

## **Final Report**

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

### **TVA, Kingston Monitoring and Analysis Project Clinch River Sediment Samples**

#### **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

April 18, 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-CRM0.0-EEUSA (CRM0.0); Clinch River reference sediment identified as BULKSED-CLINCHREFERENCE-EEUSA (CRS); and Clinch River water identified as BULKSW-CRM7.0-EEUSA (CRW). Several dilutions of CRM0.0 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 CRM0.0 treatments were compared to survival and reproduction in a CRS control with CRW. Survival and reproduction of *C. dubia* neonates in the CRM0.0 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 CRM0.0.**

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, 2011, on ice and with custody seals intact (Appendix D).

This test, which was performed on one (CRM0.0) 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 15.0 to 20.0 hours old when this test was initiated. Ten replicates of each control treatment and six CRM0.0 concentrations were prepared the day before the test was initiated. CRM0.0 dilutions were prepared with CRS. CRM0.0 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 February 17, 2011, at 1400 and completed February 23 at 1205.

## MATERIALS AND METHODS

*C. dubia* was cultured and maintained in moderately hard synthetic freshwater 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 CRM0.0, CRS, and CRW samples were delivered to EE USA and stored at 0.1 to 6°C (Appendix D & Table 2). Two 1-liter containers each of CRM0.0 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 February 16<sup>th</sup> (Day -1), the density of each sediment, LCS, CRS, and CRM0.0, 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 CRM0.0 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 11<sup>th</sup> 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-CRM0.0-EEUSA Composite Sample, Site Sediment (CRM0.0)	E-087-11	February 7, 2011 @ 1014	
BULKSED-CLINCHREFERENCE-EEUSA Composite Sample, Clinch Reference Sediment (CRS)	E-095-11	February 9, 2011 @ 0940	February 12, 2011 @ 1240
BULKSW-CRM7.0-EEUSA Grab Sample, Clinch River Water (CRW)	E-086-11	February 11, 2011 @ 1057	

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 CRM0.0 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 11<sup>th</sup> 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 February 17 – 23, 2011						Mean
% Sample	Temperature, °C		Dissolved Oxygen, mg/L		Min	Max
	Initial	Final	Initial	Final		
MHSW	24.9	24.9	7.4	7.4		
	24.5 25.4	24.5 25.1	7.0 7.6	7.2 7.6		
LCS + MHSW	24.9	24.9	7.4	7.4		
	24.3 25.4	24.6 25.0	7.0 7.6	7.2 7.6		
LCS + CRW	24.9	24.8	7.4	7.3		
	24.3 25.4	24.5 25.1	7.0 7.6	7.0 7.5		
CRS + CRW	25.0	24.8	6.2	6.2		
	24.3 25.5	24.4 25.1	6.0 6.5	5.7 6.7		
10.0	24.9	24.9	6.0	5.6		
	24.2 25.3	24.3 25.3	5.5 6.6	5.1 6.0		
20.0	25.0	24.8	5.9	5.7		
	24.4 25.4	24.4 25.0	5.5 6.4	5.3 6.3		
40.0	24.9	24.8	5.8	5.6		
	24.3 25.4	24.3 25.3	5.2 6.2	5.0 6.3		
60.0	25.0	24.8	5.8	5.7		
	24.4 25.6	24.4 25.2	5.4 6.1	5.3 6.4		
80.0	25.0	24.8	5.8	5.6		
	24.4 25.4	24.4 25.2	5.6 6.1	5.3 6.2		
100.0	25.0	24.8	5.9	5.5		
	24.6 25.4	24.4 25.1	5.6 6.1	5.0 6.1		

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

	CRW	MHSW	MHSW	MHSW
Collected	2/11/2011			
Batch Number	BULKSW-CRM7.0-EEUSA	FW-016-11 <sup>1</sup>	FW-018-11 <sup>2</sup>	FW-019-11 <sup>3</sup>
Alkalinity, mg/l	140	60	76	64
Hardness, mg/l	140	100	84	96
Conductivity, µmhos/cm	313	299	320	312
pH, su	8.2	8.1	8.1	8.2
Dissolved Oxygen, mg/l	8.3	8.3	8.3	8.3
TRC, mg/l	0.04	0.0	0.0	0.0
Total Ammonia, mg/l	0.04	< 0.02	< 0.02	< 0.02
		<sup>1</sup> used 02/16-18/2011		
		<sup>2</sup> used 02/19-21/2011		
		<sup>3</sup> used 02/22/2011		

The test was initiated February 17<sup>th</sup> (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 1-5, 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 six 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 six days. Fisher's Exact test was used to test for a significant difference between survival in the CRS + CRW control and each CRM0.0 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, CD1102, with potassium chloride (Sigma Chemical, Lot 099K0202). The most recent SRT test was initiated on February 10, 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. All (100%) of the control females produced three broods; the mean brood size was 24.3.

The No Observed Effect Concentration (NOEC) for survival was 100% CRM0.0. The Lowest Observed Effect Concentration (LOEC) was  $>$  100.0% CRM0.0. The IC<sub>25</sub>, a point estimate of the concentration that causes a 25% reduction in survival was  $>$  100.0% CRM0.0 (Appendix B, page 1 & Table 5).

The NOEC for reproduction was 100.0% CRM0.0. The LOEC was  $>$  100.0% CRM0.0. The Minimum Significant Difference percent for this reproduction data set was 11.4% (Appendix B, page 2). The IC<sub>25</sub>, a point estimate of the concentration that causes a 25% reduction in reproduction was  $>$  100.0% CRM0.0 (Appendix B, page 2 & Table 5).

**Table 5. Summary of Percent Survival, Mean Reproduction, and Survival and Reproduction NOECs, LOECs, and IC<sub>25</sub>s for CRM0.0.**

	LCS + CRW	CRS + CRW	10% CRM0.0	20% CRM0.0	40% CRM0.0	60% CRM0.0	80% CRM0.0	100% CRM0.0
<b>% Survival</b>	100	100	100	100	100	100	100	100
<b>Mean Reproduction</b>	29.9	33.3	37	35.1	35.9	36.4	34.8	34.8
	<b>NOEC</b>		<b>LOEC</b>			<b>IC<sub>25</sub></b>		
<b>Survival</b>	100% CRM0.0		> 100% CRM0.0			> 100% CRM0.0		
<b>Reproduction</b>	100% CRM0.0		> 100% CRM0.0			> 100% CRM0.0		

In summary, *C. dubia* survival and reproduction were not significantly reduced in any control or CRM0.0 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 IC<sub>25</sub> with ± 2 SD Control Limits
- Survival IC<sub>25</sub> %CV with 75<sup>th</sup> and 90<sup>th</sup> Percentile Warning Limits
- Survival PMSD
- Reproduction IC<sub>25</sub> with ± SD Control Limits
- Reproduction IC<sub>25</sub> %CV with 75<sup>th</sup> and 90<sup>th</sup> 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, 4<sup>th</sup> 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

Veronica McNew 04/19/2011  
Veronica McNew

Effluents Testing Supervisor

Mark A. O'Neil 4/19/2011  
Mark A. O'Neil

QA/QC Supervisor

David L. Daniel 4/18/2011  
David L. Daniel

Laboratory Director

**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**  
**CRM 0.0 Sediment & CRS Reference Sediment**

	Density		
	LCS	CRS	CRM 0.0
1	72.58 g/40 ml	63.41 g/40 ml	50.30 g/40 ml
2	72.97 g/40 ml	63.49 g/40 ml	50.44 g/40 ml
3	72.84 g/40 ml	63.14 g/40 ml	50.63 g/40 ml
MEAN	72.80 g/40 ml	63.35 g/40 ml	50.46 g/40 ml
g/ml	1.82	1.58	1.26
g/5 ml	9.10	7.92	6.31
Initial	0.00 JG	0.00 JG	0.00 JG

2/16/11

Balance ID N7  
DOD 3/9/11**Test Concentrations, % CRM 0.0**

Ceriodaphnia <i>dubia</i>	Total Sediment Vol./ Conc., ml	CRM 0.0 ml / gram	CRS ml / gram	LCS ml / gram	grams sediment/ replicate	CRW/rep		MHSW/rep	
						Day 0	Days 1 - 6	Day 0	Days 1 - 6
100.0%	400	400 / 504.6	0	0	6.31	20	15	0	0
80.0%	400	320 / 403.7	80 / 126.7	0	6.63	20	15	0	0
60.0%	400	240 / 302.8	160 / 253.4	0	6.95	20	15	0	0
40.0%	400	160 / 201.6	240 / 380.1	0	7.30	20	15	0	0
20.0%	400	80 / 100.9	320 / 506.8	0	7.60	20	15	0	0
10.0%	400	40 / 50.5	360 / 570.2	0	7.76	20	15	0	0
CRS w CRW	400	0	400 / 633.5	0	7.92	20	15	0	0
LCS w CRW	400	0	0	400 / 728.0	9.10	20	15	0	0
LCS w MHSW	400	0	0	400 / 728.0	9.10	0	0	20	15
MHSW	n/a	0	0	0	n/a	0	0	20	15

Data pages &amp; Calculations by:

QA/QC Check by:

Overlying waters + sediments dispensed on 2/16/11, Day -1.

