

Final Report

***Ceriodaphnia dubia* Whole Sediment Survival and Reproduction Toxicity Test Results**

TVA, Kingston Monitoring and Analysis Project Clinch River Sediment Sample CRM4.5

Prepared for:

Rick M. Sherrard, Ph.D.
Senior Toxicologist
Tennessee Valley Authority
1101 Market Street, PSC 1X-C
Chattanooga, TN 37402

Prepared by:



58485 Pearl Acres Road, Suite D
Slidell, LA 70461
1 (800) 966-2788

May 19, 2011

EXECUTIVE SUMMARY

A whole sediment toxicity test was conducted by Environmental Enterprises USA, Inc. (EE USA) to determine potential toxicity of a Clinch River site sediment sample to *Ceriodaphnia dubia* neonates. Three samples were used in this test: Clinch River site sediment identified as BULKSED-CRM4.5-EEUSA (CRM4.5); Clinch River reference sediment identified as BULKSED-CLINCHREFERENCE-EEUSA (CRS); and Clinch River water identified as BULKSW-CRM7.0-EEUSA (CRW). Several dilutions of CRM4.5 prepared with CRS were tested with four concurrent controls. A moderately hard synthetic freshwater (MHSW) only control was included to assess test organism health. Separate laboratory control sediment (LCS) exposures with either MHSW or CRW were included to assess test acceptability requirements.

C. dubia survival and reproduction in the CRM4.5 treatments were compared to survival and reproduction in a CRS control with CRW. Survival and reproduction of *C. dubia* neonates in the CRM4.5 treatments were not reduced when compared to survival and reproduction in the CRS control. Test results are shown in Table 1.

Table 1. *Ceriodaphnia dubia* Chronic Survival and Reproduction Test Results for CRM4.5.

SURVIVAL	REPRODUCTION
NOEC / LOEC = 100.0 / > 100.0%	NOEC / LOEC = 100.0 / > 100.0%
IC_{25} > 100.0%	IC_{25} > 100.0%

INTRODUCTION

EE USA was contracted by Tennessee Valley Authority (TVA) to complete whole sediment toxicity tests with Clinch River sediment and water samples using *C. dubia* neonates. The project is described in TVA's Sediment Toxicity Study Design [1]. Site sediment samples were collected from eight representative locations on the Clinch River. Clinch River reference sediment samples and river water samples were collected upstream of the site sediment locations. The two reference sediment samples were mixed together 50:50 and homogenized in the field. TVA's contractors, Jacobs Engineering and Restoration Services, Inc., coordinated sample collection in the field and delivery of the samples to EE USA. The samples were delivered to EE USA on February 12 and March 22, 2011, on ice and with custody seals intact (Appendix D).

This test, which was performed on one (CRM4.5) of the eight sediment samples obtained from the Clinch River, was conducted in accordance with American Society for Testing and Materials (ASTM) [2] and U. S. EPA [3] toxicity testing methods. Test organisms were cultured at EE USA and were 3.75 to 9.75 hours old when this test was initiated. Ten replicates of each control treatment and six CRM4.5 concentrations were prepared the day before the test was initiated. CRM4.5 dilutions were prepared with CRS. CRM4.5 concentrations tested were 10.0, 20.0, 40.0, 60.0, 80.0, and 100.0%. A portion of the overlying water in each replicate was replaced daily. This test was initiated March 29, 2011, at 1545 and completed April 5 at 1315.

MATERIALS AND METHODS

C. dubia was cultured and maintained in MHSW at $25 \pm 1^\circ\text{C}$. Test organisms were selected from adults producing at least ten in their third or subsequent brood. Only ten neonates from any one adult were used so that one replicate in each treatment was populated with a neonate from the same adult. Test organisms were fed *Selenastrum capricornutum* (SCAP) and Yeast-Cerophyl-digested Tetramin (YCT) daily at the rate of 0.1 mL each per 15 mL of water.

ENVIRONMENTAL ENTERPRISES USA, INC.

On February 12, 2011, the CRM4.5 and CRS samples were delivered to EE USA and stored at 0.1 to 6°C. On March 22, 2011, the CRW sample was delivered to EE USA and stored at 0.1 to 6°C (Appendix D & Table 2). Two 1-liter containers each of CRM4.5 and CRS were put into separate mixing bowls and large bark pieces, rocks, and leaves were removed with forceps. Each 2-liter sediment sample was mixed for approximately five minutes with a KitchenAid Model KHM7TGCS hand-held mixer set at position "3", 580 ± 5 rpm. On March 28th (Day -1), the density of each sediment, LCS, CRS, and CRM4.5, was measured and the test treatments were prepared (Appendix A, page 1). Eight-dram shell vials were used as test chambers. The vials were washed with soap and water and rinsed with acetone, 10% HCl, deionized water, and MHSW prior to being used as test chambers. Test chambers were labeled with test concentration, replicate, and EE USA's project number. Dilutions of CRM4.5 were made with CRS according to the calculations on page 1 of Appendix A. For each treatment, 400 mL of sediment was prepared, homogenized, and then 5 mL were transferred to 11 test replicates. The 11th replicate of each treatment was used for water quality only.

Table 2. Clinch River Site Sediment, Reference Sediment, and Water Samples.

TVA Sample ID	EE USA Sample ID	Date Collected	Date Received
BULKSED-CRM4.5-EEUSA Composite Sample, Site Sediment (CRM4.5)	E-094-11	February 8, 2011 @ 1058	February 12, 2011 @ 1240
BULKSED-CLINCHREFERENCE-EEUSA Composite Sample, Clinch Reference Sediment (CRS)	E-095-11	February 9, 2011 @ 0940	
BULKSW-CRM7.0-EEUSA Grab Sample, Clinch River Water (CRW)	E-189-11	March 21, 2011 @ 1302	March 22, 2011 @ 0830

SCAP and YCT were added to aliquots of the overlying waters, MHSW and CRW; 6.0 mL each of SCAP and YCT was added to 900 mL of MHSW and 12 mL each of SCAP and YCT was added to 1800 mL CRW. The MHSW and CRW aliquots were warmed up to 25 ± 1°C. Twenty mL of MHSW were transferred to 11 test replicates of the MHSW only control. LCS, No. 5 sand supplied by EE USA and wetted to saturation with MHSW, was homogenized with a stainless steel spoon and five mL were transferred to 22 test replicates. Eleven LCS + MHSW replicates received 20 mL MHSW and 11 LCS + CRW replicates received 20 mL CRW. Twenty mL of CRW were added to each replicate of the CRS and CRM4.5 treatments.

After dispensing the sediments and water, the test chambers were placed in an environmental chamber at 25 ± 1°C with a photoperiod of 16 hours light and 8 hours dark. The test was not aerated. Initial water quality parameters (dissolved oxygen (DO) and temperature) were measured daily in the 11th replicate of each treatment. At the end of each 24-hour exposure period, prior to renewal, the ending DO and temperature in each treatment were recorded (Appendix A, pages 9 – 12 & Table 3). Alkalinity, hardness, conductivity, pH, DO, total residual chlorine, and ammonia were measured in CRW and each batch of MHSW (Appendix A, page 2 & Table 4).

**Table 3. Initial and Final Temperature and Dissolved Oxygen Data for Each Treatment:
Mean, Minimum, and Maximum.**

Water Quality Summary for Test Exposures March 29 – April 5, 2011						Mean Min Max		
% Sample	Temperature, °C		Dissolved Oxygen, mg/L					
	Initial	Final	Initial	Final	Initial	Final		
MHSW	24.7 24.4 25.1	24.8 24.5 25.1	7.1 6.7 7.3	6.6 6.5 6.9				
LCS + MHSW	24.7 24.4 25.0	24.8 24.4 25.2	7.1 6.8 7.3	6.5 6.3 6.8				
LCS + CRW	24.7 24.5 24.9	24.9 24.5 25.2	7.1 6.8 7.4	6.2 6.0 6.4				
CRS + CRW	24.7 24.5 24.9	24.8 24.7 25.1	6.4 6.2 6.9	5.9 5.4 6.4				
10.0	24.7 24.5 25.1	24.7 24.5 25.0	6.3 5.9 6.6	5.7 5.2 6.2				
20.0	24.7 24.4 25.1	24.8 24.3 25.1	6.2 5.8 6.5	5.3 5.0 5.6				
40.0	24.7 24.4 25.2	24.8 24.5 25.0	6.3 5.9 6.5	5.2 4.8 5.5				
60.0	24.6 24.4 25.0	24.8 24.5 25.2	6.3 5.9 6.6	5.4 5.0 6.0				
80.0	24.6 24.4 25.0	24.8 24.5 25.1	6.4 6.1 6.7	5.4 4.9 6.0				
100.0	24.6 24.4 25.0	24.7 24.4 25.0	6.3 6.0 6.7	5.4 5.1 5.6				

Table 4. Water Quality Data for CRW and Each Batch of MHSW.

	CRW	MHSW	MHSW	MHSW	MHSW
Collected	3/21/2011				
Batch Number	BULKSW-CRM7.0-EEUSA	FW-032-11 ¹	FW-033-11 ²	FW-034-11 ³	FW-035-11 ⁴
Alkalinity, mg/l	132	60	68	72	76
Hardness, mg/l	120	88	108	92	96
Conductivity, µmhos/cm	307	321	331	334	348
pH, su	8.1	7.9	7.8	8.1	8.2
Dissolved Oxygen, mg/l	8.4	8.2	8.2	8.1	8.2
TRC, mg/l	0.00	0.00	0.00	0.00	0.00
Total Ammonia, mg/l	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
		¹ used 03/28-30/2011			
		² used 03/31/2011			
		³ used 04/01-02/2011			
		⁴ used 04/03-04/2011			

The test was initiated March 29th (Day 0) after 15 mL of water were removed from each replicate of each treatment and replaced with water into which proper aliquots of food had been added. One *C. dubia* neonate was transferred to each replicate, and then the test chambers were placed in an environmental chamber. On Days 1-6, the test exposures were renewed as follows:

1. The *C. dubia* in each replicate and approximately 5 mL of the water in the replicate were transferred to a 30-mL disposable plastic cup.
2. Additional water equivalent to a total of 15 mL was removed from the replicate.
3. 15 mL of fresh MHSW or CRW as appropriate was transferred to the replicate.
4. The *C. dubia* was transferred back to the replicate.

Water was removed from and added to each replicate with a 25-mL pipette. *C. dubia* were transferred with disposable 3.5-mL transfer pipettes. Survival was recorded daily (Appendix A, pages 3 - 8). Reproduction was also recorded and newly produced neonates discarded before renewal. The test was terminated after seven days, after \geq 60% of each set of control organisms released their third brood.

The endpoints for the chronic test were survival and neonate production. The test acceptability criteria were 80% or greater survival in the LCS + MHSW control and an average of 15 or more young per surviving female in the control solutions (60% of surviving control females must produce three broods).

