

August 25, 2008

Ronald C. Hall, KFP 1A-KST

**KINGSTON FOSSIL (KIF) PLANT NPDES PERMIT TN0005452: TOXICITY  
TESTING OF OUTFALL 002 - AUGUST, 2008**

Attached are two copies of the toxicity report. Chronic testing of fathead minnows (*Pimephales promelas*) and daphnids (*Ceriodaphnia dubia*), using KIF discharge samples collected August 3-8, showed no toxicity to test organisms. IC<sub>25</sub> values for both species were > 100 percent effluent. The results for both species exposed to intake samples were not significantly different from control results.

Please call me at (256) 386-2755 if you have comments or questions following your review.

Cynthia L. Russell  
Biologist  
Environmental Engineering Services-West  
CTR 2L-M

CLR Attachment  
cc (Attachments)  
C. W. McCowan , KFP 1A-KST  
L. P. Johnson, LP 5D-C  
R. M. Sherrard, PSC 1X-C  
Files, RSO&E-EDMS-Muscle Shoals

KIF August 2008M

**TENNESSEE VALLEY AUTHORITY  
TOXICITY TEST REPORT**

**INTRODUCTION / EXECUTIVE SUMMARY**

Report Date: August 25, 2008

1. Facility / Discharger: Kingston Fossil Plant / TVA
2. County / State: Roane / Tennessee
3. NPDES Permit #: TN0005452
4. Type of Facility: Fossil-Fueled Steam Electric Generating Plant
5. Receiving Stream: Clinch River
6. 1Q10: 156
7. Sample Tested: 002
8. Dates Sampled: August 3-8, 2008
9. Average Flow on Days Sampled (MGD): 1351.2, 1357.1, 1357.1
10. Pertinent Site Conditions: No unusual conditions reported. (Production / operation data will be provided upon request).
11. Test Dates: August 5-12, 2008
12. Test Type: Short-term Chronic Definitive
13. Test Species: Fathead Minnows (*Pimephales promelas*)  
Daphnids (*Ceriodaphnia dubia*)
14. Concentrations Tested (%):  
*Pimephales promelas*: Outfall 002: 6.25, 12.5, 25, 50, 100  
Intake: 100  
  
*Ceriodaphnia dubia*: Outfall 002: 6.25, 12.5, 25, 50, 100  
Intake: 100

15. Permit Limit Endpoint (%): Outfall 002: IC<sub>25</sub> = 100%
16. Test Results: Outfall 002: *Pimephales promelas*: IC<sub>25</sub> > 100%  
*Ceriodaphnia dubia*: IC<sub>25</sub> > 100%
17. Facility Contact: Cynthia W. McCowan Phone #: (865) 717-2180
18. Consulting / Testing Lab: Environmental Testing Solutions, Inc.
19. Lab Contact: Jim Sumner Phone #: (828) 350-9364
20. TVA Contact: Cynthia L. Russell Phone #: (256) 386-2755
21. Notes: Outfall 002 samples collected August 3-8, 2008, showed no toxic effects to fathead minnows or daphnids. The resulting IC<sub>25</sub> values, for both species, were > 100 percent. Exposure of fathead minnows and daphnids to intake samples resulted in no significant differences for growth or reproduction from controls based on Homoscedastic t-Tests.

**METHODS SUMMARY**

**Samples:**

1. Sampling Point: Outfall 002, Intake
2. Sample Type: Outfall 002: Composite  
Intake: Composite

3. Sample Information:

ID	Date/Time* Collected (MM-DD-YY/Time <sup>‡</sup> )	Date/Time Received (MM-DD-YY/Time)	Arrival Temp. (°C)	Initial TRC <sup>†</sup> (mg/L)	Date/Time Of Last Use (MM-DD-YY/Time)
002	08-03-08 / 0900 08-04-08 / 0800	08-04-08 / 1400	1.4	< 0.10	08-05-08 / 1215 08-06-08 / 1124
Intake	08-03-08 / 0900 08-04-08 / 0800	08-04-08 / 1400	2.0	< 0.10	08-05-08 / 1215 08-06-08 / 1124
002	08-05-08 / 0900 08-06-08 / 0800	08-06-08 / 1415	1.9	< 0.10	08-07-08 / 1121 08-08-08 / 1120
Intake	08-05-08 / 0900 08-06-08 / 0800	08-06-08 / 1415	2.4	< 0.10	08-07-08 / 1121 08-08-08 / 1120
002	08-07-08 / 0900 08-08-08 / 0800	08-08-08 / 1420	0.8	< 0.10	08-09-08 / 1117 08-10-08 / 1120 08-11-08 / 1123
Intake	08-07-08 / 0900 08-08-08 / 0800	08-08-08 / 1420	0.7	< 0.10	08-09-08 / 1117 08-10-08 / 1120 08-11-08 / 1123

\* Represents the period of time during which the 24-hourly aliquots were collected.

‡ All times listed are in ET.

† Total residual chlorine.

4. Sample Manipulation: Samples from Outfall 002 and intake were warmed to test temperature (25.0 ± 1.0°C) in a warm water bath.

Test Organisms: *Pimephales promelas* *Ceriodaphnia dubia*

1. Source: Aquatox, Inc. In-house Cultures

2. Age: 19 hours old <24-hours old

Test Method Summary:

1. Test Conditions: Static, Renewal Static, Renewal

2. Test Duration: 7 days Until at least 60% of control females have 3 broods

3. Control / Dilution Water: Moderately Hard Synthetic Moderately Hard Synthetic

4. Number of Replicates: 4 10

5. Organisms per Replicate: 10 1

6. Test Initiation: (Date/Time): 08-05-08 / 1100 ET 08-05-08 / 1215 ET

7. Test Termination: (Date/Time): 08-12-08 / 1000 ET 08-12-08 / 1153 ET

8. Test Temperature: Mean = 24.6°C Mean = 24.8°C  
(24.1 - 25.0°C) (24.5 - 25.3°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 B for Bench Sheets)

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

Conducted August 5-12, 2008 using effluent from Outfall 002.

Test Solutions (% Effluent)	Percent Surviving (time interval used – days)						
	1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
6.25%	100	100	100	100	100	100	100
12.5%	100	100	100	100	100	100	100
25%	100	100	100	100	100	100	100
50%	100	100	100	98	98	98	98
100%	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	0.938	0.829	0.781	0.688	0.809
6.25%	0.908	0.854	0.828	0.731	0.830
12.5%	0.831	0.753	0.705	0.833	0.781
25%	0.824	0.702	0.713	0.668	0.727
50%	0.809	0.861	0.714	0.745	0.782
100%	0.778	0.752	0.823	0.780	0.783
Intake	0.667	0.816	0.716	0.824	0.756

IC <sub>25</sub> Value: <u>≥ 100%</u> Permit Limit: <u>100%</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>1.0 TUc</u>
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\*TU<sub>a</sub> = 100/LC<sub>50</sub>; TU<sub>c</sub> = 100/ IC<sub>25</sub>

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

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

Conducted August 5-12, 2008 using effluent from Outfall 002.

Test Solutions (% Effluent)	Percent Surviving (time interval used – days)						
	1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
6.25%	100	100	100	100	100	100	100
12.5%	100	100	100	100	100	100	100
25%	100	100	100	100	100	100	100
50%	100	100	100	100	100	100	100
100%	100	100	100	100	100	100	100

Test Solutions (% Effluent)	Reproduction (#young/female/7 days) Data (replicate number)										
	1	2	3	4	5	6	7	8	9	10	Mean
Control	27	28	29	29	30	29	30	29	31	32	29.4
6.25%	28	33	32	33	32	30	30	33	29	32	31.2
12.5%	33	33	34	35	35	36	32	33	34	33	33.8
25%	35	38	33	33	35	34	34	34	34	34	34.4
50%	37	37	38	34	39	32	29	36	35	35	35.2
100%	33	40	38	42	34	41	37	36	34	39	37.4

IC <sub>25</sub> Value: <u>&gt; 100%</u> Permit Limit: <u>100%</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>1.0 TUc</u>
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\*TU<sub>a</sub> = 100/LC<sub>50</sub>; TU<sub>c</sub> = 100/ IC<sub>25</sub>

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

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

Conducted August 5-12, 2008 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	27	29	28	30	31	30	28	30	27	32	29.2
Intake	39	40	33	36	35	34	35	37	38	36	36.3
IC <sub>25</sub> Value: <u>&gt; 100%</u> Permit Limit: <u>NA</u>						Calculated TU Estimates: <u>&lt; 1.0 TU<sub>c</sub>*</u> Permit Limit: <u>NA</u>					
95% Confidence Limits: Upper Limit: <u>NA</u> Lower Limit: <u>NA</u>											

\*TU<sub>a</sub> = 100/LC<sub>50</sub>; TU<sub>c</sub> = 100/ IC<sub>25</sub>

**REFERENCE TOXICANT TEST RESULTS** (See Appendices A and C)

Species	Date	Time	Duration	Toxicant	Results (LC <sub>50</sub> /IC <sub>25</sub> )
<i>Pimephales promelas</i>	08-05-08	1130	7 days	KCl	0.69 g/L (IC <sub>25</sub> )
<i>Ceriodaphnia dubia</i>	08-05-08	1200	7 days	NaCl	1.09 g/L (IC <sub>25</sub> )



**PHYSICAL/CHEMICAL SUMMARY**

Water Chemistry Mean Values and Ranges for *Pimephales promelas* and *Ceriodaphnia dubia* Tests of TVA / Kingston Fossil Plant - Outfall 002 performed August 5-12, 2008.

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	24.7	24.2	7.7	7.5	7.62	7.38	307	62	91	-
		24.6 - 24.8	24.1 - 24.5	7.5 - 8.0	6.6 - 7.9	7.43 - 7.87	7.16 - 7.54	290 - 324	59 - 63	86 - 94	-
	6.25%	24.7	24.4	7.8	7.5	7.51	7.37	302	-	-	-
		24.6 - 24.9	24.1 - 24.7	7.6 - 8.2	6.5 - 7.9	7.32 - 7.61	7.14 - 7.52	292 - 312	-	-	-
	12.5%	24.8	24.4	7.8	7.5	7.50	7.39	303	-	-	-
		24.7 - 24.9	24.2 - 24.6	7.6 - 8.2	6.5 - 7.9	7.32 - 7.59	7.16 - 7.55	295 - 319	-	-	-
	25%	24.8	24.4	7.8	7.5	7.50	7.42	299	-	-	-
		24.7 - 24.9	24.2 - 24.6	7.6 - 8.1	6.5 - 7.9	7.29 - 7.60	7.27 - 7.58	294 - 314	-	-	-
	50%	24.8	24.3	7.9	7.5	7.52	7.46	297	-	-	-
		24.7 - 24.9	24.1 - 24.5	7.7 - 8.1	6.7 - 7.9	7.25 - 7.62	7.32 - 7.58	293 - 312	-	-	-
100%	24.8	24.4	7.9	7.5	7.53	7.58	295	115	137	<0.10	
	24.7 - 24.9	24.3 - 24.5	7.8 - 8.1	6.8 - 7.9	7.17 - 7.63	7.43 - 7.72	288 - 304	111 - 118	131 - 146	<0.10 - <0.10	
<i>Ceriodaphnia dubia</i>	Intake	24.8	24.3	8.0	7.6	7.40	7.49	220	81	98	<0.10
		24.6 - 25.0	24.1 - 24.5	7.8 - 8.2	6.9 - 8.1	7.18 - 7.59	7.23 - 7.64	203 - 234	72 - 89	95 - 99	<0.10 - <0.10
	Control	24.7	25.0	7.7	7.9	7.62	7.50	307	62	91	-
		24.6 - 24.9	24.7 - 25.2	7.5 - 8.0	7.8 - 8.2	7.43 - 7.87	7.32 - 7.66	290 - 324	59 - 63	86 - 94	-
	6.25%	24.8	25.0	7.8	7.9	7.51	7.50	302	-	-	-
		24.7 - 24.9	24.6 - 25.2	7.6 - 8.2	7.8 - 8.1	7.32 - 7.61	7.32 - 7.65	292 - 312	-	-	-
	12.5%	24.8	24.9	7.8	7.8	7.50	7.51	303	-	-	-
		24.7 - 24.9	24.7 - 25.1	7.6 - 8.2	7.7 - 8.0	7.32 - 7.59	7.33 - 7.65	295 - 319	-	-	-
	25%	24.8	24.9	7.8	7.9	7.50	7.52	299	-	-	-
		24.7 - 24.9	24.7 - 25.3	7.6 - 8.1	7.7 - 8.0	7.29 - 7.60	7.37 - 7.65	294 - 314	-	-	-
50%	24.8	24.8	7.9	7.9	7.52	7.58	297	-	-	-	
	24.7 - 24.9	24.6 - 25.2	7.7 - 8.1	7.7 - 8.0	7.25 - 7.62	7.43 - 7.74	293 - 312	-	-	-	
100%	24.9	24.8	7.9	7.9	7.53	7.69	295	115	137	<0.10	
	24.7 - 25.0	24.6 - 25.2	7.8 - 8.1	7.8 - 8.0	7.17 - 7.63	7.54 - 7.85	288 - 304	111 - 118	131 - 146	<0.10 - <0.10	
Intake	24.8	24.9	8.0	7.8	7.40	7.58	220	81	98	<0.10	
	24.5 - 25.0	24.6 - 25.2	7.8 - 8.2	7.7 - 8.2	7.18 - 7.59	7.44 - 7.69	203 - 234	72 - 89	95 - 99	<0.10 - <0.10	

Overall temperature (°C)	Average	Minimum	Maximum
<i>Pimephales promelas</i>	24.6	24.1	25.0
<i>Ceriodaphnia dubia</i>	24.8	24.5	25.3

## **SUMMARY/CONCLUSIONS**

Outfall 002 samples collected August 3-8, 2008, showed no toxic effects to fathead minnows or daphnids. The resulting IC<sub>25</sub> values, for both species, were > 100 percent. Exposure of fathead minnows and daphnids to intake samples resulted in no significant differences for growth or reproduction from controls based on Homoscedastic t-Tests.

## Appendix A

### ADDITIONAL TOXICITY TEST INFORMATION

#### SUMMARY OF METHODS

1. *Pimephales promelas*

Tests were conducted according to EPA-821-R-02-013 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 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 C 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. TN0005452.
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, 20<sup>th</sup> Edition, 1998.
4. Quality Assurance Program: Standard Operating Procedures, Environmental Testing Solutions, Inc (most current version).

**Kingston Fossil Plant Biomonitoring  
August 5-12, 2008**

**Appendix B**

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

<b>Client:</b> TVA	<b>Delivered By (Circle One):</b>
<b>Project Name:</b> Kingston Fossil Plant	FedEx    UPS    Bus    Client
<b>P.O. Number:</b> N/A	<b>Other (specify):</b> <u>Courier Service</u>
<b>Facility Sampled:</b> KIF	<b>General Comments:</b>
<b>NPDES Number:</b> TN0005452	<b>Note:</b> Diss. Mtls. Bottles filtered & collected onsite during each sampling event @ each sample location. Diss. Metls. are then stored at TVA 4200 Greenway Dr.
<b>Collected By:</b> Larry Shelton	<b>Samples</b> are collected and shipped on ice to the contract laboratory.
	<b>All times</b> are Eastern.

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Flow (MGD)	Rain Event? (Mark as Appropriate)		Laboratory Use						
		Date	Time		Yes	If Yes, Inches	Trace	ETS Log Number	Arrival Temp. (°C)	By	Time	Appearance	
KIF - Outfall 002	Comp	08/04/08	0840 am	1351.2		<input checked="" type="radio"/>			Project 3092	14°C	J	1100	A
KIF - Intake	Comp	08/04/08	0945 am							20°C	J	1100	A
Outfall 001 Start:		08/03/08	0900 am										
Outfall 001 Finish:		08/04/08	0800 am										
Intake Start:		08/03/08	0900 am										
Intake Finish:		08/04/08	0800 am										

Sample Custody - Fill In From Top Down

<b>Relinquished By (Signature):</b>	<b>Date/Time</b>	<b>Received By (Signature):</b>	<b>Date/Time</b>
<i>Larry Shelton</i>	8/4/08 1045	<i>John Garland</i>	8/4/08 10:45
<i>John Garland</i>	8/4/08 1400	<i>John</i>	08-04-08 1400

**Instructions:** Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing 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.

**BIOMONITORING CHAIN OF CUSTODY RECORD**

Client: **TVA**  
 Project Name: **Kingston Fossil Plant**  
 P.O. Number: **N/A**  
 Facility Sampled: **KIF**  
 NPDES Number: **TN0005452**  
 Collected By: **Larry Shelton**

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   
 Other (specify): Courier Service

General Comments:  
 Note: Diss. Mtls. Bottles filtered & collected onsite during each sampling event @ each sample location. Diss. Metls. are then stored at TVA 4200 Greenway Dr.  
 Samples are collected and shipped on ice to the contract laboratory.  
 All times are Eastern.

Field Identification / Sample Description	Grab/Comp.	Collection Date/Time		Flow (MGD)	Rain Event? (Mark as Appropriate)		Laboratory Use							
		Date	Time		Yes	If Yes, Inches	Trace	ETS Log Number	Arrival Temp (°C)	By	Date	Appearance		
KIF - Outfall 002	Comp	08/06/08	0820 am	1357.1		<input checked="" type="radio"/>			3092	19°C	JL	08/06/08	1415	+
KIF - Intake	Comp	08/06/08	0955 am							24°C	JL	08/06/08	1415	+
Outfall 001 Start:		08/05/08	0900 am											
Outfall 001 Finish:		08/06/08	0800 am											
Intake Start:		08/05/08	0900 am											
Intake Finish:		08/06/08	0800 am											

Sample Custody - Fill In From Top Down

Relinquished By (Signature): *Larry Shelton* Date/Time: 8/6/08 1100 EDT  
 Received By (Signature): *Jim Jumper* Date/Time: 08-06-08 1415  
 ETS: 1415

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 4°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.



*Security Seals in place when picked up by courier.*

**BIOMONITORING CHAIN OF CUSTODY RECORD**

Client: TVA  
 Project Name: Kingston Fossil Plant  
 P.O. Number: N/A  
 Facility Sampled: KIF  
 NPDES Number: TN0005452  
 Collected By: Larry Shelton

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  
 Other (specify): Courier Service

General Comments:  
 Note: Diss. Mtls. Bottles filtered & collected onsite during each sampling event @ each sample location. Diss. Metls. are then stored at TVA 4200 Greenway Dr.  
 Samples are collected and shipped on ice to the contract laboratory.  
 All times are Eastern.

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	No	Trace	EIS Log Number	Arrival Temp (°C)	By	Date	Apparatus		
KIF - Outfall 002	Comp	08/08/08	0835 am	(1) 5 GCT	1357.1		0.11				08080800	0836	JS	08/08/08	*
KIF - Intake	Comp	08/08/08	0945 am	(1) 2.5 GCT							08080807	0946	JS	08/08/08	*
Outfall 001 Start:		08/07/08	0900 am												
Outfall 001 Finish:		08/08/08	0800 am												
Intake Start:		08/07/08	0900 am												
Intake Finish:		08/08/08	0800 am												

Sample Custody - Fill In From Top Down

Relinquished By (Signature):	Date/Time	Received By (Signature):	Date/Time
<i>Larry Shelton</i>	8/8/08 1000	<i>Ken Farland</i>	08/08/08 1100
<i>Ken Farland</i>	08-08-08 1420	<i>Jm/jum</i>	08-08-08 1420

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 4°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.