DOD 3/9/11

MHSW = Moderately Hard Synthetic Freshwater  
 CRW = Clinch River Reference Water

	MHSW	MHSW	MHSW	CRW	Meter #
Date	02/16/2011	2/19/2011	2/22/2011	2/16/2011	III
Batch #	FW-016 -11	FW-018 -11	FW-019 -11	Delivered 02/12/2011	
Alkalinity	60	76	64	140	
Hardness	100	84	96	140	
Conductivity	299	320	312	313	A46
pH	8.1 54	8.1 54	8.2 54	8.2 54	Q8
DO	8.3 mg/l	8.3 mg/l	8.3 mg/l	8.3 mg/l	57
TRC	0.0 mg/l	0.0 mg/l	0.0 mg/l	0.04 mg/l	
Ammonia	<0.02 mg/l	<0.02 mg/l	<0.02 mg/l	0.04 mg/l	
Initial	0.0	0.0	0.0	0.0	

TRC: mg/l Alkalinity: mg/l as CaCO<sub>3</sub> Conductivity: µS/cm Hardness: mg/l as CaCO<sub>3</sub>

S 1002/16/11

Comments: CRM &0 sandy w/ some bark and a few leaves.

CRM 0.0 very clean and smooth.

**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**  
**CRM 0.0 Sediment & CRS Reference Sediment**

Test Organisms Age: 15.0 - 20.0 Hours Old

Test Organisms Source: EE USA Test Initiation At: 1400 on 2/17/2011  
 Counted by: David L. Daniel QC/QA by: Jeanne V. S.

Loaded by: David L. Daniel Organism Lot # C0021111-03

Exposure Chamber: 8 dram vials. Feeding: 0.1 ml *S. capricornutum* (Lot # S1-11) &  
 0.1 ml YCT (Lot # Y2-11) / 15 ml.

***C. dubia* Daily Survival & Reproduction Data**

Treatment: MHSW only.

REP	1	2	3	4	5	6	7	8	9	10	% Sur.	No. of Neonates Per Day	Tech
0	0	0	0	0	0	0	0	0	0	0	III	III	DUD
1	0	0	0	0	0	0	0	0	0	0	100	0	DUD
2	0	0	0	0	0	0	0	0	0	0	100	0	DUD
3	4	4	5	5	5	3	5	5	5	100	45	0	DUD
4	0	0	0	0	0	12	0	0	0	0	100	12	DUD
5	8	7	9	8	8	10	8	8	6	8	100	80	DUD
6	12	8	10	9	12	3	11	10	9	10	100	74	DUD
7						(A)							
	3rd Brood Reproduction Per Replicate										Mean	CV %	
	24	19	23	22	25	30	22	23	20	23	23.1	13.0	

Comments: (A) split brood. dud 2/21/11

**C. dubia Daily Survival & Reproduction Data Cont.**

Treatment: LCS w MHSW.														
	REP	11	12	13	14	15	16	17	18	19	20	% Sur.	No. of Neonates Per Day	Tech
0	0	0	0	0	0	0	0	0	0	0	0	100	100	D4D
1	0	0	0	0	0	0	0	0	0	0	0	100	0	D4D
2	0	0	0	0	0	0	0	0	0	0	0	100	0	D4D
3	5	6	6	6	6	4	6	5	6	6	100	56	D4D	
4	0	0	0	0	0	10	0	0	0	0	0	100	10	D4D
5	6	9	8	8	10	0	9	8	9	8	100	75	D4D	
6	9	10	9	10	12	12	8	12	11	9	100	102	D4D	
7														
	3rd Brood Reproduction Per Replicate											Mean	CV %	
	20	25	23	24	28	26	23	25	26	23	24.3	9.11		

Treatment: LCS w CRW.														
	REP	21	22	23	24	25	26	27	28	29	30	% Sur.	No. of Neonates Per Day	Tech
0	0	0	0	0	0	0	0	0	0	0	0	100	100	D4D
1	0	0	0	0	0	0	0	0	0	0	0	100	0	D4D
2	0	0	0	0	0	0	0	0	0	0	0	100	0	D4D
3	6	6	5	5	6	6	6	5	6	6	100	57	D4D	
4	0	0	0	0	0	0	0	0	0	0	0	100	0	D4D
5	11	10	10	5	8	10	12	9	10	10	10	100	95	D4D
6	15	18	16	15	16	13	14	15	14	11	100	147	D4D	
7														
	3rd Brood Reproduction Per Replicate											Mean	CV %	
	32	34	31	25	30	29	32	29	30	27	29.9	8.70		

Comments:

TVA, CRM 0.0, Site Sediment  
TVA, CRS, Reference Sediment  
TVA, CRW, River Water

E-087-11  
E-095-11  
E-086-11

**C. dubia Daily Survival & Reproduction Data Cont.**

	REP	Treatment: CRS w CRW.												% Sur.	No. of Neonates Per Day	Tech
		31	32	33	34	35	36	37	38	39	40					
	0	0	0	0	0	0	0	0	0	0	0		///	///	DUP	
	1	0	0	0	0	0	0	0	0	0	0		100	0	DUP	
	2	0	0	0	0	0	0	0	0	0	0		100	0	DUP	
	3	5	5	5	6	6	4	0	6	5	6		100	48	DUP	
	4	0	0	0	0	0	13	7	0	0	0		100	20	DUP	
	5	12	11	14	11	12	0	11	14	12	13		100	110	DUP	
	6	18	15	17	20	18	22	15	0	16	14		100	155	DUP	
	7															
	3rd Brood Reproduction Per Replicate												Mean	CV %	//////	
	35	31	36	37	36	39	33	20	33	33	33	33	15.1			

	REP	Treatment: 10% CRM 0.0 w CRW.												% Sur.	No. of Neonates Per Day	Tech
		41	42	43	44	45	46	47	48	49	50					
	0	0	0	0	0	0	0	0	0	0	0		///	///	DUP	
	1	0	0	0	0	0	0	0	0	0	0		100	0	DUP	
	2	0	0	0	0	0	0	0	0	0	0		100	0	DUP	
	3	6	5	7	5	5	4	6	5	6	6		100	55	DUP	
	4	0	0	0	0	0	10	0	0	0	0		100	10	DUP	
	5	10	15	12	13	12	0	13	11	14	14		100	114	DUP	
	6	22	20	24	18	16	21	19	15	18	18		100	191	DUP	
	7															
	3rd Brood Reproduction Per Replicate												Mean	CV %	//////	
	38	40	43	36	33	35	38	31	38	38	320		9.28			

**Comments:**

TVA, CRM 0.0, Site Sediment  
 TVA, CRS, Reference Sediment  
 TVA, CRW, River Water

E-087-11  
 E-095-11  
 E-086-11

**C. dubia Daily Survival & Reproduction Data Cont.****Treatment: 20% CRM 0.0 w CRW.**

	REP	51	52	53	54	55	56	57	58	59	60	% Sur.	No. of Neonates Per Day	Tech
		0	0	0	0	0	0	0	0	0	0	100	0	040
1	0	0	0	0	0	0	0	0	0	0	0	100	0	040
2	0	0	0	0	0	0	0	0	0	0	0	100	0	040
3	6	6	6	5	5	4	6	6	5	5	5	100	54	040
4	0	0	0	0	0	10	0	0	0	0	0	100	10	040
5	13	12	14	10	12	15	14	11	14	10	10	100	125	040
6	21	21	18	20	17	0	16	16	15	18	100	162	D4P	
7														
3rd Brood Reproduction Per Replicate												Mean	CV %	111111
	40	39	38	35	34	29	36	33	34	33	35.1	9.35		

**Treatment: 40% CRM 0.0 w CRW.**

	REP	61	62	63	64	65	66	67	68	69	70	% Sur.	No. of Neonates Per Day	Tech
		0	0	0	0	0	0	0	0	0	0	100	0	040
1	0	0	0	0	0	0	0	0	0	0	0	100	0	040
2	0	0	0	0	0	0	0	0	0	0	0	100	0	040
3	7	5	6	6	5	6	7	6	6	7	7	100	61	040
4	0	0	0	1	0	12 <sup>A</sup>	0	0	0	0	0	100	14	040
5	11	10	12	9	10	14	14	11	13	11	100	115	040	
6	18	14	15	25	21	0	20	18	18	20	100	749169	D4P	
7													(B)	
3rd Brood Reproduction Per Replicate												Mean	CV %	111111
	36	29	33	41	36	33	41	35	37	38	33.9	(B) 10.3		

35.9

Comments: (A) 13 040 2/21/11

(B) wrong data 36-022811

*C. dubia* Daily Survival & Reproduction Data Cont.

