Final Report

Ceriodaphnia dubia
Whole Sediment Survival and Reproduction Toxicity Test Results

TVA, Kingston Monitoring and Analysis Project
Emory River Sediment Sample ERM1.0

Prepared for:

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August 22, 2011

EXECUTIVE SUMMARY

A whole sediment toxicity test was conducted by Environmental Enterprises USA, Inc. (EE USA) to determine potential toxicity of an Emory River site sediment sample to *Ceriodaphnia dubia* neonates. Three samples were used in this test: Emory River site sediment identified as BULKSED-ERM1.0-EEUSA-052311 (ERM1.0); Emory River reference sediment identified as BULKSED-EMORYREFERENCE-EEUSA-052511 (ERS); and Emory River water identified as BULKSW-ERM9.0-EEUSA-061511 (ERW). Several dilutions of ERM1.0 prepared with ERS 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 ERW were included to assess test acceptability requirements.

C. dubia survival and reproduction in the ERM1.0 treatments were compared to survival and reproduction in an ERS control with ERW. Survival and reproduction of *C. dubia* neonates in the ERM1.0 treatments were not reduced when compared to survival and reproduction in the ERS control. Test results are shown in Table 1.

Table 1. *Ceriodaphnia dubia* Chronic Survival and Reproduction Test Results for ERM1.0.

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 Emory 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 Emory River. Emory 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 June 7 and 16, 2011, on ice and with custody seals intact (Appendix D).

This test, which was performed on one (ERM1.0) of the eight sediment samples obtained from the Emory 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 6.0 to 14.0 hours old when this test was initiated. Ten replicates of each control treatment and six ERM1.0 concentrations were prepared the day before the test was initiated. ERM1.0 dilutions were prepared with ERS. ERM1.0 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 June 18, 2011, at 1300 and completed June 25 at 1210.

MATERIALS AND METHODS

C. dubia was cultured and maintained in MHSW at 25 ± 1°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.

On June 7, 2011, the ERM1.0 and ERS samples were delivered to EE USA and stored at 0.1 to 6°C. On June 16, 2011, the ERW sample was delivered to EE USA and stored at 0.1 to 6°C (Appendix D & Table 2). Two 1-liter containers each of ERM1.0 and ERS were put into separate mixing bowls. 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 June 17th (Day -1), the density of each sediment, LCS, ERS, and ERM1.0, 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 ERM1.0 were made with ERS 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. Emory River Site Sediment, Reference Sediment, and Water Samples.

TVA Sample ID	EE USA Sample ID	Date Collected	Date Received
BULKSED-ERM1.0-EEUSA-052311 Composite Sample, Site Sediment (ERM1.0)	E-390-11	May 23, 2011 @ 0850	June 7, 2011 @ 0740
BULKSED-EMORYREFERENCE-EEUSA-052511 Composite Sample, Emory Reference Sediment (ERS)	E-382-11	May 25, 2011 @ 0855	
BULKSW-ERM9.0-EEUSA-061511 Grab Sample, Emory River Water (ERW)	E-440-11	June 15, 2011 @ 1021	June 16, 2011 @ 0825

SCAP and YCT were added to aliquots of the overlying waters, MHSW and ERW; 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 ERW. The MHSW and ERW 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 + ERW replicates received 20 mL ERW. Twenty mL of ERW were added to each replicate of the ERS and ERM1.0 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 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 ERW 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 June 18 – 25, 2011					Mean Min Max
% Sample	Temperature, °C		Dissolved Oxygen, mg/L		
	Initial	Final	Initial	Final	
MHSW	24.4 23.7 25.2	24.7 24.4 25.1	7.6 7.4 7.9	7.3 6.3 8.2	
LCS + MHSW	24.4 24.0 25.0	24.7 24.2 25.2	7.3 7.1 7.6	7.1 6.4 8.1	
LCS + ERW	24.5 24.0 25.0	24.7 24.3 25.2	7.1 6.6 7.5	6.6 5.6 7.9	
ERS + ERW	24.5 24.0 25.3	24.7 24.3 25.1	5.9 4.8 6.6	5.8 5.0 6.9	
10.0	24.5 24.0 25.1	24.7 24.3 25.1	6.1 5.3 7.0	5.7 5.4 6.1	
20.0	24.5 24.0 25.0	24.7 24.2 25.0	6.0 5.0 6.7	5.6 5.2 6.0	
40.0	24.5 24.0 25.4	24.6 24.2 25.0	6.1 5.2 6.8	5.6 5.2 6.2	
60.0	24.5 24.0 25.3	24.6 24.2 24.9	6.2 5.2 7.1	5.6 4.3 6.1	
80.0	24.5 24.0 25.2	24.6 24.2 24.9	6.1 5.5 7.3	5.5 4.6 6.2	
100.0	24.5 24.0 25.0	24.6 24.1 24.9	6.0 5.1 7.1	5.4 5.1 6.0	