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

Client: TVA  
 Facility: Kingston Fossil Plant  
 NPDES #: TN 0005452  
 Project #: 3A2

County: Roane  
 Treatment: Non-treated  
 Outfall: 002

Dilution preparation information:						Comments:
Dilution prep (%)	6.25	12.5	25	50	100	
Effluent volume (mL)	156.25	312.5	625	1250	2500	
Diluent volume (mL)	2343.75	2187.5	1875	1250	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism information:		Test information:	
Organism age:	19 HOURS OLD	Randomizing template:	BLUE
Date and times organisms were born between:	08-04-08 1600	Incubator number:	3B
Organism source:	ATOX BATCH Pp 08-04-08	Artemia lot number:	861004 W
Transfer bowl information:	pH = 7.66      Temperature = 25.3 °C	Total drying time:	24-HOURS
Average transfer volume:	0.1384 mL	Date / Time in:	08-12-08 1035
		Date / Time out:	08-13-08 1040
		Oven temperature:	60°C

**Daily feeding and renewal information:**

Day	Date	Morning feeding time	Afternoon feeding time	Test initiation, renewal, or termination time	Control water batch used	Sample numbers used	Analyst
0	08-05-08	—	1550	1100	MHSW	080804.01 + 02	dl
1	08-06-08	0800	1400	1003	07-30-08B	080804.01 + 02	dl
2	08-07-08	0800	1410	1005	08-04-08A	080806.20 + 21	dl
3	08-08-08	0800	1413	1007	08-04-08A	080806.20 + 21	dl
4	08-09-08	0750	1400	1009	08-04-08B	080808.06 + 07	dl
5	08-10-08	0745	1400	1003	08-04-08B	080808.06 + 07	dl
6	08-11-08	0730	1335	1002	08-04-08B	080808.06 + 07	dl
7	08-12-08			1000			dl

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	0%	≤ 20%	7-day LC <sub>50</sub>	> 100%
Average weight per initial larvae:	0.809		NOEC	100%
Average weight per surviving larvae:	0.809	≥ 0.25 mg/larvae	LOEC	> 100%
			ChV	> 100%
			IC <sub>25</sub>	> 100%

Species: *Pimephales promelas*


Date: 08-05-08

Client: TVA / Kingston Fossil Plant - Non-treated

**Survival and Growth Data**

Day	CONTROL				6.25%				12.5%						
	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	<sup>1L</sup> 10	10	10	<sup>1SM</sup> 10	<sup>1L</sup> 10	10	10	<sup>1SM</sup> 10	10	10	<sup>1SM</sup> 10	10			
A = Pan weight (mg) Tray color code: <u>1H blue</u> Analyst: <u>MM</u>		13.44	14.78	15.12	14.05	13.23	15.75	15.39	14.41	13.71	14.60	14.48	14.45		
B = Pan + Larvae weight (mg) Analyst: <u>BSC</u>		22.82	23.07	22.93	20.93	22.31	24.29	23.67	21.70	22.12	22.13	21.53	22.78		
Larvae weight (mg) = A - B		9.38	8.29	7.81	6.88	9.08	8.54	8.28	7.31	8.31	7.53	7.05	8.33		
Weight per initial number of larvae (mg) = C / Initial number of larvae		0.938	0.829	0.781	0.688	0.908	0.854	0.828	0.731	0.831	0.753	0.705	0.833		
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	0.809				0.830				- 2.67.		0.781		3.57.	

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.

Calculations and data reviewed: 

**Comments:**

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Species: *Pimephales promelas*

Client: TVA / Kingston Fossil Plant - Non-treated

Date: 08-05-08

**Survival and Growth Data**

Day	25%				50%				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	9 <sup>d</sup>	10	10	10	10	10												
5	10	10	10	10	10	10	9	10	10	10	10	10												
6	10	10	10	10	10	10	9	10	10	10	10	10												
7	10	10 <sup>sm</sup>	10 <sup>sm</sup>	10 <sup>sm</sup>	10	10	9	10	10	10	10	10												
A = Pan weight (mg) Tray color code: <u>14.614</u> Analyst: <u>NM</u>																								
B = Pan + Larvae weight (mg) Analyst: <u>RSC</u>																								
Larvae weight (mg) = A - B																								
Weight per initial number of larvae (mg) = C / Initial number of larvae																								
Average weight per initial number of larvae (mg)   Percent reduction from control (%)																								
<table border="0" style="width:100%; text-align:center;"> <tr> <td colspan="2">0.727</td> <td colspan="2">10.2%</td> <td colspan="2">0.762</td> <td colspan="2">3.3%</td> <td colspan="2">0.783</td> <td colspan="2">3.2%</td> </tr> </table>													0.727		10.2%		0.762		3.3%		0.783		3.2%	
0.727		10.2%		0.762		3.3%		0.783		3.2%														

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.

Calculations and data reviewed: *HL*

**Comments:**

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Species: *Pimephales promelas*

Client: TVA / Kingston Fossil Plant - Non-treated

Date: 08-05-08

*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 <sup>lg</sup>	10	10 <sup>lg</sup>	10
A = Pan weight (mg) Tray color code: <u>H-blue</u> Analyst: <u>MD</u>				
	14.61	14.41	14.37	14.28
B = Pan + Larvae weight (mg) Analyst: <u>BEL</u>				
	21.28	22.57	21.53	22.52
Larvae weight (mg) = A - B				
	6.67	8.16	7.16	8.24
Weight per initial number of larvae (mg) = C / Initial number of larvae				
	0.667	0.816	0.716	0.824
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		
		0.756		6.6%

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.

Calculations and data reviewed: SP

Comments:

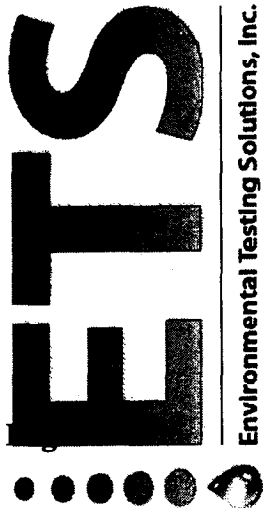
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*Pimephales promelas* Chronic Whole Effluent Toxicity Test  
EPA-821-R-02-013, Method 1000.0

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

Environmental Testing Solutions, Inc.

Project number: 3992

Reviewed by: *J. J. J.*

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 (three weigh 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 (three weigh per surviving number of larvae) (%)					
Control	A	10	10	13.44	22.82	9.38	0.938		0.938		0.809	12.9		
	B	10	10	14.78	23.07	8.29	0.829		0.829		0.809			
	C	10	10	15.12	22.93	7.81	0.781		0.781		0.809			
	D	10	10	14.05	20.93	6.88	0.688		0.688		0.809			
6.25%	E	10	10	13.23	22.31	9.08	0.908		0.908		0.830	8.9		
	F	10	10	15.75	24.29	8.54	0.854		0.854		0.830			
	G	10	10	15.39	23.67	8.28	0.828		0.828		0.830			
	H	10	10	14.41	21.72	7.31	0.731		0.731		0.830			
12.5%	I	10	10	13.71	22.02	8.31	0.831		0.831		0.781	8.0		
	J	10	10	14.60	22.13	7.53	0.753		0.753		0.781			
	K	10	10	14.48	21.53	7.05	0.705		0.705		0.781			
	L	10	10	14.45	22.78	8.33	0.833		0.833		0.781			
25%	M	10	10	14.59	22.83	8.24	0.824		0.824		0.727	9.3		
	N	10	10	14.21	21.23	7.02	0.702		0.702		0.727			
	O	10	10	13.41	20.54	7.13	0.713		0.713		0.727			
	P	10	10	14.81	21.49	6.68	0.668		0.668		0.727			
50%	Q	10	10	14.46	22.55	8.09	0.809		0.809		0.802	6.0		
	R	10	10	13.10	21.71	8.61	0.861		0.861		0.802			
	S	10	9	15.86	23.00	7.14	0.793		0.793		0.802			
	T	10	10	13.88	21.33	7.45	0.745		0.745		0.802			
100%	U	10	10	15.62	23.40	7.78	0.778		0.778		0.783	3.8		
	V	10	10	14.95	22.47	7.52	0.752		0.752		0.783			
	W	10	10	13.38	21.61	8.23	0.823		0.823		0.783			
	X	10	10	12.92	20.72	7.80	0.780		0.780		0.783			
100% Intake	Y	10	10	14.61	21.28	6.67	0.667		0.667		0.756	10.2		
	Z	10	10	14.41	22.57	8.16	0.816		0.816		0.756			
	AA	10	10	14.37	21.53	7.16	0.716		0.716		0.756			
	BB	10	10	14.28	22.52	8.24	0.824		0.824		0.756			

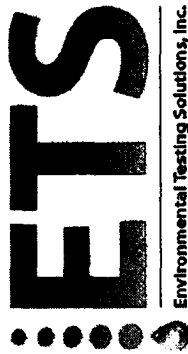
Outfall 002:  
Dunnett's MSD value: 0.1205  
PMSD: 14.9

Intake:  
Dunnett's MSD value: 0.1257  
PMSD: 15.5

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

Lower PMSD bound determined by USEPA (10th percentile) = 12%  
Upper PMSD bound determined by USEPA (90th percentile) = 30%  
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).

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



# TVA / Kingston Fossil Plant, Outfall 002

## August 05-12, 2008

### Statistical Analyses

Environmental Testing Solutions, Inc.

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

Start Date: 8/5/2008      Test ID: PpFRCR      Sample ID: TVA / Kingston Fossil Plant, Outfall 002  
 End Date: 8/12/2008      Lab ID: ETS-Envir. Testing Sol.      Sample Type: DMR-Discharge Monitoring Report  
 Sample Date:      Protocol: FWCHR-EPA-821-R-02-013      Test Species: PP-Pimephales promelas

Comments: Non-treated

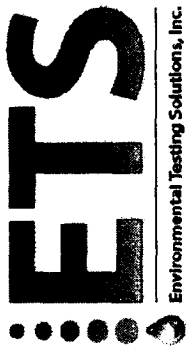
Conc-%	1	2	3	4
D-Control	0.9380	0.8290	0.7810	0.6880
6.25	0.9080	0.8540	0.8280	0.7310
12.5	0.8310	0.7530	0.7050	0.8330
25	0.8240	0.7020	0.7130	0.6680
50	0.8090	0.8610	0.7140	0.7450
100	0.7780	0.7520	0.8230	0.7800

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	I-Tailed		Mean	N-Mean
			Min	Max	CV%	Critical			MSD			
D-Control	0.8090	1.0000	0.6880	0.9380	12.859	4	-0.425	2.410	0.1205	0.8196	1.0000	
6.25	0.8303	1.0263	0.7310	0.9080	8.923	4	0.570	2.410	0.1205	0.8196	1.0000	
12.5	0.7805	0.9648	0.7050	0.8330	8.023	4	1.645	2.410	0.1205	0.7805	0.9523	
25	0.7268	0.8983	0.6680	0.8240	9.302	4	0.535	2.410	0.1205	0.7641	0.9322	
50	0.7823	0.9669	0.7140	0.8610	8.403	4	0.515	2.410	0.1205	0.7641	0.9322	
100	0.7833	0.9682	0.7520	0.8230	3.755	4			0.1205	0.7641	0.9322	

Auxiliary Tests		Statistic		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)		0.98911625	0.884	0.10828099	-0.3313661
Bartlett's Test indicates equal variances (p = 0.60)		3.62678957	15.0862722		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	MSDp	MSB
Dunnnett's Test	100	>100	1	0.12047456	0.00483747
Treatments vs D-Control				0.14891788	0.00499789
					0.46326116
					5, 18

#### Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	13.913			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



**TVA / Kingston Fossil Plant, Intake**  
**August 05-12, 2008**  
**Statistical Analyses**

Larval Fish Growth and Survival Test-7 Day Growth

Test ID: PpFRCR      Sample ID: TVA / Kingston Fossil Plant, Intake  
 Lab ID: ETS-Envir. Testing Sol.      Sample Type: DMR-Discharge Monitoring Report  
 Protocol: FWCHR-EPA-821-R-02-013      Test Species: PP-Pimephales promelas

Start Date: 8/5/2008  
 End Date: 8/12/2008  
 Sample Date:  
 Comments: Non-treated

Conc-%	1	2	3	4
D-Control	0.9380	0.8290	0.7810	0.6880
100	0.6670	0.8160	0.7160	0.8240

Conc-%	Mean	N-Mean	Transform: Untransformed				t-Stat	1-Tailed Critical	MSD	Mean	N-Mean
			Min	Max	CV%	N					
D-Control	0.8090	1.0000	0.6880	0.9380	12.859	4			0.8090	1.0000	
100	0.7558	0.9342	0.6670	0.8240	10.176	4	1.943	0.1257	0.7558	0.9342	

**Auxiliary Tests**

Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )  
 F-Test indicates equal variances ( $p = 0.63$ )  
 Hypothesis Test (1-tail, 0.05)  
 Homoscedastic t Test indicates no significant differences  
 Treatments vs D-Control

Statistic	Critical	Skew	Kurt
0.97357935	0.749	0.04262499	-0.9586298
1.82961154	47.4672279		
MSDu	MSB	MSE	F-Prob
0.12569582	0.15537184	0.00836846	0.44187054
			1, 6

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	75.962			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

\* indicates IC estimate less than the lowest concentration



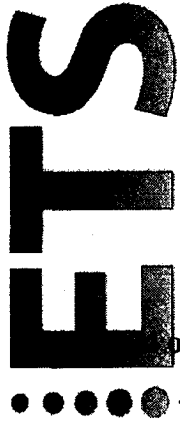
TVA / Kingston Fossil Plant, Outfall 002  
August 05-12, 2008

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

Daily Chemical Analyses

Project number: 3992

Reviewed by: *J. J. J.*



Environmental Testing Solutions, Inc.

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)	7.43	7.37	7.82	7.26	7.53	7.39	7.72	7.54	7.87	7.50	7.43	7.45	7.53	7.16
	DO (mg/L)	7.6	7.7	7.4	7.4	7.6	7.5	7.6	7.8	7.5	7.9	8.0	7.6	7.6	6.6
	Conductivity (µmhos/cm)	310	324	305	302	305	302	312	308	308	302	290			
	Alkalinity (mg/L CaCO <sub>3</sub> )	59		63		63				63					
	Hardness (mg/L CaCO <sub>3</sub> )	92		94		94				86					
	Temperature (°C)	24.7	24.3	24.6	24.2	24.7	24.1	24.7	24.1	24.8	24.2	24.6	24.2	24.2	24.8
6.25%	pH (SU)	7.48	7.36	7.43	7.27	7.32	7.39	7.59	7.52	7.61	7.50	7.57	7.44	7.55	7.14
	DO (mg/L)	7.6	7.6	7.7	7.4	7.7	7.6	7.7	7.7	8.0	7.9	8.2	7.5	7.6	6.5
	Conductivity (µmhos/cm)	300	312	302	302	302	307	307	304	304	292	292	292	300	300
	Temperature (°C)	24.7	24.6	24.8	24.2	24.8	24.5	24.7	24.2	24.8	24.1	24.7	24.5	24.5	24.9
	pH (SU)	7.46	7.36	7.45	7.29	7.32	7.39	7.56	7.55	7.59	7.51	7.57	7.45	7.54	7.16
	DO (mg/L)	7.6	7.6	7.7	7.4	7.7	7.6	7.7	7.8	8.0	7.9	8.2	7.5	7.5	7.7
12.5%	Conductivity (µmhos/cm)	300	319	302	302	302	307	307	296	296	295	295	295	300	300
	Temperature (°C)	24.7	24.5	24.7	24.5	24.8	24.3	24.8	24.2	24.8	24.3	24.7	24.6	24.6	24.3
	pH (SU)	7.47	7.38	7.46	7.29	7.29	7.39	7.56	7.58	7.60	7.53	7.58	7.50	7.55	7.27
	DO (mg/L)	7.6	7.6	7.7	7.4	7.7	7.6	7.8	7.8	8.0	7.9	8.1	7.6	7.6	6.5
	Conductivity (µmhos/cm)	294	314	298	298	298	302	302	295	295	294	294	294	298	294
	Temperature (°C)	24.8	24.5	24.7	24.4	24.8	24.5	24.8	24.4	24.8	24.2	24.7	24.3	24.3	24.6
25%	pH (SU)	7.50	7.39	7.52	7.32	7.25	7.48	7.60	7.58	7.59	7.58	7.62	7.52	7.58	7.32
	DO (mg/L)	7.7	7.7	7.7	7.5	7.8	7.6	7.9	7.7	8.0	7.9	8.1	7.6	7.8	6.7
	Conductivity (µmhos/cm)	293	312	295	295	295	300	300	293	293	293	293	293	294	294
	Temperature (°C)	24.8	24.5	24.7	24.4	24.8	24.4	24.8	24.4	24.8	24.2	24.7	24.3	24.3	24.6
	pH (SU)	7.54	7.53	7.58	7.43	7.17	7.58	7.63	7.72	7.59	7.68	7.58	7.64	7.59	7.48
	DO (mg/L)	7.8	7.6	7.9	7.5	7.9	7.7	7.9	7.7	8.0	7.9	8.1	7.6	7.8	6.8
50%	Conductivity (µmhos/cm)	292	304	294	294	294	296	296	288	288	294	294	294	294	294
	Temperature (°C)	111	118	118	118	118	116	116	116	116	116	116	116	116	116
	Alkalinity (mg/L CaCO <sub>3</sub> )	131	135	135	135	135	146	146	146	146	146	146	146	146	146
	Hardness (mg/L CaCO <sub>3</sub> )	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	Temperature (°C)	24.8	24.4	24.7	24.3	24.8	24.5	24.7	24.3	24.9	24.5	24.7	24.4	24.9	24.3
	pH (SU)	7.42	7.52	7.49	7.48	7.18	7.64	7.59	7.58	7.36	7.50	7.33	7.47	7.40	7.23
100%	DO (mg/L)	7.8	7.6	8.0	7.5	7.4	7.4	7.9	7.8	8.1	8.1	8.2	7.6	7.9	6.9
	Conductivity (µmhos/cm)	226	234	234	234	234	233	233	203	203	206	206	206	206	206
	Temperature (°C)	83	89	89	89	89	72	72	72	72	72	72	72	72	72
	Alkalinity (mg/L CaCO <sub>3</sub> )	99	99	99	99	99	95	95	95	95	95	95	95	95	95
	Hardness (mg/L CaCO <sub>3</sub> )	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	Temperature (°C)	24.8	24.4	24.7	24.5	24.9	24.5	24.8	24.2	24.7	24.1	24.6	24.4	25.0	24.3

Species: *Pimephales promelas*  
 Client: TVA / Kingston Fossil Plant - Non-treated

Date: 08-05-08

Daily Chemistry:

		Day					
		0		1		2	
Analyst		JK	Mon	Mon	JK	JK	JK
Concentration	Parameter						
CONTROL	pH (S.U.)	7.43	7.43	7.82	7.26	7.53	7.39
	DO (mg/L)	7.6	7.7	7.7	7.4	7.6	7.5
	Conductivity (µmhos/cm)	310		324		305	
	Alkalinity (mg CaCO <sub>3</sub> /L)	59				63	
	Hardness (mg CaCO <sub>3</sub> /L)	92				94	
	Temperature (°C)	24.7	24.3	24.6	24.2	24.7	24.1
6.25%	pH (S.U.)	7.48	7.36	7.43	7.27	7.32	7.39
	DO (mg/L)	7.6	7.6	7.7	7.4	7.7	7.6
	Conductivity (µmhos/cm)	300		312		302	
	Temperature (°C)	24.7	24.6	24.6	24.2	24.8	24.5
12.5%	pH (S.U.)	7.46	7.36	7.45	7.29	7.32	7.39
	DO (mg/L)	7.6	7.6	7.7	7.4	7.7	7.6
	Conductivity (µmhos/cm)	300		319		302	
	Temperature (°C)	24.7	24.5	24.7	24.5	24.8	24.3
25%	pH (S.U.)	7.47	7.38	7.46	7.29	7.29	7.39
	DO (mg/L)	7.6	7.6	7.7	7.4	7.7	7.6
	Conductivity (µmhos/cm)	294		314		298	
	Temperature (°C)	24.8	24.5	24.7	24.4	24.8	24.5
50%	pH (S.U.)	7.50	7.39	7.52	7.32	7.25	7.48
	DO (mg/L)	7.7	7.7	7.7	7.5	7.8	7.6
	Conductivity (µmhos/cm)	293		312		295	
	Temperature (°C)	24.8	24.5	24.7	24.4	24.8	24.4
100%	pH (S.U.)	7.54	7.53	7.58	7.43	7.17	7.58
	DO (mg/L)	7.8	7.6	7.9	7.5	7.9	7.7
	Conductivity (µmhos/cm)	292		304		294	
	Alkalinity (mg CaCO <sub>3</sub> /L)	111				118	
	Hardness (mg CaCO <sub>3</sub> /L)	131				135	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	24.8	24.4	24.7	24.3	24.8	24.5
	Temperature (°C)	24.8	24.4	24.7	24.3	24.8	24.5
100% Intake	pH (S.U.)	7.42	7.52	7.49	7.48	7.18	7.64
	DO (mg/L)	7.8	7.6	8.0	7.5	8.0	7.4
	Conductivity (µmhos/cm)	226		234		234	
	Alkalinity (mg CaCO <sub>3</sub> /L)	83				89	
	Hardness (mg CaCO <sub>3</sub> /L)	99				99	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	24.8	24.4	24.7	24.5	24.9	24.5
	Temperature (°C)	24.8	24.4	24.7	24.5	24.9	24.5
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*  
 Client: TVA / Kingston Fossil Plant - Non-treated

Date: 08-05-08

		Day							
		3		4		5		6	
Analyst		A	UAB	UAB	A/BSC	A/BSC	A	A	KEL
Concentration	Parameter								
CONTROL	pH (S.U.)	7.72	7.54	7.87	7.50	7.43	7.45	7.53	7.16
	DO (mg/L)	7.6	7.8	7.5	7.9	8.0	7.6	7.6	6.6
	Conductivity (µmhos/cm)	312		308		302		290	
	Alkalinity (mg CaCO <sub>3</sub> /L)			63					
	Hardness (mg CaCO <sub>3</sub> /L)			86					
	Temperature (°C)	24.7	24.1	24.8	24.2	24.6	24.2	24.8	24.5
6.25%	pH (S.U.)	7.59	7.52	7.61	7.50	7.57	7.44	7.55	7.14
	DO (mg/L)	7.7	7.7	8.0	7.9	8.2	7.5	7.6	6.5
	Conductivity (µmhos/cm)	307		304		292		300	
	Temperature (°C)	24.7	24.2	24.8	24.1	24.7	24.5	24.9	24.7
12.5%	pH (S.U.)	7.56	7.55	7.59	7.51	7.57	7.45	7.54	7.16
	DO (mg/L)	7.7	7.8	8.0	7.9	8.2	7.5	7.7	6.5
	Conductivity (µmhos/cm)	307		296		295		300	
	Temperature (°C)	24.8	24.2	24.8	24.3	24.7	24.6	24.9	24.3
25%	pH (S.U.)	7.56	7.58	7.60	7.53	7.58	7.50	7.55	7.27
	DO (mg/L)	7.8	7.8	8.0	7.9	8.1	7.6	7.8	6.5
	Conductivity (µmhos/cm)	302		295		294 294 294		298	
	Temperature (°C)	24.8	24.4	24.8	24.2	24.7	24.3	24.9	24.6
50%	pH (S.U.)	7.60	7.58	7.59	7.58	7.62	7.52	7.58	7.32
	DO (mg/L)	7.9	7.7	8.0	7.9	8.1	7.6	7.8	6.7
	Conductivity (µmhos/cm)	300		293		293		294	
	Temperature (°C)	24.8	24.1	24.9	24.2	24.7	24.5	24.9	24.3
100%	pH (S.U.)	7.63	7.72	7.59	7.68	7.58	7.64	7.59	7.48
	DO (mg/L)	7.9	7.7	8.0	7.9	8.1	7.6	7.8	6.8
	Conductivity (µmhos/cm)	296		288		294		294	
	Alkalinity (mg CaCO <sub>3</sub> /L)			116					
	Hardness (mg CaCO <sub>3</sub> /L)			146					
	TR chlorine (mg/L)			<0.10					
	Temperature (°C)	24.7	24.3	24.9	24.5	24.7	24.4	24.9	24.3
100% Intake	pH (S.U.)	7.59	7.58	7.36	7.50	7.33	7.47	7.40	7.23
	DO (mg/L)	7.9	7.8	8.1	8.1	8.2	7.6	7.9	6.9
	Conductivity (µmhos/cm)	233		203		206		206	
	Alkalinity (mg CaCO <sub>3</sub> /L)			72					
	Hardness (mg CaCO <sub>3</sub> /L)			95					
	TR chlorine (mg/L)			<0.10					
	Temperature (°C)	24.8	24.2	24.7	24.1	24.6	24.4	25.0	24.3
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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

Client: TVA  
 Facility: Kingston Fossil Plant  
 NPDES #: TN 0005452  
 Project #: 399Z

County: Roane  
 Treatment: Non-treated  
 Outfall: 002

Dilution preparation information:						Comments:
Dilution prep (%)	6.25	12.5	25	50	100	
Effluent volume (mL)	156.25	312.5	625	1250	2500	
Diluent volume (mL)	2343.75	2187.5	1875	1250	0	
Total volume (mL)	2500	2500	2500	2500	2500	

Test organism source information:											Test information:	
Organism age:	< 24-hours old										Randomizing template color:	GREEN
Date and times organisms were born between:	08-05-08 0600 TO 1030										Incubator number and shelf location:	282
Culture board:	07-29-08 B										YWT batch:	07-01-08
Replicate number:	1	2	3	4	5	6	7	8	9	10	Selenastrum batch:	07-25-08
Culture board cup number:	2	5	6	9	11	12	15	16	17	21		
Transfer bowl information:	pH = 7.70 SU Temperature = 24.9 °C											

**Daily renewal information:**

Day	Date	Test initiation, renewal, or termination time	Control water batch used MHSW	Sample numbers used	Analyst
0	08-05-08	1215	07-30-08 B	080804.01 + 02	dl
1	08-06-08	1124	07-30-08 B	080804.01 + 02	dl
2	08-07-08	1121	08-04-08 A	080806.20 + 21	dl
3	08-08-08	1120	08-04-08 A	080806.20 + 21	dl
4	08-09-08	1117	08-04-08 B	080808.06 + 07	dl
5	08-10-08	1120	08-04-08 B	080808.06 + 07	dl
6	08-11-08	1123	08-04-08 B	080808.06 + 07	dl
7	08-12-08	1153			dl

Control information:	1	2	Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	0%	0%	≤ 20%	7-day LC50	> 100%
% Adults having 3 <sup>rd</sup> Broods:	100%	100%	≥ 80%	NOEC	100%
% Mortality:	0%	0%	≤ 20%	LOEC	> 100%
Mean Offspring/Female:	29.4	29.2	≥ 15.0 offspring/female	ChV	> 100%
% CV:	4.9%	5.8%	< 40.0 %	IC25	> 100%

Species: *Ceriodaphnia dubia*  
Client: Kingston Fossil Plant - Non-treated

Date: 08-05-08

**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	3	3	4	3	4	3	3	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	11	0	11	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	10	10	10	10	13	0	10	0	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	15	15	16	13	15	17	15	16	16
Total young produced		27	28	29	29	30	29	30	29	31	32
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

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	29.4

**CONC: 6.25%**

*Survival and Reproduction Data*

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	3	5	3	4	4	3	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	13	12	10	0	11	11	13	0	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	10	0	0	0	12	0	0	0	12	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	17	17	18	17	15	15	17	14	16
Total young produced		28	33	32	33	32	30	30	33	29	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	31.2
% Reduction from Control:	-6.1%

Species: *Ceriodaphnia dubia*

Client: Kingston Fossil Plant - Non-treated

Date: 02-05-08

CONC: 12.5%

*Survival and Reproduction Data*

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	4	3	5	3	3	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	12	13	13	0	11	13	11	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	12	10	0	0	0	12	0	0	0	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	19	18	19	17	21	18	16	19	17
Total young produced		33	33	34	35	35	36	32	33	34	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	33.8
% Reduction from Control:	-15.0%

CONC: 25%

*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	3	3	5	4	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	14	0	12	0	11	14	11	0	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	10	0	12	0	0	0	13	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	20	20	18	18	19	16	20	18	17
Total young produced		35	38	33	33	35	34	34	34	34	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	34.4
% Reduction from Control:	-17.0%

Species: *Ceriodaphnia dubia*

Client: Kingston Fossil Plant - Non-treated

Date: 08-05-08

CONC: 50%

*Survival and Reproduction Data*

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	3	4	4	3	4	4	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	14	14	13	14	12	0	12	12	14
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	12	0	0	0	0	0	10	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	20	20	19	17	21	17	15	20	18	17
Total young produced		37	37	38	34	39	32	29	36	35	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	35.2
% Reduction from Control:	-19.17%

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	4	5	4	6	4	5	4	4	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	15	13	16	0	14	15	12	0	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	11	0	0	0	13	0	0	0	11	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	20	21	20	17	22	18	20	20	21
Total young produced		33	40	38	42	34	41	37	36	34	39
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	37.4
% Reduction from Control:	-27.2%

Species: *Ceriodaphnia dubia*

Client: Kingston Fossil Plant - Non-treated

Date: 08-05-08

**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	3	3	4	3	5	3	3	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	12	0	10	0	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	11	10	10	13	12	0	11	0	10	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	16	14	14	14	15	14	17	13	17
Total young produced		27	29	28	30	31	30	28	30	27	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	29.2

**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	5	4	3	4	3	3	5	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	16	0	12	12	0	11	13	13	15
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	15	0	12	0	0	13	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	20	19	17	21	19	18	21	19	22	18
Total young produced		39	40	33	36	35	34	35	37	38	36
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

<b>Concentration:</b>	
% Mortality:	07.
Mean Offspring/Female:	36.3
% Reduction from Control:	-24.3%





TVA / Kingston Fossil Plant, Outfall 002  
 August 05-12, 2008  
 Verification of *Ceriodaphnia* Reproduction Totals

Environmental Testing Solutions, Inc.

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	3	3	4	3	4	3	3	3	4	4	33
5	0	0	0	0	0	11	0	11	0	0	22
6	10	10	10	10	13	0	10	0	12	12	87
7	14	15	15	16	13	15	17	15	16	16	152
Total	27	28	29	29	30	29	30	29	31	32	294

50%

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	4	4	3	4	4	5	4	41
5	0	14	14	13	14	12	0	12	12	14	105
6	12	0	0	0	0	0	10	0	0	0	22
7	20	20	19	17	21	17	15	20	18	17	184
Total	37	37	38	34	39	32	29	36	35	35	352

6.25%

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	3	3	5	3	4	4	3	3	3	35
5	0	13	12	10	0	11	11	13	0	13	83
6	10	0	0	0	12	0	0	12	0	0	34
7	14	17	17	18	17	15	15	17	14	16	160
Total	28	33	32	33	32	30	30	33	29	32	312

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	4	5	4	6	4	5	4	4	3	5	44
5	0	15	13	16	0	14	15	12	0	13	98
6	11	0	0	0	13	0	0	0	11	0	35
7	18	20	21	20	17	22	18	20	20	21	197
Total	33	40	38	42	34	41	37	36	34	39	374

12.5%

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	3	5	3	3	4	4	4	39
5	0	0	12	13	13	0	11	13	11	0	73
6	12	10	0	0	0	12	0	0	0	12	46
7	16	19	18	19	17	21	18	16	19	17	180
Total	33	33	34	35	35	36	32	33	34	33	338

Control-2

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	3	4	3	5	3	3	3	4	4	35
5	0	0	0	0	0	12	0	10	0	11	33
6	11	10	10	13	12	0	11	0	10	0	77
7	13	16	14	14	14	15	14	17	13	17	147
Total	27	29	28	30	31	30	28	30	27	32	292

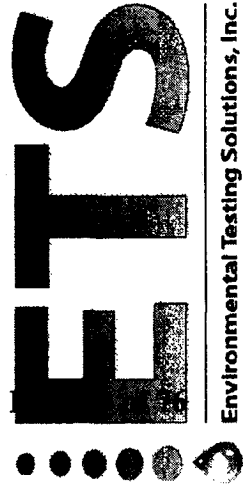
25%

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	3	3	5	4	4	3	3	4	37
5	12	14	0	12	0	11	14	11	0	13	87
6	0	0	10	0	12	0	0	0	13	0	35
7	19	20	20	18	18	19	16	20	18	17	185
Total	35	38	33	33	35	34	34	34	34	34	344

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	5	4	3	4	3	3	5	3	3	37
5	0	16	0	12	12	0	11	13	13	15	92
6	15	0	12	0	0	13	0	0	0	0	40
7	20	19	17	21	19	18	21	19	22	18	194
Total	39	40	33	36	35	34	35	37	38	36	363

TVA / Kingston Fossil Plant, Outfall 002  
August 05-12, 2008



*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

3992

Project number:

Received by: *J. J. J.*

Concentration (%)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from pooled controls (%)
	1	2	3	4	5	6	7	8	9	10				
Control - 1	27	28	29	29	30	29	30	29	31	32	100	29.4	4.9	Not applicable
6.25%	28	33	32	33	32	30	30	33	29	32	100	31.2	5.8	-6.1
12.5%	33	33	34	35	35	36	32	33	34	33	100	33.8	3.6	-15.0
25%	35	38	33	33	35	34	34	34	34	34	100	34.4	4.2	-17.0
50%	37	37	38	34	39	32	29	36	35	35	100	35.2	8.4	-19.7
100%	33	40	38	42	34	41	37	36	34	39	100	37.4	8.4	-27.2
Control - 2	27	29	28	30	31	30	28	30	27	32	100	29.2	5.8	Not applicable
100% Intake	39	40	33	36	35	34	35	37	38	36	100	36.3	6.1	-24.3

**Outfall 002:**

Dunnnett's MSD value: 2.192  
PMSD: 7.5

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

**Intake:**

Dunnnett's MSD value: 1.526  
PMSD: 5.2

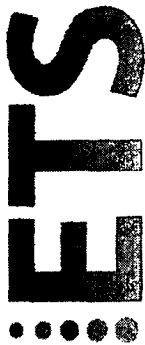
Lower PMSD bound determined by USEPA (10<sup>th</sup> percentile) = 13%.

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

Lower and upper PMSD bounds were determined from the 10<sup>th</sup> and 90<sup>th</sup> 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.



Environmental Testing Solutions, Inc.

# TVA / Kingston Fossil Plant, Outfall 002

## August 05-12, 2008

### Statistical Analyses

**Start Date:** 8/5/2008      **Test ID:** C4FRCR      **Sample ID:** TVA / Kingston Fossil Plant, Outfall 002  
**End Date:** 8/12/2008      **Lab ID:** ETS-Envir. Testing Sol.      **Sample Type:** DMR-Discharge Monitoring Report  
**Sample Date:**      **Protocol:** FWCHR-EPA-821-R-02-013      **Test Species:** CD-Ceriodaphnia dubia  
**Comments:**

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	27.000	28.000	29.000	29.000	30.000	29.000	30.000	29.000	31.000	32.000
6.25	28.000	33.000	32.000	33.000	32.000	30.000	30.000	33.000	29.000	32.000
12.5	33.000	33.000	34.000	35.000	35.000	36.000	32.000	33.000	34.000	33.000
25	35.000	38.000	33.000	33.000	35.000	34.000	34.000	34.000	34.000	34.000
50	37.000	37.000	38.000	34.000	39.000	32.000	29.000	36.000	35.000	35.000
100	33.000	40.000	38.000	42.000	34.000	41.000	37.000	36.000	34.000	39.000

Conc-%	Mean	N-Mean	Transform: Untransformed				CV%	N	t-Stat	1-Tailed		Isotonic
			Min	Max	MSD	Critical				Mean	N-Mean	
D-Control	29.400	1.0000	29.400	27.000	32.000	4.863	10	-1.878	2.287	2.192	33.567	1.0000
6.25	31.200	1.0612	31.200	28.000	33.000	5.813	10	-4.591	2.287	2.192	33.567	1.0000
12.5	33.800	1.1497	33.800	32.000	36.000	3.637	10	-5.217	2.287	2.192	33.567	1.0000
25	34.400	1.1701	34.400	33.000	38.000	4.157	10	-6.052	2.287	2.192	33.567	1.0000
50	35.200	1.1973	35.200	29.000	39.000	8.449	10	-8.347	2.287	2.192	33.567	1.0000
100	37.400	1.2721	37.400	33.000	42.000	8.380	10		2.287	2.192	33.567	1.0000

**Auxiliary Tests**

Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ( $p > 0.01$ )	0.76377356	-0.3247167	0.72868278
Bartlett's Test indicates equal variances ( $p = 0.01$ )	14.2226963		
Hypothesis Test (1-tail, 0.05)	MSDp	MSB	MSE
Dunnnett's Test	MSDu	MSDp	MSE
Treatments vs D-Control	TU	MSB	MSE
	1	82.1466667	4.59259259
		2.0E-10	5, 54

**Treatments vs D-Control**

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

Linear Interpolation (200 Resamples)



Environmental Testing Solutions, Inc.

