.776	15.7	8.0	0.12	15.9	12.2
15	02-07-12	100	0.662	0.768	10.5	8.2	0.09	13.4	12.3
16	02-14-12	97.5	0.658	0.761	5.2	8.0	0.07	10.1	12.1
17	03-06-12	100	0.854	0.767	3.5	7.8	0.09	10.8	12.1
18	03-06-12	100	0.873	0.773	5.1	7.6	0.10	11.4	12.0
19	04-12-12	97.5	0.706	0.769	7.0	7.6	0.06	8.7	11.8
20	05-08-12	100	0.613	0.761	8.1	7.6	0.08	13.1	11.9

**Note:**

CV = Coefficient of variation for control growth.

Lower CV bound determined by USEPA (10<sup>th</sup> percentile) = 3.5%.

Upper CV bound determined by USEPA (90<sup>th</sup> 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 (10<sup>th</sup> percentile) = 12%.

Upper PMSD bound determined by USEPA (90<sup>th</sup> percentile) = 30%.

CT = Central Tendency (mean Control Growth, CV, or PMSD)

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program.

EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

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.



**Potassium Chloride Chronic Reference Toxicant Test  
(EPA-821-R-02-013 Method 1000.0)**

Species: *Pimephales promelas*

PpKClCR Test Number: 248

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 1046				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Milli-Q water.				
Dilution prep (mg/L)	450	600	750	900	1050	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	991	988	985	982	979	
Total volume (mL)	1000	1000	1000	1000	1000	

Test organism information:			Test information:	
Organism age:	70.6 HOURS OLD		Randomizing template:	GREEN
Date and times organisms were born between:	05-07-12 1600		Incubator number and shelf location:	3D
Organism source:	ATOX BATCH P 05-07-12		Artemia CHM number:	CHM 652
			Drying information for weight determination:	
Transfer vessel information:	pH = 7.79 Temperature = 24.8 °C		Date / Time in oven:	05-15-12 1140
Average transfer volume:	0.1209 mL		Initial oven temperature:	60 °C
			Date / Time out of oven:	05-16-12 1140
			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		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	05-08-12	—	—	1545	JL	1236	JL	05-03-12 A
1	05-09-12	0800	JL	1400	JL	1138	JL	05-03-12 B
2	05-10-12	0830	JL	1430	JL	1150	JL	05-07-12
3	05-11-12	0800	JL	1400	JL	1137	JL	05-09-12 A
4	05-12-12	0800	JL	1400	JL	1210	JL	05-10-12 A
5	05-13-12	0800	JL	1400	JL	1208	JL	05-10-12 B
6	05-14-12	0800	JL	1400	JL	1137	JL	05-10-12 B
7	05-15-12					1136	JL	

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	0%	≤ 20%	7-day LC <sub>50</sub>	669.8
Average weight per initial larvae:	0.613		NOEC	600
Average weight per surviving larvae:	0.613	≥ 0.25 mg/larvae	LOEC	750
			ChV	670.8
			IC <sub>25</sub>	774.3

Species: *Pimephales promelas*

PpKCICR Test Number: 248

**Survival and Growth Data**

Day	Control				450 mg KCl/L				600 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>Dark Blue (breakers)</u> Analyst: <u>MLT</u> Date: <u>05-04-12</u>												
B = Pan + Larvae weight (mg) Analyst: <u>CDJ</u> Date: <u>05-13-12</u>												
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>Jum</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>Jum</u>												
Average weight per initial number of larvae (mg)      Percent reduction from control (%)												
0.613      0.633      -3.37      0.651      -6.27												

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:


Species: *Pimephales promelas*

PpKCICR Test Number: 748

**Survival and Growth Data**

Day	750 mg KCl/L				900 mg KCl/L				1050 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	8 <sup>2d</sup>	9 <sup>1d</sup>	9 <sup>1d</sup>	9 <sup>1d</sup>	6 <sup>2d</sup>	6 <sup>2d</sup>	8 <sup>2d</sup>	6 <sup>2d</sup>	5 <sup>2d</sup>	5 <sup>2d</sup>	5 <sup>2d</sup>	5 <sup>2d</sup>
2	8	9	9	9	6	6	8	5 <sup>1d</sup>	3 <sup>2d</sup>	3 <sup>2d</sup>	3 <sup>2d</sup>	3 <sup>2d</sup>
3	8	8 <sup>1d</sup>	9	9	5 <sup>1d</sup>	5 <sup>1d</sup>	7 <sup>1d</sup>	5	2 <sup>1d</sup>	2 <sup>1d</sup>	3	3
4	8	8	9	9	5	5	6 <sup>1d</sup>	5	1 <sup>1d</sup>	2	3	3
5	8	8	9	9	4 <sup>1d</sup>	5	6	5	1	2	3	1 <sup>2d</sup>
6	8	8	9	8 <sup>1d</sup>	4	5	5 <sup>1d</sup>	5	1	2	2 <sup>1d</sup>	1
7	8	8	8 <sup>1d</sup>	7 <sup>1d</sup>	4 <sup>1d</sup>	5	5	5	1	1 <sup>1d</sup>	1 <sup>1d</sup>	1 <sup>1d</sup>
A = Pan weight (mg) Tray color code:: <u>Dark Blue (broken)</u> Analyst: <u>NHG</u> Date: <u>05-04-12</u>												
B = Pan + Larvae weight (mg) Analyst: <u>CDJ</u> Date: <u>05-17-12</u>												
C = Larvae weight (mg) = B - A Hand calculated. Analyst: <u>Jim</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Hand calculated. Analyst: <u>Jim</u>												
Average weight per initial number of larvae (mg)      Percent reduction from control (%)												
0.495      19.27      0.365      40.47      0.081      86.77												

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:




*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: 248  
Test dates: May 08-15, 2012