	REP	Treatment: 60% CRM 0.0 w CRW.										% Sur.	No. of Neonates Per Day	Tech
		71	72	73	74	75	76	77	78	79	80			
	0	0	0	0	0	0	0	0	0	0	0	100	100	D40
	1	0	0	0	0	0	0	0	0	0	0	100	0	D40
	2	0	0	0	0	0	0	0	0	0	0	100	0	D40
	3	5	5	6	6	6	6	5	5	6	100	(B) 56	AP	
	4	0	0	0	0	0	12	0	0	0	0	100	12	D40
	5	10	14	11	10	13	0	14	10	12	12	100	106	D40
	6	17	17	23	20	15	24	18	18	21	17	100	190	D40
	7													
	3rd Brood Reproduction Per Replicate										Mean	CV %	////////	
	32	36	40	36	34	42	38	33	38	35	35.8	(B) 8.61		
											36.4			

	REP	Treatment: 80% CRM 0.0 w CRW.										% Sur.	No. of Neonates Per Day	Tech
		81	82	83	84	85	86	87	88	89	90			
	0	0	0	0	0	0	0	0	0	0	0	100	100	D40
	1	0	0	0	0	0	0	0	0	0	0	100	0	D40
	2	0	0	0	0	0	0	0	0	0	0	100	0	D40
	3	5	5	6	6	5	6	7	5	5	6	100	56	D40
	4	0	0	0	0	0	11	0	0	0	0	100	11	D40
	5	8	10	12	10	11	(9)	10	12	13	11	100	106	D40
	6	20	17	15	18	15	(11)	23	18	20	18	100	175	D40
	7						(A)							
	3rd Brood Reproduction Per Replicate										Mean	CV %	////////	
	33	32	33	34	31	37	40	35	38	35	34.8	8.11		

Comments: (A) split brood D40 2/23/11

(B) wrong data DC-022811

## C. dubia Daily Survival &amp; Reproduction Data Cont.

Treatment: 100% CRM 0.0 w CRW.														
	REP	91	92	93	94	95	96	97	98	99	100	% Sur.	No. of Neonates Per Day	Tech
	0	0	0	0	0	0	0	0	0	0	0	111	111	040
	1	0	0	0	0	0	0	0	0	0	0	100	0	040
	2	0	0	0	0	0	0	0	0	0	0	100	0	040
	3	5	6	7	6	6	6	5	6	7	100	(A) 54 (60)	040	1112
	4	0	0	0	0	0	12	0	0	0	0	100	12	040
	5	10	10	12	10	10	10	12	10	10	11	100	105	040
	6	19	15	17	21	20	0	17	22	22	18	100	171	040
	7													
	3rd Brood Reproduction Per Replicate												Mean	CV %
	34	31	36	37	36	28	35	37	38	36	34.2	34.8	8.86	11111

Calculations by: Beth R. SmithQA/QC by: Jennifer GriffithData Entry by: Beth R. SmithDouble Data Entry by: Beth R. Smith orQA/QC Officer: n/a

Comments: Day 0: water very clear prior to renewal/turbid after but settles out fast. 040

Day 1: no problem finding neonates. 040

(A) wrong data JG-022811

**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 % CRM 0.0						Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	
02/16/11	MHSW	LCS + MHSW									
DO	8.1	8.1	8.3	7.5	7.2	7.7	7.8	7.6	7.4	7.1	57
Temp	25.4	25.4	24.6	24.4	24.4	24.5	24.5	24.5	24.6	24.5	A46
Tech. Initials	Initials: D40										
Times	Initial Time: 1605										

Day 0	Controls				Treatment % CRM 0.0						Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	
02/17/11	MHSW	LCS + MHSW									
DO	F 7.6	7.6	7.4	6.5	6.4	6.4	6.1	5.7	5.8	6.3	57
	I 7.6	7.5	7.5	6.5	6.6	6.4	6.2	6.1	6.0	6.1	57
Temp	F 24.8	25.0	24.7	24.9	24.7	24.6	24.5	24.6	24.6	24.5	A46
	I 25.1	25.1	25.1	25.3	25.3	25.1	25.4	25.4	25.2	25.1	A46
Tech. Initials	Finals: D40										
Times	Final Time: 1300										Initial Time: 1520

Day 1	Controls				Treatment % CRM 0.0						Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	
02/18/11	MHSW	LCS + MHSW									
DO	F 7.4	7.4	7.3	6.4	5.8	5.7	5.7	5.5	5.4	5.8	57
	I 7.5	7.4	7.6	6.2	6.0	6.0	6.0	5.4	5.7	6.1	57
Temp	F 25.0	24.8	24.7	25.1	25.3	25.0	25.0	25.2	24.9	25.1	A46
	I 24.8	24.7	24.8	24.8	24.6	24.6	24.4	24.1	24.8	24.8	A46
Tech. Initials	Finals: D40										
Times	Final Time: 0915										Initial Time: 1410

TVA, CRM 0.0, Site Sediment  
TVA, CRS, Reference Sediment  
TVA, CRW, River Water

E-087-11  
E-095-11  
E-086-11

**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 % CRM 0.0							Meter #
	MHSW		Clinch River Water									
02/19/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%		
DO F	7.5	7.6	7.5	6.0	6.0	5.8	5.4	5.4	5.5	5.4	57	
I	7.6	7.6	7.4	6.3	5.8	5.5	5.2	5.5	5.6	5.6	57	
Temp F	25.0	24.9	25.1	25.1	24.8	24.8	24.7	24.9	25.2	25.0	A46	
I	25.2	25.3	25.3	25.4	25.3	25.4	25.4	25.4	25.3	25.3	A46	
Tech. Initials	Finals: D4P									Initials: D4P		
Times	Final Time: 0810									Initial Time: 1505		

Day 3	Controls				Treatment % CRM 0.0							Meter #
	MHSW		Clinch River Water									
02/20/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%		
DO F	7.6	7.4	7.4	6.2	5.9	6.3	6.3	6.4	6.2	6.1	57	
I	7.4	7.4	7.0	6.0	5.5	5.5	5.5	5.7	5.7	5.9	57	
Temp F	24.9	25.0	24.6	24.8	25.1	25.0	24.8	24.9	24.7	24.9	A46	
I	25.4	25.4	25.4	25.5	25.3	25.4	25.3	25.6	25.4	25.4	A46	
Tech. Initials	Finals: D4P									Initials: D4P		
Times	Final Time: 0940									Initial Time: 1238		

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 % CRM 0.0							Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
02/21/11	MHSW	LCS + MHSW										
DO	F	7.2	7.3	7.2	5.7	5.4	5.4	5.6	5.7	5.3	5.1	57
	I	7.4	7.2	7.5	6.0	6.2	6.0	6.0	5.8	5.8	5.6	57
Temp	F	25.1	25.0	25.0	24.8	25.2	25.0	25.3	25.1	25.0	25.1	A46
	I	24.6	24.6	24.6	24.5	24.6	24.8	24.6	24.5	24.6	24.6	A46
Tech. Initials		Finals: D4D				Initials: D4D						
Times		Final Time: 0921				Initial Time: 1335						

Day 5	Controls				Treatment % CRM 0.0							Meter #
	MHSW		LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
02/22/11	MHSW	LCS + MHSW										
DO	F	7.2	7.2	7.3	5.9	5.1	5.3	5.4	5.8	5.7	5.0	57
	I	7.0	7.0	7.2	6.2	5.9	5.7	5.9	6.0	6.1	6.0	57
Temp	F	24.5	24.6	24.5	24.4	24.3	24.4	24.3	24.5	24.4	24.4	A46
	I	24.5	24.3	24.3	24.3	24.2	24.4	24.3	24.4	24.4	24.6	A46
Tech. Initials		Finals: D4D				Initials: DCP						
Times		Final Time: 0725				Initial Time: 1505						

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 6	Controls				Treatment % CRM 0.0						Meter #
	MHSW		Clinch River Water								
02/23/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO	F	7.3	7.3	7.0	6.7	5.5	5.5	5.0	5.3	5.4	5.6
	I										
Temp	F	24.7	25.0	25.0	24.8	24.5	24.4	24.6	24.4	24.4	24.5
	I										
Tech. Initials	Finals: DLD						Initials: M/A				
Times	Final Time: 0900						Initial Time: M/A				

Day 7	Controls				Treatment % CRM 0.0						Meter #
	MHSW		TVA Supplied Clinch River Water								
02/24/11	MHSW	LCS + MHSW	LCS + CRW	CRS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F											
Temp F											
Tech. Initials	Finals:				Initials:				<i>MM</i>		
Times	Final Time:				Initial Time:				<i>MM</i>		

**Comments:**

**Environmental Enterprises USA, Inc.**

## **APPENDIX B**

**Ceriodaphnia Survival and Reproduction Test-7 Day Survival**

Start Date: 2/17/2011 Test ID: cd08711 Sample ID: CRM0.0  
 End Date: 2/23/2011 Lab ID: EE USA Sample Type: Whole Sediment  
 Sample Date: 2/7/2011 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
IC5	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

Start Date: 2/17/2011 Test ID: cd08711 Sample ID: CRM0.0  
 End Date: 2/23/2011 Lab ID: EE USA Sample Type: Whole Sediment  
 Sample Date: 2/7/2011 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	32.000	34.000	31.000	25.000	30.000	29.000	32.000	29.000	30.000	27.000
CRS+CRW	35.000	31.000	36.000	37.000	36.000	39.000	33.000	20.000	33.000	33.000
10	38.000	40.000	43.000	36.000	33.000	35.000	38.000	31.000	38.000	38.000
20	40.000	39.000	38.000	35.000	34.000	29.000	36.000	33.000	34.000	33.000
40	36.000	29.000	33.000	41.000	36.000	33.000	41.000	35.000	37.000	38.000
60	32.000	36.000	40.000	36.000	34.000	42.000	38.000	33.000	38.000	35.000
80	33.000	32.000	33.000	34.000	31.000	37.000	40.000	35.000	38.000	35.000
100	34.000	31.000	36.000	37.000	36.000	28.000	35.000	37.000	38.000	36.000