# TVA / Kingston Fossil Plant, Intake

## August 05-12, 2008

### Statistical Analyses

#### Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 8/5/2008      Test ID: CDFRCR      Sample ID: TVA / Kingston Fossil Plant, Intake  
 End Date: 8/12/2008      Lab ID: ETS-Envir. Testing Sol.      Sample Type: DMR-Discharge Monitoring Report  
 Sample Date:      Protocol: FWCHR-EPA-821-R-02-013      Test Species: CD-Ceriodaphnia dubia  
 Comments:

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	27.000	29.000	28.000	30.000	31.000	30.000	28.000	30.000	27.000	32.000
100	39.000	40.000	33.000	36.000	35.000	34.000	35.000	37.000	38.000	36.000

Conc-%	Mean	N-Mean	Transform: Untransformed			CV%	N	t-Stat	1-Tailed		Isotonic	
			Min	Max	Mean				Critical	MSD		Mean
D-Control	29.200	1.0000	27.000	32.000	29.200	5.776	10	-8.068	1.734	1.526	32.750	1.0000
100	36.300	1.2432	33.000	40.000	36.300	6.098	10				32.750	1.0000

Auxiliary Tests		Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )		0.96861571	0.868	0.22971757	-0.7175098
F-Test indicates equal variances ( $p = 0.43$ )		1.72265625	6.54108953		
Hypothesis Test (1-tail, 0.05)		MSDu	MSB	MSE	df
Homoscedastic t Test indicates no significant differences		1.52601978	0.05226095	252.05	3.87222222
Treatments vs D-Control				2.2E-07	1, 18

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

Linear Interpolation (200 Resamples)

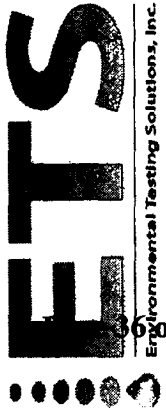
TVA / Kingston Fossil Plant, Outfall 002  
August 05-12, 2008

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

Daily Chemical Analyses

Project number: 3992

Reviewed by: *J. J. J.*



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)	7.43	7.45	7.82	7.32	7.48	7.53	7.72	7.57	7.87	7.59	7.43	7.45	7.53	7.66
	DO (mg/L)	7.6	7.8	7.7	7.8	7.8	7.6	7.9	7.9	7.5	8.2	8.0	7.9	7.6	7.9
	Conductivity (µmhos/cm)	310		324			305	312		308		302		290	
	Alkalinity (mg/L CaCO <sub>3</sub> )	59				63	63			63					
	Hardness (mg/L CaCO <sub>3</sub> )	92				94	94			86					
6.25%	Temperature (°C)	24.7	24.9	24.6	24.8	25.2	24.7	24.6	24.9	24.8	25.2	24.8	25.1	24.9	24.7
	pH (SU)	7.48	7.47	7.43	7.32	7.47	7.32	7.59	7.57	7.61	7.59	7.57	7.44	7.55	7.65
	DO (mg/L)	7.6	7.8	7.7	7.8	7.8	7.7	7.7	7.8	8.0	8.1	8.2	7.8	7.6	7.9
	Conductivity (µmhos/cm)	300		312			302	307		304		292		300	
	Temperature (°C)	24.7	24.9	24.8	24.6	25.0	24.7	24.7	24.9	24.8	25.2	24.7	25.1	24.9	25.2
12.5%	pH (SU)	7.46	7.49	7.45	7.33	7.47	7.32	7.56	7.57	7.59	7.59	7.57	7.44	7.54	7.65
	DO (mg/L)	7.6	7.8	7.7	7.7	7.9	7.7	7.7	7.8	8.0	8.0	8.2	7.8	7.7	7.9
	Conductivity (µmhos/cm)	300		319			302	307		296		295		300	
	Temperature (°C)	24.8	24.8	24.8	24.9	25.1	24.7	24.7	24.7	24.8	24.8	24.7	25.0	24.9	25.0
	pH (SU)	7.47	7.49	7.46	7.37	7.48	7.29	7.56	7.60	7.60	7.60	7.58	7.47	7.55	7.65
25%	DO (mg/L)	7.6	7.9	7.7	7.7	7.9	7.7	7.8	7.8	8.0	8.0	8.1	7.8	7.8	7.9
	Conductivity (µmhos/cm)	294		314			298	302		295		294		298	
	Temperature (°C)	24.8	24.8	24.8	24.7	25.3	24.8	24.7	24.8	24.9	24.7	24.7	25.0	24.9	24.9
	pH (SU)	7.50	7.52	7.52	7.43	7.53	7.25	7.60	7.66	7.59	7.66	7.62	7.53	7.58	7.74
	DO (mg/L)	7.7	7.9	7.7	7.7	7.9	7.8	7.9	7.9	8.0	8.0	8.1	7.8	7.8	7.9
50%	Conductivity (µmhos/cm)	293		312			295	300		293		293		294	
	Temperature (°C)	24.8	24.6	24.8	24.7	25.2	24.8	24.7	25.0	24.9	24.8	24.7	24.7	24.9	24.9
	pH (SU)	7.54	7.57	7.58	7.54	7.65	7.17	7.63	7.78	7.59	7.74	7.58	7.67	7.59	7.85
	DO (mg/L)	7.8	7.9	7.9	7.8	7.9	7.9	7.9	7.9	8.0	8.0	8.1	7.9	7.8	7.8
	Conductivity (µmhos/cm)	292		304			294	296		288		294		294	
100%	Temperature (°C)	111					118			116					
	Alkalinity (mg/L CaCO <sub>3</sub> )	131					135			146					
	Hardness (mg/L CaCO <sub>3</sub> )	<0.10					<0.10			<0.10					
	Temperature (°C)	24.9	24.9	24.9	24.7	25.2	24.8	24.7	24.7	24.9	24.6	24.7	24.9	24.9	24.7
	pH (SU)	7.42	7.55	7.49	7.53	7.69	7.18	7.59	7.67	7.36	7.55	7.33	7.44	7.40	7.64
100% Intake	DO (mg/L)	7.8	7.8	8.0	7.7	7.7	8.0	7.9	7.7	8.1	8.2	8.2	8.0	7.9	7.8
	Conductivity (µmhos/cm)	226		234			234	233		203		206		206	
	Alkalinity (mg/L CaCO <sub>3</sub> )	83					89			72					
	Hardness (mg/L CaCO <sub>3</sub> )	99					99			95					
	Temperature (°C)	24.8	24.9	24.9	24.6	25.2	24.9	24.7	24.9	24.8	24.9	24.5	24.9	24.9	24.9

Species: *Ceriodaphnia dubia*  
Client: Kingston Fossil Plant - Non-treated

Date: 08-05-08

Daily Chemistry:

		Day					
		0		1		2	
Analyst		dl	ML	ML	dl	dl	dl
Concentration	Parameter						
CONTROL	pH (S.U.)	7.43	7.45	7.82	7.32	7.53	7.48
	DO (mg/L)	7.6	7.8	7.7	7.8	7.6	7.8
	Conductivity (µmhos/cm)	310		324		305	
	Alkalinity (mg CaCO <sub>3</sub> /L)	59				63	
	Hardness (mg CaCO <sub>3</sub> /L)	92				94	
	Temperature (°C)	24.7	24.9	24.6	24.8	24.7	25.2
6.25%	pH (S.U.)	7.48	7.47	7.43	7.32	7.32	7.47
	DO (mg/L)	7.6	7.8	7.7	7.8	7.7	7.8
	Conductivity (µmhos/cm)	300		312		302	
	Temperature (°C)	24.7	24.9	24.8	24.6	24.7	25.0
12.5%	pH (S.U.)	7.46	7.49	7.45	7.33	7.32	7.47
	DO (mg/L)	7.6	7.8	7.7	7.7	7.7	7.9
	Conductivity (µmhos/cm)	300		319		302	
	Temperature (°C)	24.8	24.8	24.8	24.9	24.7	25.1
25%	pH (S.U.)	7.47	7.49	7.46	7.37	7.29	7.48
	DO (mg/L)	7.6	7.9	7.7	7.7	7.7	7.9
	Conductivity (µmhos/cm)	294		314		298	
	Temperature (°C)	24.8	24.8	24.8	24.7	24.8	25.3
50%	pH (S.U.)	7.50	7.52	7.52	7.43	7.25	7.53
	DO (mg/L)	7.7	7.9	7.7	7.7	7.8	7.9
	Conductivity (µmhos/cm)	293		312		295	
	Temperature (°C)	24.8	24.6	24.8	24.7	24.8	25.2
100%	pH (S.U.)	7.54	7.57	7.58	7.54	7.17	7.65
	DO (mg/L)	7.8	7.9	7.9	7.8	7.9	7.9
	Conductivity (µmhos/cm)	292		304		294	
	Alkalinity (mg CaCO <sub>3</sub> /L)	111				118	
	Hardness (mg CaCO <sub>3</sub> /L)	131				135	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	24.9	24.9	24.9	24.7	24.8	25.2
100% Intake	pH (S.U.)	7.42	7.62	7.49	7.53	7.18	7.69
	DO (mg/L)	7.8	7.8	8.0	7.7	8.0	7.7
	Conductivity (µmhos/cm)	226		224		234	
	Alkalinity (mg CaCO <sub>3</sub> /L)	83				89	
	Hardness (mg CaCO <sub>3</sub> /L)	99				99	
	TR chlorine (mg/L)	<0.10				<0.10	
	Temperature (°C)	24.8	24.9	24.9	24.6	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: *Ceriodaphnia dubia*  
Client: Kingston Fossil Plant - Non-treated

Date: 08-05-08

		Day							
		3		4		5		6	
Analyst		JL	LFB	LFB	A/BSL	A/BSL	JL	JL	KAL
Concentration	Parameter								
CONTROL	pH (S.U.)	7.72	7.57	7.87	7.59	7.43	7.45	7.53	7.66
	DO (mg/L)	7.6	7.4	7.5	8.2	8.0	7.9	7.6	7.9
	Conductivity (µmhos/cm)	312		308		302		290	
	Alkalinity (mg CaCO <sub>3</sub> /L)			63					
	Hardness (mg CaCO <sub>3</sub> /L)			86					
	Temperature (°C)	24.6	24.9	24.8	25.2	24.8	25.1	24.9	24.7
6.25%	pH (S.U.)	7.59	7.57	7.61	7.59	7.57	7.44	7.55	7.65
	DO (mg/L)	7.7	7.8	8.0	8.1	8.2	7.8	7.6	7.9
	Conductivity (µmhos/cm)	307		304		292		300	
	Temperature (°C)	24.7	24.9	24.8	25.2	24.7	25.1	24.9	25.2
12.5%	pH (S.U.)	7.56	7.57	7.59	7.59	7.57	7.44	7.54	7.65
	DO (mg/L)	7.7	7.8	8.0	8.0	8.2	7.8	7.7	7.9
	Conductivity (µmhos/cm)	307		296		295		300	
	Temperature (°C)	24.7	24.7	24.8	24.8	24.7	25.0	24.9	25.0
25%	pH (S.U.)	7.56	7.60	7.60	7.60	7.58	7.47	7.55	7.65
	DO (mg/L)	7.8	7.8	8.0	8.0	8.1	7.8	7.8	7.9
	Conductivity (µmhos/cm)	302		296		294		298	
	Temperature (°C)	24.7	24.8	24.9	24.7	24.7	25.0	24.9	24.9
50%	pH (S.U.)	7.60	7.66	7.59	7.66	7.62	7.53	7.58	7.74
	DO (mg/L)	7.9	7.9	8.0	8.0	8.1	7.8	7.8	7.9
	Conductivity (µmhos/cm)	300		293		293		294	
	Temperature (°C)	24.7	25.0	24.9	24.8	24.7	24.7	24.9	24.9
100%	pH (S.U.)	7.63	7.78	7.59	7.74	7.58	7.67	7.59	7.85
	DO (mg/L)	7.9	7.9	8.0	8.0	8.1	7.9	7.8	7.8
	Conductivity (µmhos/cm)	296		288		294		294	
	Alkalinity (mg CaCO <sub>3</sub> /L)			116					
	Hardness (mg CaCO <sub>3</sub> /L)			146					
	TR chlorine (mg/L)			<0.10					
100% Intake	Temperature (°C)	24.7	24.9	25.0	24.6	24.7	24.9	25.0	24.7
	pH (S.U.)	7.59	7.67	7.36	7.55	7.33	7.44	7.40	7.64
	DO (mg/L)	7.9	7.7	8.1	8.2	8.2	8.0	7.9	7.8
	Conductivity (µmhos/cm)	233		203		206		206	
	Alkalinity (mg CaCO <sub>3</sub> /L)			72					
	Hardness (mg CaCO <sub>3</sub> /L)			95					
	TR chlorine (mg/L)			<0.10					
Temperature (°C)	24.7	24.9	24.8	24.9	24.5	24.9	25.0	24.9	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final



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

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

Analyst: LAB  
 Date analyzed: 08-05-08

Iodide reagent: INR310  
 Acid reagent: INR300

**Calibration:**

	0.10 mg/L	1.00 mg/L
Reference standard number	INSS579	INSS579

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%)
INSS579	0.50	0.502	100.4%

**Duplicate sample precision:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\frac{(S - D)}{((S+D)/2)} \times 100$ (acceptable range = ± 10%)
080805.07	Cape Fear-Up/Int Duplicate	cloudy/particles, tan	S < 0.0190 D 0.0166	LB

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	Blank (should be = < 0.10 mg/L)		< 0.008816
080805.06	Cape Fear-out 007	slightly cloudy w/ part, pale tan	< 0.0117
080805.03	Sutton Plant	clear w/ particles, pale tan	< 0.00197
080805.02	Chattanooga	cloudy w/ particles, gold	< 0.000113
080805.04	PCS-out 007	clear w/ few particles, NO color	< 0.000740
080805.05	↓ out 100	clear w/ part., NO color	< 0.000404
080805.08	OWASA	clear, NO color	< 0.000620
080805.01	Foxwood Hills	clear, NO color	< 0.0000284
080804.02	KIF INTAKE	LAB LIGHT TAN, CLEAR	< 0.000712
080804.01	KIF OUTFALL 002	LIGHT TAN, CLEAR	< 0.000563

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%)
INSS579	0.50	0.477	95.4%

Reviewed by: JH  
 Date reviewed: 08-05-08





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

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

Analyst: KEN  
Date analyzed: 08.07.08

Iodide reagent: INR310  
Acid reagent: INR304

**Calibration:**

	0.10 mg/L	1.00 mg/L
Reference standard number	<u>INSS579</u>	<u>INSS579</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>INSS579</u>	<u>0.50</u>	<u>0.505</u>	<u>101.0%</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 = ± 10%)
<u>080807.01</u>	<u>Foxwood Hills</u>	<u>colorless</u>	<u>S 10.00326</u>	
<u>↓</u>	<u>Duplicate</u>		<u>D 10.00512</u>	<u>~</u>

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	<u>Blank (should be = &lt; 0.10 mg/L)</u>		<u>10.00059</u>
<u>080807.02</u>	<u>Chattanooga</u>	<u>yellow-brown, slightly cloudy</u>	<u>10.00307</u>
<u>080807.03</u>	<u>Washington</u>	<u>no color, clear</u>	<u>10.000134</u>
<u>080807.04</u>	<u>Washington</u>	<u>no color, clear</u>	<u>10.000134</u>
<u>080806.20</u>	<u>TVA-KIF-002</u>	<u>colorless, clear</u>	<u>10.000306</u>
<u>080806.21</u>	<u>↓ intake</u>	<u>no color, clear</u>	<u>10.000331</u>
<u>080806.19</u>	<u>Marshall WWTP</u>	<u>no color, clear</u>	<u>10.000328</u>
<u>1/2</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>INSS579</u>	<u>0.50</u>	<u>0.515</u>	<u>103.0%</u>

Reviewed by: JL  
Date reviewed: 08-07-08



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

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

Analyst LAB  
Date analyzed 08-09-08

Iodide reagent: INR2310  
Acid reagent: INR2306

**Calibration:**

	0.10 mg/L	1.00 mg/L
Reference standard number	IN55579	IN55579

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 = ± 10%)
IN55579	0.50	0.498	99.6%

**Duplicate sample precision:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)	%RPD = $\frac{(S - D)}{((S+D)/2)} \times 100$ (acceptable range = ± 10%)
080809.05	AAF McQuay	slightly cloudy w/ particles, pale tan	S < 0.000193	
↓	Duplicate		D < 0.000127	LB

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	Blank (should be = < 0.10 mg/L)		< 0.00837
080809.06	Belews Creek	clear, NO COLOR	0.00750
080809.10	Wilson WWTP	clear, pale tan	< 0.000735
080809.02	Southcary WWTP	clear, pale tan	0.000303
080809.03	Foxwood Hills	clear, pale tan	< 0.000121
080809.01	Scarlett Acres	cloudy w/ part, pale grey	0.000792
080809.17	JP Stevens	opaque w/ part, orange	0.000936
080809.16	Roseboro WWTP	clear, NO COLOR	0.00719
080809.15	Rockingham WWTP	slightly cloudy w/ part, pale yellow	< 0.00109
080809.08	Marshall SS	clear, NO COLOR	0.0445

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%)
	0.50		LB

Reviewed by JL  
Date reviewed 08-09-08



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

Matrix: Water, RL = 0.10 mg/L

Meter: Accumet Model AR25 pH/Ion Meter

Analyst LAB  
Date analyzed 08-09-08

Iodide reagent:   
Acid reagent:

**Calibration:**

	0.10 mg/L	1.00 mg/L
Reference standard number		<u>LB</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>IN55819</u>	<u>0.50</u>	<u>0.474</u>	<u>94.8%</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 = ± 10%)
<u>080809.12</u>	<u>Elementis</u>	<u>NO COLOR</u> <u>clear w/part</u>	<u>S 0.000593</u>	
<u>↓</u>	<u>Duplicate</u>		<u>D 0.000198</u>	<u>LB</u>

**Sample measurements:**

Sample number	Sample ID	Sample characteristics	Residual chlorine (mg/L)
	<u>Blank (should be = &lt; 0.10 mg/L)</u>		<u>LB</u>
<u>080809.14</u>	<u>Hercules</u>	<u>cloudy w/particles, tan</u>	<u>0.000859</u>
<u>080809.07</u>	<u>Bladenboro WWTP</u>	<u>cloudy w/particles, tan</u>	<u>0.0408</u>
<u>080809.09</u>	<u>Raleigh CC</u>	<u>clear w/particles, NO COLOR</u>	<u>0.00394</u>
<u>080808.14</u>	<u>Waynesville WWTP</u>	<u>slightly cloudy w/part, tan</u>	<u>0.000128</u>
<u>080808.03</u>	<u>Cape Fear up/INT</u>	<u>cloudy w/part, tan</u>	<u>0.0526</u>
<u>080808.02</u>	<u>Cape Fear out 007</u>	<u>slightly cloudy w/part, tan</u>	<u>0.0208</u>
<u>080808.05</u>	<u>GWASA</u>	<u>clear, NO COLOR</u>	<u>0.00144</u>
<u>080809.13</u>	<u>Lincoln CT</u>	<u>clear w/particles, NO COLOR</u>	<u>0.0141</u>
<u>080809.11</u>	<u>Craven Co. Wood</u>	<u>clear, NO COLOR</u>	<u>0.00130</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>IN55819</u>	<u>0.50</u>		<u>LB</u>

Reviewed by JL  
Date reviewed 08-09-08



## Alkalinity (SM 2320 B)

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

Analyst BSC  
Date analyzed 08-03-08

Time initiated 10:43  
Time completed 11:21

Titrate samples to  
pH = 4.50 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
10.5	INR301	INSS569	0.2	12.0	11.8	0.0212	10.6

Bk Correction 0.0 - ~~0.2~~ = 0 ml  
Laboratory control standard: 0.2

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%)
INSS535	100	100	12.0	21.2	9.2	10.6	97.5	97.5%

**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%)
0730-00A	MHS H <sub>2</sub> O	100	41.5	44.8	56	10.6	S 59	— BSC
↓	Duplicate (B)	1	26.8	32.4	56	1	D 59	

**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 535	50 <sup>K</sup> 100	100	32.4	41.9	9.5	10.6	101 <sup>N</sup> 100

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%)
59	101 - 59 = 42 <sup>N</sup>	84% (101/120)

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
0730-00B	MHS H <sub>2</sub> O	100	41.9	47.5	56	10.6	59
50533	EPS# 070044	1	0.0	2.2	2.2	1	23
50534	070045	1	2.2	4.4	2.2	1	23
50535	070046	1	4.4	4.8	0.4	1	4.2

**Alkalinity**  
**(SM 2320 B)**

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

Analyst RSC  
Date analyzed 08-10-08

Time initiated 1244  
Time completed 1445

**Titrate samples to**  
**pH = 4.50 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
4.98	JNR301	INSS 607	0.1	11.6	11.5	0.0217	10.9

Blk. Correction 0.0 → 0.1

**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 607	100	100	11.5	22.6	9.1	10.9	99.2	99.2%

**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%)
08-04-08B	MHS H <sub>2</sub> O	100	20.6	26.4	58	10.9	<sup>S</sup> 63	
J	Duplicate (B)	L	26.4	32.3	59	L	<sup>D</sup> 64	1.6%

**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 607	100	100	26.4	44.9	15.5	10.9	169 = 170

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) (mg CaCO <sub>3</sub> /L) MV = A - B	% R = MV / SV x 100 (acceptable range = 75 to 125%)
64	105 = 106	105% = 106%

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
08-07-00	MHS H <sub>2</sub> O	100	47.9	47.5	56	10.9	61
08-09-08	J		50	57	5.7		62
08-07-00	SSW H <sub>2</sub> O		57	56	2.9		32
08-04-08A	MHS H <sub>2</sub> O		20.9	20.9	58		63
08-04-08A	SAIT SW		30.9	30.9	10.0		110
08-04-08B	J		30.9	41.3	10.4		110
08-09-08	J		10.7	10.7	120		120
08-09-08	Washington		10.7	30.0	19.3		210
50631	EES* 086801		30.0	32.5	2.5		27

## Alkalinity (SM 2320 B)

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

Analyst BSC  
Date analyzed 08-10-08

Time initiated 12:44  
Time completed 1:15

Titrate samples to  
pH = 4.50 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>BSC</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%)
JN55 607	100	100	225	422	97	10.9	106	106%