Concentration (mg/L KO)	Replicate	Initial number of larvae	Final number of larvae	A = Pen weight (mg)	B = Pen + Larvae weight (mg)	Larvae weight (mg) = A - B	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.68	21.38	6.70	0.670	0.613	8.1	0.670	100.0	0.613	8.1	Not applicable
	B	10	10	13.56	19.07	5.51	0.551			0.551				
	C	10	10	13.13	19.39	6.26	0.626			0.626				
	D	10	10	13.72	19.75	6.03	0.603			0.603				
450	E	10	10	13.79	20.10	6.31	0.631	0.633	9.6	0.631	100.0	0.633	9.6	-3.3
	F	10	10	12.95	20.14	7.19	0.719			0.719				
	G	10	10	13.39	19.36	5.97	0.597			0.597				
	H	10	10	14.47	20.31	5.84	0.584			0.584				
600	I	10	10	13.92	20.48	6.56	0.656	0.651	6.8	0.656	100.0	0.651	6.8	-6.2
	J	10	10	15.17	21.36	6.19	0.619			0.619				
	K	10	10	13.62	19.79	6.17	0.617			0.617				
	L	10	10	13.51	20.62	7.11	0.711			0.711				
750	M	10	8	15.29	20.82	5.53	0.691	0.639	6.8	0.553	77.5	0.495	8.4	19.2
	N	10	8	13.50	18.46	4.96	0.620			0.496				
	O	10	8	13.74	18.47	4.73	0.591			0.473				
	P	10	7	12.70	17.28	4.58	0.654			0.458				
900	Q	10	4	14.62	18.04	3.42	0.855	0.773	8.3	0.342	47.5	0.365	6.3	40.4
	R	10	5	13.32	16.82	3.50	0.700			0.350				
	S	10	5	14.05	17.83	3.78	0.756			0.378				
	T	10	5	14.05	17.96	3.91	0.782			0.391				
1050	U	10	1	13.91	14.64	0.73	0.730	0.813	14.7	0.073	10.0	0.081	14.7	86.7
	V	10	1	13.44	14.28	0.84	0.840			0.084				
	W	10	1	14.89	15.60	0.71	0.710			0.071				
	X	10	1	14.29	15.26	0.97	0.970			0.097				

Dunnett's MSD value: 0.0800  
PMDS: 13.1

MSD = Minimum Significant Difference  
PMDS = Percent Minimum Significant Difference

PMDS is a measure of test precision. The PMDS is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.  
Lower PMDS bound determined by USEPA (10th percentile) = 12%.  
Upper PMDS bound determined by USEPA (90th percentile) = 30%.

Lower and upper PMDS bounds were determined from the 10th and 90th percentile, respectively, of PMDS 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.



## Statistical Analyses

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

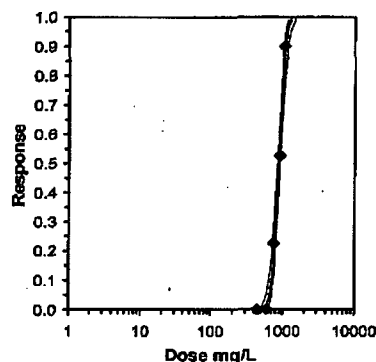
Start Date: 5/8/2012 Test ID: PpKCICR Sample ID: REF-Ref Toxicant  
End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride  
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	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
780	0.8000	0.8000	0.8000	0.7000
*900	0.4000	0.5000	0.5000	0.5000
*1050	0.1000	0.1000	0.1000	0.1000

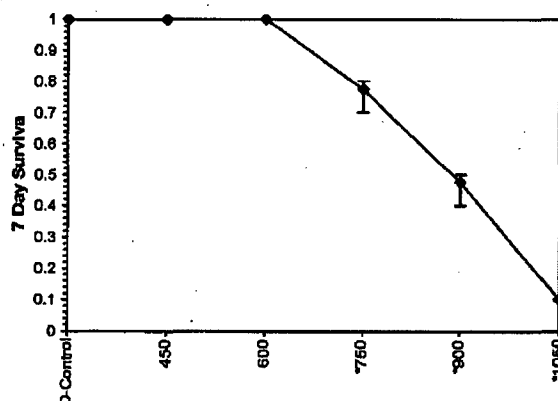
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.7750	0.7750	1.0782	0.9912	1.1071	5.379	4	10.00	10.00	9	40
*900	0.4750	0.4750	0.7802	0.6847	0.7854	6.622	4	10.00	10.00	21	40
*1050	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	38	40

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.63088	0.884	-2.1517	6.4494
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					600	750	670.82	
Treatments vs D-Control								

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu
Slope	14.4814	1.87334	10.6137	18.3492	0	1.78643	7.81472	0.61789	2.93943
Intercept	-37.667	5.80045	-48.936	-26.198					0.06905
TSCR									
Point	Probits	mg/L	95% Fiducial Limits						
EC01	2.674	600.876	521.385	654.429					
EC05	3.355	689.845	602.697	714.916					
EC10	3.718	709.467	650.476	750.155					
EC15	3.984	737.684	684.352	775.454					
EC20	4.158	760.871	712.091	796.859					
EC25	4.328	781.362	736.336	815.805					
EC40	4.747	835.476	798.142	889.388					
EC50	5.000	869.818	834.745	906.603					
EC60	5.253	905.673	870.247	948.43					
EC75	5.674	988.289	927.107	1028.37					
EC80	5.842	994.385	949.312	1063.47					
EC85	6.038	1025.65	975.202	1106.65					
EC90	6.282	1066.41	1008.03	1164.36					
EC95	6.845	1129.83	1057.85	1256.74					
EC99	7.326	1259.13	1155.35	1452.86					



Dose-Response Plot



# Statistical Analyses

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

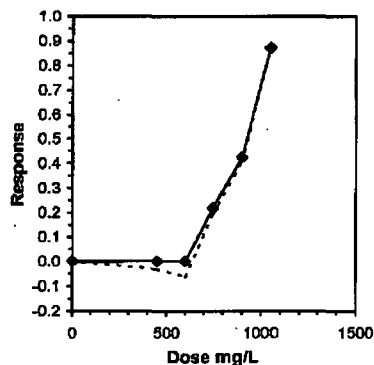
Start Date: 5/8/2012	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 5/15/2012	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas
Comments:		

Conc-mg/L	1	2	3	4
D-Control	0.6700	0.5510	0.6280	0.6030
450	0.6310	0.7190	0.5970	0.5840
600	0.6580	0.8190	0.6170	0.7110
750	0.6530	0.4980	0.4730	0.4580
900	0.3420	0.3500	0.3780	0.3910
1050	0.0730	0.0840	0.0710	0.0970

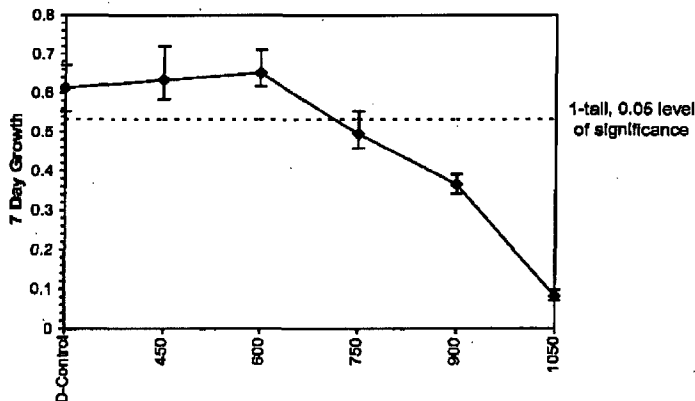
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.6125	1.0000	0.6125	0.5510	0.6700	8.087	4				0.6320	1.0000
450	0.6328	1.0331	0.6328	0.5840	0.7190	9.612	4	-0.552	2.180	0.0800	0.6320	1.0000
600	0.6508	1.0824	0.6508	0.6170	0.7110	6.760	4	-1.042	2.180	0.0800	0.6320	1.0000
750	0.4950	0.8082	0.4950	0.4580	0.5530	8.425	4				0.4950	0.7832
900	0.3653	0.5963	0.3653	0.3420	0.3910	6.320	4				0.3653	0.5779
1050	0.0813	0.1327	0.0813	0.0710	0.0970	14.714	4				0.0813	0.1286