The response used in the statistical analysis of the survival data was the proportion of test organisms surviving in each treatment chamber after seven days. Fisher's Exact test was used to test for a significant difference between survival in the CRS + CRW control and each CRM4.5 concentration. The response used in the reproduction data analysis was the total number of neonates produced per replicate. Reproduction data were tested for normal distribution and homogeneity of variance using the Kolmogorov D and Bartlett's tests, respectively. Reproduction data were normally distributed, equal in variance, and evaluated by Dunnett's Test. The statistical tests were performed using ToxCalc Version 5.0.32 at a probability level of 0.05 [4].

Sensitivity of test organisms to a known toxicant was determined by performing a chronic Standard Reference Toxicant (SRT) test, CD1103, with potassium chloride (Sigma Chemical, Lot 099K0202). The most recent SRT test was initiated on March 24, 2011, with less than 24-hour-old *C. dubia* neonates.

RESULTS AND DISCUSSION

The control *C. dubia* met the test acceptability criteria of 80% or greater survival and an average of 15 or more young per surviving female in the LCS + MHSW control solution. One hundred percent survival occurred in the LCS + MHSW control. Ten out of ten (100%) of the control females produced three broods; the mean brood size was 27.1.

The No Observed Effect Concentration (NOEC) for survival was 100% CRM4.5. The Lowest Observed Effect Concentration (LOEC) was > 100.0% CRM4.5. The IC₂₅, a point estimate of the concentration that causes a 25% reduction in survival was > 100.0% CRM4.5 (Appendix B, page 1 & Table 5).

The NOEC for reproduction was 100.0% CRM4.5. The LOEC was > 100.0% CRM4.5. The Minimum Significant Difference percent for this reproduction data set was 7.7% (Appendix B, page 2). The IC₂₅, a point estimate of the concentration that causes a 25% reduction in reproduction was > 100.0% CRM4.5 (Appendix B, page 2 & Table 5).

Table 5. Summary of Percent Survival, Mean Reproduction, and Survival and Reproduction NOECs, LOECs, and IC₂₅s for CRM4.5.

	LCS + CRW	CRS + CRW	10% CRM4.5	20% CRM4.5	40% CRM4.5	60% CRM4.5	80% CRM4.5	100% CRM4.5
% Survival	100	100	100	100	100	100	100	100
Mean Reproduction	28.9	29.9	29.0	28.8	30.1	28.2	29.0	28.3
	NOEC		LOEC			IC ₂₅		
Survival	100% CRM4.5		> 100% CRM4.5			> 100% CRM4.5		
Reproduction	100% CRM4.5		> 100% CRM4.5			> 100% CRM4.5		

In summary, *C. dubia* survival and reproduction were not significantly reduced in any control or CRM4.5 treatment. Survival and reproduction statistical data for the MHSW only, LCS + MHSW, and LCS + CRW controls are presented on pages 3 and 4 of Appendix B.

The neonates used in the potassium chloride SRT met all of the quality control test parameters. The following SRT control charts are given in Appendix C:

- Survival IC₂₅ with ± 2 SD Control Limits
- Survival IC₂₅ %CV with 75th and 90th Percentile Warning Limits
- Survival PMSD
- Reproduction IC₂₅ with ± SD Control Limits
- Reproduction IC₂₅ %CV with 75th and 90th Percentile Warning Limits
- Control Reproduction with Lower Limit
- Control Reproduction %CV with TVA Limit
- Reproduction PMSD

REFERENCES

1. Tennessee Valley Authority. 2011. Kingston Monitoring and Analysis Project Non-Time-Critical Removal Action Sampling and Analysis Plan Sediment Toxicity Study Design. Chattanooga, TN.
2. American Society for Testing and Materials. 2005. Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates. Annex A2. Guidance for Conducting Sediment Toxicity Tests with *D. magna* and *C. dubia*. E 1706-05. West Conshohocken, PA.
3. U.S. Environmental Protection Agency. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 4th ed. EPA-821-R-02-013. Office of Water, Washington, DC.
4. Tidepool Scientific Software. 2007. ToxCalc™ Toxicity Data Analysis Software. Version 5.0.32. McKinleyville, CA.

ENVIRONMENTAL ENTERPRISES USA, INC.

REPORT TEST REVIEW

(for)

J. K. McNew
Veronica McNew
Effluents Testing Supervisor

5/19/2011

Mark A. O'Neil
Mark A. O'Neil
QA/QC Supervisor

5/19/2011

David L. Daniel
David L. Daniel
Laboratory Director

5/19/2011

Environmental Enterprises USA, Inc.

APPENDIX A

Cladoceran, Ceriodaphnia dubia**Whole Sediment Survival and Reproduction Test**

ASTM E 1706 – 05, Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates:
**A2. GUIDANCE FOR CONDUCTING SEDIMENT TOXICITY TESTS WITH
DAPHNIA MAGNA (*D. magna*) AND *CERIODAPHNIA DUBIA* (*C. dubia*)**

TVA, Kingston Monitoring and Analysis Project
CRM4.5 Sediment & CRS Reference Sediment

	Density		
	LCS	CRS	CRM4.5
1	69.57 g/40 ml	63.23 g/40 ml	53.99 g/40 ml
2	69.33 g/40 ml	63.06 g/40 ml	53.76 g/40 ml
3	69.85 g/40 ml	63.21 g/40 ml	53.16 g/40 ml
MEAN	69.58 g/40 ml	63.17 g/40 ml	53.63 g/40 ml
g/ml	1.74	1.58	1.34
g/5 ml	6.70	7.90	6.70
Scale ID	N7	N7	N7
Date & Time	03.28.11 1030	03.28.11 1350	03.28.11 1400
Initial	JG	JG	JG

Test Concentrations, % CRM4.5

Ceriodaphnia dubia	Total Sediment Vol./ Conc., ml	CRM4.5 ml / gram	CRS ml / gram	LCS ml / gram	grams sediment/ replicate	CRW/rep		MHSW/rep		Tech, Date, & Time	
						Day 0	Days 1 - 6	Day 0	Days 1 - 6	Sed	H2O
100.0%	400	400 / 536.3	0	0	6.70	20	15	0	0	JG 3/28/11 1525	JG 3/28/11 1525
80.0%	400	320 / 429.1	80 / 126.3	0	6.94	20	15	0	0		
60.0%	400	240 / 321.8	160 / 252.7	0	7.18	20	15	0	0		
40.0%	400	160 / 214.5	240 / 379.0	0	7.42	20	15	0	0		
20.0%	400	80 / 107.3	320 / 505.3	0	7.66	20	15	0	0		
10.0%	400	40 / 53.6	360 / 568.5	0	7.78	20	15	0	0		
CRS w CRW	400	0	400 / 631.7	0	7.90	20	15	0	0		
LCS w CRW	400	0	0	400 / 695.8	8.70	20	15	0	0		
LCS w MHSW	400	0	0	400 / 695.8	8.70	0	0	20	15		
MHSW	n/a	0	0	0	n/a	0	0	20	15		

Data pages & Calculations by: R. K. R. QA/QC Check by: Veronica McMen

MHSW = Moderately Hard Synthetic Freshwater

	MHSW	MHSW	MHSW	MHSW	Meter #
Date	03/28/2011	03/31/2011	4/1/2011	4/3/2011	III
Batch #	FW-032-11	FW-033-11	FW-034-11	FW-035-11	
Alkalinity	60	68	72	76	
Hardness	88	108	92	96	
Conductivity	321	331	334	348	A46
pH	7.9	7.8	8.1	8.2	Q8
DO	8.2	8.2	8.1	8.2	S7
TRC	0.00	0.00	0.00	0.00	A27
Ammonia	<0.02	<0.02	<0.02	<0.02	
Initial	DOP	DOP	DOP	DOP	

CRW = Clinch River Reference Water

	CRW	CRW			Meter #
Date	3/25/2011	1/2011			III
Batch #	Delivered 03/22/2011	Delivered 1/2011			
Alkalinity	132				
Hardness	120				
Conductivity	307				A46
pH	8.1				Q8
DO	8.4				S7
TRC	0.00				A27
Ammonia	<0.02				
Initial	DOP				

Alkalinity: mg/l as CaCO₃ Hardness: mg/l as CaCO₃ Conductivity: µS/cm pH: su
 TRC: mg/l Dissolved Oxygen (DO): mg/l Total Residual Chlorine (TRC): mg/l Ammonia, Total: mg/l

Comments: CRS - Sandy with very few pieces of bark or leaves.

CRM 4.5 - very clean with a slight odor.

DOP 3/28/11

Cladoceran, Ceriodaphnia dubia**Whole Sediment Survival and Reproduction Test**

ASTM E 1706 – 05, Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates:
 A2. GUIDANCE FOR CONDUCTING SEDIMENT TOXICITY TESTS WITH
 DAPHNIA MAGNA (D. magna) AND CERIODAPHNIA DUBIA (C. dubia)

TVA, Kingston Monitoring and Analysis Project
CRM4.5 Sediment & CRS Reference Sediment

Test Organisms Age: 3.75 - 9.75 Hours Old

Test Organisms Source: EE> Test Initiation At: 1545 on 3/29/2011

Counted by: David L. Daniel QC/QA by: Veronica McNew

Loaded by: David L. Daniel Organism Lot # CDO32911-03

Exposure Chamber: 8 dram vials. Feeding: 0.1 ml *S. capricornutum* (Lot # S2-11) &
 0.1 ml YCT (Lot # Y3-11) / 15 ml.

C. dubia Daily Survival & Reproduction Data

Treatment: MHSW only.														
DAY	REP	1	2	3	4	5	6	7	8	9	10	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	D0 Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	D0 Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	D0 TK
	3	0	0	0	0	0	0	0	0	0	0	100	0	D0 TK
	4	4	3	4	4	4	4	4	3	4	100	38	D0 TK	
	5	0	10	10	8	8	0	10	0	9	0	100	55	D0 TK
	6	11	0	13	12	0	10	0	12	14	12	100	71	D0 Vh
	7	9	12	0	0	14	13	12	0	14	14	100	88	D0 -
	3rd Brood Reproduction Per Replicate											Mean	CV %	////////
	24	25	27	24	26	27	26	16	27	30	25.2	14.6		

Comments: *split brood. D0 4/6/11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: LCS w MHSW.														
DAY	REP	11	12	13	14	15	16	17	18	19	20	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	111	111	000 VH
	1	0	0	0	0	0	0	0	0	0	0	100	0	000 VH
	2	0	0	0	0	0	0	0	0	0	0	100	0	000 TK
	3	0	0	0	0	0	0	0	0	0	0	100	0	000 TK
	4	4	4	4	3	4	4	4	4	4	4	100	39	000 TK
	5	0	10	0	1	0	0	10	0	0	10	100	39	000 TK
	6	12	0	13	0	11	12	0	14	12	0	100	74	000 VH
	7	15	12	11	13	12	14	11	10	14	7	100	119	000 /
3rd Brood Reproduction Per Replicate												Mean	CV %	111111
	31	26	24	2s	27	30	25	28	30	21	27.1	11.1		