**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%)
50032	EES# 080002	100	422	445	2.3	10.9	<sup>S</sup> 25	
50032	Duplicate (B)	100	0.0	2.1	2.1	1	<sup>D</sup> 23	91% (8.3%)

**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)
JN55 607	100	100	225	12.2	12.2	10.9	133 = 130

Sample alkalinity (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) (mg CaCO <sub>3</sub> /L) MV = A - B	% R = MV / SV x 100 (acceptable range = 75 to 125%)
23	110 (107)	110% (107%)

**Sample measurements:**

Sample number	Sample ID	Sample volume (ml)	Begin ml	End ml	Total ml	Multiplier	Alkalinity (mg CaCO <sub>3</sub> /L)
50033	EES# 080003	100	12.2	17.2	1.7	10.9	18
080805.01	Foxwood 1	1	12.9	22.0	8.1		88
080807.01	2	1	22.0	29.1	7.1		77
080809.03	3	1	29.1	37.3	8.2		89
080805.02	Chattanooga 1	25	37.3	40.2	8.9 (4)		390
080807.02	2	1	0.0	10.0	10.0		110 = 110
080809.04	3	1	10.0	20.0	10.0		110 = 110
080806.01	TA KIF002 1	50	20.6	25.7	5.1 (2)		110 = 111
080806.20	2	1	25.7	31.1	5.4		120 = 110

Retrieved by: km

Date reviewed: 08-11-08

**Alkalinity**  
**(SM 2320 B)**

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

Analyst BCL  
Date analyzed 08-10-08

Time initiated 12:44  
Time completed 1:14:45

Titrate samples to  
pH = 4.50 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>BCL</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>JNS5607</u>	<u>100</u>	<u>100</u>	<u>31.1</u>	<u>46.5</u>	<u>9.4</u>	<u>10.9</u>	<u>102</u>	<u>102%</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>080804.02</u>	<u>TVA KIF 002 3</u>	<u>50</u>	<u>0.0</u>	<u>5.3</u>	<u>5.3</u>	<u>(2) 10.9</u>	<u>116</u>	
<u>↓</u>	<u>Duplicate (B)</u>	<u>50</u>	<u>5.3</u>	<u>10.7</u>	<u>5.4</u>	<u>↓ ↓</u>	<u>118</u>	<u>1.7%</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>JNS5607</u>	<u>200</u>	<u>50</u>	<u>5.3</u>	<u>21.3</u>	<u>15.7</u>	<u>(2) 10.9</u>	<u>378</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>118</u>	<u>219</u>	<u>109%</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>080804.02</u>	<u>TVA KIF INT 1</u>	<u>50</u>	<u>2.7</u>	<u>24.5</u>	<u>3.8</u>	<u>(2) 10.9</u>	<u>83</u>
<u>080806.21</u>	<u>↓ 2</u>	<u>↓</u>	<u>24.5</u>	<u>28.6</u>	<u>4.1</u>	<u>↓ ↓</u>	<u>89</u>
<u>080808.07</u>	<u>↓ 3</u>	<u>↓</u>	<u>28.6</u>	<u>31.9</u>	<u>3.3</u>	<u>↓ ↓</u>	<u>72</u>
<u>KE</u>							



### Total Hardness (SM 2340 C)

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

Analyst BSC  
Date analyzed 08-03-08

Time initiated 0948  
Time completed 1035

**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
INA 308	INSS 513	0.0	10.0	10.0	0.020	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 566	40	50	10.0	12.1	2.1	20.0	42	105%

**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
07-20-08A	MHS H <sub>2</sub> O	50	12.1	16.6	4.5	20.0	<sup>S</sup> 90.8	
J	Duplicate (B)	J	16.6	21.1	4.5	J	<sup>D</sup> 90	(100%) 100% - BSC

**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 566	40	50	16.6	21.1	4.5	20.0	130

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) (mg CaCO <sub>3</sub> /L) MV = A - B	% R = MV / SV x 100 (acceptable range = 75 to 125%)
90	130 - 90 = 40	100%

**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	23.1	23.2	0.1	20.0	2.0
07-20-08B	MHS H <sub>2</sub> O	J	23.2	27.5	4.3	J	110-BSC 92
<div style="border: 1px solid black; width: 100%; height: 100%; transform: rotate(45deg); opacity: 0.5;"></div>							
BSC							

Page 48 of 76

**Total Hardness (SM 2340 C)**

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

Analyst BSC  
Date analyzed 08-10-08

Time initiated 0933  
Time completed 1117 1020

**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
JNR308	JNSS 513	0.0	9.8	9.8	0.0204	20.4

**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%)
JNSS 566	40	50	9.8	11.3	2.0	20.4	40.8 = 41	102% (102.5%)

**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
08-04-00B	MHS H <sub>2</sub> O	50	11.3	16.0	4.2	20.4	S 86	
J	Duplicate (B)	50	16.0	20.4	4.4	20.4	D 90	45%

**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)
JNSS 566	40	50	16.0	20.4	4.3	20.4	128 = 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%)
88 (90)	40	100%

**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	0.1	0.1	20.4	2.0
08-07-00	MHS H <sub>2</sub> O		0.1	4.1	4.0		82
08-09-00	J		4.1	8.6	4.5		92
08-07-00	SSW H <sub>2</sub> O		8.6	10.7	2.1		43
08-04-00A	MHS H <sub>2</sub> O		10.7	15.3	4.6		94
08085.01	Popwood 1		15.3	17.8	2.5		51
08087.01	J 2		17.8	21.7	3.9		80
08089.03	J 3		21.7	24.8	3.1		63
08019.0576	Washinton	24.8	29.9	5.1	100		
	Waynesville	29.9	45.2	15.3	310	4	

**Total Hardness (SM 2340 C)**

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

Analyst BSC  
Date analyzed 02-10-08

Time initiated 07:33 1020  
Time completed 11:15

**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
IN2308	INSS Si3	0.1	16.2	16.1	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 <del>516</del> <u>516</u>	40	50	<del>10.2</del> <u>12.2</u>	12.2	2.0	19.8	<del>400</del> <u>39.6</u>	<del>99.0</del> <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
080805.02	Chattanooga 1	25	12.2	16.8	4.6	(2) 19.8	S 182	<del>2.2</del> <u>5.4</u>
↓	Duplicate (B)	25	16.8	21.5	4.7	1	D 186	

**Matrix spike recovery: \* I accidentally dumped out sample before spiking**

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 <del>516</del> <u>516</u>	<del>40</del> <u>50</u>	50	21.5	28.5	7.0	(2) <del>19.8</del> <u>19.8</u>	<del>277</del> <u>277</u>

Sample hardness (B) (mg CaCO <sub>3</sub> /L)	Measured spike value (MV) (mg CaCO <sub>3</sub> /L)	% R = MV / SV x 100 (acceptable range = 75 to 125%)
<del>155</del> <u>190</u>	<del>135</del> <u>90</u>	<del>135</del> <u>90</u>

**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)						
080807.02	Chattanooga 2	25	28.5	33.7	5.2	(2) 19.8	210
080809.04	↓ 3	↓	33.7	37.7	4.0	↓	160
080804.01	TVA-KIF002 1	50	37.7	44.3	6.6	↓	130 = 131
080804.20	↓ 2	↓	44.3	50.9	6.6	↓	140 = 135
080808.06	↓ 3	↓	6.7	14.1	7.4	↓	150 = 146
080804.02	INT 1	↓	44.1	19.1	5.0	↓	100 = 99
080806.21	↓ 2	↓	19.1	24.1	5.0	↓	100 = 99
080801.0176	↓ 3	↓	24.1	28.1	4.0	↓	95

Note: If >15ml of titrant is used, sample must be diluted

Reviewed by 12/2/08

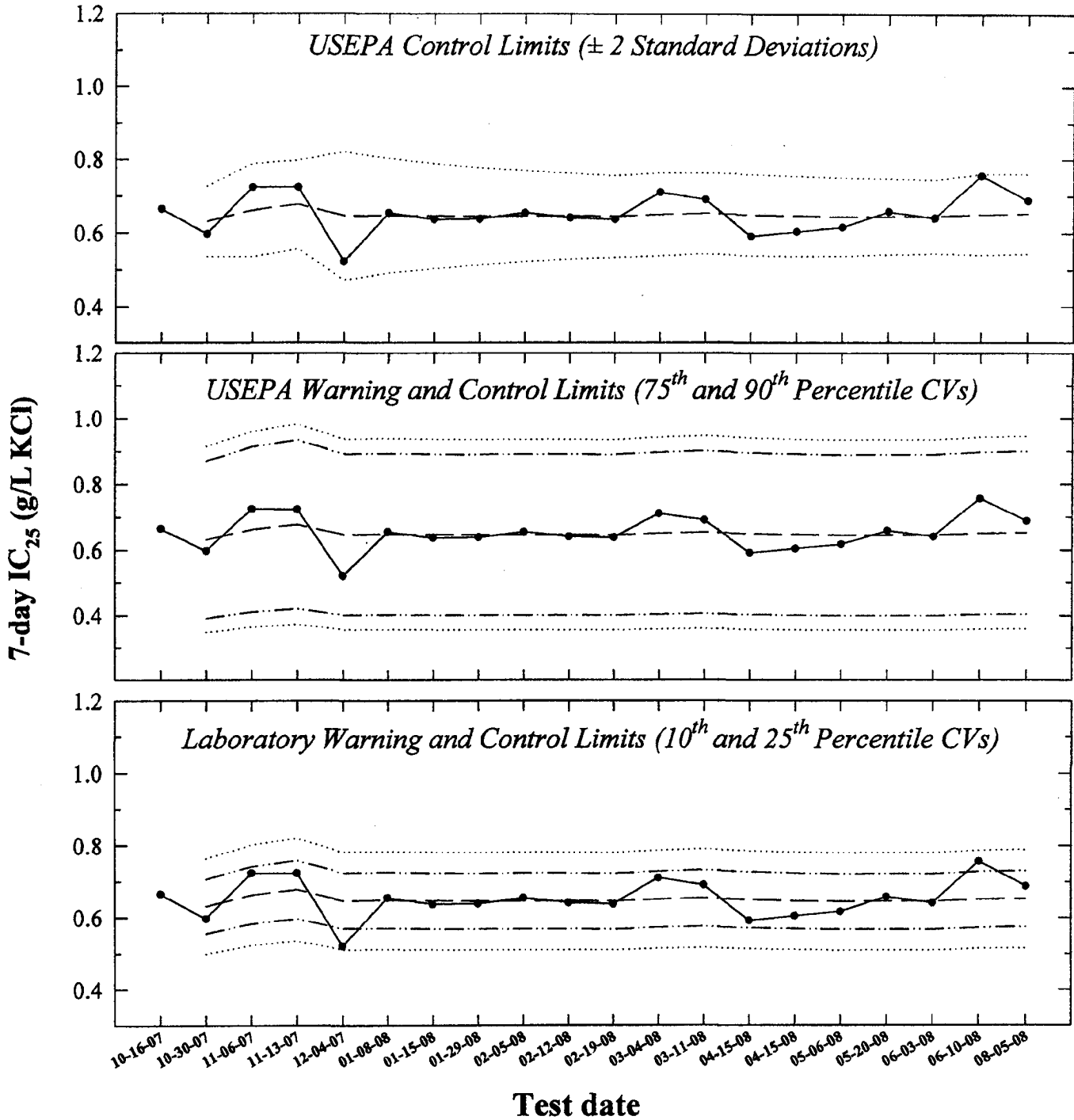
Date reviewed 02-11-08

**Kingston Fossil Plant Biomonitoring  
August 5-12, 2008**

**Appendix C**

**Reference Toxicant Test and  
Control Chart**

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



—●— 7-day IC<sub>25</sub> = 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<sub>25</sub>)

— · — · Warning Limits (mean IC<sub>25</sub> ± S<sub>A.10</sub> or S<sub>A.75</sub>)

····· Control Limits (mean IC<sub>25</sub> ± S<sub>A.25</sub>, S<sub>A.90</sub>, or 2 Standard Deviations)



Environmental Testing Solutions, Inc.

*Pimephales promelas*  
Chronic Reference Toxicant Control Chart

Test number	Test date	7-day IC <sub>25</sub> (g/L KCl)	CT (g/L KCl)	S	State and USEPA Control Limits		Laboratory Warning Limits		S <sub>A,10</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	10-16-07	0.66	0.63	0.05	0.54	0.73	0.56	0.71	0.08	0.50	0.76	0.24	0.39	0.87	0.28	0.35	0.91	0.08
2	10-30-07	0.60	0.66	0.06	0.53	0.79	0.58	0.74	0.08	0.52	0.80	0.25	0.41	0.91	0.30	0.36	0.96	0.10
3	11-06-07	0.72	0.68	0.06	0.56	0.80	0.60	0.76	0.08	0.54	0.82	0.26	0.42	0.93	0.30	0.37	0.98	0.09
4	11-13-07	0.72	0.65	0.09	0.47	0.82	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.36	0.94	0.14
5	12-04-07	0.52	0.65	0.08	0.49	0.80	0.57	0.73	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.36	0.94	0.12
6	01-08-08	0.65	0.65	0.07	0.50	0.79	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.36	0.94	0.11
7	01-15-08	0.64	0.65	0.07	0.51	0.78	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.35	0.93	0.10
8	01-29-08	0.64	0.64	0.06	0.52	0.77	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.36	0.94	0.10
9	02-05-08	0.65	0.65	0.06	0.53	0.76	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.35	0.94	0.09
10	02-12-08	0.64	0.64	0.06	0.53	0.76	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.35	0.93	0.09
11	02-19-08	0.64	0.64	0.06	0.54	0.76	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.35	0.93	0.09
12	03-04-08	0.71	0.65	0.06	0.54	0.76	0.57	0.73	0.08	0.51	0.79	0.25	0.40	0.90	0.29	0.36	0.94	0.09
13	03-11-08	0.69	0.65	0.06	0.54	0.76	0.57	0.73	0.08	0.52	0.79	0.25	0.40	0.90	0.29	0.36	0.95	0.08
14	04-15-08	0.59	0.65	0.06	0.54	0.76	0.57	0.73	0.08	0.51	0.78	0.25	0.40	0.90	0.29	0.36	0.94	0.09
15	04-15-08	0.60	0.65	0.05	0.54	0.75	0.57	0.72	0.08	0.51	0.78	0.25	0.40	0.89	0.29	0.36	0.94	0.08
16	05-06-08	0.62	0.64	0.05	0.54	0.75	0.57	0.72	0.08	0.51	0.78	0.24	0.40	0.89	0.29	0.35	0.93	0.08
17	05-20-08	0.66	0.64	0.05	0.54	0.75	0.57	0.72	0.08	0.51	0.78	0.24	0.40	0.89	0.29	0.35	0.93	0.08
18	06-03-08	0.64	0.64	0.05	0.54	0.74	0.57	0.72	0.08	0.51	0.78	0.24	0.40	0.89	0.29	0.35	0.93	0.08
19	06-10-08	0.76	0.65	0.06	0.54	0.76	0.57	0.73	0.08	0.51	0.79	0.25	0.40	0.90	0.29	0.36	0.94	0.08
20	08-05-08	0.69	0.65	0.05	0.54	0.76	0.57	0.73	0.08	0.52	0.79	0.25	0.40	0.90	0.29	0.36	0.95	0.08

Note: 7-d IC<sub>25</sub> = 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<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> = 0.12)

S<sub>A,25</sub> = Standard deviation corresponding to the 25<sup>th</sup> percentile CV. (S<sub>A,25</sub> = 0.21)

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> = 0.38)

S<sub>A,90</sub> = Standard deviation corresponding to the 90<sup>th</sup> percentile CV. (S<sub>A,90</sub> = 0.45)

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.

Organisms obtained from Aquatox, Inc.

atx08-05-08



## Precision of Endpoint Measurements

### *Pimephales promelas* Chronic Reference Toxicant Data

Test number	Test date	Control Survival (%)	Control Mean Growth (mg/larvae)	CT for Control Growth (mg/larvae)	CV (%)	CT for Control Growth CV (%)	MSD	PMSD (%)	CT for PMSD (%)
1	10-16-07	100	0.662		13.6		0.08	12.8	
2	10-30-07	100	0.771	0.716	12.0	12.8	0.16	20.1	16.5
3	11-06-07	100	0.624	0.685	6.4	10.7	0.09	14.7	15.9
4	11-13-07	100	0.603	0.665	14.2	11.6	0.10	16.8	16.1
5	12-04-07	100	0.756	0.683	7.9	10.8	0.11	14.5	15.8
6	01-08-08	100	0.821	0.706	2.7	9.5	0.03	3.4	13.7
7	01-15-08	100	0.842	0.725	10.5	9.6	0.14	17.0	14.2
8	01-29-08	97.5	0.728	0.726	5.4	9.1	0.07	9.0	13.5
9	02-05-08	100	0.818	0.736	7.2	8.9	0.07	9.1	13.0
10	02-12-08	100	0.709	0.733	2.4	8.2	0.05	6.8	12.4
11	02-19-08	100	0.833	0.742	6.1	8.0	0.13	15.5	12.7
12	03-04-08	100	0.731	0.741	6.0	7.9	0.07	9.0	12.4
13	03-11-08	97.5	0.695	0.738	5.1	7.7	0.07	10.5	12.2
14	04-15-08	100	0.999	0.756	9.0	7.8	0.11	11.1	12.2
15	04-15-08	100	0.898	0.766	9.1	7.8	0.08	9.1	11.9
16	05-06-08	100	0.857	0.772	4.4	7.6	0.16	18.2	12.3
17	05-20-08	100	0.844	0.776	19.2	8.3	0.16	18.8	12.7
18	06-03-08	100	0.918	0.784	6.1	8.2	0.09	9.4	12.5
19	06-10-08	97.5	0.724	0.781	10.9	8.3	0.11	15.7	12.7
20	08-05-08	100	0.854	0.784	15.3	8.7	0.13	15.0	12.8

**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>m</sup> percentile) = 12%.  
 Upper PMSD bound determined by USEPA (90<sup>th</sup> percentile) = 30%.  
 CT = Central Tendency (mean Control Growth, CV, or PMSD)

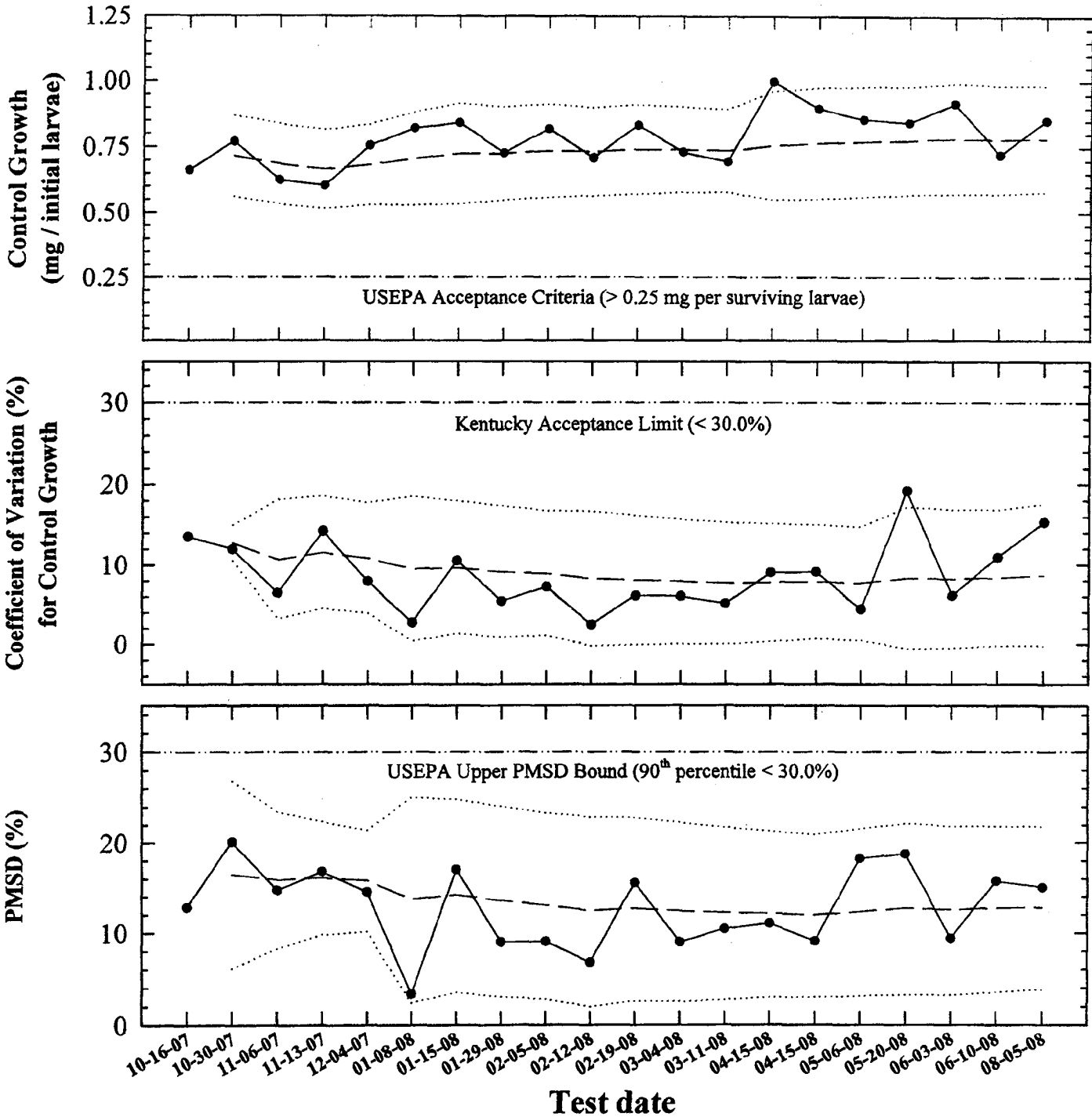
USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

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.