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)				0.92935	0.805	0.61878	-0.6846
Bartlett's Test indicates equal variances (p = 0.87)				0.28584	9.21035		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnett's Test				600	>600		
Treatments vs D-Control				0.08004	0.13067	0.00146	0.0027
				0.69672	2, 9		

Linear Interpolation (200 Resamples)						
Point	mg/L	SD	95% CL(Exp)	Skew		
IC05	834.60	28.01	593.31	661.72	-7.8928	
IC10	869.20	14.86	633.19	723.43	0.9209	
IC15	703.80	18.42	666.12	781.73	0.9450	
IC20	738.39	20.46	694.18	808.57	0.4813	
IC25	774.28	20.26	718.07	838.43	0.1832	
IC40	883.87	12.92	841.40	918.61	-0.1823	
IC50	926.01	6.72	908.42	943.16	-0.0795	



Dose-Response Plot



Species: *Pimephales promelas*

PpKCICR Test Number: 248

**Daily Chemistry:**

Analyst		Day					
		0		1		2	
		CDT	CDT	CDT	CDT	CDT	LAB
Concentration	Parameter						
CONTROL	pH (S.U.)	7.85	7.88	7.92	7.74	7.95	7.60
	DO (mg/L)	7.6	7.8	7.8	7.6	7.7	6.5
	Conductivity (µmhos/cm)	327		310		303	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		63	
	Hardness (mg CaCO <sub>3</sub> /L)	88		88		89	
	Temperature (°C)	24.7	25.0	24.9	24.6	24.8	24.6
450 mg KCl/L	pH (S.U.)	7.86	7.86	7.94	7.77	7.95	7.67
	DO (mg/L)	7.7	7.7	7.8	7.6	7.7	7.0
	Conductivity (µmhos/cm)	1100		1100		1040	
	Temperature (°C)	24.9	24.7	25.0	24.5	24.7	24.6
600 mg KCl/L	pH (S.U.)	7.85	7.84	7.92	7.76	7.97	7.65
	DO (mg/L)	7.7	7.7	7.9	7.6	7.7	6.9
	Conductivity (µmhos/cm)	1390		1370		1310	
	Temperature (°C)	24.9	24.7	25.0	24.5	24.7	24.5
750 mg KCl/L	pH (S.U.)	7.85	7.83	7.94	7.77	7.97	7.70
	DO (mg/L)	9.7	7.7	7.9	7.5	7.7	7.1
	Conductivity (µmhos/cm)	1630		1600		1520	
	Temperature (°C)	24.9	24.7	25.0	24.7	24.7	24.5
900 mg KCl/L	pH (S.U.)	7.86	7.83	7.95	7.81	7.97	7.70
	DO (mg/L)	7.8	7.6	7.9	7.5	7.7	7.1
	Conductivity (µmhos/cm)	1910		1860		1810	
	Temperature (°C)	24.9	24.8	25.0	24.7	24.7	24.5
1050 mg KCl/L	pH (S.U.)	7.85	7.81	7.95	7.77	7.96	7.69
	DO (mg/L)	7.8	7.7	8.0	7.6	7.7	7.0
	Conductivity (µmhos/cm)	2170		2110		2030	
	Temperature (°C)	24.9	24.5	25.1	24.6	24.7	24.5
STOCK	Conductivity (µmhos/cm)	65800					
		Initial	Final	Initial	Final	Initial	Final

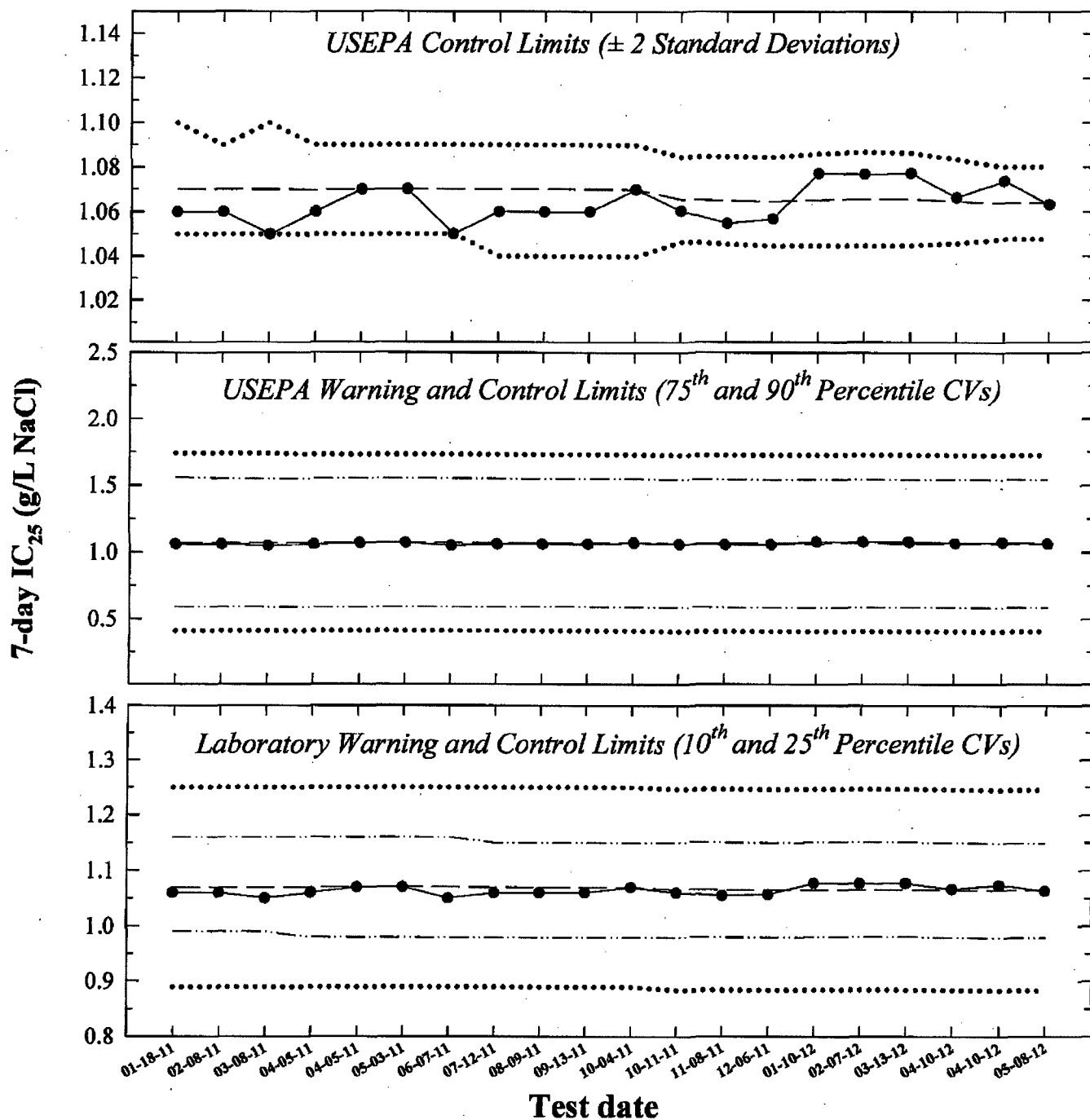
Independent Review by  
Kathy L. Krasinski  
ETS