Conc-%	Transform: Untransformed							1-Tailed		Isotonic		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
LCS+CRW	29.900	0.8979	29.900	25.000	34.000	8.700	10	*			35.540	1.0000
CRS+CRW	33.300	1.0000	33.300	20.000	39.000	15.703	10					
10	37.000	1.1111	37.000	31.000	43.000	9.275	10	-2.297	2.347	3.781	35.540	1.0000
20	35.100	1.0541	35.100	29.000	40.000	9.348	10	-1.117	2.347	3.781	35.540	1.0000
40	35.900	1.0781	35.900	29.000	41.000	10.293	10	-1.614	2.347	3.781	35.540	1.0000
60	36.400	1.0931	36.400	32.000	42.000	8.610	10	-1.924	2.347	3.781	35.540	1.0000
80	34.800	1.0450	34.800	31.000	40.000	8.105	10	-0.931	2.347	3.781	34.800	0.9792
100	34.800	1.0450	34.800	28.000	38.000	8.862	10	-0.931	2.347	3.781	34.800	0.9792

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution ( $p > 0.05$ )	0.631363	0.895	-0.87844	2.167536						
Bartlett's Test indicates equal variances ( $p = 0.56$ )	4.882866	16.81189								
The control means are not significantly different ( $p = 0.08$ )	1.840903	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	3.781187	0.113549	14.99048	12.97619	0.341603	6, 63

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-7 Day Survival**

Start Date:	2/17/2011	Test ID:	cd08711	Sample ID:	CRM0.0					
End Date:	2/23/2011	Lab ID:	EE USA	Sample Type:	Whole Sediment					
Sample Date:	2/7/2011	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	Not Resp			N	Fisher's Exact P	1-Tailed Critical
			Resp	Not Resp	Total		*	
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: 2/17/2011      Test ID: cd08711      Sample ID: CRM0.0  
 End Date: 2/23/2011      Lab ID: EE USA      Sample Type: Whole Sediment  
 Sample Date: 2/7/2011      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	19.000	23.000	22.000	25.000	30.000	22.000	23.000	20.000	23.000
LCS+MHSW	20.000	25.000	23.000	24.000	28.000	26.000	23.000	25.000	26.000	23.000
LCS+CRW	32.000	34.000	31.000	25.000	30.000	29.000	32.000	29.000	30.000	27.000

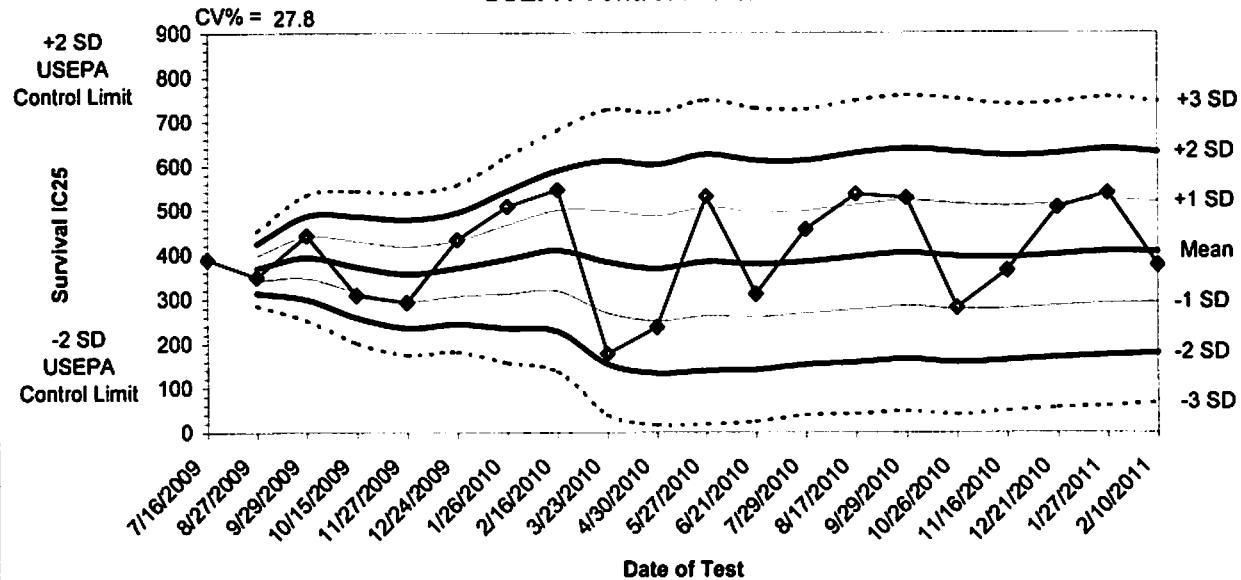
Conc-%	Transform: Untransformed							1-Tailed		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
MHSW	23.100	0.9506	23.100	19.000	30.000	12.979	10			
LCS+MHSW	24.300	1.0000	24.300	20.000	28.000	9.109	10	*		
LCS+CRW	29.900	1.2305	29.900	25.000	34.000	8.700	10	-5.185	1.734	1.873

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.968709	0.905	-0.35867	0.071586
F-Test indicates equal variances (p = 0.64)	1.380952	6.54109		
The control means are not significantly different (p = 0.32)	1.018234	2.100922		
Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE
Homoscedastic t Test indicates no significant differences	1.873003	0.077078	156.8	5.833333
Treatments vs LCS+MHSW				6.2E-05
			1, 18	

**Environmental Enterprises USA, Inc.**

## **APPENDIX C**

**C. dubia SRT, Survival IC25, mg/L KCl**  
**USEPA Control Limits**



Test #	Test Date	Survival IC25	Mean IC25	-1 SD	-2 SD	+1 SD	+2 SD	-3 SD	+3 SD	Toxicant Lot #
CD0909	7/16/2009	389								029K0050
CD0910	8/27/2009	350	370	342	314	397	425	287	452	029K0050
CD0911	9/29/2009	443	394	347	301	441	487	254	534	029K0050
CD0912	10/15/2009	310	373	316	260	430	486	203	543	029K0050
CD0913	11/27/2009	294	357	297	236	418	478	176	539	049K0305
CD0915	12/24/2009	433	370	307	245	432	495	183	557	049K0305
CD1002	1/26/2010	508	390	312	235	467	544	158	621	079K0011
CD1003	2/16/2010	546	409	319	228	500	590	138	680	079K0011
CD1004	3/23/2010	178	383	269	155	498	612	40	727	049K0305
CD1006	4/30/2010	238	369	252	134	486	603	17	721	049K0305
CD1007	5/27/2010	531	384	262	141	505	627	19	748	049K0305
CD1009	6/21/2010	312	378	260	142	495	613	25	731	049K0305
CD1010	7/29/2010	456	384	269	154	498	613	39	728	079K0011
CD1011	8/17/2010	536	395	277	160	512	630	42	747	079K0011
CD1012	9/29/2010	527	403	285	167	522	640	49	758	079K0011
CD1013	10/26/2010	281	396	277	159	514	632	41	751	099K0202
CD1014	11/16/2010	364	394	279	164	509	623	49	738	099K0202
CD1015	12/21/2010	506	400	286	171	515	629	57	744	099K0202
CD1101	1/27/2011	539	407	292	176	523	639	60	755	099K0202
CD1102	2/10/2011	376	406	293	180	519	632	67	744	099K0202

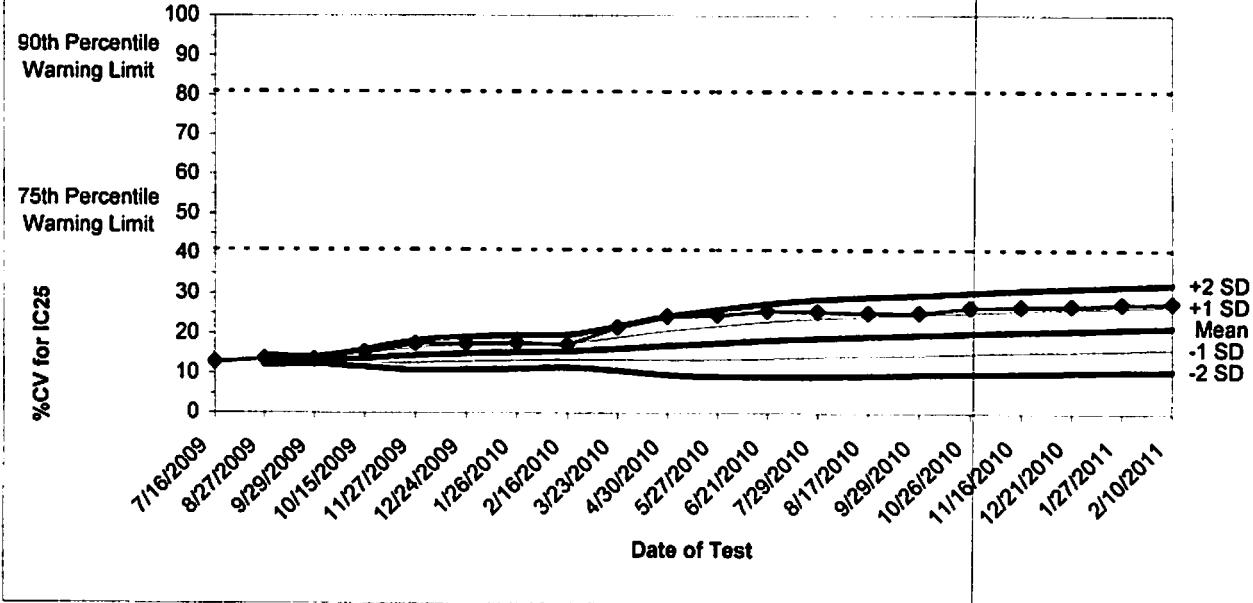
CD1005 - IC25 less than lowest concentration tested and could not be graphed