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

	ERW	MHSW	MHSW	MHSW	MHSW
Collected	6/15/2011	/////	/////	/////	/////
Batch Number	BULKSW-ERM9.0-EEUSA-061511	FW-058-11[1]	FW-059-11[2]	FW-061-11[3]	
Alkalinity, mg/l	44	76	72	80	
Hardness, mg/l	72	84	84	100	
Conductivity, µmhos/cm	122.4	347	314	322	
pH, su	7.8	8.3	8.2	8.2	
Dissolved Oxygen, mg/l	4.7	8.0	7.9	7.9	
TRC, mg/l	0.00	0.00	0.00	0.00	
Total Ammonia, mg/l	0.2	< 0.02	< 0.02	< 0.02	
		[1] used 06/17-19/2011			
		[2] used 06/20-22/2011			
		[3] used 06/23-24/2011			

The test was initiated June 18th (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 ERW 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 ≥ 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 ERS + ERW control and each ERM1.0 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 and 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, CD1106, with potassium chloride (Sigma Chemical, Lot 060M0116V). The most recent SRT test was initiated on May 26, 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 29.0.

The No Observed Effect Concentration (NOEC) for survival was 100% ERM1.0. The Lowest Observed Effect Concentration (LOEC) was > 100.0% ERM1.0. The IC_{25}, a point estimate of the concentration that causes a 25% reduction in survival was > 100.0% ERM1.0 (Appendix B, page 1 & Table 5).

The NOEC for reproduction was 100.0% ERM1.0. The LOEC was > 100.0% ERM1.0. The Minimum Significant Difference percent for this reproduction data set was 9.4% (Appendix B, page 2). The IC_{25}, a point estimate of the concentration that causes a 25% reduction in reproduction was > 100.0% ERM1.0 (Appendix B, page 2 & Table 5).

Table 5. Summary of Percent Survival, Mean Reproduction, and Survival and Reproduction NOECs, LOECs, and IC_{25}s for ERM1.0.

	LCS + ERW	ERS + ERW	10% ERM1.0	20% ERM1.0	40% ERM1.0	60% ERM1.0	80% ERM1.0	100% ERM1.0
% Survival	100	100	100	100	100	100	100	100
Mean Reproduction	28.7	31.8	32.6	32.1	30.9	31.4	32.2	32.8

	NOEC	LOEC	IC_{25}
Survival	100% ERM1.0	> 100% ERM1.0	> 100% ERM1.0
Reproduction	100% ERM1.0	> 100% ERM1.0	> 100% ERM1.0

In summary, *C. dubia* survival and reproduction were not significantly reduced in any control or ERM1.0 treatment. Survival and reproduction statistical data for the MHSW only, LCS + MHSW, and LCS + ERW 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.

REPORT TEST REVIEW

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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

ERM1.0 Sediment & ERS Reference Sediment

	Density		
	LCS	**ERS**	**ERM1.0**
1	71.65 g/40 ml	52.25 g/40 ml	51.82 g/40 ml
2	71.64 g/40 ml	52.83 g/40 ml	51.78 g/40 ml
3	71.59 g/40 ml	52.46 g/40 ml	51.90 g/40 ml
MEAN	71.62 g/40 ml	52.51 g/40 ml	51.83 g/40 ml
g/ml	1.791	1.313	1.296
g/5 ml	8.95	6.56	6.48
Scale ID	N7	N7	N7
Date & Time	6/17/11 0815	6/17/11 0822	6/17/11 0830
Initial	DW JG	DW JG	DW JG