Species: *Pimephales promelas*

PpKCICR Test Number: 248

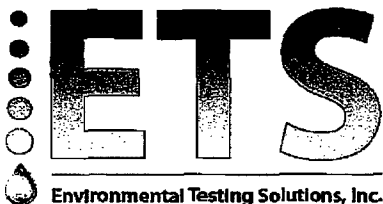
		Day							
		3		4		5		6	
		LAB	MW	MW	MW	MW	MW	MW	MW
Concentration	Parameter								
CONTROL	pH (S.U.)	7.98	7.57	8.02	7.58	7.97	7.56	8.00	7.41
	DO (mg/L)	7.4	6.9	7.8	6.7	7.6	6.3	7.6	6.0
	Conductivity (µmhos/cm)	294		309		313		312	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		62		<del>62</del>	
	Hardness (mg CaCO <sub>3</sub> /L)	87		89		89			
	Temperature (°C)	24.7	24.6	24.8	24.3	24.7	24.2	24.7	24.7
450 mg KCl/L	pH (S.U.)	8.01	7.62	7.94	7.52	7.94	7.54	8.04	7.45
	DO (mg/L)	7.4	7.1	7.6	6.7	7.7	6.5	7.5	6.2
	Conductivity (µmhos/cm)	1050		1050		1100		1080	
	Temperature (°C)	24.8	24.2	24.7	24.2	24.7	24.2	24.7	24.7
600 mg KCl/L	pH (S.U.)	8.01	7.61	7.93	7.51	7.94	7.57	8.03	7.44
	DO (mg/L)	7.3	7.1	7.6	7.1	7.7	6.1	7.5	6.3
	Conductivity (µmhos/cm)	1310		1310		1390		1340	
	Temperature (°C)	24.8	24.5	24.7	24.6	24.7	24.5	24.7	24.7
750 mg KCl/L	pH (S.U.)	8.00	7.54	7.94	7.59	7.93	7.56	8.04	7.45
	DO (mg/L)	7.3	7.0	7.5	7.1	7.7	6.2	7.6	6.3
	Conductivity (µmhos/cm)	1560		1580		1600		1580	
	Temperature (°C)	24.8	24.7	24.7	24.3	24.7	24.5	24.8	24.5
900 mg KCl/L	pH (S.U.)	8.00	7.55	7.94	7.49	7.93	7.55	8.04	7.49
	DO (mg/L)	7.4	6.7	7.4	7.1	7.6	6.4	7.6	6.4
	Conductivity (µmhos/cm)	1830		1820		1880		1830	
	Temperature (°C)	24.8	24.4	24.7	24.2	24.7	24.6	24.8	24.6
1050 mg KCl/L	pH (S.U.)	8.00	7.58	7.93	7.54	7.93	7.57	8.04	7.57
	DO (mg/L)	7.4	6.9	7.4	6.8	7.6	6.0	7.5	6.4
	Conductivity (µmhos/cm)	2050		2060		2150		2070	
	Temperature (°C)	24.8	24.4	24.7	24.2	24.7	24.5	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

*Ceriodaphnia dubia*  
**Chronic Reference Toxicant Control Chart**



- 7-day  $IC_{25}$  = 25% inhibition concentration. An estimation of the concentration of sodium chloride that would cause a 25% reduction in *Ceriodaphnia* reproduction for the test population.
- — — Central Tendency (mean  $IC_{25}$ )
- - - - - Warning Limits (mean  $IC_{25} \pm S_{A.10}$  or  $S_{A.75}$ )
- ..... Control Limits (mean  $IC_{25} \pm S_{A.25}$ ,  $S_{A.90}$ , or 2 Standard Deviations)