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

CD1001 - Training lab tech

CD0914 - Training lab tech

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



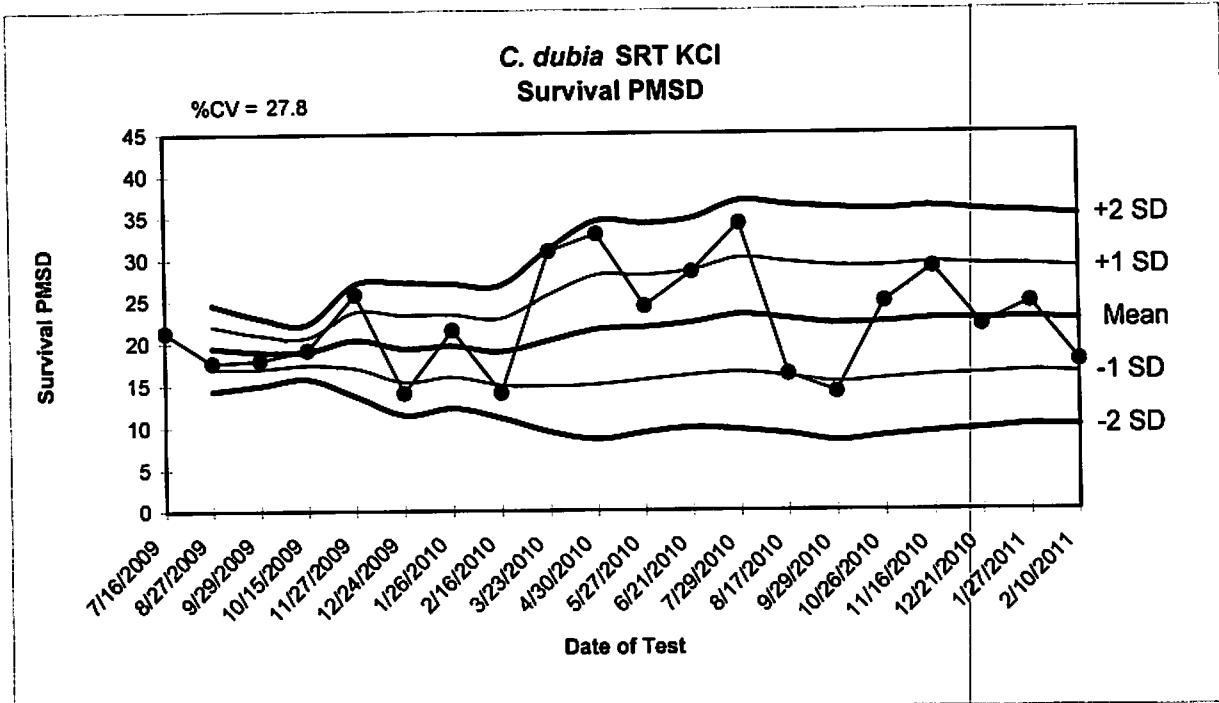
Test #	Test Date	%CV for IC25	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
CD0909	7/16/2009	12.8						41.0	81.0	029K0050
CD0910	8/27/2009	13.6	13.2	12.6	12.0	13.8	14.4	41.0	81.0	029K0050
CD0911	9/29/2009	13.4	13.3	12.8	12.4	13.7	14.1	41.0	81.0	029K0050
CD0912	10/15/2009	15.3	13.8	12.7	11.6	14.9	15.9	41.0	81.0	029K0050
CD0913	11/27/2009	17.4	14.5	12.6	10.8	16.3	18.2	41.0	81.0	049K0305
CD0915	12/24/2009	17.4	15.0	12.9	10.9	17.0	19.0	41.0	81.0	049K0305
CD1002	1/26/2010	17.6	15.3	13.2	11.1	17.5	19.6	41.0	81.0	079K0011
CD1003	2/16/2010	17.2	15.6	13.5	11.5	17.6	19.7	41.0	81.0	079K0011
CD1004	3/23/2010	21.5	16.2	13.5	10.7	19.0	21.7	41.0	81.0	049K0305
CD1006	4/30/2010	24.4	17.0	13.4	9.7	20.7	24.4	41.0	81.0	049K0305
CD1007	5/27/2010	24.7	17.7	13.6	9.4	21.9	26.1	41.0	81.0	049K0305
CD1009	6/21/2010	25.7	18.4	13.8	9.2	23.0	27.6	41.0	81.0	049K0305
CD1010	7/29/2010	25.5	19.0	14.1	9.3	23.8	28.6	41.0	81.0	079K0011
CD1011	8/17/2010	25.2	19.4	14.5	9.6	24.3	29.2	41.0	81.0	079K0011
CD1012	9/29/2010	25.3	19.8	14.8	9.8	24.8	29.8	41.0	81.0	079K0011
CD1013	10/26/2010	26.6	20.2	15.1	10.0	25.3	30.4	41.0	81.0	099K0202
CD1014	11/16/2010	26.8	20.6	15.4	10.2	25.8	31.0	41.0	81.0	099K0202
CD1015	12/21/2010	27.1	21.0	15.7	10.4	26.2	31.5	41.0	81.0	099K0202
CD1101	1/27/2011	27.6	21.3	16.0	10.6	26.7	32.0	41.0	81.0	099K0202
CD1102	2/10/2011	27.8	21.6	16.2	10.8	27.0	32.4	41.0	81.0	099K0202

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

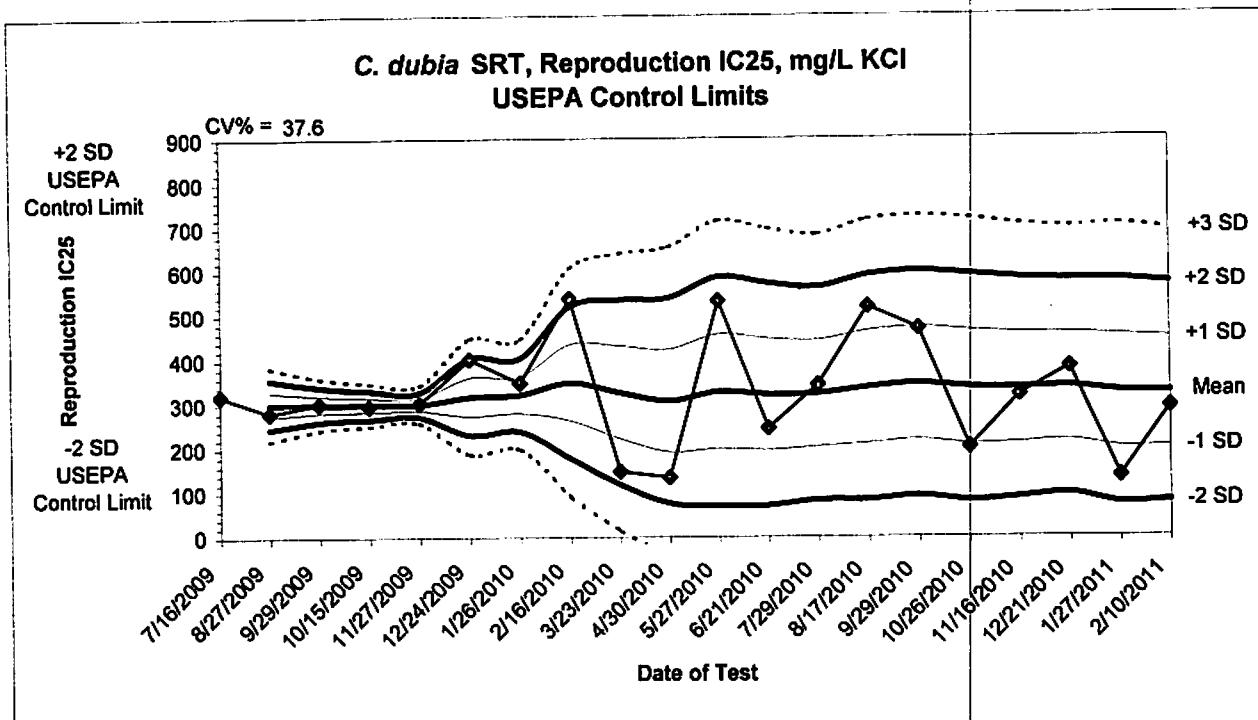
CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl

CD1001 - Training lab tech

CD0914 - Training lab tech



Test #	Test Date	Survival PMSD	Mean	-1 SD	-2 SD	+1 SD	+2 SD	Toxicant Lot #
CD0909	7/16/2009	21.3						029K0050
CD0910	8/27/2009	17.7	19.5000	16.9544	14.4088	22.0456	24.5912	029K0050
CD0911	9/29/2009	18.0	19.0000	17.0025	15.0050	20.9975	22.9950	029K0050
CD0912	10/15/2009	19.2	19.0500	17.4160	15.7820	20.6840	22.3180	029K0050
CD0913	11/27/2009	25.8	20.4000	17.0661	13.7322	23.7339	27.0678	049K0305
CD0915	12/24/2009	14.1	19.3500	15.4121	11.4742	23.2879	27.2258	049K0305
CD1002	1/26/2010	21.5	19.6571	15.9717	12.2862	23.3426	27.0281	079K0011
CD1003	2/16/2010	14.1	18.9625	15.0252	11.0878	22.8998	26.8372	079K0011
CD1004	3/23/2010	30.9	20.2889	14.8668	9.4448	25.7109	31.1330	049K0305
CD1006	4/30/2010	33.0	21.5600	15.0570	8.5540	28.0630	34.5660	049K0305
CD1007	5/27/2010	24.3	21.8091	15.5847	9.3603	28.0335	34.2579	049K0305
CD1009	6/21/2010	28.4	22.3583	16.1261	9.8939	28.5906	34.8228	049K0305
CD1010	7/29/2010	34.2	23.2692	16.4582	9.6471	30.0803	36.8914	079K0011
CD1011	8/17/2010	16.2	22.7643	15.9531	9.1420	29.5754	36.3866	079K0011
CD1012	9/29/2010	14.1	22.1867	15.2525	8.3183	29.1208	36.0550	079K0011
CD1013	10/26/2010	24.8	22.3500	15.6192	8.8884	29.0808	35.8116	099K0202
CD1014	11/16/2010	28.9	22.7353	16.0274	9.3195	29.4432	36.1511	099K0202
CD1015	12/21/2010	22.0	22.6944	16.1845	9.6746	29.2044	35.7143	099K0202
CD1101	1/27/2011	24.7	22.8000	16.4568	10.1135	29.1432	35.4865	099K0202
CD1102	2/10/2011	17.7	22.5450	16.2665	9.9880	28.8235	35.1020	099K0202



Test #	Test Date	Repro. IC25	Mean IC25	-1 SD	-2 SD	+1 SD	+2 SD	-3 SD	+3 SD	Toxicant Lot #
CD0909	7/16/2009	321								029K0050
CD0910	8/27/2009	282	302	274	246	329	357	219	384	029K0050
CD0911	9/29/2009	303	302	282	263	322	341	243	361	029K0050
CD0912	10/15/2009	297	301	285	268	317	333	252	349	029K0050
CD0913	11/27/2009	304	301	287	273	315	329	259	344	049K0305
CD0915	12/24/2009	403	318	275	232	362	405	188	448	049K0305
CD1002	1/26/2010	349	323	281	240	364	405	199	446	079K0011
CD1003	2/16/2010	539	350	264	179	435	521	93	606	079K0011
CD1004	3/23/2010	149	327	223	119	432	536	15	640	049K0305
CD1006	4/30/2010	135	308	193	77	424	539	-39	655	049K0305
CD1007	5/27/2010	533	329	200	71	458	586	-58	715	049K0305
CD1009	6/21/2010	246	322	197	71	447	572	-54	697	049K0305
CD1010	7/29/2010	343	323	203	83	443	563	-37	683	079K0011
CD1011	8/17/2010	518	337	211	84	464	590	-42	717	079K0011
CD1012	9/29/2010	469	346	220	93	473	599	-34	726	079K0011
CD1013	10/26/2010	202	337	210	82	465	592	-45	719	099K0202
CD1014	11/16/2010	320	336	213	89	460	583	-34	707	099K0202
CD1015	12/21/2010	382	339	218	98	459	579	-22	699	099K0202
CD1101	1/27/2011	136	328	202	76	454	580	-49	705	099K0202
CD1102	2/10/2011	292	326	203	81	449	572	-42	694	099K0202

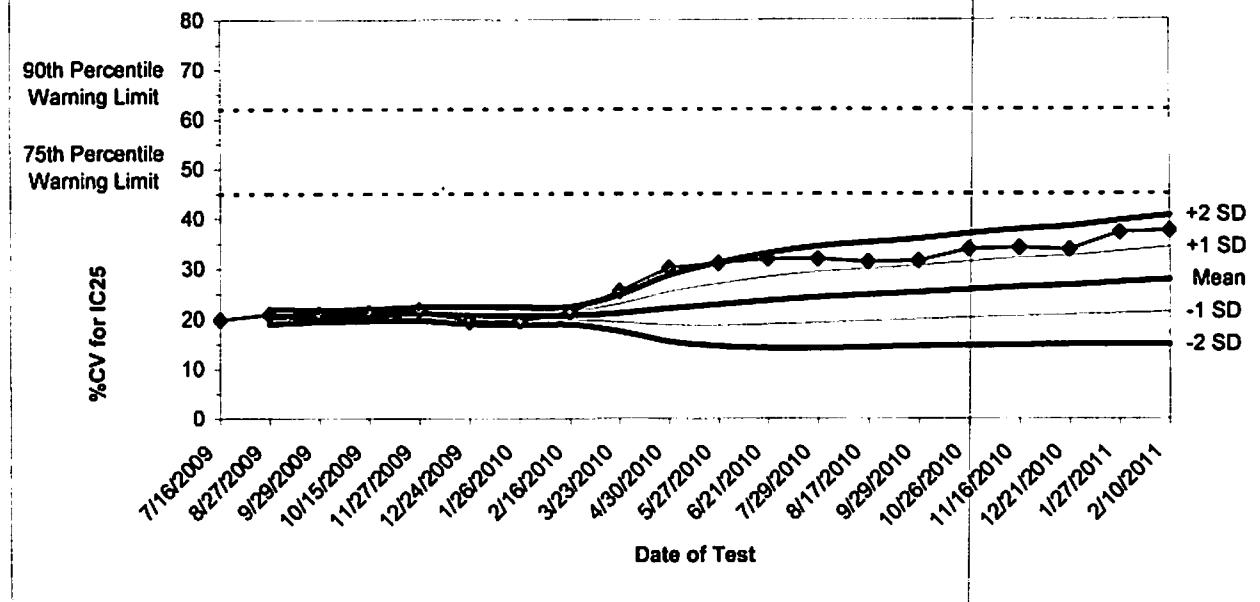
CD1005 - IC25 less than lowest concentration tested and could not be graphed

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

CD1001 - Training lab tech

CD0914 - Training lab tech

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



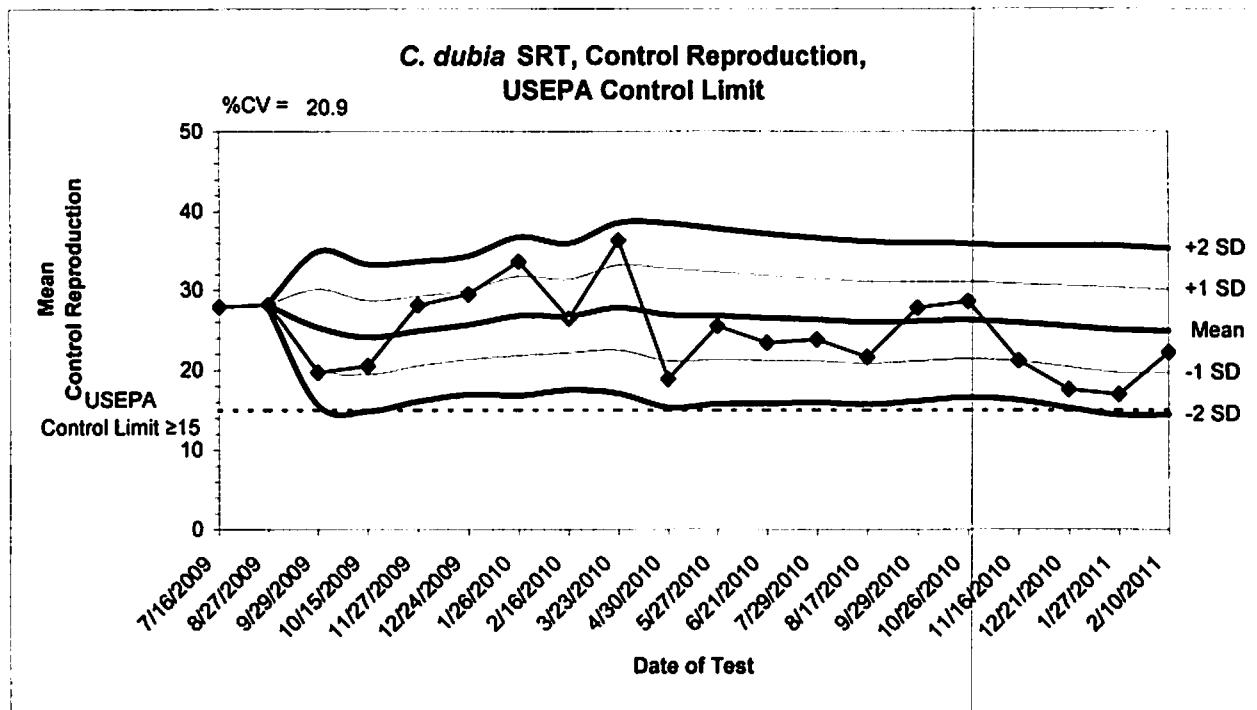
Test #	Test Date	%CV for IC25	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
CD0909	7/16/2009	19.9						45.0	62.0	029K0050
CD0910	8/27/2009	21.0	20.4	19.7	19.0	21.2	21.9	45.0	62.0	029K0050
CD0911	9/29/2009	21.0	20.6	20.0	19.4	21.2	21.8	45.0	62.0	029K0050
CD0912	10/15/2009	21.3	20.8	20.2	19.6	21.4	21.9	45.0	62.0	029K0050
CD0913	11/27/2009	21.8	21.0	20.3	19.6	21.7	22.3	45.0	62.0	049K0305
CD0915	12/24/2009	19.4	20.7	19.9	19.0	21.6	22.5	45.0	62.0	049K0305
CD1002	1/26/2010	19.6	20.6	19.6	18.7	21.5	22.4	45.0	62.0	079K0011
CD1003	2/16/2010	21.3	20.7	19.8	18.9	21.5	22.4	45.0	62.0	079K0011
CD1004	3/23/2010	25.5	21.2	19.4	17.6	23.0	24.8	45.0	62.0	049K0305
CD1006	4/30/2010	30.1	22.1	18.8	15.5	25.4	28.7	45.0	62.0	049K0305
CD1007	5/27/2010	31.1	22.9	18.8	14.6	27.0	31.2	45.0	62.0	049K0305
CD1009	6/21/2010	31.9	23.7	18.9	14.2	28.4	33.1	45.0	62.0	049K0305
CD1010	7/29/2010	31.9	24.3	19.2	14.1	29.4	34.4	45.0	62.0	079K0011
CD1011	8/17/2010	31.3	24.8	19.6	14.3	30.0	35.2	45.0	62.0	079K0011
CD1012	9/29/2010	31.6	25.2	19.9	14.6	30.6	35.9	45.0	62.0	079K0011
CD1013	10/26/2010	33.9	25.8	20.2	14.6	31.4	36.9	45.0	62.0	099K0202
CD1014	11/16/2010	34.1	26.3	20.5	14.7	32.0	37.8	45.0	62.0	099K0202
CD1015	12/21/2010	33.8	26.7	20.8	14.9	32.6	38.4	45.0	62.0	099K0202
CD1101	1/27/2011	37.2	27.2	21.0	14.9	33.4	39.6	45.0	62.0	099K0202
CD1102	2/10/2011	37.6	27.8	21.3	14.8	34.2	40.7	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