Treatment: LCS w CRW.														
DAY	REP	21	22	23	24	25	26	27	28	29	30	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	111	111	000 VH
	1	0	0	0	0	0	0	0	0	0	0	100	0	000 VH
	2	0	0	0	0	0	0	0	0	0	0	100	0	000 TK
	3	3	4	0	0	0	0	0	0	0	0	100	7	000 TK
	4	0	0	4	4	4	4	3	4	4	4	100	31	000 TK
	5	10	11	0	0	0	0	12	9	12	10	100	64	000 TK
	6	0	0	12	14	12	12	0	0	0	0	100	50	000 VH
	7	14	12	13	17	16	15	13	10	15	12	100	137	000 /
3rd Brood Reproduction Per Replicate												Mean	CV %	111111
	27	27	29	35	32	31	28	23	31	26	28.9	11.9		

Comments:

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: CRS w CRW.														
DAY	REP	31	32	33	34	35	36	37	38	39	40	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	00 Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	00 Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	00 TK
	3	0	0	0	0	0	0	4	0	0	0	100	4	00 TK
	4	4	3	5	5	4	4	0	5	4	4	100	38	00 TK
	5	0	12	*11	10	0	11	11	0	0	13	100	68	00 -TK
	6	9	0	1	0	11	0	12	10	10	0	100	53	00 Vh
	7	18	15	15	15	13	14	0	16	16	14	100	136	00 /
3rd Brood Reproduction Per Replicate												Mean	CV %	////////
31 30 32 30 28 29 27 31 30 31 29.9 5.1														

Treatment: 10% CRM4.5 w CRW.														
DAY	REP	41	42	43	44	45	46	47	48	49	50	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	00 Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	00 Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	00 TK
	3	0	0	0	0	0	0	0	0	0	0	100	0	00 TK
	4	6	4	4	5	4	3	4	4	4	5	100	43	00 TK
	5	11	10	6	0	9	12	10	12	0	12	100	73	00 TK
	6	0	0	9	10	0	0	0	3	10	0	100	32	00 Vh
	7	16	13	15	13	13	12	16	15	15	14	100	142	00 /
3rd Brood Reproduction Per Replicate												Mean	CV %	////////
33 27 28 28 26 27 30 31 29 31 29.0 7.62														

Comments: *split broods. 00 4/1/11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: 20% CRM4.5 w CRW.														
DAY	REP	51	52	53	54	55	56	57	58	59	60	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	III	III	DD Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	DD Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	DD TK
	3	0	0	4	4	4	0	0	0	0	0	100	12	DD TK
	4	4	4	0	0	0	4	5	4	5	4	100	30	DD TK
	5	0	0	10	10	10	9	10	0	0	0	100	49	DD TK
	6	11	9	0	0	0	0	0	12	14	12	100	58	DD Vh
	7	16	14	13	17	14	15	12	13	11	14	100	139	DD /
3rd Brood Reproduction Per Replicate												Mean	CV %	////////
		31	27	21	31	28	24	27	29	30	30	28.8	5.62	

Treatment: 40% CRM4.5 w CRW.														
DAY	REP	61	62	63	64	65	66	67	68	69	70	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	III	III	DD Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	DD Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	DD TK
	3	0	0	0	0	4	0	0	0	0	0	100	44④	DD TK
	4	5	4	5	4	0	5	4	5	4	4	100	40	DD TK
	5	0	12	0	12	10	0	10	10	0	11	100	65	DD TK
	6	12	0	12	0	0	10	0	0	13	0	100	47	DD Vh
	7	10	16	15	14	16	13	15	14	15	17	100	145	DD /
3rd Brood Reproduction Per Replicate												Mean	CV %	////////
		21	32	32	30	30	28	29	29	32	32	30.1	6.16	

Comments: ④ wrong data DD 4/1/11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: 60% CRM4.5 w CRW.														
DAY	REP	71	72	73	74	75	76	77	78	79	80	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	111	111	D00 Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	D-0 Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	D-0 TK
	3	0	0	0	0	0	0	0	0	0	0	100	0	D00 TK
	4	4	4	3	4	5	3	4	4	4	5	100	40	D00 TK
	5	13	11	12	10	10	0	9	0	0	12	100	77	D00 TK
	6	0	0	0	0	0	13	0	11	10	0	100	34	D00 Vh
	7	15	14	10	14	11	11	16	13	12	15	100	131	D00 -
3rd Brood Reproduction Per Replicate													Mean	CV %
	32	29	25	28	26	27	29	28	26	32	28.2	8.49		

Treatment: 80% CRM4.5 w CRW.														
DAY	REP	81	82	83	84	85	86	87	88	89	90	% Sur.	No. of Neonates Per Day	Tech CD H2O
	0	0	0	0	0	0	0	0	0	0	0	111	111	D00 Vh
	1	0	0	0	0	0	0	0	0	0	0	100	0	D00 Vh
	2	0	0	0	0	0	0	0	0	0	0	100	0	D00 TK
	3	0	0	0	0	0	0	0	4	4	0	100	8	D00 TK
	4	4	6	5	5	6	4	4	0	0	4	100	38	D00 TK
	5	0	13	0	10	0	0	0	12	11	0	100	46	D00 TK
	6	10	0	12	0	13	11	10	0	0	10	100	66	D00 Vh
	7	12	15	9	14	14	13	13	14	16	12	100	132	D00 -
3rd Brood Reproduction Per Replicate													Mean	CV %
	26	34	26	29	33	28	27	30	31	26	29.0	10.2		

Comments:

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: 100% CRM4.5 w CRW.

DAY	REP	91	92	93	94	95	96	97	98	99	100	% Sur.	No. of Neonates Per Day	Tech		
		CD	H2O	Time												
1	0	0	0	0	0	0	0	0	0	0	100	100	0	DUP	Vhr	1545
	1	0	0	0	0	0	0	0	0	0	100	100	0	DUP	Vhr	1040
	2	0	0	0	0	0	0	0	0	0	100	100	0	DUP	TK	1450
	3	0	0	0	0	5	0	0	0	0	100	100	5	DUP	TK	1100
	4	4	6	6	S	0	S	S	S	4	4	100	44	DUP	TK	0900
	5	10	0	12	0	14	(9)	0	0	12	10	100	67	DUP	TK	0915
	6	0	11	0	12	0	(1)	10	9	0	0	100	43	DUP	Vhr	1210
	7	14	10	9	8	15	13	12	13	14	16	100	124	DUP	—	1315
3rd Brood Reproduction Per Replicate												Mean	CV %	//////////		
	28	27	27	25	34	28	27	27	30	30	28.3	8.82				

Calculations by: Beth QA/QC by: Veronica McNewData Entry by: BethDouble Data Entry by: Beth orQA/QC Officer: MK

Comments: * split brood 4/6/11

C. dubia Water Quality Data
 All Treatments: Initial Temp.: 23.5 to 26.4°C; Initial DO: 4.0 to 8.3 mg/l

Day -1	Controls				Treatment % CRM4.5						Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	
03/28/11	MHSW	LCS + MHSW									
DO	7.1	7.4	7.4	6.8	6.2	6.2	6.2	6.1	6.1	6.1	57
Temp	24.7	24.5	24.5	24.6	24.5	24.5	24.7	24.8	24.7	24.5	A46
Tech. Initials	Initials: DCD										
Times	Initial Time: 16:20										

Day 0	Controls				Treatment % CRM4.5						Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	
03/29/11	MHSW	LCS + MHSW									
DO											
F											
	7.3	7.3	7.4	6.4	6.3	6.2	6.4	6.6	6.6	6.7	57
Temp											
F											
	24.7	24.7	24.6	24.7	24.7	24.8	24.8	24.7	24.6	24.6	A46
Tech. Initials	Finals: Data not recorded. DCD Initials: DCD										
Times	Final Time: n/a Initial Time: 11:15										

Day 1	Controls				Treatment % CRM4.5						Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	
03/30/11	MHSW	LCS + MHSW									
DO											
F	6.9	6.8	6.3	5.4	5.2	5.3	5.5	5.7	5.5	5.4	57
	7.2	7.3	7.1	6.3	5.9	6.1	6.3	6.2	6.2	6.0	57
Temp											
F	24.9	24.9	25.0	24.9	25.0	25.1	25.0	25.2	25.1	25.0	A46
	25.1	25.0	24.9	24.9	25.1	25.1	25.2	25.0	25.0	25.0	A46
Tech. Initials	Finals: DCD Initials: DCD										
Times	Final Time: 08:30 Initial Time: 11:15										

TVA, CRM4.5, Site Sediment
 TVA, CRS, Reference Sediment
 TVA, CRW, River Water

C. dubia 7-day Chronic.
 9 of 12

E-094-11
 E-095-11
 E-189-11

C. dubia Water Quality Data Cont.
 All Treatments: Initial Temp.: 23.5 to 26.4°C; Initial DO: 4.0 to 8.3 mg/l

Day 2	Controls				Treatment % CRM4.5						Meter #
	MHSW		Clinch River Water								
03/31/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO	F	6.6	6.6	6.4	6.4	6.2	5.6	5.5	6.0	6.0	5.6
	I	7.1	6.9	7.0	6.5	6.5	6.2	6.2	6.2	6.3	5.7
Temp	F	24.3	24.6	24.8	24.7	24.5	24.3	24.6	24.5	24.5	A46
	I	24.6	24.8	24.7	24.6	24.6	24.4	24.6	24.5	24.6	A46
Tech. Initials	Finals: D44						Initials: D44				
Times	Final Time: 0830						Initial Time: 1530				

Day 3	Controls				Treatment % CRM4.5						Meter #
	MHSW		Clinch River Water								
04/01/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO	F	6.8	6.6	6.3	5.6	6.0	5.4	5.4	5.1	5.4	5.4
	I	7.0	6.9	7.1	6.5	6.6	6.4	6.4	6.4	6.5	6.4
Temp	F	24.8	24.8	24.8	24.7	24.6	24.8	24.9	24.8	24.7	A46
	I	24.6	24.4	24.5	24.5	24.5	24.5	24.5	24.4	24.5	A46
Tech. Initials	Finals: D44						Initials: D44				
Times	Final Time: 0850						Initial Time: 1430				

Comments:

C. dubia Water Quality Data Cont.

All Treatments: Initial Temp.: 23.5 to 26.4°C; Initial DO: 4.0 to 8.3 mg/l

Day 4	Controls				Treatment % CRM4.5						Meter #
	MHSW		Clinch River Water								
04/02/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO	F	6.5	6.3	6.1	6.1	6.0	5.5	5.4	5.6	5.5	5.5
	I	7.2	7.1	7.1	6.9	6.6	6.5	6.5	6.5	6.3	6.3
Temp	F	25.1	25.2	25.2	25.1	25.0	24.9	25.0	25.1	25.1	25.0
	I	24.6	24.5	24.5	24.7	24.6	24.7	24.7	24.6	24.7	24.6
Tech. Initials	Finals: <i>DWD</i>						Initials: <i>DWD</i>				
Times	Final Time: 0750						Initial Time: 1135				

Day 5	Controls				Treatment % CRM4.5						Meter #
	MHSW		Clinch River Water								
04/03/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO	F	6.5	6.3	6.0	5.7	5.5	5.0	4.9	5.0	5.2	5.6
	I	7.0	7.1	6.9	6.2	6.3	6.5	6.5	6.6	6.7	6.2
Temp	F	25.0	25.0	24.9	24.8	24.7	25.0	24.9	24.7	24.6	24.6
	I	24.6	24.8	24.8	24.5	24.5	24.5	24.7	24.5	24.4	24.4
Tech. Initials	Finals: <u>020</u>					Initials: <u>DAD</u>					
Times	Final Time: 07/10					Initial Time: 12/10					

Comments:

TVA, CRM4.5, Site Sediment
TVA, CRS, Reference Sediment
TVA, CRW, River Water

C. dubia 7-day Chronic.
11 of 12

C. dubia Water Quality Data Cont.

Day 6	Controls				Treatment % CRM4.5						Meter #
	MHSW		Clinch River Water								
04/04/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO	F	6.5	6.4	6.3	6.0	5.5	5.3	5.0	5.1	4.9	5.1
	I	6.7	6.8	6.8	6.2	6.0	5.8	5.9	5.9	6.1	6.0
Temp	F	24.7	24.8	24.8	24.8	24.8	24.8	24.9	24.9	24.8	24.8
	I	24.4	24.6	24.6	24.7	24.8	24.8	24.8	24.8	24.7	24.7
Tech. Initials		Finals: Dp				Initials: Dp					
Times		Final Time: 0820				Initial Time: 165					

Comments:

TVA, CRM4.5, Site Sediment
TVA, CRS, Reference Sediment
TVA, CRW, River Water

C. dubia 7-day Chronic.
12 of 12

E-094-11
E-095-11
E-189-11

Environmental Enterprises USA, Inc.