## Ceriodaphnia dubia Chronic Reference Toxicant Control Chart

Test number	Test date	7-day IC <sub>25</sub> (g/L NaCl)	CT (g/L NaCl)	S	State and USEPA Control Limits		S <sub>A,10</sub>	Laboratory Warning Limits		S <sub>A,25</sub>	Laboratory Control Limits		S <sub>A,75</sub>	USEPA Warning Limits		S <sub>A,90</sub>	USEPA Control Limits		CV
					CT - 2S	CT + 2S		CT - S <sub>A,10</sub>	CT + S <sub>A,10</sub>		CT - S <sub>A,25</sub>	CT + S <sub>A,25</sub>		CT - S <sub>A,75</sub>	CT + S <sub>A,75</sub>		CT - S <sub>A,90</sub>	CT + S <sub>A,90</sub>	
1	01-18-11	1.06	1.07	0.01	1.05	1.10	0.09	0.99	1.16	0.18	0.89	1.25	0.48	0.59	1.56	0.66	0.41	1.74	0.01
2	02-08-11	1.06	1.07	0.01	1.05	1.09	0.09	0.99	1.16	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.74	0.01
3	03-08-11	1.05	1.07	0.01	1.05	1.10	0.09	0.99	1.16	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.74	0.01
4	04-05-11	1.06	1.07	0.01	1.05	1.09	0.09	0.98	1.16	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
5	04-05-11	1.07	1.07	0.01	1.05	1.09	0.09	0.98	1.16	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
6	05-03-11	1.07	1.07	0.01	1.05	1.09	0.09	0.98	1.16	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
7	06-07-11	1.05	1.07	0.01	1.05	1.09	0.09	0.98	1.16	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
8	07-12-11	1.06	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
9	08-09-11	1.06	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
10	09-13-11	1.06	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
11	10-04-11	1.07	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.89	1.25	0.48	0.59	1.55	0.66	0.41	1.73	0.01
12	10-11-11	1.06	1.07	0.01	1.05	1.08	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.55	0.66	0.40	1.73	0.01
13	11-08-11	1.05	1.07	0.01	1.05	1.08	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.54	0.66	0.40	1.73	0.01
14	12-06-11	1.06	1.06	0.01	1.04	1.08	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.54	0.66	0.40	1.72	0.01
15	01-10-12	1.08	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.54	0.66	0.40	1.73	0.01
16	02-07-12	1.08	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.55	0.66	0.40	1.73	0.01
17	03-13-12	1.08	1.07	0.01	1.04	1.09	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.55	0.66	0.40	1.73	0.01
18	04-10-12	1.07	1.06	0.01	1.05	1.08	0.09	0.98	1.15	0.18	0.88	1.25	0.48	0.59	1.54	0.66	0.40	1.72	0.01
19	04-10-12	1.07	1.06	0.01	1.05	1.08	0.09	0.98	1.15	0.18	0.88	1.24	0.48	0.59	1.54	0.66	0.40	1.72	0.01
20	05-08-12	1.06	1.06	0.01	1.05	1.08	0.09	0.98	1.15	0.18	0.88	1.24	0.48	0.59	1.54	0.66	0.40	1.72	0.01

Note: 7-d IC<sub>25</sub> = 7-day 25% inhibition concentration. An estimation of the concentration of sodium chloride that would cause a 25% reduction in *Ceriodaphnia* reproduction for the test population.

CT = Central tendency (mean IC<sub>25</sub>).

S = Standard deviation of the IC<sub>25</sub> values.

#### Laboratory Control and Warning Limits

Laboratory control and warning limits were established using the standard deviation of the IC<sub>25</sub> values corresponding to the 10th and 25th percentile CVs. These ranges are more stringent than the control and warning limits recommended by USEPA for the test method and endpoint.

S<sub>A,10</sub> = Standard deviation corresponding to the 10<sup>th</sup> percentile CV. (S<sub>A,10</sub> = 0.08)

S<sub>A,25</sub> = Standard deviation corresponding to the 25<sup>th</sup> percentile CV. (S<sub>A,25</sub> = 0.17)

#### USEPA Control and Warning Limits

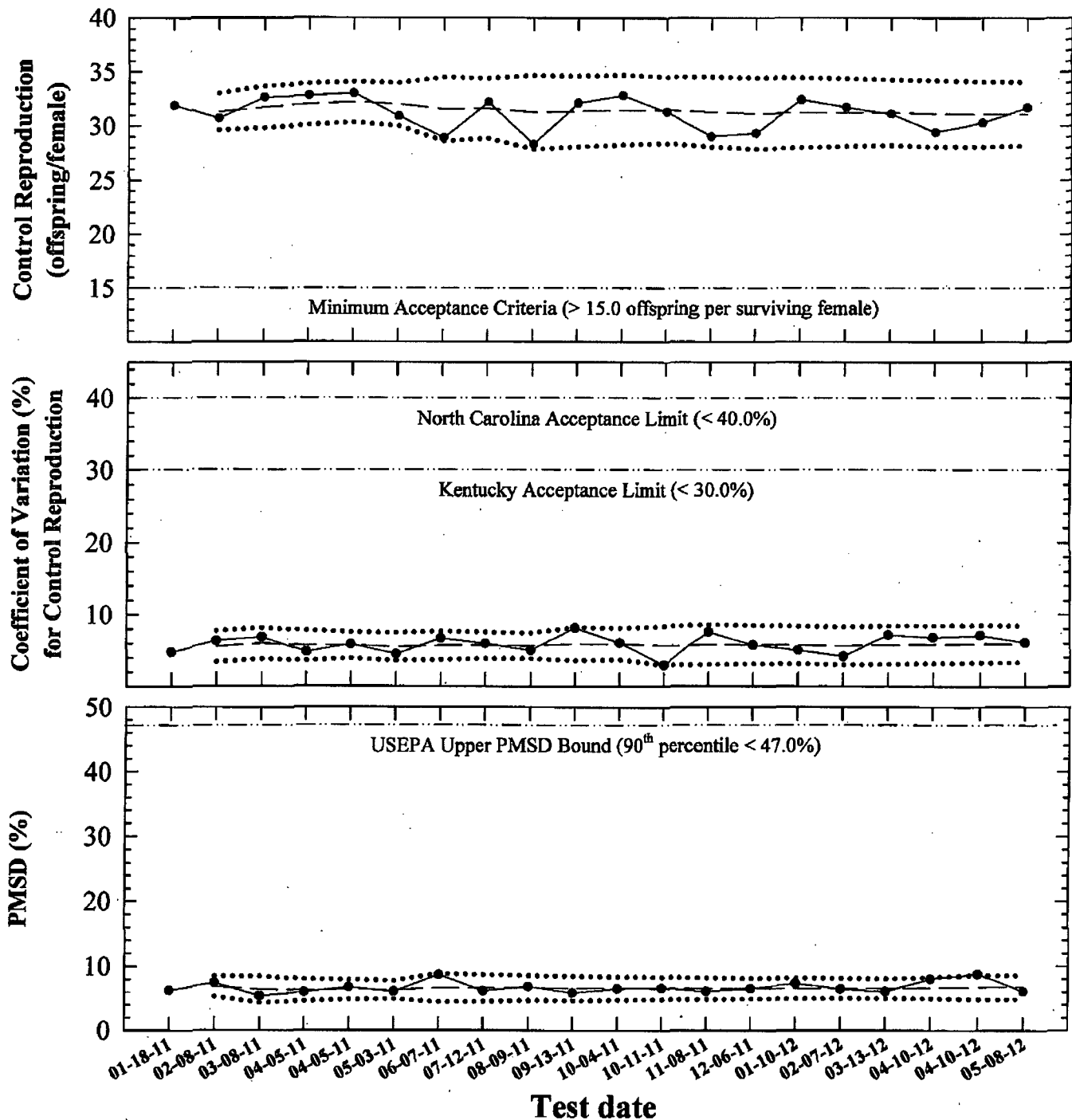
S<sub>A,75</sub> = Standard deviation corresponding to the 75<sup>th</sup> percentile CV. (S<sub>A,75</sub> = 0.45)

S<sub>A,90</sub> = Standard deviation corresponding to the 90<sup>th</sup> percentile CV. (S<sub>A,90</sub> = 0.62)