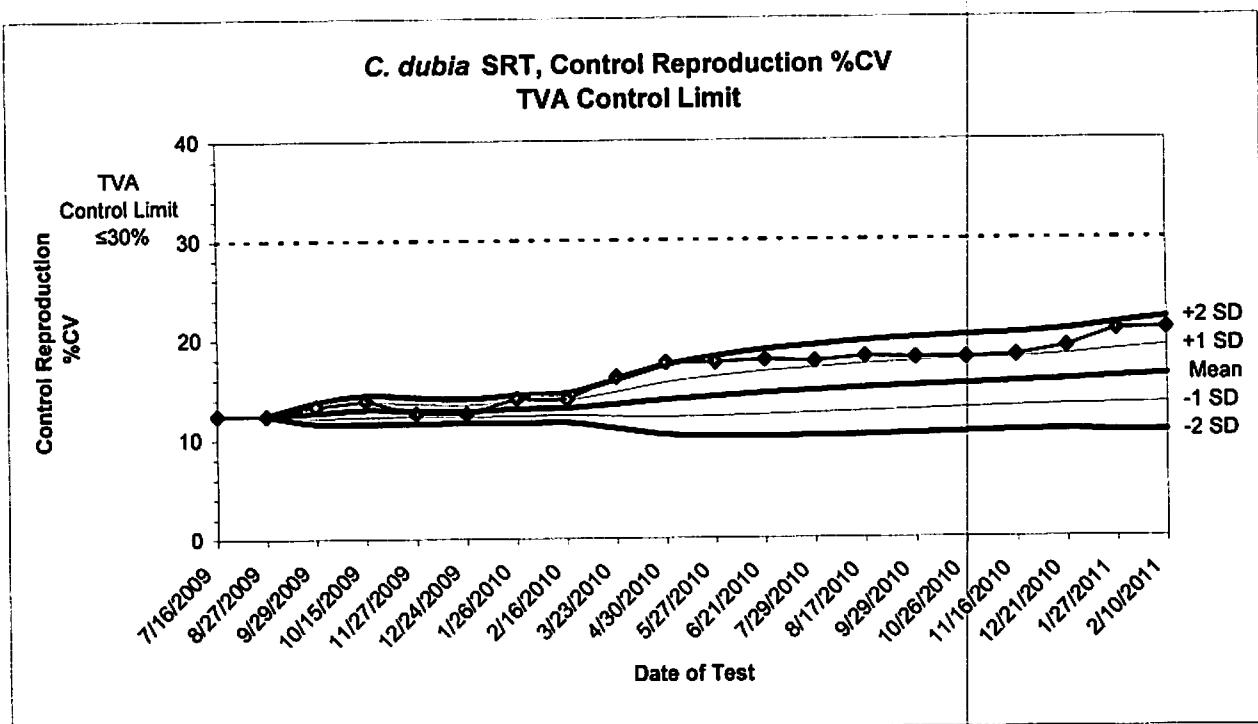


Test #	Test Date	Mean Control Repro.	Mean	-1 SD	-2 SD	+1 SD	+2 SD		Control Limit	Toxicant Lot #
CD0909	7/16/2009	27.9							15.0	029K0050
CD0910	8/27/2009	28.2	28.1	27.8	27.6	28.3	28.5		15.0	029K0050
CD0911	9/29/2009	19.7	25.3	20.4	15.6	30.1	34.9		15.0	029K0050
CD0912	10/15/2009	20.5	24.1	19.5	14.9	28.7	33.3		15.0	029K0050
CD0913	11/27/2009	28.1	24.9	20.5	16.1	29.3	33.6		15.0	049K0305
CD0915	12/24/2009	29.5	25.7	21.3	17.0	30.0	34.3		15.0	049K0305
CD1002	1/26/2010	33.6	26.8	21.8	16.8	31.8	36.7		15.0	079K0011
CD1003	2/16/2010	26.4	26.7	22.1	17.5	31.3	36.0		15.0	079K0011
CD1004	3/23/2010	36.3	27.8	22.4	17.1	33.2	38.5		15.0	049K0305
CD1006	4/30/2010	18.9	26.9	21.1	15.3	32.7	38.5		15.0	049K0305
CD1007	5/27/2010	25.5	26.8	21.3	15.8	32.3	37.8		15.0	049K0305
CD1009	6/21/2010	23.4	26.5	21.2	15.8	31.8	37.2		15.0	049K0305
CD1010	7/29/2010	23.8	26.3	21.1	16.0	31.5	36.6		15.0	079K0011
CD1011	8/17/2010	21.6	26.0	20.8	15.7	31.1	36.2		15.0	079K0011
CD1012	9/29/2010	27.8	26.1	21.1	16.2	31.0	36.0		15.0	079K0011
CD1013	10/26/2010	28.6	26.2	21.4	16.6	31.1	35.9		15.0	099K0202
CD1014	11/16/2010	21.2	25.9	21.1	16.3	30.8	35.6		15.0	099K0202
CD1015	12/21/2010	17.6	25.5	20.4	15.3	30.6	35.6		15.0	099K0202
CD1101	1/27/2011	17.0	25.0	19.7	14.4	30.3	35.7		15.0	099K0202
CD1102	2/10/2011	22.2	24.9	19.7	14.5	30.1	35.3		15.0	099K0202

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

CD1001 - Training lab tech

CD0914 - Training lab tech



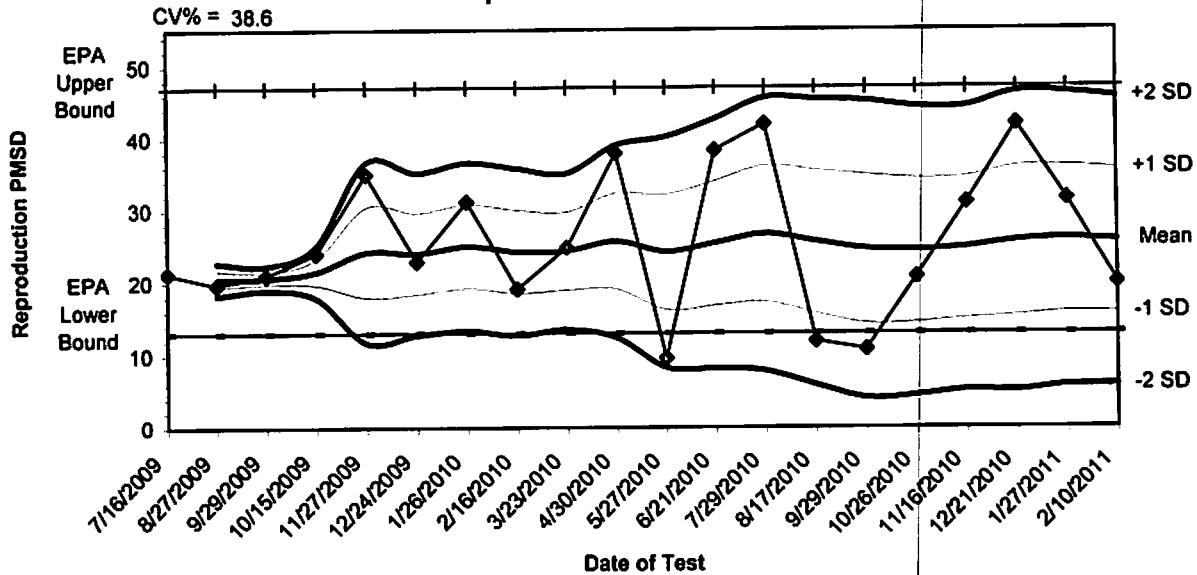
Test #	Test Date	Control Repro. %CV	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD		Control Limit	Toxicant Lot #
CD0909	7/16/2009	12.4							30.0	029K0050
CD0910	8/27/2009	12.4	12.4	12.4	12.3	12.4	12.4		30.0	029K0050
CD0911	9/29/2009	13.3	12.7	12.1	11.6	13.2	13.8		30.0	029K0050
CD0912	10/15/2009	13.8	13.0	12.3	11.5	13.7	14.4		30.0	029K0050
CD0913	11/27/2009	12.6	12.9	12.2	11.6	13.6	14.2		30.0	049K0305
CD0915	12/24/2009	12.5	12.8	12.2	11.6	13.4	14.0		30.0	049K0305
CD1002	1/26/2010	14.1	13.0	12.3	11.6	13.7	14.5		30.0	079K0011
CD1003	2/16/2010	14.0	13.1	12.4	11.6	13.9	14.6		30.0	079K0011
CD1004	3/23/2010	16.2	13.5	12.2	11.0	14.7	15.9		30.0	049K0305
CD1006	4/30/2010	17.6	13.9	12.1	10.4	15.6	17.4		30.0	049K0305
CD1007	5/27/2010	17.6	14.2	12.2	10.2	16.2	18.2		30.0	049K0305
CD1009	6/21/2010	17.9	14.5	12.3	10.2	16.7	18.9		30.0	049K0305
CD1010	7/29/2010	17.7	14.8	12.5	10.2	17.0	19.3		30.0	079K0011
CD1011	8/17/2010	18.2	15.0	12.7	10.3	17.4	19.7		30.0	079K0011
CD1012	9/29/2010	18.0	15.2	12.8	10.4	17.6	20.0		30.0	079K0011
CD1013	10/26/2010	18.0	15.4	13.0	10.5	17.8	20.2		30.0	099K0202
CD1014	11/16/2010	18.2	15.6	13.1	10.7	18.0	20.5		30.0	099K0202
CD1015	12/21/2010	19.1	15.8	13.2	10.7	18.3	20.8		30.0	099K0202
CD1101	1/27/2011	20.8	16.0	13.3	10.6	18.7	21.4		30.0	099K0202
CD1102	2/10/2011	20.9	16.3	13.4	10.6	19.1	22.0		30.0	099K0202