Environmental Testing Solutions, Inc.

*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.

— — — Reference Frequency (mean Control Growth, CV, or PMSD)

..... Control Limits (mean Control Growth, CV, or PMSD ± 2 Standard Deviations)



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

PpKCICR Test Number: 156

<i>Dilution preparation information:</i>						<i>Comments:</i>
KCl CHM number:		CHM 349				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized 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	

<i>Test organism information:</i>		<i>Test information:</i>	
Organism age:	19.5 HOURS OLD	Randomizing template:	GREEN
Date and times organisms were born between:	08-04-08 1600	Incubator number and shelf location:	3D
Organism source:	ATOX BATCH Pp 08-04-08	Artemia lot number:	86-1004W
Transfer bowl information:	pH = 7.66 SU Temperature = 25.3 °C	Total drying time:	24-HOURS
Average transfer volume:	0.1384 L	Date / Time in:	08-12-08 1035
		Date / Time out:	08-13-08 1040
		Oven temperature:	60°C

*Daily feeding and renewal information:*

Day	Date	Morning feeding time	Afternoon feeding time	Test initiation, renewal, or termination time	MHS batch used	Analyst
0	08-05-08	—	1550	1130	07-30-08B	dl
1	08-06-08	0800	1400	1032	07-30-08B	dl
2	08-07-08	0800	1410	1033	08-04-08A	dl
3	08-08-08	0800	1413	1031	08-04-08A	dl
4	08-09-08	0750	1400	1035	08-04-08B	dl
5	08-10-08	0745	1400	1040	08-04-08B	dl
6	08-11-08	0730	1335	1036	08-04-08B	dl
7	08-12-08			1033		dl

<i>Control information:</i>		<i>Acceptance criteria</i>	<i>Summary of test endpoints:</i>	
% Mortality:	0%	≤ 20%	7-day LC <sub>50</sub>	772.9
Average weight per initial larvae:	0.854		NOEC	600
Average weight per surviving larvae:	0.854	≥ 0.25 mg/larvae	LOEC	750
			ChV	670.8
			IC <sub>25</sub>	687.6

Species: *Pimephales promelas*

PpKCICR Test Number: 156

**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	9 <sup>fg</sup>	10	10	10	10	10	10	10
5	10	10	10	10	9	10	10	10	10	10	10	10
6	10	10	10	10	9	10	10	10	10	10	10	10
7	10 <sup>2SM</sup>	10	10	10 <sup>16</sup>	9 <sup>16</sup>	10 <sup>16</sup>	10	10	10	10	10 <sup>15</sup>	10
A = Pan weight (mg) Tray color code: <u>wed</u> Analyst: <u>MM</u>												
B = Pan + Larvae weight (mg) Analyst: <u>BGL</u>												
C = Larvae weight (mg) = A - B												
Weight per initial number of larvae (mg) = C / Initial number of larvae												
Average weight per initial number of larvae (mg)		0.854		0.901		-5.6%		0.839		1.8%		
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.

Calculations and data reviewed: *[Signature]*

**Comments:**

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Species: *Pimephales promelas*

PpKCICR Test Number: 156

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>	7 <sup>3d</sup>	8 <sup>2d</sup>	7 <sup>3d</sup>	3 <sup>7d</sup>	6 <sup>4d</sup>	4 <sup>6d</sup>	4 <sup>6d</sup>	3 <sup>7d</sup>	2 <sup>8d</sup>	2 <sup>6d</sup>	3 <sup>7d</sup>																										
2	8	7	8	7	3	6	4	4	3	2	1 <sup>d</sup>	2 <sup>id</sup>																										
3	8	7	8	7	3	6	4	4	3	2	1	2																										
4	7 <sup>id</sup>	6 <sup>id</sup>	8	7	2 <sup>id</sup>	4 <sup>2d</sup>	3 <sup>id</sup>	3 <sup>id</sup>	1 <sup>2d</sup>	2	0 <sup>id</sup>	0 <sup>2d</sup>																										
5	6 <sup>id</sup>	6	8	6 <sup>id</sup>	1 <sup>id</sup>	2 <sup>2d</sup>	2 <sup>id</sup>	1 <sup>2d</sup>	0 <sup>id</sup>	0 <sup>2d</sup>	0	0																										
6	6	6	8	6	1	2	2	1	0	0	0	0																										
7	6	6	7 <sup>id</sup>	6 <sup>15d</sup>	1 <sup>lg</sup>	1 <sup>id</sup>	1 <sup>id</sup>	1 <sup>lg</sup>	0	0	0	0																										
A = Pan weight (mg) Tray color code: <u>red</u> Analyst: <u>MM</u>																																						
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:12.5%;"></td> <td style="width:12.5%;">13.57</td> <td style="width:12.5%;">14.13</td> <td style="width:12.5%;">14.28</td> <td style="width:12.5%;">13.99</td> <td style="width:12.5%;">14.09</td> <td style="width:12.5%;">14.58</td> <td style="width:12.5%;">13.75</td> <td style="width:12.5%;">13.64</td> <td style="width:12.5%;">14.13</td> <td style="width:12.5%;">15.21</td> <td style="width:12.5%;">15.76</td> <td style="width:12.5%;">14.47</td> </tr> <tr> <td></td> <td>14.13</td> <td>14.11</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>														13.57	14.13	14.28	13.99	14.09	14.58	13.75	13.64	14.13	15.21	15.76	14.47		14.13	14.11										
	13.57	14.13	14.28	13.99	14.09	14.58	13.75	13.64	14.13	15.21	15.76	14.47																										
	14.13	14.11																																				
B = Pan + Larvae weight (mg) Analyst: <u>BSC</u>																																						
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:12.5%;"></td> <td style="width:12.5%;">19.51</td> <td style="width:12.5%;">19.10</td> <td style="width:12.5%;">20.58</td> <td style="width:12.5%;">18.51</td> <td style="width:12.5%;">15.08</td> <td style="width:12.5%;">15.44</td> <td style="width:12.5%;">14.47</td> <td style="width:12.5%;">14.72</td> <td colspan="4" rowspan="2" style="text-align: center; vertical-align: middle;"> </td> </tr> <tr> <td></td> <td>5.58</td> <td>4.99</td> <td>6.30</td> <td>4.52</td> <td>0.99</td> <td>0.86</td> <td>0.72</td> <td>1.08</td> </tr> </table>														19.51	19.10	20.58	18.51	15.08	15.44	14.47	14.72						5.58	4.99	6.30	4.52	0.99	0.86	0.72	1.08				
	19.51	19.10	20.58	18.51	15.08	15.44	14.47	14.72																														
	5.58	4.99	6.30	4.52	0.99	0.86	0.72	1.08																														
C = Larvae weight (mg) = A - B																																						
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:12.5%;"></td> <td style="width:12.5%;">0.538</td> <td style="width:12.5%;">0.999</td> <td style="width:12.5%;">0.630</td> <td style="width:12.5%;">0.452</td> <td style="width:12.5%;">0.099</td> <td style="width:12.5%;">0.086</td> <td style="width:12.5%;">0.072</td> <td style="width:12.5%;">0.108</td> <td style="width:12.5%;">0</td> <td style="width:12.5%;">0</td> <td style="width:12.5%;">0</td> <td style="width:12.5%;">0</td> </tr> </table>														0.538	0.999	0.630	0.452	0.099	0.086	0.072	0.108	0	0	0	0													
	0.538	0.999	0.630	0.452	0.099	0.086	0.072	0.108	0	0	0	0																										
Weight per initial number of larvae (mg) = C / Initial number of larvae																																						
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:12.5%;"></td> <td style="width:12.5%;">0.530</td> <td style="width:12.5%;">38.07</td> <td style="width:12.5%;">0.091</td> <td style="width:12.5%;">89.37</td> <td style="width:12.5%;">0</td> <td style="width:12.5%;">1007</td> <td colspan="6"></td> </tr> </table>														0.530	38.07	0.091	89.37	0	1007																			
	0.530	38.07	0.091	89.37	0	1007																																
Average weight per initial number of larvae (mg)		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.

Calculations and data reviewed: A

**Comments:**

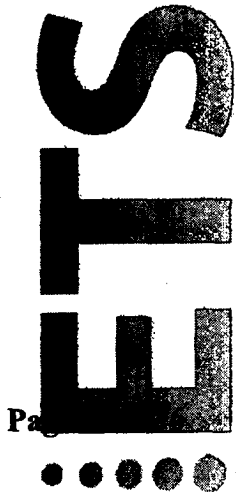
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Environmental Testing Solutions, Inc.

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

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

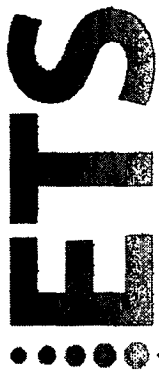
Test number: PPKCGR #156  
Test dates: August 05-12, 2008  
Reviewed by: *J. [Signature]*

Concentration (mg/L KO)	Replicate	Initial number of larvae	Final number of larvae	A = Pass weight (mg)	B = Pass + 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	14.60	21.54	6.94	0.694	0.854	15.3	0.694	100.0	0.854	15.3	Not applicable
	B	10	10	15.86	24.57	8.71	0.871			0.871				
	C	10	10	14.44	22.81	8.37	0.837			0.837				
450	D	10	10	14.57	24.70	10.13	1.013			1.013				
	E	10	9	13.90	22.77	8.87	0.986			0.887	97.5	0.901	3.9	-5.6
	F	10	10	14.91	24.44	9.53	0.953			0.953				
600	G	10	10	14.79	23.53	8.74	0.874			0.874				
	H	10	10	14.80	23.71	8.91	0.891			0.891				
	I	10	10	12.74	21.01	8.27	0.827			0.827	100.0	0.839	5.7	1.8
750	J	10	10	14.14	22.63	8.49	0.849			0.849				
	K	10	10	14.06	21.87	7.81	0.781			0.781				
	L	10	10	13.52	22.49	8.97	0.897			0.897				
900	M	10	6	14.13	19.51	5.38	0.897			0.538	62.5	0.530	14.3	38.0
	N	10	6	14.11	19.10	4.99	0.832			0.499				
	O	10	7	14.28	20.58	6.30	0.900			0.630				
1050	P	10	6	13.99	18.51	4.52	0.753			0.452				
	Q	10	1	14.09	15.08	0.99	0.990			0.099	10.0	0.091	17.2	89.3
	R	10	1	14.58	15.44	0.86	0.860			0.086				
1050	S	10	1	13.75	14.47	0.72	0.720			0.072				
	T	10	1	13.64	14.72	1.08	1.080			0.108				
	U	10	0	0.00	0.00	0.00	0.000			0.000	0.0	0.000	#DIV/0!	100.0
1050	V	10	0	0.00	0.00	0.00	0.000			0.000				
	W	10	0	0.00	0.00	0.00	0.000			0.000				
	X	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1281  
PMSD: 15.0  
MSD = Minimum Significant Difference  
PMSD = Percent Minimum Significant Difference

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

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a, USEPA, 2001b). USEPA, 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.



Environmental Testing Solutions, Inc.

# Statistical Analyses

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

Start Date: 8/5/2008 Test ID: PpKClCR REF-Ref Toxicant  
 End Date: 8/12/2008 Lab ID: ETS-Envir. Testing Sol. Sample ID: 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	0.9000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.6000	0.6000	0.7000	0.6000
900	0.1000	0.1000	0.1000	0.1000
1050	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	0.000	4		0	40	
450	0.9750	0.9750	1.3713	1.2490	5.942	4	16.00	10.00	40	
600	1.0000	1.0000	1.4120	1.4120	0.000	4	18.00	10.00	40	
*750	0.6250	0.6250	0.9123	0.8861	5.759	4	10.00	10.00	40	
*900	0.1000	0.1000	0.3218	0.3218	0.000	4	10.00	10.00	40	
1050	0.0000	0.0000	0.1588	0.1588	0.000	4			40	

### Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution ( $p \leq 0.01$ )  
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)

Steel's Many-One Rank Test

Treatments vs D-Control

NOEC LOEC ChV TU

600 750 670.820393

Statistic

0.77513826

Critical

0.868

Skew

-1.2150016

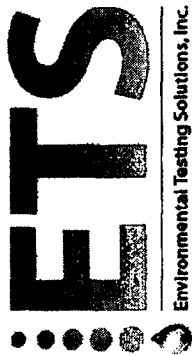
Kurt

5.36244191

Trim Level EC50 95% CL

Trim Level	EC50	95% CL
0.0%		
5.0%	772.32	743.62 802.13
10.0%	773.40	741.91 806.23
20.0%	777.93	733.16 825.44
Auto-1.3%	772.86	745.55 801.16

Trimmed Spearman-Kärber



# Statistical Analyses

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

Start Date: 8/5/2008 Test ID: PpKClCR REF-Ref Toxicant  
 End Date: 8/12/2008 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride  
 Sample Date: FWCHR-EPA-821-R-02-013 Protocol: PP-Pimephales promelas Test Species:

Comments:

Conc-mg/L	1	2	3	4
D-Control	0.6940	0.8710	0.8370	1.0130
450	0.8870	0.9530	0.8740	0.8910
600	0.8270	0.8490	0.7810	0.8970
750	0.5380	0.4990	0.6300	0.4520
900	0.0990	0.0860	0.0720	0.1080
1050	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Transform: Untransformed				1-Tailed			Isotonic		
	Mean	N-Mean	Max	CV%	t-Stat	Critical	MSD	Mean	N-Mean	
D-Control	0.8538	1.0000	0.6940	15.340	-0.808	2.180	0.1281	0.8775	1.0000	
450	0.9013	1.0556	0.8740	3.912	0.260	2.180	0.1281	0.8775	1.0000	
600	0.8385	0.9821	0.7810	5.749				0.8385	0.9556	
750	0.5298	0.6205	0.4520	14.255				0.5298	0.6037	
900	0.0913	0.1069	0.0720	17.197				0.0913	0.1040	
1050	0.0000	0.0000	0.0000	0.000				0.0000	0.0000	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92262149	0.805	0.02218568	2.50557331
Bartlett's Test indicates equal variances (p = 0.08)	4.95044184	9.2103405		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnnett's Test	600	>600		
Treatments vs D-Control	0.12810631	0.15005131	0.00428425	0.0069065
			0.55926651	2.9

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	602.37	66.80	246.83	633.13
IC10	623.68	27.61	481.66	655.87
IC15	645.00	17.06	584.71	679.82
IC20	666.32	16.08	610.64	704.72
IC25	687.63	16.22	634.67	729.38
IC40	751.11	14.52	695.32	782.62
IC50	781.13	10.77	736.87	807.17

Organisms obtained from Aquatox, Inc.

Species: *Pimephales promelas*

PpKCICR Test Number: 156

Daily Chemistry:

		Day					
		0		1		2	
Analyst		<i>dl</i>	<i>blx</i>	<i>nlx</i>	<i>dl</i>	<i>dl</i>	<i>dl</i>
Concentration	Parameter						
CONTROL	pH (S.U.)	7.43	7.68	7.82	7.57	7.53	7.43
	DO (mg/L)	7.6	7.6	7.7	7.5	7.6	7.6
	Conductivity (µmhos/cm)	310		324		305	
	Alkalinity (mg CaCO <sub>3</sub> /L)	59				63	
	Hardness (mg CaCO <sub>3</sub> /L)	92				94	
	Temperature (°C)	24.3	24.7	24.6	24.3	24.5	24.2
450 mg KCl/L	pH (S.U.)	7.57	7.68	7.83	7.51	7.63	7.53
	DO (mg/L)	7.6	7.6	7.8	7.5	7.6	7.6
	Conductivity (µmhos/cm)	1070		1110		1070	
	Temperature (°C)	24.5	24.7	24.5	24.6	24.5	24.2
600 mg KCl/L	pH (S.U.)	7.61	7.64	7.83	7.43	7.64	7.52
	DO (mg/L)	7.6	7.6	7.8	7.4	7.7	7.5
	Conductivity (µmhos/cm)	1330		1410		1320	
	Temperature (°C)	24.4	24.6	24.5	24.3	24.6	24.3
750 mg KCl/L	pH (S.U.)	7.62	7.64	7.82	7.44	7.65	7.53
	DO (mg/L)	7.7	7.6	7.7	7.4	7.7	7.5
	Conductivity (µmhos/cm)	1580		1650		1560	
	Temperature (°C)	24.4	24.6	24.5	24.4	24.5	24.1
900 mg KCl/L	pH (S.U.)	7.65	7.65	7.82	7.47	7.66	7.53
	DO (mg/L)	7.7	7.5	7.7	7.5	7.7	7.5
	Conductivity (µmhos/cm)	1850		1900		1800	
	Temperature (°C)	24.5	24.5	24.5	24.5	24.5	24.1
1050 mg KCl/L	pH (S.U.)	7.64	7.65	7.80	7.50	7.65	7.62
	DO (mg/L)	7.7	7.6	7.7	7.4	7.7	7.6
	Conductivity (µmhos/cm)	2100		2160		2070	
	Temperature (°C)	24.5	24.7	24.5	24.5	24.5	24.3
STOCK Page 62 of 76	Conductivity (µmhos/cm)	70700		—x		—x	
		Initial	Final	Initial	Final	Initial	Final