APPENDIX B

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date: 3/29/2011 Test ID: cd09411 Sample ID: CRM4.5
 End Date: 4/5/2011 Lab ID: EE USA Sample Type: Whole Sediment
 Sample Date: 2/8/2011 Protocol: ASTM E1706-05 Annex A2 Test Species: CD-Ceriodaphnia dubia
 Comments: LCS=Lab Control Sediment; CRW=Clinch River Water; CRS=Clinch Reference Sediment

Conc-%	1	2	3	4	5	6	7	8	9	10
LCS+CRW	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
CRS+CRW	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
60	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's	1-Tailed	Isotonic	
							Exact P	Critical	Mean	N-Mean
LCS+CRW	1.0000	1.0000	0	10	10	10	0.6238	*	1.0000	1.0000
CRS+CRW	1.0000	1.0000	0	10	10	10			1.0000	1.0000
10	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
20	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
40	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
60	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
80	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000
100	1.0000	1.0000	0	10	10	10	1.0000	0.0500	1.0000	1.0000

Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU

Fisher's Exact Test 100 >100 1

Treatments vs CRS+CRW

Linear Interpolation (200 Resamples)

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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 3/29/2011 Test ID: cd09411 Sample ID: CRM4.5
 End Date: 4/5/2011 Lab ID: EE USA Sample Type: Whole Sediment
 Sample Date: 2/8/2011 Protocol: ASTM E1706-05 Annex A2 Test Species: CD-Ceriodaphnia dubia
 Comments: LCS=Lab Control Sediment; CRW=Clinch River Water; CRS=Clinch Reference Sediment

Conc-%	1	2	3	4	5	6	7	8	9	10
LCS+CRW	27.000	27.000	29.000	35.000	32.000	31.000	28.000	23.000	31.000	26.000
CRS+CRW	31.000	30.000	32.000	30.000	28.000	29.000	27.000	31.000	30.000	31.000
10	33.000	27.000	28.000	28.000	26.000	27.000	30.000	31.000	29.000	31.000
20	31.000	27.000	27.000	31.000	28.000	28.000	27.000	29.000	30.000	30.000
40	27.000	32.000	32.000	30.000	30.000	28.000	29.000	29.000	32.000	32.000
60	32.000	29.000	25.000	28.000	26.000	27.000	29.000	28.000	26.000	32.000
80	26.000	34.000	26.000	29.000	33.000	28.000	27.000	30.000	31.000	26.000
100	28.000	27.000	27.000	25.000	34.000	28.000	27.000	27.000	30.000	30.000

Conc-%	Transform: Untransformed							t-Stat	1-Tailed Critical	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N			Mean	N-Mean
LCS+CRW	28.900	0.9666	28.900	23.000	35.000	11.925	10				
CRS+CRW	29.900	1.0000	29.900	27.000	32.000	5.097	10	*		29.900	1.0000
10	29.000	0.9699	29.000	26.000	33.000	7.624	10	0.914	2.347	2.310	29.300 0.9799
20	28.800	0.9632	28.800	27.000	31.000	5.623	10	1.118	2.347	2.310	29.300 0.9799
40	30.100	1.0067	30.100	27.000	32.000	6.156	10	-0.203	2.347	2.310	29.300 0.9799
60	28.200	0.9431	28.200	25.000	32.000	8.491	10	1.727	2.347	2.310	28.600 0.9565
80	29.000	0.9699	29.000	26.000	34.000	10.151	10	0.914	2.347	2.310	28.600 0.9565
100	28.300	0.9465	28.300	25.000	34.000	8.822	10	1.626	2.347	2.310	28.300 0.9465

Auxiliary Tests

Kolmogorov D Test indicates normal distribution ($p > 0.05$)	0.770119	0.895	0.517368	-0.14767
Bartlett's Test indicates equal variances ($p = 0.44$)	5.869729	16.81189		
The control means are not significantly different ($p = 0.41$)	0.839181	2.100922		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	2.309965	0.077256	5.295238	4.842857	0.376228	6, 63

Treatments vs CRS+CRW

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL	Skew
IC05	93.000			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

Ceriodaphnia Survival and Reproduction Test: 7 Day Survival

Start Date: 3/29/2011 Test ID: cd09411 Sample ID: CRM4.5
 End Date: 4/5/2011 Lab ID: EE USA Sample Type: Whole Sediment
 Sample Date: 2/8/2011 Protocol: ASTM E1706-05 Annex A2 Test Species: CD-Ceriodaphnia dubia
 Comments: MHSW=Mod Hard Synthetic Water; LCS=Lab Control Sediment; CRW=Clinch River Water

Conc-%	1	2	3	4	5	6	7	8	9	10
MHSW	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
LCS+MHSW	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
LCS+CRW	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's	1-Tailed
							Exact P	Critical
MHSW	1.0000	1.0000	0	10	10	10	0.6238	*
LCS+MHSW	1.0000	1.0000	0	10	10	10		
LCS+CRW	1.0000	1.0000	0	10	10	10	1.0000	0.0500

Hypothesis Test (1-tail, 0.05)

Fisher's Exact Test indicates no significant differences

Treatments vs LCS+MHSW

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 3/29/2011 Test ID: cd09411 Sample ID: CRM4.5
 End Date: 4/5/2011 Lab ID: EE USA Sample Type: Whole Sediment
 Sample Date: 2/8/2011 Protocol: ASTM E1706-05 Annex A2 Test Species: CD-Ceriodaphnia dubia
 Comments: MHSW=Mod Hard Synthetic Water; LCS=Lab Control Sediment; CRW=Clinch River Water

Conc-%	1	2	3	4	5	6	7	8	9	10
MHSW	24.000	25.000	27.000	24.000	26.000	27.000	26.000	16.000	27.000	30.000
LCS+MHSW	31.000	26.000	28.000	25.000	27.000	30.000	25.000	28.000	30.000	21.000
LCS+CRW	27.000	27.000	29.000	35.000	32.000	31.000	28.000	23.000	31.000	26.000

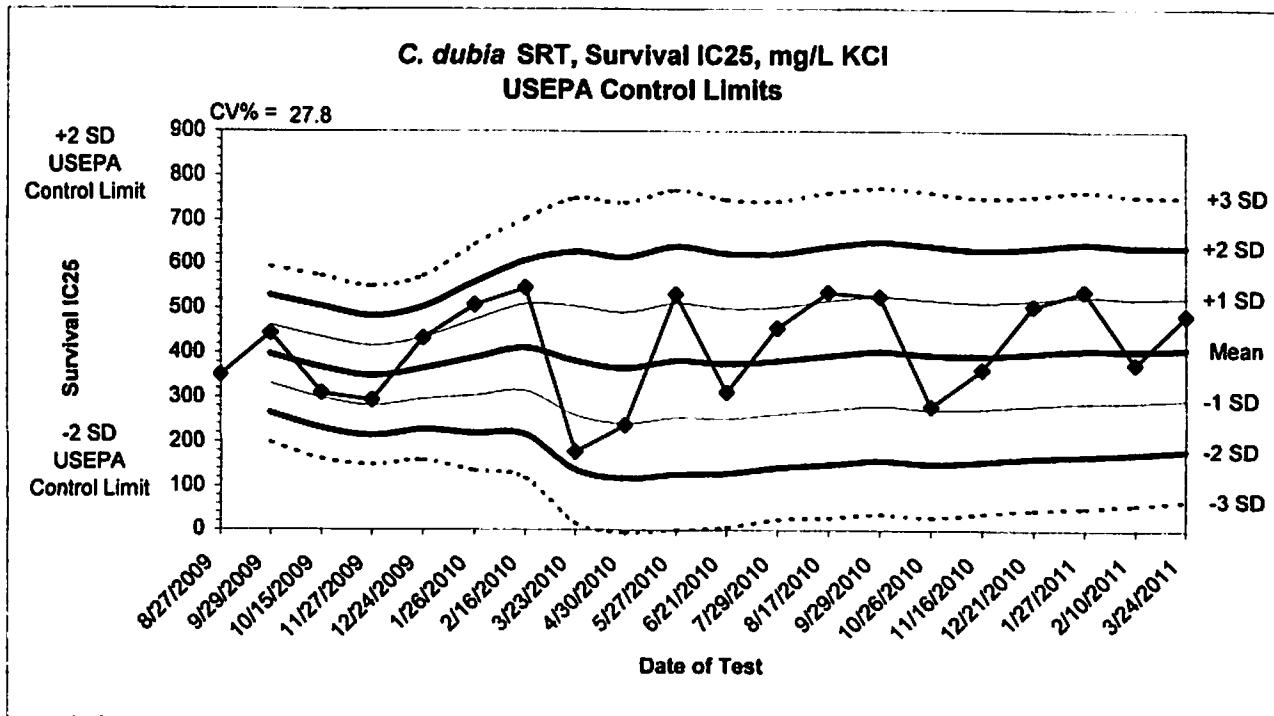
Conc-%	Transform: Untransformed							1-Tailed		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
MHSW	25.200	0.9299	25.200	16.000	30.000	14.586	10			
LCS+MHSW	27.100	1.0000	27.100	21.000	31.000	11.063	10	*		
LCS+CRW	28.900	1.0664	28.900	23.000	35.000	11.925	10	-1.246	1.734	2.505

Auxiliary Tests

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.05$)	0.971385	0.905	-0.19986	-0.12009
F-Test indicates equal variances ($p = 0.68$)	1.321384	6.54109		
The control means are not significantly different ($p = 0.22$)	1.266667	2.100922		
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE
Homoscedastic t Test indicates no significant differences	2.504907	0.092432	16.2	10.43333
Treatments vs LCS+MHSW			F-Prob	df
			0.22871	1, 18

Environmental Enterprises USA, Inc.