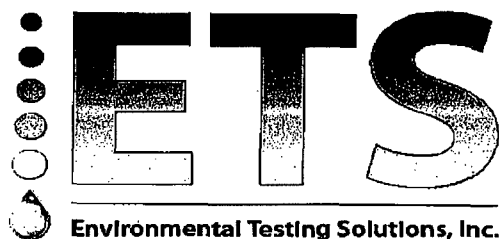
CV = Coefficient of variation of the IC<sub>25</sub> values.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

*Ceriodaphnia dubia*  
**Chronic Reference Toxicant Control Chart**  
**Precision of Endpoint Measurements**



- Control Reproduction, Coefficient of Variation (CV), or Percent Minimum Significant Difference (PMSD) PMSD is the minimum significant difference between the control and treatment that can be declared statistically significant.
- Central Tendency (mean Control Reproduction, CV, or PMSD)
- ..... Control Limits (mean Control Reproduction, CV, or PMSD  $\pm$  2 Standard Deviations)



## Precision of Endpoint Measurements

### *Ceriodaphnia dubia* Chronic Reference Toxicant Data

Test number	Test date	Control Survival (%)	Control Mean Reproduction (offspring/female)	CT for Control Mean Reproduction (offspring/female)	CV (%)	CT for Control Reproduction CV (%)	MSD	PMSD (%)	CT for PMSD (%)
1	01-18-11	100	31.9		4.8		2.0	6.3	
2	02-08-11	100	30.7	31.3	6.3	5.6	2.3	7.4	6.9
3	03-08-11	100	32.6	31.7	6.9	6.0	1.8	5.4	6.4
4	04-05-11	100	32.8	32.0	4.8	5.7	2.0	6.0	6.3
5	04-05-11	100	33.0	32.2	5.9	5.8	2.2	6.7	6.4
6	05-03-11	100	30.9	32.0	4.5	5.5	1.9	6.1	6.3
7	06-07-11	100	28.9	31.5	6.7	5.7	2.5	8.7	6.6
8	07-12-11	100	32.2	31.6	6.0	5.7	2.0	6.2	6.6
9	08-09-11	100	28.3	31.3	5.0	5.7	1.9	6.8	6.6
10	09-13-11	100	32.1	31.3	8.2	5.9	1.9	5.8	6.5
11	10-04-11	100	32.8	31.5	6.1	5.9	2.1	6.5	6.5
12	10-11-11	100	31.3	31.5	3.0	5.7	2.1	6.6	6.5
13	11-08-11	100	29.0	31.3	7.6	5.8	1.8	6.1	6.5
14	12-06-11	100	29.3	31.1	5.8	5.8	1.9	6.5	6.5
15	01-10-12	100	32.4	31.2	5.1	5.8	2.4	7.4	6.6
16	02-07-12	100	31.7	31.2	4.2	5.7	2.1	6.5	6.6
17	03-13-12	100	31.1	31.2	7.2	5.8	1.9	6.1	6.5
18	04-10-12	100	29.4	31.1	6.8	5.8	2.4	8.0	6.6
19	04-10-12	100	30.3	31.1	7.1	5.9	2.7	8.8	6.7
20	05-08-12	100	31.7	31.1	6.1	5.9	1.9	6.1	6.7

Note:

CV = Coefficient of variation for control reproduction.

Lower CV bound determined by USEPA (10<sup>th</sup> percentile) = 8.9%.

Upper CV bound determined by USEPA (90<sup>th</sup> percentile) = 42%

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 (10<sup>th</sup> percentile) = 13%.

Upper PMSD bound determined by USEPA (90<sup>th</sup> percentile) = 47%.

CT = Central Tendency (Mean Control Reproduction, CV, or PMSD)

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

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.





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

CdNaClCR #: 134

Dilution preparation information:					Comments:
NaCl Stock INSS number:	<u>1055 1059</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:		Blue			
Date and times organisms were born between:				05-08-12 0640 TO 0900								Incubator number and shelf location:		2B2			
Culture board:				05-01-12 A								YWT batch:		05-01-12			
Replicate number:				1	2	3	4	5	6	7	8	9	10	Selenastrum batch:		04-30-12	
Culture board cup number:				3	5	6	10	12	13	15	17	21	22				
Transfer vessel information:				pH = 7.88 S.U. Temperature = 25.1 °C													
Average transfer volume (mL):				0.0527 mL													

**Daily renewal information:**

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	MHSW batch used	Analyst
0	<u>05-08-12</u>	<u>0908</u>	<u>05-05-12A</u>	<u>JL</u>
1	<u>05-09-12</u>	<u>0810</u>	<u>05-05-12B</u>	<u>JL</u>
2	<u>05-10-12</u>	<u>0810</u>	<u>05-07-12</u>	<u>JL</u>
3	<u>05-11-12</u>	<u>0809</u>	<u>05-09-12A</u>	<u>JL</u>
4	<u>05-12-12</u>	<u>0810</u>	<u>05-10-12A</u>	<u>JL</u>
5	<u>05-13-12</u>	<u>0812</u>	<u>05-10-12B</u>	<u>JL</u>
6	<u>05-14-12</u>	<u>0810</u>	<u>05-10-12B</u>	<u>JL</u>
7	<u>05-15-12</u>	<u>0809</u>		<u>JL</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	<u>07.</u>	≤ 20%	7-day LC <sub>50</sub>	<u>&gt;1400</u>
% Adults having 3 <sup>rd</sup> Broods:	<u>1007.</u>	≥ 80%	NOEC	<u>1000</u>
% Mortality:	<u>01.</u>	≤ 20%	LOEC	<u>1200</u>
Mean Offspring/Female:	<u>31.7</u>	≥ 15.0 offspring/female	ChV	<u>1095.5</u>
% CV:	<u>6.17.</u>	< 40.0 %	IC <sub>25</sub>	<u>1063.4</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 134

**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	4	5	6	5	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	10	11	10	11	10	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	17	17	15	13	15	18	15	18	16	17
Total young produced		34	33	29	29	31	34	30	33	31	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 <sup>rd</sup> 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: 31.7

**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	4	5	4	4	5	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	10	10	10	12	11	10	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	16	15	15	18	17	18	16	16	15
Total young produced		33	32	30	29	32	34	33	30	34	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: 31.7

% Reduction from Control: 0.07.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 134

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	5	4	5	5	4	4	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	10	13	11	12	10	10	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	16	14	14	16	15	13	18	16	17
Total young produced		33	32	28	32	32	31	27	33	32	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:	31.1
% Reduction from Control:	1.97.