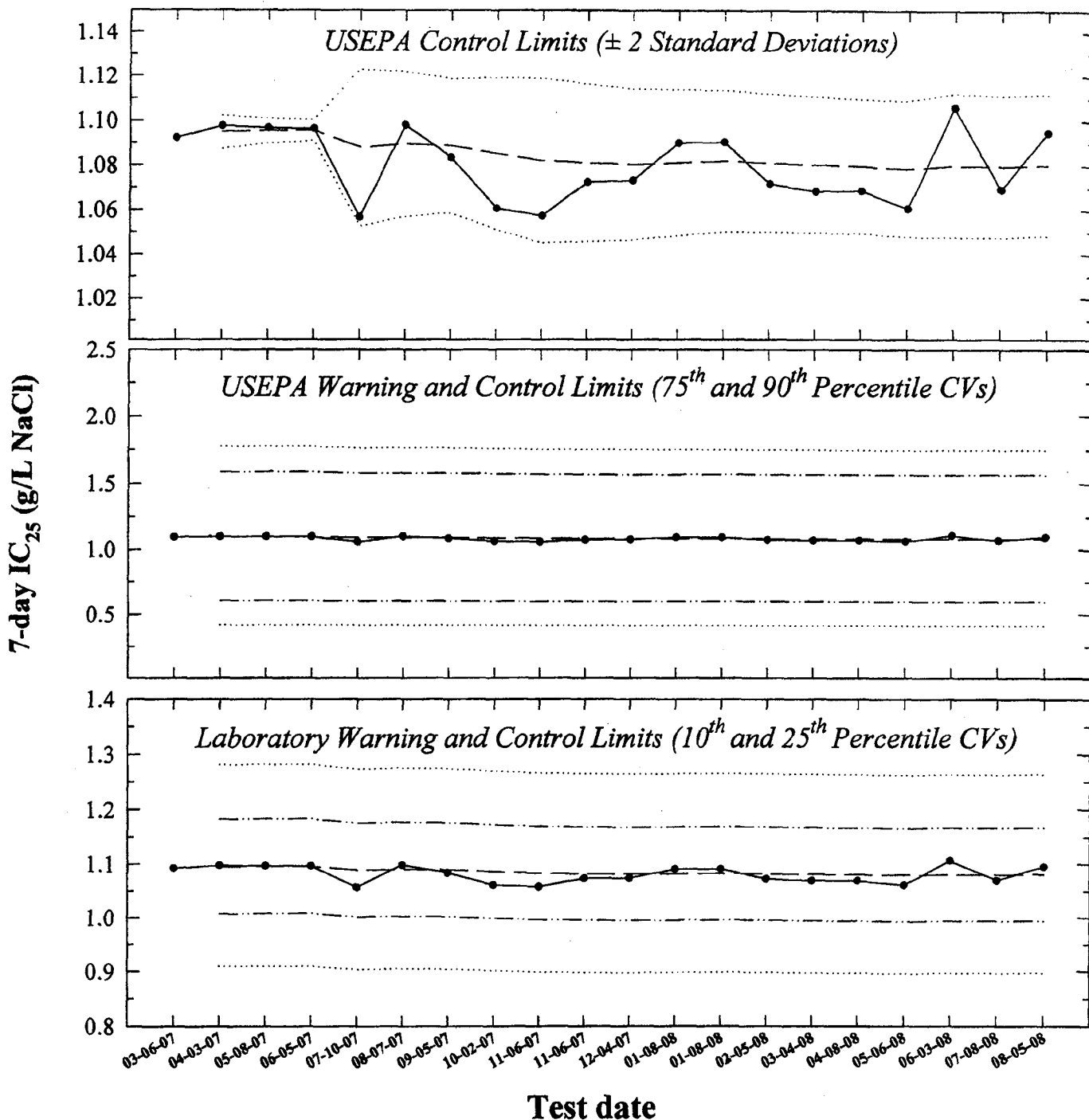
Species: *Pimephales promelas*

PpKCICR Test Number: 156

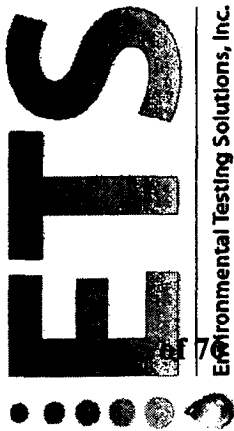
		Day							
		3		4		5		6	
Analyst		JL	UAB	UAB	JL/BS	JL/BS	JL	JL	MSL
Concentration	Parameter								
CONTROL	pH (S.U.)	7.72	7.60	7.87	7.48	7.43	7.58	7.53	7.39
	DO (mg/L)	7.6	7.7	7.5	7.7	8.0	7.4	7.6	6.5
	Conductivity (µmhos/cm)	312		308		302		290	
	Alkalinity (mg CaCO <sub>3</sub> /L)			63					
	Hardness (mg CaCO <sub>3</sub> /L)			86					
	Temperature (°C)	24.5	24.7	24.8	24.2	24.6	24.5	24.8	24.1
450 mg KCl/L	pH (S.U.)	7.80	7.60	7.90	7.55	7.61	7.61	7.68	7.36
	DO (mg/L)	7.6	7.5	7.5	7.8	7.9	7.4	7.6	6.2
	Conductivity (µmhos/cm)	1090		1060		1060		1060	
	Temperature (°C)	24.5	24.3	24.8	24.2	24.5	24.4	24.9	24.2
600 mg KCl/L	pH (S.U.)	7.81	7.64	7.90	7.60	7.65	7.61	7.70	7.39
	DO (mg/L)	7.7	7.6	7.6	7.7	8.0	7.4	7.6	6.2
	Conductivity (µmhos/cm)	1340		1300		1330		1310	
	Temperature (°C)	24.5	24.2	24.8	24.1	24.5	24.4	24.9	24.2
750 mg KCl/L	pH (S.U.)	7.82	7.64	7.90	7.60	7.65	7.61	7.71	7.39
	DO (mg/L)	7.7	7.7	7.7	7.7	8.0	7.5	7.6	6.3
	Conductivity (µmhos/cm)	1590		1560		1570		1550	
	Temperature (°C)	24.6	24.2	24.8	24.1	24.5	24.2	24.9	24.3
900 mg KCl/L	pH (S.U.)	7.82	7.63	7.90	7.60	7.68	7.61	7.72	7.47
	DO (mg/L)	7.7	7.6	7.7	7.8	8.0	7.4	7.6	6.3
	Conductivity (µmhos/cm)	1830		1800		1820		1790	
	Temperature (°C)	24.6	24.5	24.8	24.2	24.6	24.3 24.6	24.8	24.3
1050 mg KCl/L	pH (S.U.)	7.82	7.70	7.91	7.61	7.70			
	DO (mg/L)	7.7	7.8	7.9	7.7	7.9			
	Conductivity (µmhos/cm)	2100		2060		2060			
	Temperature (°C)	24.6	24.4	24.8	24.0	24.5	24.3	24.8	
STOCK	Conductivity (µmhos/cm)	—H		—K		—G		—X	
Page 63 of 76		Initial	Final	Initial	Final	Initial	Final	Initial	Final



*Ceriodaphnia dubia*  
Chronic Reference Toxicant Control Chart



- 7-day IC<sub>25</sub> = 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<sub>25</sub>)
- · — · — Warning Limits (mean IC<sub>25</sub>  $\pm$  S<sub>A.10</sub> or S<sub>A.75</sub>)
- Control Limits (mean IC<sub>25</sub>  $\pm$  S<sub>A.25</sub>, S<sub>A.90</sub>, or 2 Standard Deviations)



**Ceriodaphnia dubia**  
Chronic Reference Toxicant Control Chart

Environmental Testing Solutions, Inc.

Test number	Test date	7-day IC <sub>25</sub> (g/L NaCl)	CT (g/L NaCl)	S	State and USEPA Control Limits		Laboratory Warning Limits		S <sub>A,10</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	03-06-07	1.09	1.09	0.00	1.09	1.10	1.01	1.18	0.19	0.91	1.28	0.49	0.60	1.59	0.68	0.42	1.77	0.00
2	04-03-07	1.10	1.10	0.00	1.09	1.10	1.01	1.18	0.19	0.91	1.28	0.49	0.60	1.59	0.68	0.42	1.77	0.00
3	05-08-07	1.10	1.10	0.00	1.09	1.10	1.01	1.18	0.19	0.91	1.28	0.49	0.60	1.59	0.68	0.42	1.77	0.00
4	06-05-07	1.10	1.10	0.00	1.09	1.10	1.01	1.18	0.19	0.91	1.28	0.49	0.60	1.59	0.68	0.42	1.77	0.00
5	07-10-07	1.06	1.09	0.02	1.05	1.12	1.00	1.17	0.18	0.90	1.27	0.49	0.60	1.58	0.67	0.41	1.76	0.02
6	08-07-07	1.10	1.09	0.02	1.06	1.12	1.00	1.18	0.19	0.90	1.27	0.49	0.60	1.58	0.68	0.41	1.76	0.01
7	09-05-07	1.08	1.09	0.02	1.06	1.12	1.00	1.18	0.19	0.90	1.27	0.49	0.60	1.58	0.67	0.41	1.76	0.01
8	10-02-07	1.06	1.09	0.02	1.05	1.12	1.00	1.17	0.18	0.90	1.27	0.49	0.60	1.57	0.67	0.41	1.76	0.02
9	11-06-07	1.06	1.08	0.02	1.05	1.12	1.00	1.17	0.18	0.90	1.27	0.49	0.60	1.57	0.67	0.41	1.75	0.02
10	11-06-07	1.07	1.08	0.02	1.05	1.12	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.02
11	12-04-07	1.07	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.02
12	01-08-08	1.09	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.02
13	01-08-08	1.09	1.08	0.02	1.05	1.11	1.00	1.17	0.18	0.90	1.27	0.49	0.60	1.57	0.67	0.41	1.75	0.01
14	02-05-08	1.07	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.01
15	03-04-08	1.07	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.01
16	04-08-08	1.07	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.01
17	05-06-08	1.06	1.08	0.02	1.05	1.11	0.99	1.16	0.18	0.90	1.26	0.49	0.59	1.56	0.67	0.41	1.75	0.01
18	06-03-08	1.11	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.01
19	07-08-08	1.07	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	0.01
20	08-05-08	1.09	1.08	0.02	1.05	1.11	0.99	1.17	0.18	0.90	1.26	0.49	0.59	1.57	0.67	0.41	1.75	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)

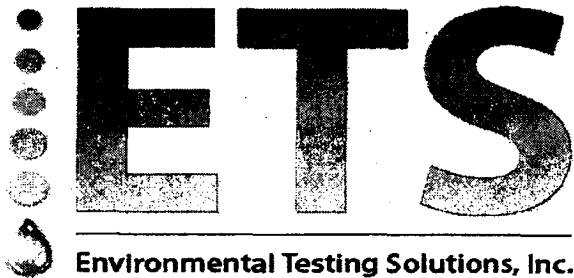
S<sub>A,25</sub> = Standard deviation corresponding to the 25<sup>th</sup> percentile CV. (S<sub>A,25</sub> = 0.17)

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.



# 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	03-06-07	100	30.1		6.0		2.6	8.7	
2	04-03-07	100	33.1	31.6	4.4	5.2	2.2	6.6	7.7
3	05-08-07	100	32.0	31.7	8.5	6.3	2.7	8.4	7.9
4	06-05-07	100	28.4	30.9	7.5	6.6	2.5	8.7	8.1
5	07-10-07	100	29.3	30.6	6.0	6.5	2.5	8.5	8.2
6	08-07-07	100	28.7	30.3	7.5	6.6	2.9	10.0	8.5
7	09-05-07	100	29.7	30.2	5.7	6.5	2.5	8.4	8.5
8	10-02-07	100	29.5	30.1	9.4	6.9	2.4	8.2	8.4
9	11-06-07	100	28.0	29.9	6.1	6.8	2.5	8.8	8.5
10	11-06-07	100	30.4	29.9	5.6	6.7	2.2	7.2	8.4
11	12-04-07	100	31.8	30.1	5.5	6.6	2.5	7.9	8.3
12	01-08-08	100	30.3	30.1	6.6	6.6	2.2	7.3	8.2
13	01-08-08	100	31.8	30.2	4.9	6.4	2.4	7.7	8.2
14	02-05-08	100	31.3	30.3	6.4	6.4	2.8	9.1	8.2
15	03-04-08	100	31.5	30.4	8.0	6.5	2.7	8.5	8.3
16	04-08-08	100	29.6	30.3	7.7	6.6	2.6	8.8	8.3
17	05-06-08	100	32.1	30.4	6.3	6.6	2.5	7.9	8.3
18	06-03-08	100	30.5	30.5	7.9	6.7	3.0	9.9	8.4
19	07-08-08	100	30.9	30.5	6.7	6.7	2.4	7.7	8.3
20	08-05-08	100	29.4	30.4	7.9	6.7	2.2	7.6	8.3

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)

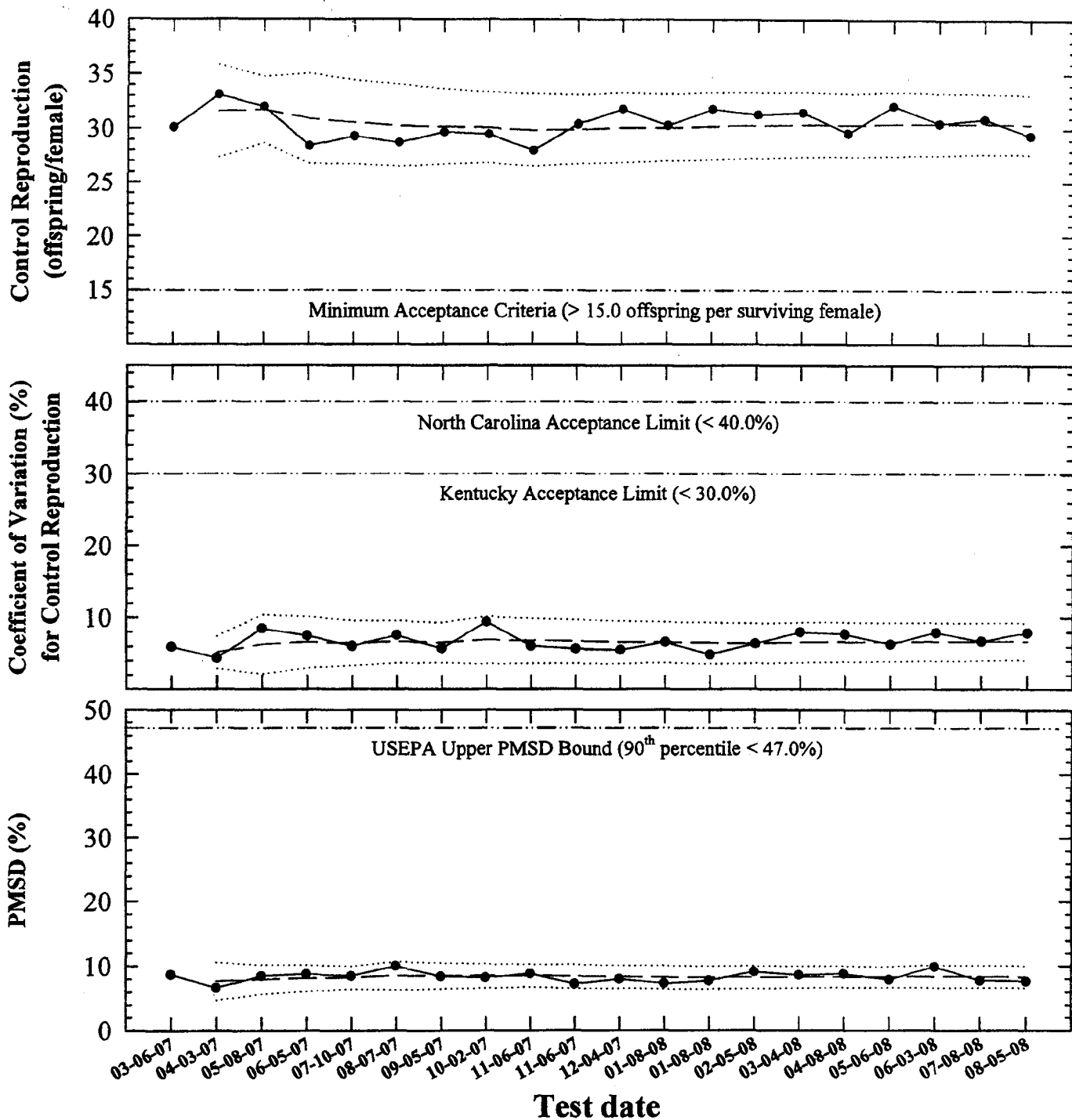
USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

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.



Environmental Testing Solutions, Inc.