Test Concentrations, % ERM1.0											
Ceriodaphnia dubia	Total Sediment Vol./ Conc., ml	ERM1.0 ml / gram	ERS ml / gram	LCS ml / gram	grams sediment/ replicate	ERW/rep Day 0	ERW/rep Days 1 - 6	MHSW/rep Day 0	MHSW/rep Days 1 - 6	Tech, Date, & Time Sed	Tech, Date, & Time H2O
100.0%	400	400 / 518.3	0	0	6.48	20	15	0	0	JG 06-17-11 0935	DW 6/17/11 0945
80.0%	400	320 / 414.7	80 / 105.0	0	6.50	20	15	0	0		
60.0%	400	240 / 311.0	160 / 210.1	0	6.51	20	15	0	0		
40.0%	400	160 / 207.3	240 / 315.1	0	6.53	20	15	0	0		
20.0%	400	80 / 103.7	320 / 420.1	0	6.55	20	15	0	0		
10.0%	400	40 / 51.8	360 / 472.6	0	6.56	20	15	0	0		
ERS w ERW	400	0	400 / 525.1	0	6.56	20	15	0	0		
LCS w ERW	400	0	0	400 / 716.3	8.95	20	15	0	0		
LCS w MHSW	400	0	0	400 / 716.3	8.95	0	0	20	15		
MHSW	n/a	0	0	0	n/a	0	0	20	15		

Data pages & Calculations by: [signature] QA/QC Check by: [signature]

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

MHSW = Moderately Hard Synthetic Freshwater

	MHSW	MHSW	MHSW		Meter #
Date	06/17/2011	6/20/2011	6/23/2011		///
Batch #	FW-058-11	FW-059 -11	FW-061 -11		
Alkalinity	76	72	80		
Hardness	84	84	100		
Conductivity	347	314	322		A46
pH	8.3	8.2	8.2		Q8
DO	8.0	7.9	7.9		S7
TRC	0.0	0.0	0.0		A27
Ammonia	<0.02	<0.02	<0.02		
Initial	DW	DW	DW		

ERW = Emory River Reference Water

	ERW	ERW			Meter #
Date	06/16/2011	/2011			///
Batch #	Delivered 06/16/2011	Delivered / /2011			
Alkalinity	44				
Hardness	72				
Conductivity	122.4				A46
pH	7.8				Q8
DO	4.7				S7
TRC	0.0				A27
Ammonia	0.2				
Initial	VR				

Alkalinity: mg/l as $CaCO_3$ Hardness: mg/l as $CaCO_3$ Conductivity: µS/cm pH: su
TRC: mg/l Dissolved Oxygen (DO): mg/l Total Residual Chlorine (TRC): mg/l Ammonia, Total: mg/l

Comments: ERS very clean.

ERM 1.0 clean but strong H_2S-like odor DW 6/23/11

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

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

ERM1.0 Sediment & ERS Reference Sediment

Test Organisms Age: 6.0 - 14.0 Hours Old

Test Organisms Source: EEUSA Test Initiation At: 1300 on 6 / 18 /2011

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

Loaded by: David L. Daniel Organism Lot # CD061811-02

Exposure Chamber: 8 dram vials. **Feeding**: 0.1 ml *S. capricornutum* (Lot # 54-11 & 55-11 *) & 0.1 ml YCT (Lot # 42-11) / 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	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DLD	DLD
	1	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	2	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	3	0	0	0	0	0	0	0	0	0	0	100	0	DLD	JG
	4	6	6	6	4	6	6	5	5	6	4	100	54	DLD	JG
	5	10	10	13	14	11	11 **	8	11	10	13	100	~~121~~ Ⓐ ///	DLD	JG
	6	0	0	0	0	0	0	4	0	0	0	100	4	DLD	DLD
	7	14	16	12	13	10	17	14	14	10	16	100	136	DLD	NA
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		30	32	31	31	27	34	31	30	26	33	30.5	8.07		

Comments: * 54-11 used 6/17/11 only. DLD

** split brood 6/24/11 DLD

Ⓐ wrong data JG 062711

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	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DWD	DWD
	1	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD
	2	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD
	3	0	0	0	0	0	0	0	0	0	0	100	0	DWD	JG
	4	6	6	5	6	5	6	4	5	6	4	100	53	DWD	JG
	5	11	10	10	9	10	11	10	11	10	10	100	102	DWD	JG
	6	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD
	7	12	13	12	16	12	13	17	13	14	13	100	~~137~~ (A) 135	DWD	NA
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		29	29	27	31	27	30	31	29	30	27	29.0	5.39		

Treatment: LCS w ERW.