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	4	4	5	4	4	4	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	11	13	11	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	15	16	14	15	16	13	17	15	13	15
Total young produced		31	30	30	32	31	27	33	31	28	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:	30.3
% Reduction from Control:	4.47.

Species: Ceriodaphnia dubia  
1200 mg NaCl/L

CdNaClCR #: 134

**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	0	1	1	2	1	3	3	1	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	3	5	2	3	3	0	0	2	4	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	5	2	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	7	6	4	6	5	5	7	4	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		10	12	7	11	9	13	12	7	8	8
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:	9.7
% Reduction from Control:	64.47.

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	0	0	1	0	1	0	1	1	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	1	0	1	0	2	0	0	3	2
	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	5	3	0	3	4	1	3	0	0	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		5	4	1	4	5	3	4	1	3	4
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:	3.4
% Reduction from Control:	89.37.





Environmental Testing Solutions, Inc.

## Verification of *Ceriodaphnia* Reproduction Totals

### Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	5	6	5	5	4	4	4	46
5	12	12	10	11	10	11	10	11	11	12	110
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	15	13	15	18	15	18	16	17	161
Total	34	33	29	29	31	34	30	33	31	33	317

### 600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	4	4	5	4	4	6	4	44
5	12	12	10	10	10	12	11	10	12	11	110
6	0	0	0	0	0	0	0	0	0	0	0
7	17	16	15	15	18	17	18	16	16	15	163
Total	33	32	30	29	32	34	33	30	34	30	317

### 800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	5	5	4	4	5	4	4	45
5	10	11	10	13	11	12	10	10	12	10	109
6	0	0	0	0	0	0	0	0	0	0	0
7	18	16	14	14	16	15	13	18	16	17	157
Total	33	32	28	32	32	31	27	33	32	31	311

### 1000 mg NaCl/L

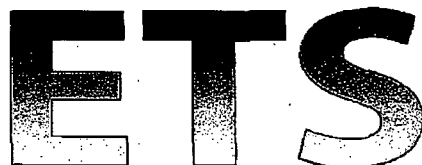
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	4	4	4	4	4	5	5	43
5	12	10	11	13	11	10	12	12	10	10	111
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	14	15	16	13	17	15	13	15	149
Total	31	30	30	32	31	27	33	31	28	30	303

### 1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	1	1	2	1	3	3	1	1	2	15
5	3	5	2	3	3	0	0	2	4	1	23
6	0	0	0	0	0	5	2	0	0	0	7
7	7	6	4	6	5	5	7	4	3	5	52
Total	10	12	7	11	9	13	12	7	8	8	97

### 1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	1	0	1	0	1	1	0	0	4
5	0	1	0	1	0	2	0	0	3	2	9
6	0	0	0	0	0	0	0	0	0	0	0
7	5	3	0	3	4	1	3	0	0	2	21
Total	5	4	1	4	5	3	4	1	3	4	34



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: CdNaCICR #134

Test dates: May 08-15, 2012

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	34	33	29	29	31	34	30	33	31	33	100	31.7	6.1	Not applicable
600	33	32	30	29	32	34	33	30	34	30	100	31.7	5.8	0.0
800	33	32	28	32	32	31	27	33	32	31	100	31.1	6.5	1.9
1000	31	30	30	32	31	27	33	31	28	30	100	30.3	5.8	4.4
1200	10	12	7	11	9	13	12	7	8	8	100	9.7	22.8	69.4
1400	5	4	1	4	5	3	4	1	3	4	100	3.4	42.1	89.3

Dunnett's MSD value: 1.927

PMDS: 6.1

MSD = Minimum Significant Difference

PMDS = Percent Minimum Significant Difference

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

Lower PMDS bound determined by USEPA (10<sup>th</sup> percentile) = 13%.

Upper PMDS bound determined by USEPA (90<sup>th</sup> percentile) = 47%.

Lower and upper PMDS bounds were determined from the 10th and 90th percentile, respectively, of PMDS 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.

File: CdNaCICR\_050812.xlsx  
Table populated from associated "Verification of *Ceriodaphnia* Reproduction Totals" spreadsheet.  
Spreadsheet entered by: J. Sumner  
Reviewed by:

## Statistical Analyses

### Ceriodaphnia Survival and Reproduction Test-Reproduction

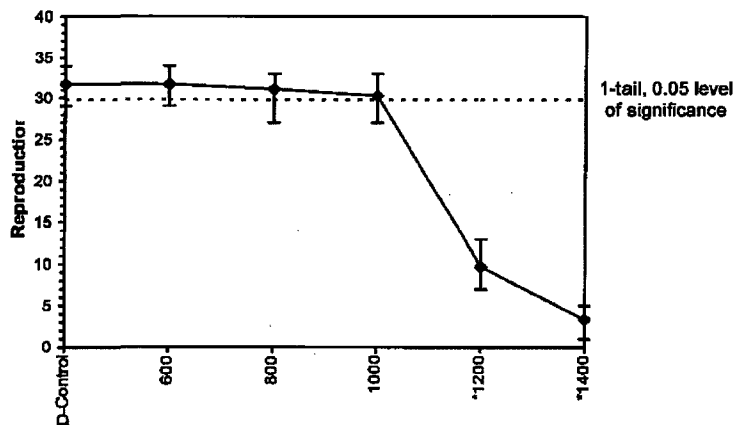
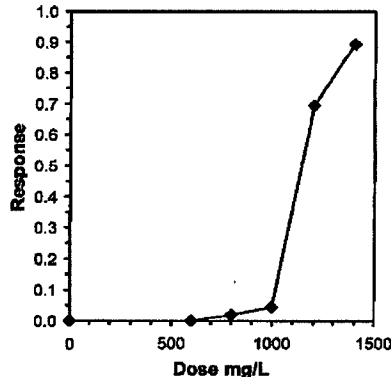
Start Date: 5/8/2012 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant  
End Date: 5/15/2012 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride  
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia  
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	33.000	29.000	29.000	31.000	34.000	30.000	33.000	31.000	33.000
600	33.000	32.000	30.000	29.000	32.000	34.000	33.000	30.000	34.000	30.000
800	33.000	32.000	28.000	32.000	32.000	31.000	27.000	33.000	32.000	31.000
1000	31.000	30.000	30.000	32.000	31.000	27.000	33.000	31.000	28.000	30.000
1200	10.000	12.000	7.000	11.000	9.000	13.000	12.000	7.000	8.000	8.000
1400	5.000	4.000	1.000	4.000	5.000	3.000	4.000	1.000	3.000	4.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
D-Control	31.700	1.0000	31.700	29.000	34.000	6.140	10				31.700	1.0000
600	31.700	1.0000	31.700	29.000	34.000	5.789	10	0.000	2.287	1.927	31.700	1.0000
800	31.100	0.9811	31.100	27.000	33.000	6.511	10	0.712	2.287	1.927	31.100	0.9811
1000	30.300	0.9558	30.300	27.000	33.000	5.832	10	1.661	2.287	1.927	30.300	0.9558
*1200	9.700	0.3060	9.700	7.000	13.000	22.821	10	26.109	2.287	1.927	9.700	0.3060
*1400	3.400	0.1073	3.400	1.000	5.000	42.054	10	33.586	2.287	1.927	3.400	0.1073