APPENDIX C



Test #	Test Date	Survival IC25	Mean IC25	-1 SD	-2 SD	+1 SD	+2 SD	-3 SD	+3 SD	Toxicant Lot #
CD0910	8/27/2009	350								029K0050
CD0911	9/29/2009	443	397	331	265	462	528	199	594	029K0050
CD0912	10/15/2009	310	368	299	231	436	504	163	572	029K0050
CD0913	11/27/2009	294	349	282	216	416	483	149	550	049K0305
CD0915	12/24/2009	433	366	297	228	435	504	159	573	049K0305
CD1002	1/26/2010	508	390	305	220	474	559	136	644	079K0011
CD1003	2/16/2010	546	412	315	217	509	607	120	704	079K0011
CD1004	3/23/2010	178	383	260	138	505	627	16	750	049K0305
CD1006	4/30/2010	238	367	243	118	491	615	-6	739	049K0305
CD1007	5/27/2010	531	383	255	127	511	639	-1	767	049K0305
CD1009	6/21/2010	312	377	253	130	500	623	7	747	049K0305
CD1010	7/29/2010	456	383	263	144	503	623	24	743	079K0011
CD1011	8/17/2010	536	395	273	150	517	640	28	762	079K0011
CD1012	9/29/2010	527	404	282	159	527	650	36	772	079K0011
CD1013	10/26/2010	281	396	274	151	519	641	29	764	099K0202
CD1014	11/16/2010	364	394	276	157	513	631	38	750	099K0202
CD1015	12/21/2010	506	401	283	165	519	637	47	755	099K0202
CD1101	1/27/2011	539	408	289	170	527	646	51	765	099K0202
CD1102	2/10/2011	376	407	291	175	523	638	59	754	099K0202
CD1103	3/24/2011	485	411	297	182	525	639	68	753	099K0202

CD1005 - IC25 less than lowest concentration tested and could not be graphed

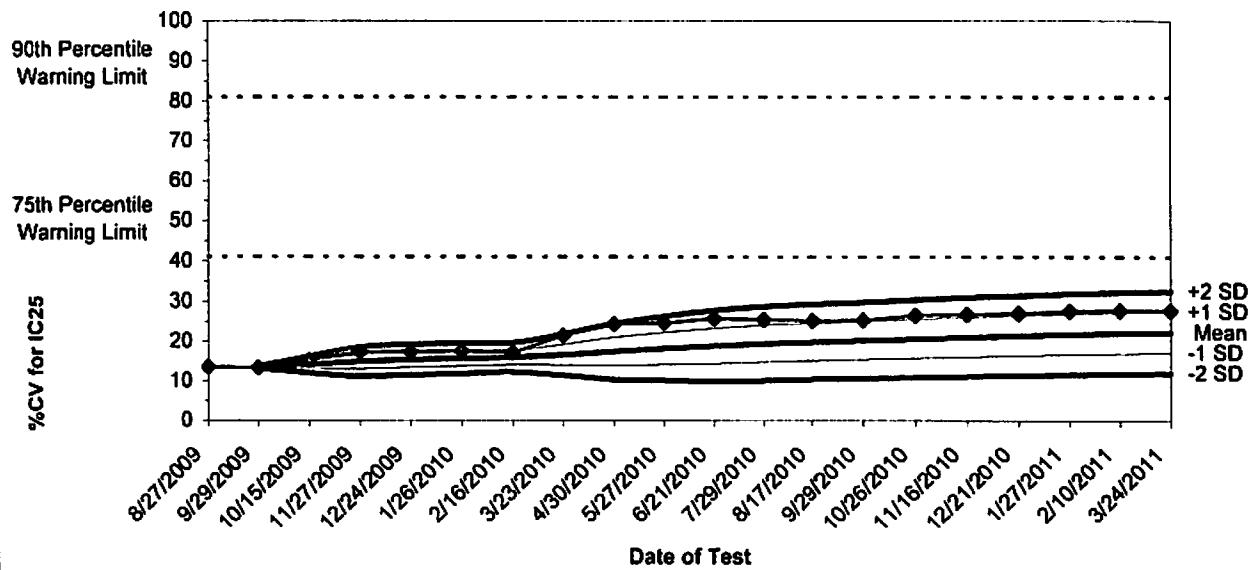
CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

CD0914 - Training lab tech

QA QC by: MWD 4/15/11

**C. dubia SRT, Survival IC25,
USEPA Within Lab %CV Warning and Control Limits**



Test #	Test Date	%CV for IC25	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
CD0910	8/27/2009	13.6						41.0	81.0	029K0050
CD0911	9/29/2009	13.4	13.5	13.4	13.2	13.6	13.8	41.0	81.0	029K0050
CD0912	10/15/2009	15.3	14.1	13.1	12.0	15.2	16.2	41.0	81.0	029K0050
CD0913	11/27/2009	17.4	14.9	13.1	11.2	16.8	18.6	41.0	81.0	049K0305
CD0915	12/24/2009	17.4	15.4	13.5	11.5	17.3	19.3	41.0	81.0	049K0305
CD1002	1/26/2010	17.6	15.8	13.8	11.9	17.7	19.7	41.0	81.0	079K0011
CD1003	2/16/2010	17.2	16.0	14.1	12.3	17.8	19.7	41.0	81.0	079K0011
CD1004	3/23/2010	21.5	16.7	14.1	11.5	19.3	21.9	41.0	81.0	049K0305
CD1006	4/30/2010	24.4	17.5	14.0	10.5	21.1	24.6	41.0	81.0	049K0305
CD1007	5/27/2010	24.7	18.2	14.2	10.2	22.3	26.3	41.0	81.0	049K0305
CD1009	6/21/2010	25.7	18.9	14.5	10.0	23.4	27.8	41.0	81.0	049K0305
CD1010	7/29/2010	25.5	19.5	14.8	10.2	24.1	28.8	41.0	81.0	079K0011
CD1011	8/17/2010	25.2	19.9	15.2	10.5	24.6	29.4	41.0	81.0	079K0011
CD1012	9/29/2010	25.3	20.3	15.5	10.8	25.1	29.8	41.0	81.0	079K0011
CD1013	10/26/2010	26.6	20.7	15.8	11.0	25.6	30.5	41.0	81.0	099K0202
CD1014	11/16/2010	26.8	21.1	16.2	11.2	26.0	31.0	41.0	81.0	099K0202
CD1015	12/21/2010	27.1	21.5	16.4	11.4	26.5	31.5	41.0	81.0	099K0202
CD1101	1/27/2011	27.6	21.8	16.7	11.7	26.9	31.9	41.0	81.0	099K0202
CD1102	2/10/2011	27.8	22.1	17.0	11.9	27.2	32.3	41.0	81.0	099K0202
CD1103	3/24/2011	27.8	22.4	17.3	12.1	27.5	32.7	41.0	81.0	099K0202

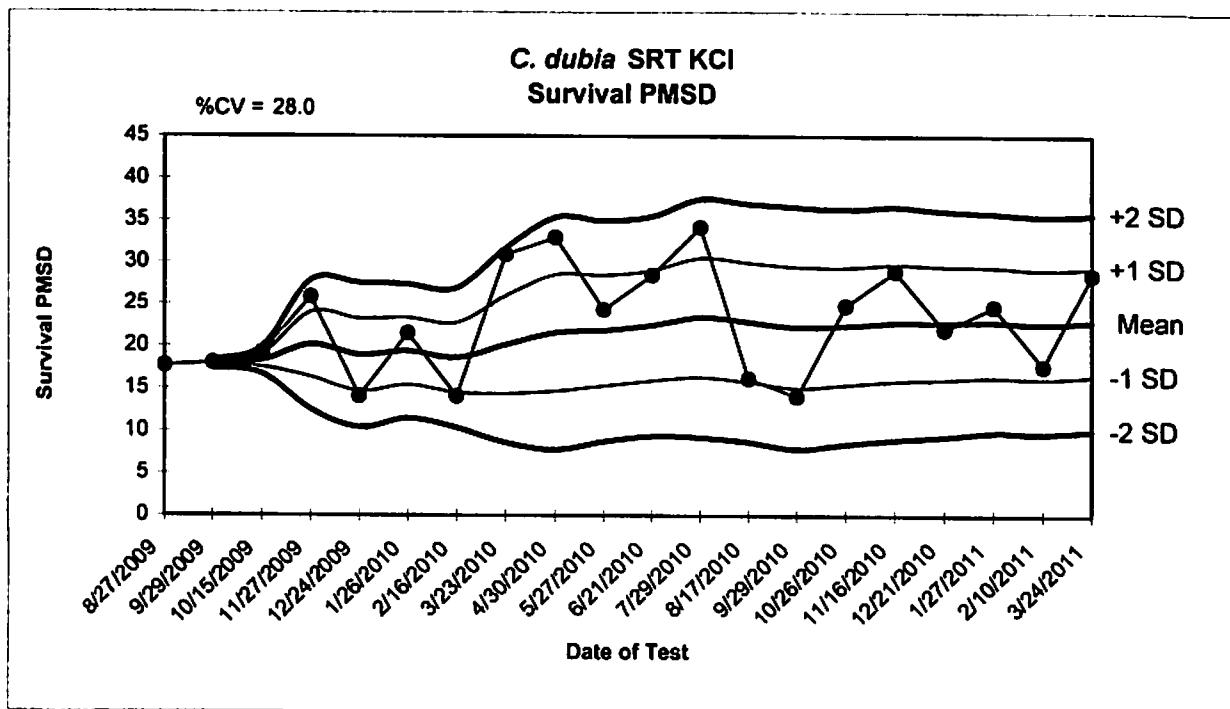
CD1005 - IC25 less than lowest concentration tested and could not be graphed

CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

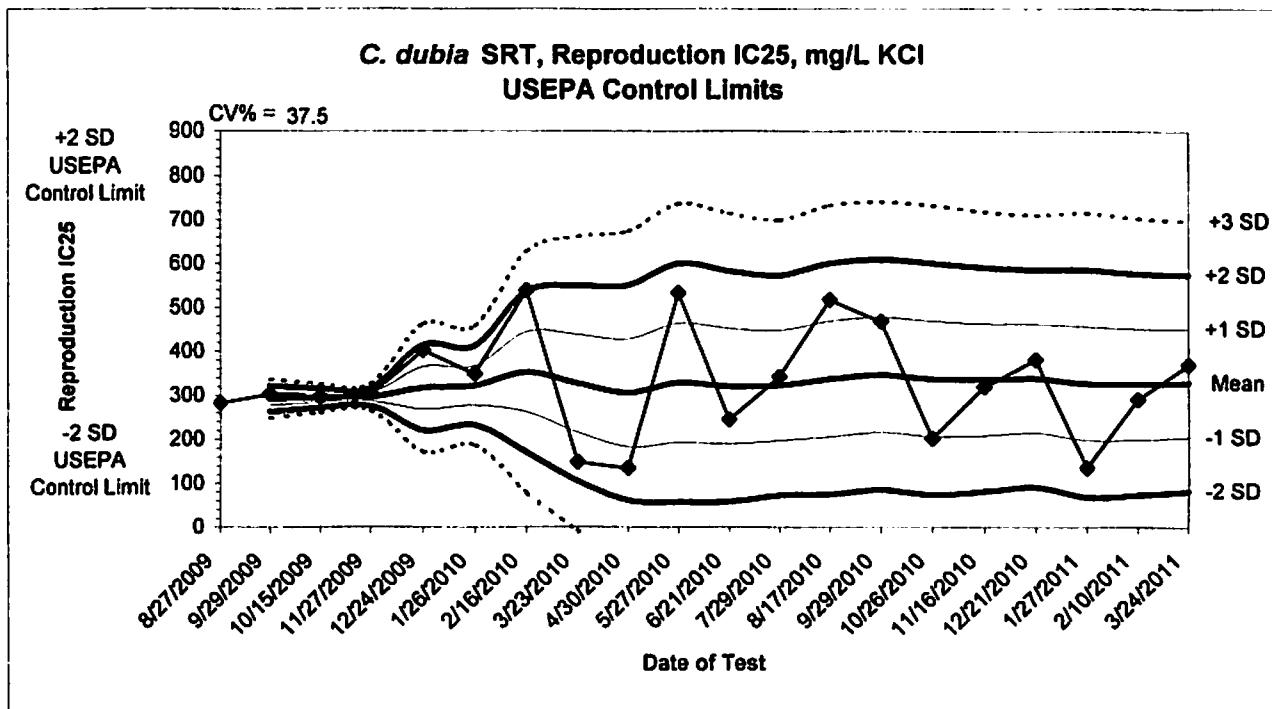
CD0914 - Training lab tech

MAA by: MHO 4/15/11



Test #	Test Date	Survival PMSD	Mean	-1 SD	-2 SD	+1 SD	+2 SD	Toxicant Lot #
CD0910	8/27/2009	17.7						029K0050
CD0911	9/29/2009	18.0	17.8500	17.6379	17.4257	18.0621	18.2743	029K0050
CD0912	10/15/2009	19.2	18.3000	17.5063	16.7125	19.0937	19.8875	029K0050
CD0913	11/27/2009	25.8	20.1750	16.3694	12.5638	23.9806	27.7862	049K0305
CD0915	12/24/2009	14.1	18.9600	14.6888	10.4176	23.2312	27.5024	049K0305
CD1002	1/26/2010	21.5	19.3833	15.4248	11.4663	23.3418	27.3003	079K0011
CD1003	2/16/2010	14.1	18.6286	14.4999	10.3713	22.7572	26.8859	079K0011
CD1004	3/23/2010	30.9	20.1625	14.3803	8.5981	25.9447	31.7269	049K0305
CD1006	4/30/2010	33.0	21.5889	14.6921	7.7953	28.4857	35.3825	049K0305
CD1007	5/27/2010	24.3	21.8600	15.3013	8.7427	28.4187	34.9773	049K0305
CD1009	6/21/2010	28.4	22.4545	15.9275	9.4004	28.9816	35.5087	049K0305
CD1010	7/29/2010	34.2	23.4333	16.3463	9.2593	30.5204	37.6074	079K0011
CD1011	8/17/2010	16.2	22.8769	15.8012	8.7256	29.9526	37.0283	079K0011
CD1012	9/29/2010	14.1	22.2500	15.0586	7.8672	29.4414	36.6328	079K0011
CD1013	10/26/2010	24.8	22.4200	15.4590	8.4979	29.3810	36.3421	099K0202
CD1014	11/16/2010	28.9	22.8250	15.9076	8.9903	29.7424	36.6597	099K0202
CD1015	12/21/2010	22.0	22.7765	16.0758	9.3751	29.4772	36.1779	099K0202
CD1101	1/27/2011	24.7	22.8833	16.3669	9.8505	29.3998	35.9162	099K0202
CD1102	2/10/2011	17.7	22.6105	16.1670	9.7235	29.0540	35.4975	099K0202
CD1103	3/24/2011	28.5	22.9050	16.4966	10.0882	29.3134	35.7218	099K0202

QAQC by: AKO 4/15/11



Test #	Test Date	Repro. IC25	Mean IC25	-1 SD	-2 SD	+1 SD	+2 SD	-3 SD	+3 SD	Toxicant Lot #
CD0910	8/27/2009	282								029K0050
CD0911	9/29/2009	303	293	278	263	307	322	248	337	029K0050
CD0912	10/15/2009	297	294	283	272	305	316	262	326	029K0050
CD0913	11/27/2009	304	297	286	276	307	317	266	327	049K0305
CD0915	12/24/2009	403	318	269	221	366	415	173	463	049K0305
CD1002	1/26/2010	349	323	278	233	368	413	188	458	079K0011
CD1003	2/16/2010	539	354	262	171	445	537	79	628	079K0011
CD1004	3/23/2010	149	328	217	105	440	551	-6	663	049K0305
CD1006	4/30/2010	135	307	184	62	429	552	-61	674	049K0305
CD1007	5/27/2010	533	329	194	58	465	601	-78	737	049K0305
CD1009	6/21/2010	246	322	190	59	453	584	-72	716	049K0305
CD1010	7/29/2010	343	324	198	73	449	574	-53	700	079K0011
CD1011	8/17/2010	518	339	207	75	470	602	-56	733	079K0011
CD1012	9/29/2010	469	348	217	86	479	610	-46	741	079K0011
CD1013	10/26/2010	202	338	206	74	470	602	-57	734	099K0202
CD1014	11/16/2010	320	337	210	82	464	592	-45	719	099K0202
CD1015	12/21/2010	382	340	216	92	464	587	-32	711	099K0202
CD1101	1/27/2011	136	328	199	69	458	587	-60	717	099K0202
CD1102	2/10/2011	292	326	200	74	452	579	-52	705	099K0202
CD1103	3/24/2011	370	329	206	82	452	575	-41	698	099K0202

CD1005 - IC25 less than lowest concentration tested and could not be graphed

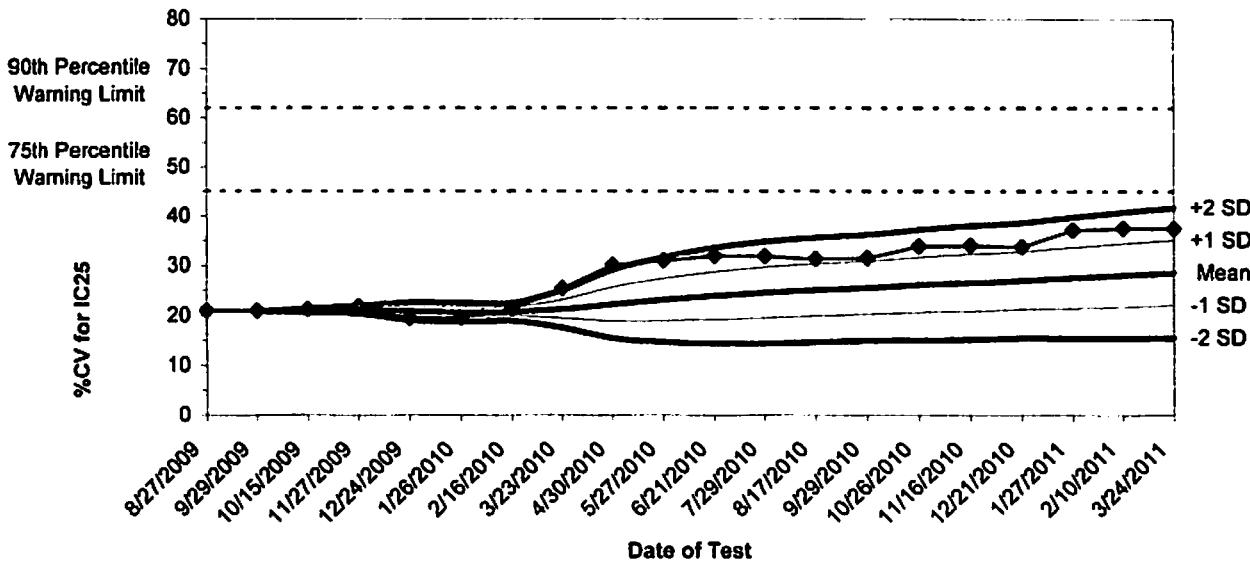
CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

CD0914 - Training lab tech

QA QC by: MAO 4/15/11

C. dubia SRT, Reproduction IC25
USEPA Within Lab %CV Warning and Control Limits



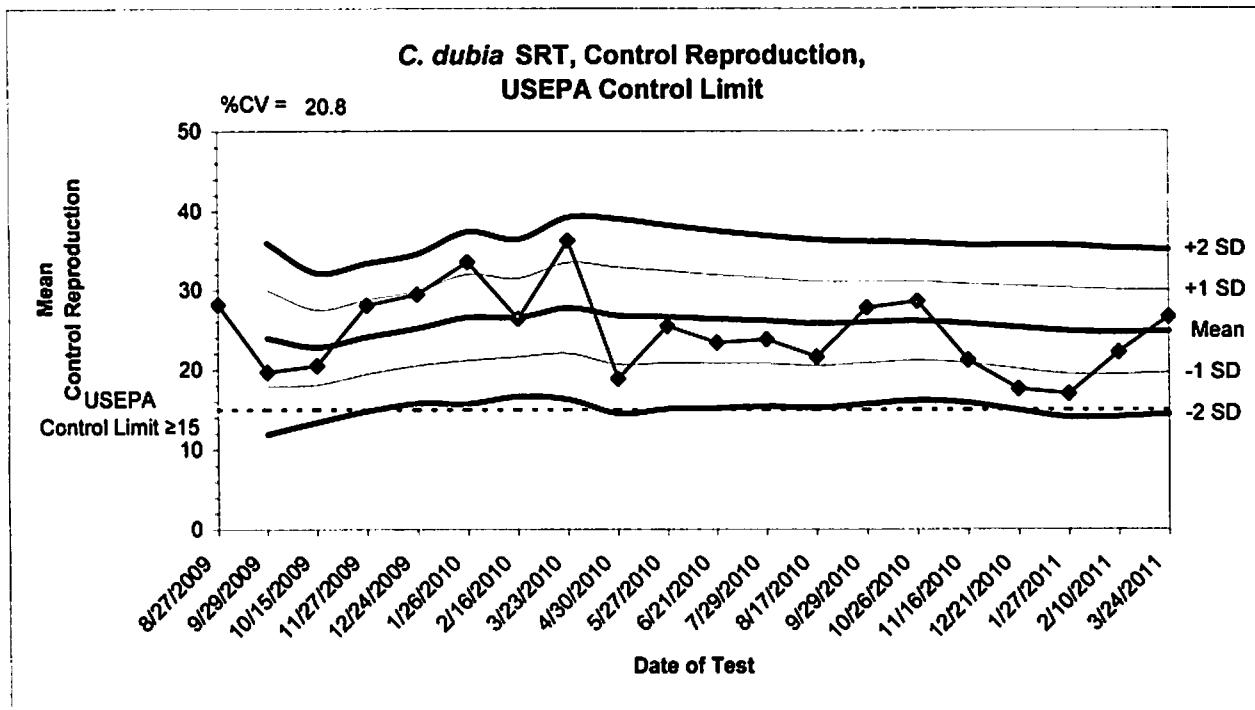
Test #	Test Date	%CV for IC25	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
CD0910	8/27/2009	21.0						45.0	62.0	029K0050
CD0911	9/29/2009	21.0	21.0	21.0	20.9	21.0	21.0	45.0	62.0	029K0050
CD0912	10/15/2009	21.3	21.1	20.9	20.7	21.2	21.4	45.0	62.0	029K0050
CD0913	11/27/2009	21.8	21.2	20.8	20.5	21.6	22.0	45.0	62.0	049K0305
CD0915	12/24/2009	19.4	20.9	20.0	19.1	21.8	22.6	45.0	62.0	049K0305
CD1002	1/26/2010	19.6	20.7	19.7	18.8	21.6	22.6	45.0	62.0	079K0011
CD1003	2/16/2010	21.3	20.8	19.9	19.0	21.7	22.6	45.0	62.0	079K0011
CD1004	3/23/2010	25.5	21.4	19.5	17.6	23.2	25.1	45.0	62.0	049K0305
CD1006	4/30/2010	30.1	22.3	18.9	15.5	25.7	29.1	45.0	62.0	049K0305
CD1007	5/27/2010	31.1	23.2	19.0	14.7	27.4	31.7	45.0	62.0	049K0305
CD1009	6/21/2010	31.9	24.0	19.2	14.4	28.8	33.6	45.0	62.0	049K0305
CD1010	7/29/2010	31.9	24.7	19.5	14.4	29.8	34.9	45.0	62.0	079K0011
CD1011	8/17/2010	31.3	25.2	19.9	14.7	30.4	35.6	45.0	62.0	079K0011
CD1012	9/29/2010	31.6	25.6	20.3	15.0	30.9	36.3	45.0	62.0	079K0011
CD1013	10/26/2010	33.9	26.2	20.6	15.1	31.7	37.3	45.0	62.0	099K0202
CD1014	11/16/2010	34.1	26.7	21.0	15.2	32.4	38.1	45.0	62.0	099K0202
CD1015	12/21/2010	33.8	27.1	21.3	15.5	32.9	38.7	45.0	62.0	099K0202
CD1101	1/27/2011	37.2	27.6	21.5	15.4	33.8	39.9	45.0	62.0	099K0202
CD1102	2/10/2011	37.6	28.2	21.8	15.5	34.5	40.9	45.0	62.0	099K0202
CD1103	3/24/2011	37.5	28.6	22.1	15.6	35.2	41.7	45.0	62.0	099K0202