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

CD1001 - Training lab tech

CD0914 - Training lab tech

***C. dubia* SRT KCI  
Reproduction PMSD**



Test #	Test Date	Reprod. PMSD	Mean PMSD	-1 SD	-2 SD	+1 SD	+2 SD	Upper PMSD Bound	Lower PMSD Bound	Toxicant Lot #
CD0909	7/16/2009	21.3	20.5000	19.3686	18.2373	21.6314	22.7627	47	13	029K0050
CD0910	8/27/2009	19.7	20.6667	19.8162	18.9657	21.5172	22.3676	47	13	029K0050
CD0911	9/29/2009	21.0	21.5000	19.6945	17.8889	23.3055	25.1111	47	13	029K0050
CD0912	10/15/2009	24.0	24.2000	17.9634	11.7268	30.4366	36.6732	47	13	049K0305
CD0913	11/27/2009	35.0	23.9833	18.3800	12.7766	29.5867	35.1901	47	13	049K0305
CD0915	12/24/2009	22.9	25.0143	19.2173	13.4204	30.8112	36.6082	47	13	079K0011
CD1002	1/26/2010	31.2	24.2750	18.5151	12.7552	30.0349	35.7948	47	13	079K0011
CD1003	2/16/2010	19.1	24.3333	18.9426	13.5519	29.7241	35.1148	47	13	049K0305
CD1004	3/23/2010	24.8	25.6900	19.0389	12.3879	32.3411	38.9921	47	13	049K0305
CD1006	4/30/2010	37.9	24.2273	16.2681	8.3090	32.1864	40.1456	47	13	049K0305
CD1007	5/27/2010	9.6	25.4000	16.7923	8.1846	34.0077	42.6154	47	13	049K0305
CD1009	6/21/2010	38.3	26.6692	17.2426	7.8160	36.0958	45.5224	47	13	079K0011
CD1010	7/29/2010	41.9	25.6143	15.7347	5.8551	35.4939	45.3734	47	13	079K0011
CD1011	8/17/2010	11.9	25.6143	14.3668	4.1069	34.8865	45.1464	47	13	079K0011
CD1012	9/29/2010	10.8	24.6267	14.4208	4.4603	34.3417	44.3022	47	13	099K0202
CD1013	10/26/2010	20.7	24.7706	14.9937	5.2168	34.5475	44.3243	47	13	099K0202
CD1014	11/16/2010	31.0	25.7222	15.4137	5.1052	36.0307	46.3392	47	13	099K0202
CD1015	12/21/2010	41.9	25.7222	15.4137	5.1052	36.1317	46.2371	47	13	099K0202
CD1101	1/27/2011	31.5	26.0263	15.9209	5.8156	35.6508	45.5816	47	13	099K0202
CD1102	2/10/2011	19.9	25.7200	15.7892	5.8584	35.6508	45.5816	47	13	099K0202

**Environmental Enterprises USA, Inc.**

**APPENDIX D**

Locations: TVA - KIF - NTC - TCA - 001, 002, 003

## BIOMONITORING CHAIN OF CUSTODY RECORD

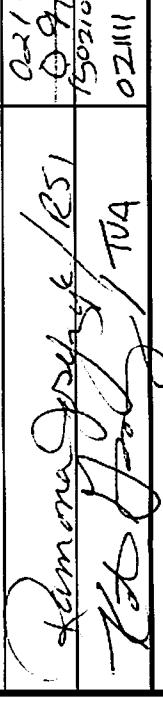
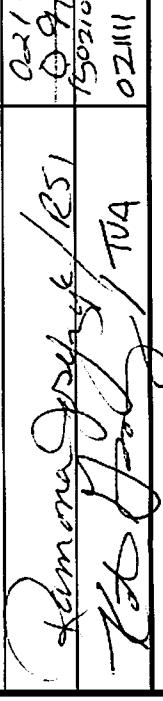
Page 1 of 1

COC No. BULKSED-021111-EEUSA

Client: TVA	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					Delivered By (Circle One):  <input checked="" type="radio"/> FedEx <input type="radio"/> UPS <input type="radio"/> Bus <input type="radio"/> Client <input checked="" type="radio"/> Courier
Project Name: KIF Ash Toxicity Study						Other (specify): _____
Date of Sample Collection: 02/07/11, 02/08/11, 02/09/11						General Comments: Homogenized sediment from the Clinch River
Location: CRM0.0, CRM1.5, CRM2.0, CRM2.5, CRM3.0, CRM3.5, CRM4.0, CRM4.5, CRM6.5, CRM7.5						"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)
Example: BULKSED-CRM0.0-EEUSA	—	Date	Time	—	Yes Inches	No
BULKSED-CRM0.0-EEUSA	G	02/07/11	1014	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM1.5-EEUSA	G	02/07/11	1243	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM2.0-EEUSA	G	02/07/11	0955	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM2.5-EEUSA	G	02/07/11	1340	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM3.0-EEUSA	G	02/08/11	0921	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM3.5-EEUSA	G	02/08/11	1000	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM4.0-EEUSA	G	02/08/11	1235	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CRM4.5-EEUSA	G	02/08/11	1058	(4) 1000 mL	0.0-0.5	N/A
BULKSED-CLINCHREFERENCE-EEUSA	C	02/09/11	0940	32 (4) 1000 mL	0.0-0.5	N/A

Sample Custody – Fill In From Top Down

Relinquished By (Signature)/Affiliation:	Date/Time	Received By (Signature)/ Affiliation:	Date/Time
	02/16/11 / 0920 7502101 02/11/11 / 1000	Katrin Johnson / TVA 	02/01/11 / 0920 02/12/11 / 1000
	02/16/11 / 0920 7502101 02/11/11 / 1000	Brian J. Boles / TVA 	02/01/11 / 0920 02/12/11 / 1000

Associated UPS Tracking #'s (if applicable):

Looabookz: TVA-KIF-NTC-TOX-#003

## CHAIN OF CUSTODY RECORD

Page 1 of 1

COC No. BULKSW-021111-EEUSA

Client: TVA	Delivered By (Circle One):							
Project Name: KIF Ash Toxicity Study	FedEx	UPS	Bus	Client	Courier			
Date of Sample Collection: 02/11/2011	Other (specify): _							
Location: CRM7.0	General Comments:							
TVA-KIF-NTC-TOX-P03	Bulk Clinch River reference water for sediment toxicity study collected in 2.5 gallon (10L) cubitainers.							
Collected By: M. Greer (RSI), C.L. Jackson (TVA), R. Josefczyk (RSI), A. Johnson (RSI), T. Walk (RSI), S. Gaze (RSI), C. Fawcett (RSI), J. Ken Kart (RSI)	TVA#1 is Batch "A"							
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		If Yes, Inches	No Trace	Log #	Arrival Temp. (°C)	By
BULKSW-CRM7.0-EEUSA	G	02/11/2011 1057	(2) 10L cubitainers	8.10	NA NA	E-086-11	2.1°C	1300
Sample Custody - Fill In From Top Down								
Relinquished By (Signature)/Affiliation:	Date/Time			Received By (Signature)/Affiliation:			Date/Time	
<u>M. Greer</u> / TVA	02/11/11 / 1230			<u>Kee Gees</u> / TVA			02/11/11 / 1230	
<u>K. Johnson</u>	02/11/11 / 1300			<u>David L. Daniel</u>			02/12/11 / 1240	
Associated UPS Tracking #'s (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 <sup>KG02111</sup> Bran kg02111 Katie Gassaway/TVA

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	