Auxillary Tests					Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution (p > 0.01)					1.02293	1.035	-0.3894	-0.8258						
Bartlett's Test indicates equal variances (p = 0.88)					1.79808	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					1000	1200	1095.45		1.92678	0.06078	1662.88	3.55	3.7E-43	5, 54
Treatments vs D-Control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	1001.8	91.4611	703.535	1009.92	-1.5251
IC10	1017.18	16.9162	1002.53	1024.94	-11.3848
IC15	1032.57	5.71867	1018.68	1040.35	-0.2299
IC20	1047.96	5.47513	1034.69	1055.49	-0.1685
IC25	1063.35	5.29693	1050.98	1071.19	-0.1022
IC40	1109.51	5.20953	1097.81	1118.78	0.0763
IC50	1140.29	5.52815	1129.3	1150.27	0.1443



File: CdNaClCR\_050812.xlsx  
Entered by: J. Sumner  
Reviewed by: *[Signature]*

Species: *Ceriodaphnia dubia*

CdNaClCR #: 134

**Daily Chemistry:**

		Day					
		0		1		2	
Analyst		CNT	CNT	CNT	CNT	CNT	LAB
Concentration	Parameter						
CONTROL	pH (S.U.)	7.85	7.95	7.92	7.98	7.95	7.98
	DO (mg/L)	7.6	8.0	7.8	7.9	7.7	7.6
	Conductivity (µmhos/cm)	327		310		303	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		63	
	Hardness (mg CaCO <sub>3</sub> /L)	88		88		89	
	Temperature (°C)	25.1	25.2	24.9	24.9	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.84	7.90	7.92	7.92	7.96	7.91
	DO (mg/L)	7.8	7.8	7.9	7.8	8.0	7.5
	Conductivity (µmhos/cm)	1420		1380		1330	
	Temperature (°C)	25.2	25.0	25.2	24.9	25.0	25.1
800 mg NaCl/L	pH (S.U.)	7.84	7.89	7.93	7.90	7.94	7.90
	DO (mg/L)	7.8	7.9	7.9	7.8	8.0	7.5
	Conductivity (µmhos/cm)	1760		1720		1670	
	Temperature (°C)	25.2	25.0	25.2	24.9	25.0	25.1
1000 mg NaCl/L	pH (S.U.)	7.84	7.88	7.93	7.91	7.95	7.91
	DO (mg/L)	7.8	8.0	8.0	7.7	8.0	7.5
	Conductivity (µmhos/cm)	2110		2050		1990	
	Temperature (°C)	24.9	25.0	25.1	24.9	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.83	7.87	7.91	7.88	7.95	7.89
	DO (mg/L)	7.8	8.0	8.0	7.7	7.9	7.5
	Conductivity (µmhos/cm)	2450		2360		2320	
	Temperature (°C)	25.0	25.1	25.1	24.9	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	7.83	7.87	7.91	7.88	7.95	7.89
	DO (mg/L)	7.8	8.0	8.0	7.7	7.9	7.5
	Conductivity (µmhos/cm)	2800		2700		2640	
	Temperature (°C)	25.0	25.1	25.1	24.9	25.0	25.0
STOCK	Conductivity (µmhos/cm)	108000					
		Initial	Final	Initial	Final	Initial	Final



Species: *Ceriodaphnia dubia*

CdNaClCR #: 134

		Day							
		3		4		5		6	
		LAB	Min	Min	Min	Min	Min	Min	Min
Analyst									
Concentration	Parameter								
CONTROL	pH (S.U.)	7.98	7.95	8.02	7.92	7.97	7.90	8.04	7.72
	DO (mg/L)	7.4	7.7	7.8	7.7	7.4	7.5	7.6	7.5
	Conductivity (µmhos/cm)	294		309		313		312	
	Alkalinity (mg CaCO <sub>3</sub> /L)	63		63		62		<del>64</del>	
	Hardness (mg CaCO <sub>3</sub> /L)	87		89		89		<del>88</del>	
	Temperature (°C)	24.8	25.1	25.0	24.9	24.7	24.8	24.8	24.8
600 mg NaCl/L	pH (S.U.)	7.93	7.90	7.98	7.85	7.93	7.85	8.03	7.71
	DO (mg/L)	7.6	7.6	7.7	7.6	7.7	7.5	7.7	7.5
	Conductivity (µmhos/cm)	1300		1350		1380		1400	
	Temperature (°C)	24.8	24.8	25.0	24.9	24.7	24.8	24.8	24.8
800 mg NaCl/L	pH (S.U.)	7.94	7.88	7.98	7.86	7.97	7.84	8.05	7.71
	DO (mg/L)	7.5	7.6	7.7	7.6	7.8	7.5	7.7	7.5
	Conductivity (µmhos/cm)	1640		1700		1720		1720	
	Temperature (°C)	24.8	24.8	25.1	24.9	24.7	25.2	24.9	24.8
1000 mg NaCl/L	pH (S.U.)	7.96	7.90	7.98	7.84	7.98	7.84	8.05	7.70
	DO (mg/L)	7.4	7.7	7.8	7.7	7.8	7.5	7.7	7.6
	Conductivity (µmhos/cm)	1970		2040		2000		2070	
	Temperature (°C)	24.8	24.8	25.1	25.0	24.7	24.8	24.9	24.8
1200 mg NaCl/L	pH (S.U.)	7.95	7.88	7.98	7.83	7.97	7.83	8.04	7.70
	DO (mg/L)	7.5	7.7	7.8	7.7	7.8	7.5	7.7	7.6
	Conductivity (µmhos/cm)	2320		2390		2410		2470	
	Temperature (°C)	24.8	24.8	25.1	25.0	24.7	24.9	24.9	25.0
1400 mg NaCl/L	pH (S.U.)	7.95	7.89	7.97	7.84	7.96	7.84	8.04	7.72
	DO (mg/L)	7.6	7.7	7.8	7.7	7.8	7.5	7.7	7.6
	Conductivity (µmhos/cm)	2600		2670		2700		2770	
	Temperature (°C)	24.8	24.8	25.1	25.0	24.7	24.9	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final