CD1005 - IC25 less than lowest concentration tested and could not be graphed

CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

CD0914 - Training lab tech

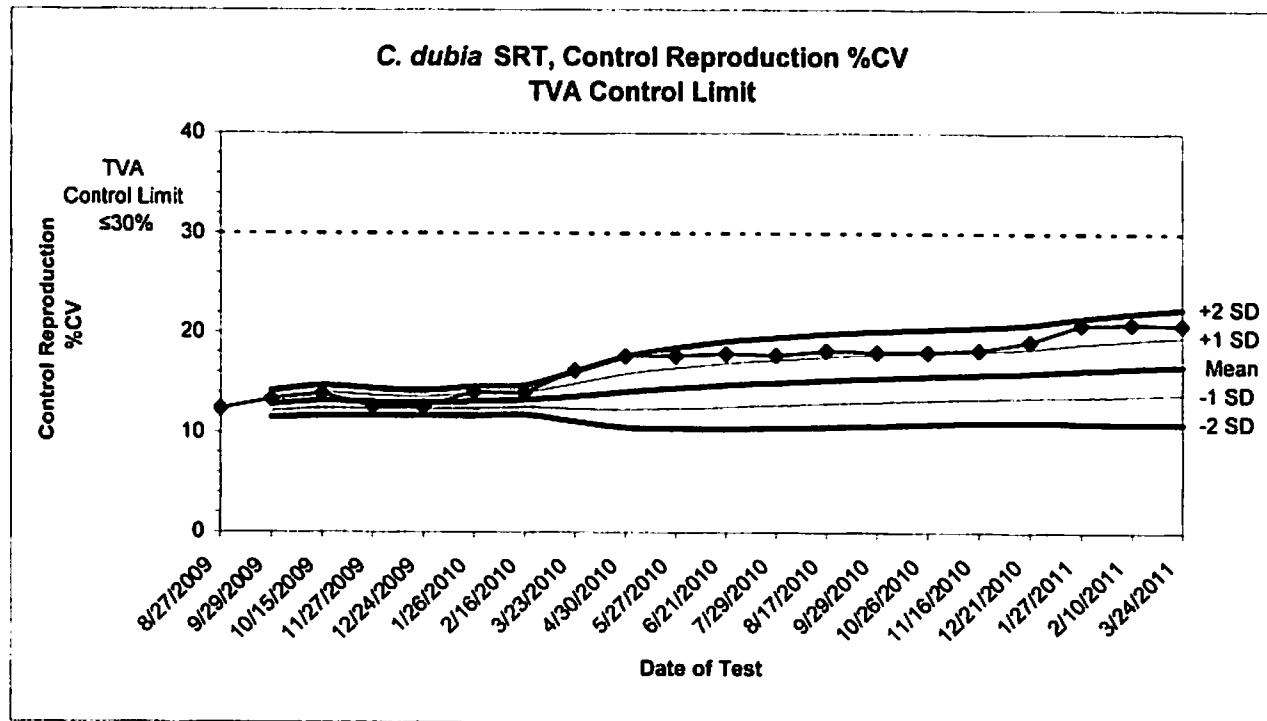


Test #	Test Date	Mean Control Repro.	Mean	-1 SD	-2 SD	+1 SD	+2 SD		Control Limit	Toxicant Lot #
CD0910	8/27/2009	28.2							15.0	029K0050
CD0911	9/29/2009	19.7	24.0	17.9	11.9	30.0	36.0		15.0	029K0050
CD0912	10/15/2009	20.5	22.8	18.1	13.4	27.5	32.2		15.0	029K0050
CD0913	11/27/2009	28.1	24.1	19.5	14.8	28.8	33.4		15.0	049K0305
CD0915	12/24/2009	29.5	25.2	20.5	15.8	29.9	34.6		15.0	049K0305
CD1002	1/26/2010	33.6	26.6	21.2	15.8	32.0	37.4		15.0	079K0011
CD1003	2/16/2010	26.4	26.6	21.6	16.7	31.5	36.5		15.0	079K0011
CD1004	3/23/2010	36.3	27.8	22.1	16.3	33.5	39.2		15.0	049K0305
CD1006	4/30/2010	18.9	26.8	20.7	14.6	32.9	39.0		15.0	049K0305
CD1007	5/27/2010	25.5	26.7	20.9	15.1	32.5	38.2		15.0	049K0305
CD1009	6/21/2010	23.4	26.4	20.8	15.2	32.0	37.5		15.0	049K0305
CD1010	7/29/2010	23.8	26.2	20.8	15.4	31.5	36.9		15.0	079K0011
CD1011	8/17/2010	21.6	25.8	20.5	15.2	31.1	36.4		15.0	079K0011
CD1012	9/29/2010	27.8	26.0	20.8	15.7	31.1	36.2		15.0	079K0011
CD1013	10/26/2010	28.6	26.1	21.1	16.2	31.1	36.1		15.0	099K0202
CD1014	11/16/2010	21.2	25.8	20.9	15.9	30.8	35.7		15.0	099K0202
CD1015	12/21/2010	17.6	25.3	20.1	14.9	30.5	35.7		15.0	099K0202
CD1101	1/27/2011	17.0	24.9	19.5	14.0	30.3	35.7		15.0	099K0202
CD1102	2/10/2011	22.2	24.7	19.4	14.1	30.0	35.3		15.0	099K0202
CD1103	3/24/2011	26.7	24.8	19.7	14.5	30.0	35.2		15.0	099K0202

CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

CD0914 - Training lab tech



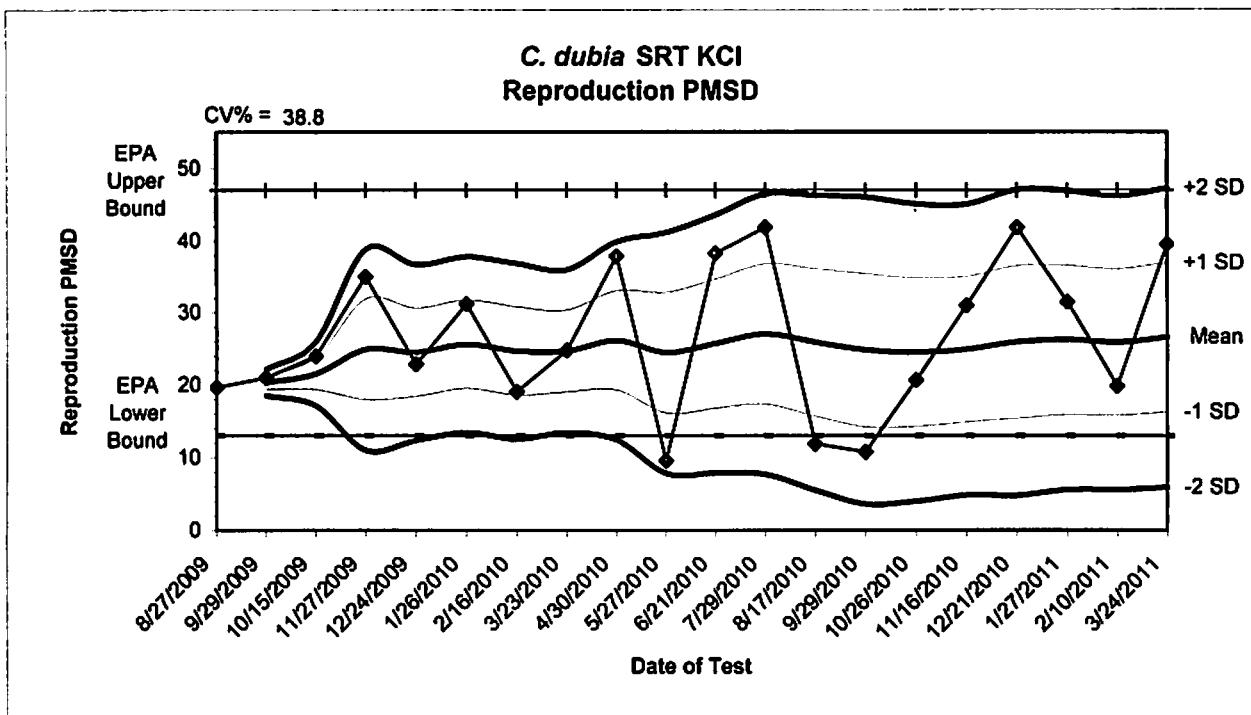
Test #	Test Date	Control Repro. %CV	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD		Control Limit	Toxicant Lot #
CD0910	8/27/2009	12.4							30.0	029K0050
CD0911	9/29/2009	13.3	12.8	12.2	11.5	13.5	14.2		30.0	029K0050
CD0912	10/15/2009	13.8	13.2	12.4	11.7	13.9	14.7		30.0	029K0050
CD0913	11/27/2009	12.6	13.0	12.3	11.6	13.7	14.4		30.0	049K0305
CD0915	12/24/2009	12.5	12.9	12.3	11.7	13.6	14.2		30.0	049K0305
CD1002	1/26/2010	14.1	13.1	12.4	11.7	13.8	14.6		30.0	079K0011
CD1003	2/16/2010	14.0	13.2	12.5	11.8	14.0	14.7		30.0	079K0011
CD1004	3/23/2010	16.2	13.6	12.4	11.1	14.8	16.1		30.0	049K0305
CD1006	4/30/2010	17.6	14.0	12.3	10.5	15.8	17.6		30.0	049K0305
CD1007	5/27/2010	17.6	14.4	12.4	10.4	16.4	18.4		30.0	049K0305
CD1009	6/21/2010	17.9	14.7	12.5	10.4	16.9	19.1		30.0	049K0305
CD1010	7/29/2010	17.7	15.0	12.7	10.5	17.2	19.5		30.0	079K0011
CD1011	8/17/2010	18.2	15.2	12.9	10.6	17.5	19.9		30.0	079K0011
CD1012	9/29/2010	18.0	15.4	13.1	10.7	17.8	20.1		30.0	079K0011
CD1013	10/26/2010	18.0	15.6	13.2	10.8	18.0	20.3		30.0	099K0202
CD1014	11/16/2010	18.2	15.8	13.4	11.0	18.1	20.5		30.0	099K0202
CD1015	12/21/2010	19.1	16.0	13.5	11.1	18.4	20.8		30.0	099K0202
CD1101	1/27/2011	20.8	16.2	13.6	11.0	18.9	21.5		30.0	099K0202
CD1102	2/10/2011	20.9	16.5	13.7	10.9	19.2	22.0		30.0	099K0202
CD1103	3/24/2011	20.8	16.7	13.8	10.9	19.6	22.4		30.0	099K0202

CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

CD0914 - Training lab tech

QAQC by: MHO 4/15/11



Test #	Test Date	Reprod. PMSD	Mean PMSD	-1 SD	-2 SD	+1 SD	+2 SD	Upper PMSD Bound	Lower PMSD Bound	Toxicant Lot #
CD0910	8/27/2009	19.7	20.3500	19.4308	18.5115	21.2692	22.1885	47	13	029K0050
CD0911	9/29/2009	21.0	21.5667	19.3614	17.1561	23.7720	25.9773	47	13	029K0050
CD0912	10/15/2009	24.0	24.9250	17.9712	11.0173	31.8788	38.8327	47	13	049K0305
CD0913	11/27/2009	35.0	24.5200	18.4301	12.3402	30.6099	36.6998	47	13	049K0305
CD0915	12/24/2009	22.9	25.6333	19.5418	13.4503	31.7249	37.8164	47	13	079K0011
CD1002	1/26/2010	31.2	24.7000	18.6156	12.5312	30.7844	36.8688	47	13	079K0011
CD1003	2/16/2010	19.1	24.7125	19.0793	13.4461	30.3457	35.9789	47	13	049K0305
CD1004	3/23/2010	24.8	26.1778	19.3156	12.4534	33.0400	39.9021	47	13	049K0305
CD1006	4/30/2010	37.9	24.5200	16.1930	7.8659	32.8470	41.1741	47	13	049K0305
CD1007	5/27/2010	9.6	25.7727	16.8470	7.9213	34.6984	43.6241	47	13	049K0305
CD1009	6/21/2010	38.3	25.9462	15.7447	5.5433	36.1476	46.3490	47	13	049K0305
CD1010	7/29/2010	41.9	27.1167	17.4162	7.7157	36.8172	46.5177	47	13	079K0011
CD1011	8/17/2010	11.9	24.8643	14.2600	3.6558	35.4685	46.0728	47	13	079K0011
CD1012	9/29/2010	10.8	24.5867	14.3117	4.0368	34.8616	45.1365	47	13	099K0202
CD1013	10/26/2010	20.7	24.9875	14.9323	4.8772	35.0427	45.0978	47	13	099K0202
CD1014	11/16/2010	31.0	24.9824	15.4177	4.8530	36.5470	47.1117	47	13	099K0202
CD1015	12/21/2010	41.9	26.2889	15.9574	5.6260	36.6203	46.9518	47	13	099K0202
CD1101	1/27/2011	31.5	26.2889	15.9574	5.6260	36.6203	46.9518	47	13	099K0202
CD1102	2/10/2011	19.9	25.9526	15.8059	5.6591	36.0994	46.2462	47	13	099K0202
CD1103	3/24/2011	39.6	26.6350	16.2981	5.9613	36.9719	47.3087	47	13	099K0202

QAQC by: MAO 5/19/11

Environmental Enterprises USA, Inc.

APPENDIX D

BIOMONITORING CHAIN OF CUSTODY RECORD

Page 1 of 1

Client: TVA	Environmental Enterprises USA, Inc. 58485 Pearl Acres Road, Suite D Slidell, LA 70461			Delivered By (Circle One): <input checked="" type="checkbox"/> Courier
Project Name: KIF Ash Toxicity Study	Date of Sample Collection: 02/07/11, 02/08/11, 02/09/11			FedEx UPS Bus Client
Location: CRM0.0, CRM1.5, CRM2.0, CRM2.5, CRM3.0, CRM3.5, CRM4.0, CRM4.5, CRM6.5, CRM7.5	Attn: David L. Daniel Office 800.966.2788 Cell 985.707.5442			Other (specify): General Comments: Homogenized sediment from the Clinch River "CLINCHREFERENCE" is a composite sample of CRM6.5 and CRM7.5.
Collected By: R. Josefczyk (RSI), L. Jackson (TVA), R. Vance (RSI), E. Arnold (RSI), M. Greer (RSI), D. Mathis (RSI)				

Field Identification / Sample Description	Grab/ Comp	Collection Date/Time	Number of Containers & Volume Collected	Depth (ft)	Rain Event? (Mark as Appropriate)		Laboratory Use (as applicable)				
					If Yes, Inches	No Trace	Log #	Arrival Temp. (°C)	By	Time	Appearance
BULKSED-CRM0.0-EEUSA	C	02/07/11 1014	(4) 1000 mL	0.0-0.5	N/A	N/A	E-007-11	1.0 + 1.1	04	1320/1450	Sediment
BULKSED-CRM1.5-EEUSA	C	02/07/11 1243	(4) 1000 mL	0.0-0.5	N/A	N/A	E-008-11	0.3 + 1.1	04	1320/1450	"
BULKSED-CRM2.0-EEUSA	C	02/07/11 0955	(4) 1000 mL	0.0-0.5	N/A	N/A	E-009-11	0.8 + 1.9	04	1325/1455	"
BULKSED-CRM2.5-EEUSA	C	02/07/11 1340	(4) 1000 mL	0.0-0.5	N/A	N/A	E-010-11	0.9 + 0.7	04	1325/1450	"
BULKSED-CRM3.0-EEUSA	G	02/08/11 0921	(4) 1000 mL	0.0-0.5	N/A	N/A	E-011-11	1.3 + 1.8	04	1320/1450	"
BULKSED-CRM3.5-EEUSA	G	02/08/11 1000	(4) 1000 mL	0.0-0.5	N/A	N/A	E-012-11	0.9	04	1425	"
BULKSED-CRM4.0-EEUSA	G	02/08/11 1235	(4) 1000 mL	0.0-0.5	N/A	N/A	E-013-11	0.8	04	1420	"
BULKSED-CRM4.5-EEUSA	G	02/08/11 1058	(4) 1000 mL	0.0-0.5	N/A	N/A	E-014-11	1.9	04	1400	"
BULKSED-CLINCHREFERENCE-EEUSA	C	02/09/11 0940	32 (4) 1000 mL	0.0-0.5	N/A	N/A	E-015-11	0.8, 2.3, + 1.6	04	*	"

Sample Custody - Fill In From Top Down

Relinquished By (Signature)/Affiliation:	Date/Time	Received By (Signature)/Affiliation:	Date/Time
Damon Joseph /ESI TVA	02/07/11 0920	Kotic Johnson /TVA	02/07/11 0920
Totie Johnson /TVA TVA	02/07/11 1100	Reichardt, S.	02/12/11 1240

Associated UPS Tracking #: (if applicable):

COURIER TRANSPORT DOCUMENTATION

DATE: 02/11/2011

COURIER COMPANY:

Sonic Subcontractor

From:	To:
TVA c/o Katie Gassaway 189 Lakeshore Drive Harriman, TN 37748 865-803-4503	Environmental Enterprises USA, Inc. 58485 Pearl Acres Road, Suite D Slidell, LA 70461 Attn: David L. Daniel 1-800-966-2788 985-707-5442

No. of Items:	Description:
7	Cooler(s) taped and custody sealed. Coolers are batched 1 of 1, 2 of 2, and 4 of 4 containing water and sediment.

Shippers Name/Company: Sonic ^{KG02111} Bonnie Gassaway / Katie Gassaway/T

Date / Time: 02/11/11 / 1400

Courier Signature/Company: R. Neal

Date / Time: 2-11-11 16100

Receipt Signature/Company: R. Neal / EE USA

Date / Time: 2/12/11 1240 David Daniel

Corresponding Chains of Custody:

BULKSED-021111-EEUSA page 1 of 1	
BULKSW-021111-EEUSA page 1 of 1	

RECORD COPY

CHAIN OF CUSTODY RECORD

Page 1 of 1

CC No. BULKSW-032111-EUUSA

Client: TVA						
Project Name: KIF Ash Toxicity Study						
Date of Sample Collection: 03/21/2011						
Location: CRM7.0						
Environmental Enterprises USA, Inc. 58485 Pearl Acres Road, Suite D Slidell, LA 70461 Attn: David L. Daniel Office 800.966.2788 Cell 985.707.5442						
Collected By: Ramona Joseczky (RSI), Lynne Jackson (TVA), Mark Greer (RSI) E. W. Jackson (RSI) T. Walls (RSI) E. Arnold (RSI) A. Johnson (RSI)						
Field Identification / Sample Description	Grab/ Comp.	Collection Date/Time	Number of Containers & Volume Collected	Depth (m)	Rain Event? (Mark as Appropriate)	Laboratory Use (as applicable)
		Date	Time		Yes If Yes: Inches	No Trace
BULKSW-CRM7.0-EUUSA-032111	G	03/21/2011	1302	(3) 10L cubitainers	9.69	NA ✓
<i>By 032111</i>						
Sample Custody – Fill In From Top Down						
Relinquished By (Signature)/Affiliation:	Date/Time	Received By (Signature)/Affiliation:	Date/Time			
<i>Ramona Joseczky (RSI)</i>	032111/1450	<i>RSI</i>	032111/1650			
<i>RSI</i>	032111/1700	<i>Taylorville EE</i>	3.22-11 0830			
UPS Tracking No: 1Z939EX21590794793 * Samples in fine condition with crusty scale intact. Associated UPS Tracking #s (if applicable): 060 3/22/11						