# 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.
- — — Page 67 of 76 Page 71 of 76 Page 76 of 76 Page 81 of 76 Page 86 of 76 Page 91 of 76 Page 96 of 76 Page 101 of 76 Page 106 of 76 Page 111 of 76 Page 116 of 76 Page 121 of 76 Page 126 of 76 Page 131 of 76 Page 136 of 76 Page 141 of 76 Page 146 of 76 Page 151 of 76 Page 156 of 76 Page 161 of 76 Page 166 of 76 Page 171 of 76 Page 176 of 76 Page 181 of 76 Page 186 of 76 Page 191 of 76 Page 196 of 76 Page 201 of 76 Page 206 of 76 Page 211 of 76 Page 216 of 76 Page 221 of 76 Page 226 of 76 Page 231 of 76 Page 236 of 76 Page 241 of 76 Page 246 of 76 Page 251 of 76 Page 256 of 76 Page 261 of 76 Page 266 of 76 Page 271 of 76 Page 276 of 76 Page 281 of 76 Page 286 of 76 Page 291 of 76 Page 296 of 76 Page 301 of 76 Page 306 of 76 Page 311 of 76 Page 316 of 76 Page 321 of 76 Page 326 of 76 Page 331 of 76 Page 336 of 76 Page 341 of 76 Page 346 of 76 Page 351 of 76 Page 356 of 76 Page 361 of 76 Page 366 of 76 Page 371 of 76 Page 376 of 76 Page 381 of 76 Page 386 of 76 Page 391 of 76 Page 396 of 76 Page 401 of 76 Page 406 of 76 Page 411 of 76 Page 416 of 76 Page 421 of 76 Page 426 of 76 Page 431 of 76 Page 436 of 76 Page 441 of 76 Page 446 of 76 Page 451 of 76 Page 456 of 76 Page 461 of 76 Page 466 of 76 Page 471 of 76 Page 476 of 76 Page 481 of 76 Page 486 of 76 Page 491 of 76 Page 496 of 76 Page 501 of 76 Page 506 of 76 Page 511 of 76 Page 516 of 76 Page 521 of 76 Page 526 of 76 Page 531 of 76 Page 536 of 76 Page 541 of 76 Page 546 of 76 Page 551 of 76 Page 556 of 76 Page 561 of 76 Page 566 of 76 Page 571 of 76 Page 576 of 76 Page 581 of 76 Page 586 of 76 Page 591 of 76 Page 596 of 76 Page 601 of 76 Page 606 of 76 Page 611 of 76 Page 616 of 76 Page 621 of 76 Page 626 of 76 Page 631 of 76 Page 636 of 76 Page 641 of 76 Page 646 of 76 Page 651 of 76 Page 656 of 76 Page 661 of 76 Page 666 of 76 Page 671 of 76 Page 676 of 76 Page 681 of 76 Page 686 of 76 Page 691 of 76 Page 696 of 76 Page 701 of 76 Page 706 of 76 Page 711 of 76 Page 716 of 76 Page 721 of 76 Page 726 of 76 Page 731 of 76 Page 736 of 76 Page 741 of 76 Page 746 of 76 Page 751 of 76 Page 756 of 76 Page 761 of 76 Page 766 of 76 Page 771 of 76 Page 776 of 76 Page 781 of 76 Page 786 of 76 Page 791 of 76 Page 796 of 76 Page 801 of 76 Page 806 of 76 Page 811 of 76 Page 816 of 76 Page 821 of 76 Page 826 of 76 Page 831 of 76 Page 836 of 76 Page 841 of 76 Page 846 of 76 Page 851 of 76 Page 856 of 76 Page 861 of 76 Page 866 of 76 Page 871 of 76 Page 876 of 76 Page 881 of 76 Page 886 of 76 Page 891 of 76 Page 896 of 76 Page 901 of 76 Page 906 of 76 Page 911 of 76 Page 916 of 76 Page 921 of 76 Page 926 of 76 Page 931 of 76 Page 936 of 76 Page 941 of 76 Page 946 of 76 Page 951 of 76 Page 956 of 76 Page 961 of 76 Page 966 of 76 Page 971 of 76 Page 976 of 76 Page 981 of 76 Page 986 of 76 Page 991 of 76 Page 996 of 76
- — — Page 71 of 76 Page 76 of 76 Page 81 of 76 Page 86 of 76 Page 91 of 76 Page 96 of 76 Page 101 of 76 Page 106 of 76 Page 111 of 76 Page 116 of 76 Page 121 of 76 Page 126 of 76 Page 131 of 76 Page 136 of 76 Page 141 of 76 Page 146 of 76 Page 151 of 76 Page 156 of 76 Page 161 of 76 Page 166 of 76 Page 171 of 76 Page 176 of 76 Page 181 of 76 Page 186 of 76 Page 191 of 76 Page 196 of 76 Page 201 of 76 Page 206 of 76 Page 211 of 76 Page 216 of 76 Page 221 of 76 Page 226 of 76 Page 231 of 76 Page 236 of 76 Page 241 of 76 Page 246 of 76 Page 251 of 76 Page 256 of 76 Page 261 of 76 Page 266 of 76 Page 271 of 76 Page 276 of 76 Page 281 of 76 Page 286 of 76 Page 291 of 76 Page 296 of 76 Page 301 of 76 Page 306 of 76 Page 311 of 76 Page 316 of 76 Page 321 of 76 Page 326 of 76 Page 331 of 76 Page 336 of 76 Page 341 of 76 Page 346 of 76 Page 351 of 76 Page 356 of 76 Page 361 of 76 Page 366 of 76 Page 371 of 76 Page 376 of 76 Page 381 of 76 Page 386 of 76 Page 391 of 76 Page 396 of 76 Page 401 of 76 Page 406 of 76 Page 411 of 76 Page 416 of 76 Page 421 of 76 Page 426 of 76 Page 431 of 76 Page 436 of 76 Page 441 of 76 Page 446 of 76 Page 451 of 76 Page 456 of 76 Page 461 of 76 Page 466 of 76 Page 471 of 76 Page 476 of 76 Page 481 of 76 Page 486 of 76 Page 491 of 76 Page 496 of 76 Page 501 of 76 Page 506 of 76 Page 511 of 76 Page 516 of 76 Page 521 of 76 Page 526 of 76 Page 531 of 76 Page 536 of 76 Page 541 of 76 Page 546 of 76 Page 551 of 76 Page 556 of 76 Page 561 of 76 Page 566 of 76 Page 571 of 76 Page 576 of 76 Page 581 of 76 Page 586 of 76 Page 591 of 76 Page 596 of 76 Page 601 of 76 Page 606 of 76 Page 611 of 76 Page 616 of 76 Page 621 of 76 Page 626 of 76 Page 631 of 76 Page 636 of 76 Page 641 of 76 Page 646 of 76 Page 651 of 76 Page 656 of 76 Page 661 of 76 Page 666 of 76 Page 671 of 76 Page 676 of 76 Page 681 of 76 Page 686 of 76 Page 691 of 76 Page 696 of 76 Page 701 of 76 Page 706 of 76 Page 711 of 76 Page 716 of 76 Page 721 of 76 Page 726 of 76 Page 731 of 76 Page 736 of 76 Page 741 of 76 Page 746 of 76 Page 751 of 76 Page 756 of 76 Page 761 of 76 Page 766 of 76 Page 771 of 76 Page 776 of 76 Page 781 of 76 Page 786 of 76 Page 791 of 76 Page 796 of 76 Page 801 of 76 Page 806 of 76 Page 811 of 76 Page 816 of 76 Page 821 of 76 Page 826 of 76 Page 831 of 76 Page 836 of 76 Page 841 of 76 Page 846 of 76 Page 851 of 76 Page 856 of 76 Page 861 of 76 Page 866 of 76 Page 871 of 76 Page 876 of 76 Page 881 of 76 Page 886 of 76 Page 891 of 76 Page 896 of 76 Page 901 of 76 Page 906 of 76 Page 911 of 76 Page 916 of 76 Page 921 of 76 Page 926 of 76 Page 931 of 76 Page 936 of 76 Page 941 of 76 Page 946 of 76 Page 951 of 76 Page 956 of 76 Page 961 of 76 Page 966 of 76 Page 971 of 76 Page 976 of 76 Page 981 of 76 Page 986 of 76 Page 991 of 76 Page 996 of 76
- ..... Control Limits (mean Control Reproduction, CV, or PMSD ± 2 Standard Deviations)

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

CdNaCLCR #: 78

Dilution preparation information:						Comments:
NaCl CHM number:		CHM120				
Stock preparation:		100 g NaCl/l (dissolve 50 g NaCl in 500 ml deionized water)				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source information:											Test information:			
Organism age:		< 24-hours old									Randomizing template color:		blue	
Date and times organisms were born between:		08-05-08 0800 TO 1050									Incubator number and shelf location:		2B1	
Culture board:		07-29-08 A									YWT batch:		07-07-08	
Replicate number:		1	2	3	4	5	6	7	8	9	10	Selenastrum batch:		07-25-08
Culture board cup number:		1	3	5	13	16	17	18	19	21	24			
Transfer bowl information:		pH = 7.70 SU Temperature = 24.9 °C												

**Daily renewal information:**

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	MHSW batch used	Analyst
0	08-05-08	1200	07-30-08 B	dl
1	08-06-08	1105	07-30-08 B	dl
2	08-07-08	1101	08-04-08 A	dl
3	08-08-08	1100	08-04-08 A	dl
4	08-09-08	1100	08-04-08 B	dl
5	08-10-08	1103	08-04-08 B	dl
6	08-11-08	1105	08-04-08 B	dl
7	08-12-08	1109		dl

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	0%	≤ 20%	7-day LC <sub>50</sub>	> 1400
% Adults having 3 <sup>rd</sup> Broods:	100%	≥ 80%	NOEC	1000
% Mortality:	0%	≤ 20%	LOEC	1200
Mean Offspring/Female:	29.4	≥ 15.0 offspring/female	ChV	1095.4
% CV:	7.9%	< 40.0 %	IC <sub>25</sub>	1094.9

Species: *Ceriodaphnia dubia*  
CONTROL

CdNaCLCR #: 78

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	3	3	3	5	4	4	3	4	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	12	11	0	13	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	10	10	0	0	13	0	10	10	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	17	15	14	14	16	13	17	15	13
Total young produced		27	30	30	<del>24</del> <sup>28</sup>	32	33	27	30	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:	0%
Mean Offspring/Female:	29.4

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	3	5	4	4	3	3	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	12	0	0	0	0	11	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	11	11	0	12	10	10	10	0	13	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	14	17	13	16	16	14	13	15	13
Total young produced		29	28	34	29	30	29	27	27	32	28
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

<b>Concentration:</b>	
% Mortality:	0%
Mean Offspring/Female:	29.3
% Reduction from Control:	0.3%

Species: *Ceriodaphnia dubia*  
800 mg NaCl/L

CdNaCLCR #: 78

*Survival and Reproduction Data*

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	3	3	3	3	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	12	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	14	11	0	10	10	11	12	10	13	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	14	16	15	17	13	14	14	14	16
Total young produced		31	28	32	28	30	27	29	28	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	29.6
% Reduction from Control:	-0.79.

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	3	5	4	4	3	3	4	4	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	10	0	0	10	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	13	12	0	10	10	0	9	11	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	14	15	12	16	13	14	13	15	11
Total young produced		31	29	30	26	30	26	26	28	31	24
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:	28.1
% Reduction from Control:	4.47.

Species: *Ceriodaphnia dubia*  
1200 mg NaCl/L

CdNaCLCR #: 78

*Survival and Reproduction Data*

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	2	4	3	3	1	4	3	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	6	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	8	5	7	0	6	9	5	5	10	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	10	3	9	9	0	6	11	0	7
Total young produced		17	17	14	18	18	10	15	19	13	14
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:	15.4
% Reduction from Control:	47.67.

1400 mg NaCl/L

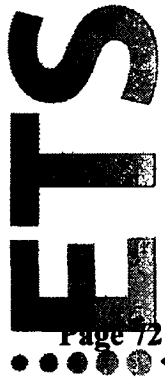
*Survival and Reproduction Data*

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	1	1	0	3	0	2	0	0	3	1
	Adult mortality	L	L	L	D	L	L	L	L	L	L
5	Young produced	0	0	0	↓	3	0	0	2	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	↓	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	3	2	2	↓	0	0	5	0	0	4
Total young produced		4	3	2	3	3	2	5	2	3	5
Final Adult Mortality		L	L	L	D	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:	107.
Mean Offspring/Female:	3.2
% Reduction from Control:	59.17.





Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

76 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	3	3	3	3	5	4	4	3	4	3	35
5	0	0	12	11	0	13	0	0	0	0	36
6	10	10	0	0	13	0	10	10	12	10	75
7	14	17	15	14	14	16	13	17	15	13	148
Total	27	30	30	28	32	33	27	30	31	26	294

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	3	5	4	4	3	3	4	4	3	37
5	0	0	10	0	0	10	0	0	0	0	20
6	13	12	0	10	10	0	9	11	12	10	87
7	14	14	15	12	16	13	14	13	15	11	137
Total	31	29	30	26	30	26	26	28	31	24	281

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	3	5	4	4	3	3	4	4	4	37
5	0	0	12	0	0	0	0	11	0	0	23
6	11	11	0	12	10	10	10	0	13	11	88
7	14	14	17	13	16	16	14	13	15	13	145
Total	29	28	34	29	30	29	27	27	32	28	293

1200 mg NaCl/L

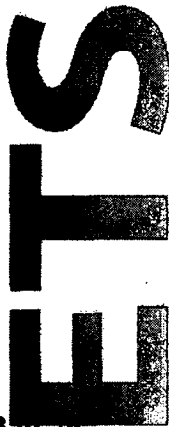
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	2	4	3	3	1	4	3	3	3	29
5	0	0	0	0	6	0	0	0	0	0	6
6	8	5	7	0	6	9	5	5	10	4	59
7	5	10	3	9	9	0	6	11	0	7	60
Total	16	17	14	18	18	10	15	19	13	14	154

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	3	3	3	3	4	4	4	35
5	0	0	12	0	0	0	0	0	0	0	12
6	14	11	0	10	10	11	12	10	13	12	103
7	13	14	16	15	17	13	14	14	14	16	146
Total	31	28	32	28	30	27	29	28	31	32	296

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	1	1	0	3	0	2	0	0	3	1	11
5	0	0	0	0	3	0	0	2	0	0	5
6	0	0	0	0	0	0	0	0	0	0	0
7	3	2	2	0	0	0	5	0	0	4	16
Total	4	3	2	3	3	2	5	2	3	5	32



Environmental Testing Solutions, Inc.

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

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

Test number: **CdNaClCR #78**  
 Test dates: **August 05-12, 2008**  
 Received by: *June*

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	27	30	30	28	32	33	27	30	31	26	100	29.4	7.9	Not applicable
600	29	28	34	29	30	29	27	27	32	28	100	29.3	7.6	0.3
800	31	28	32	28	30	27	29	28	31	32	100	29.6	6.2	-0.7
1000	31	29	30	26	30	26	26	28	31	24	100	28.1	8.8	4.4
1200	16	17	14	18	18	10	15	19	13	14	100	15.4	17.9	47.6
1400	4	3	2	3	3	2	5	2	3	5	90	3.2	35.5	89.1

Dunnett's MSD value:  $\frac{2.234}{7.6}$  MSD = Minimum Significant Difference  
 PMSD:  $\frac{7.6}{7.6}$  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 WLET 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.



Environmental Testing Solutions, Inc.

# Statistical Analyses

## Ceriodaphnia Survival and Reproduction Test - Reproduction

Start Date: 8/5/2008      Test ID: CdNaClCR      Sample ID: REF-Ref Toxicant  
 End Date: 8/12/2008      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	27.000	30.000	30.000	28.000	32.000	33.000	27.000	30.000	31.000	26.000
600	29.000	28.000	34.000	29.000	30.000	29.000	29.000	27.000	32.000	28.000
800	31.000	28.000	32.000	28.000	30.000	27.000	29.000	28.000	31.000	32.000
1000	31.000	29.000	30.000	26.000	30.000	26.000	26.000	28.000	31.000	24.000
1200	16.000	17.000	14.000	18.000	18.000	10.000	15.000	19.000	13.000	14.000
1400	4.000	3.000	2.000	3.000	3.000	2.000	5.000	2.000	3.000	5.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	CV%	t-Stat	J-Tailed		Mean	N-Mean
			Min	Max	MSD	Critical				MSD	Critical		
D-Control	29.400	1.0000	26.000	33.000	7.888	10	7.888	0.102	2.287	2.287	29.433	1.0000	
600	29.300	0.9966	27.000	34.000	7.555	10	7.555	-0.205	2.287	2.234	29.433	1.0000	
800	29.600	1.0068	27.000	32.000	6.209	10	6.209	1.330	2.287	2.234	28.100	0.9547	
1000	28.100	0.9558	24.000	31.000	8.789	10	8.789	14.327	2.287	2.234	15.400	0.5232	
*1200	15.400	0.5238	10.000	19.000	17.901	10	17.901	26.813	2.287	2.234	3.200	0.1087	
*1400	3.200	0.1088	2.000	5.000	35.478	10	35.478		2.287	2.234			

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.78750116	1.035	-0.0380093	-0.3470141
Bartlett's Test indicates equal variances (p = 0.23)	6.90396881	15.0862722		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	MSB	F-Prob
Dunnnett's Test	1000	1200	1197.04	4.77407407
Treatments vs D-Control	MSDu	MSDp	MSE	df
	2.23440775	0.07600026	4.5E-36	5, 54

Point	mg/L	SD	95% CL	Skew
IC05	1002.17848	77.7461456	779.434641	1020.05615
IC10	1025.35433	14.2887789	991.731061	1041.35951
IC15	1048.53018	10.7345501	1024.74818	1063.03383
IC20	1071.70604	10.0921623	1048.62094	1086.08067
IC25	1094.88189	9.88843252	1072.93237	1108.70826
IC40	1164.40945	11.8919414	1140.70124	1187.71569
IC50	1211.20219	12.8831181	1183.9923	1232.85271

Linear Interpolation (200 Resamples)

Species: *Ceriodaphnia dubia*

CdNaCLCR #: 78

Daily Chemistry:

		Day					
		0		1		2	
Analyst		JL	MOL	MOL	JL	JL	JL
Concentration	Parameter						
CONTROL	pH (S.U.)	7.43	7.67	7.82	7.51	7.53	7.48
	DO (mg/L)	7.6	7.8	7.7	7.7	7.6	7.6
	Conductivity (µmhos/cm)	310		324		305	
	Alkalinity (mg CaCO <sub>3</sub> /L)	59				63	
	Hardness (mg CaCO <sub>3</sub> /L)	92				94	
	Temperature (°C)	24.6	24.9	24.7	24.7	24.6	24.9
600 mg NaCl/L	pH (S.U.)	7.61	7.60	7.71	7.51	7.62	7.51
	DO (mg/L)	7.6	7.8	7.8	7.7	7.7	7.7
	Conductivity (µmhos/cm)	1380		1430		1390	
	Temperature (°C)	24.6	24.9	24.7	24.6	24.5	25.1
800 mg NaCl/L	pH (S.U.)	7.59	7.60	7.70	7.53	7.61	7.53
	DO (mg/L)	7.7	7.8	7.8	7.7	7.7	7.6
	Conductivity (µmhos/cm)	1730		1800		1750	
	Temperature (°C)	24.6	24.9	24.7	24.8	24.5	25.0
1000 mg NaCl/L	pH (S.U.)	7.59	7.59	7.68	7.52	7.61	7.54
	DO (mg/L)	7.7	7.7	7.8	7.7	7.7	7.7
	Conductivity (µmhos/cm)	2090		2160		2110	
	Temperature (°C)	24.6	24.9	24.6	24.9	24.7	25.0
1200 mg NaCl/L	pH (S.U.)	7.59	7.59	7.68	7.51	7.61	7.56
	DO (mg/L)	7.7	7.7	7.7	7.7	7.8	7.8
	Conductivity (µmhos/cm)	2420		2490		2420	
	Temperature (°C)	24.7	24.9	24.6	24.9	24.7	25.2
1400 mg NaCl/L	pH (S.U.)	7.62	7.59	7.67	7.52	7.60	7.56
	DO (mg/L)	7.7	7.7	7.7	7.7	7.8	7.8
	Conductivity (µmhos/cm)	2770		2830		2770	
	Temperature (°C)	24.7	24.8	24.6	24.9	24.6	25.1
STOCK Page 75 of 76	Conductivity (µmhos/cm)	121000		—		—	
		Initial	Final	Initial	Final	Initial	Final

Species: *Ceriodaphnia dubia*

CdNaCLCR #: 7R

		Day							
		3		4		5		6	
Analyst		A	UAB	UAB	A/BSC	A/BSC	A	A	UAB
Concentration	Parameter								
CONTROL	pH (S.U.)	7.72	<del>7.72</del>	7.87	7.51	7.43	7.46	7.53	7.68
	DO (mg/L)	7.6	8.1	7.5	8.2	8.0	7.8	7.6	7.7
	Conductivity (µmhos/cm)	312		308		302		290	
	Alkalinity (mg CaCO <sub>3</sub> /L)			63					
	Hardness (mg CaCO <sub>3</sub> /L)			86					
	Temperature (°C)	24.5	24.9	24.7	25.0	24.6	24.7	24.9	25.1
600 mg NaCl/L	pH (S.U.)	7.78	<del>7.76</del>	7.75	7.55	7.58	7.50	7.67	7.69
	DO (mg/L)	7.7	7.9 (1.4)	8.1	8.0	8.0	7.7	7.6	7.7
	Conductivity (µmhos/cm)	1390		1370		1360		1410	
	Temperature (°C)	24.7	25.0	24.9	24.7	24.6	24.9	24.8	24.7
800 mg NaCl/L	pH (S.U.)	7.76	7.66	7.74	7.59	7.60	7.50	7.67	7.72
	DO (mg/L)	7.9	7.8	<del>7.69</del> (8.0)	8.6	8.1	7.7	7.6	7.7
	Conductivity (µmhos/cm)	1760		1750		1750		1820	
	Temperature (°C)	24.5	25.0	24.8	25.1	24.5	24.8	24.8	24.6
1000 mg NaCl/L	pH (S.U.)	7.75	7.66	7.74	7.60	7.61	7.53	7.66	7.73
	DO (mg/L)	7.9	7.8	<del>7.69</del> (8.1)	8.0	8.1	7.7	7.7	7.7
	Conductivity (µmhos/cm)	2070		2090		2080		2130	
	Temperature (°C)	24.6	24.8	24.8	25.2	24.5	24.8	24.9	24.6
1200 mg NaCl/L	pH (S.U.)	7.74	7.66	7.73	7.59	7.61	7.52	7.66	7.72
	DO (mg/L)	7.9	7.8	<del>7.69</del> (8.1)	7.9	8.1	7.7	7.8	7.8
	Conductivity (µmhos/cm)	2360		2410		2420		2460	
	Temperature (°C)	24.6	25.0	24.8	24.8	24.8	24.9	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.74	7.68	7.74	7.58	7.61	7.53	7.66	7.72
	DO (mg/L)	7.9	7.9	<del>7.70</del> (8.2)	8.0	8.3	7.7	7.8	7.7
	Conductivity (µmhos/cm)	2760		2740		2750		2800	
	Temperature (°C)	24.6	25.0	24.9	24.8	24.7	25.1	24.9	24.9
STOCK	Conductivity (µmhos/cm)	—R		—K		—K		—K	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final