DAY	REP	21	22	23	24	25	26	27	28	29	30	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DWD	DWD
	1	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD
	2	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD
	3	0	0	0	0	0	0	0	0	0	0	100	0	DWD	JG
	4	6	5	6	6	5	6	4	5	6	4	100	53	DWD	JG
	5	11	10	12	10	10	8	9	12	10	10	100	102	DWD	JG
	6	0	0	0	0	0	2	0	0	0	0	100	2	DWD	DWD
	7	14	12	13	15	12	11	13	14	14	12	100	130	DWD	NA
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		31	27	31	31	27	27	26	31	30	26	28.7	7.89		

Comments: * split brood 6/24/11 DWD

(A) WRONG DATA WAS 8/8/11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: ERS w ERW.

DAY	REP	31	32	33	34	35	36	37	38	39	40	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DLD	DLD
	1	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	2	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	3	0	0	0	0	0	0	0	0	0	0	100	0	DLD	JG
	4	6	6	6	5	5	6	4	6	6	6	100	~~57~~ (A) SG	DLD	JG
	5	12	9	11	(7) *	9	12	13	12	11	10	100	106	DLD	JG
	6	0	0	0	(6)	0	0	0	0	0	0	100	6	DLD	DLD
	7	15	14	15	12	14	15	16	14	14	17	100	150	DLD	NH
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		33	29	32	30	32	33	33	32	31	33	31.8	4.40		

Treatment: 10% ERM1.0 w ERW.

DAY	REP	41	42	43	44	45	46	47	48	49	50	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DLD	DLD
	1	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	2	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	3	0	0	0	0	0	0	0	0	0	0	100	0	DLD	JG
	4	6	5	5	5	5	6	6	6	6	6	100	56	DLD	JG
	5	11	10	13	11	14	9	12	11	10	12	100	113	DLD	JG
	6	0	0	0	0	0	0	0	0	0	0	100	0	DLD	DLD
	7	17	14	16	18	17	13	15	14	17	16	100	157	DLD	NH
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		34	29	34	34	36	28	33	31	33	34	32.6	7.68		

Comments: * split brood 6/24/11 DLD

(A) wrong data JG 062711

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: 20% ERM1.0 w ERW.

DAY	REP	51	52	53	54	55	56	57	58	59	60	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DW	DW
	1	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	2	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	3	0	0	0	0	0	0	0	0	0	0	100	0	DW	JG
	4	6	5	6	4	6	6	5	5	5	6	100	54	DW	JG
	5	10	12	11	10	10	13	10	11	9	13	100	99	DW	JG
	6	0	0	0	0	0	10	0	0	0	0	100	10	DW	DW
	7	18	19	13	16	17	14	14	20	12	15	100	158	DW	NA
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		34	36	30	30	33	33	29	36	26	34	32.1	10.1		

Treatment: 40% ERM1.0 w ERW.

DAY	REP	61	62	63	64	65	66	67	68	69	70	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DW	DW
	1	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	2	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	3	0	0	0	0	0	0	0	0	0	0	100	0	DW	JG
	4	6	5	5	6	5	5	6	5	4	6	100	53	DW	JG
	5	11	11	9	10	12	9	6	9	10	13	100	100	DW	JG
	6	0	0	0	0	0	0	6	0	0	0	100	6	DW	DW
	7	17	18	14	16	16	12	14	12	15	16	100	150	DW	M+
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		34	34	28	32	33	26	32	26	29	35	30.9	10.9		

Comments: * split broods 6/24/11 DW

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: 60% ERM1.0 w ERW.

DAY	REP	71	72	73	74	75	76	77	78	79	80	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DW	DW
	1	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	2	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	3	0	0	0	0	0	0	0	0	0	0	100	0	DW	JG
	4	5	5	6	5	5	5	6	4	5	6	100	52	DW	JG
	5	10	10	9	10	10	11	12	8	9	12	100	101	DW	JG
	6	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	7	19	16	16	15	16	12	15	16	17	19	100	161	DW	NA
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		34	31	31	30	31	28	33	28	31	37	31.4	8.65		

Treatment: 80% ERM1.0 w ERW.

DAY	REP	81	82	83	84	85	86	87	88	89	90	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O
	0	0	0	0	0	0	0	0	0	0	0	///	///	DW	DW
	1	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	2	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	3	0	0	0	0	0	0	0	0	0	0	100	0	DW	JG
	4	6	6	4	5	6	4	5	6	5	5	100	52	DW	JG
	5	9	11	12	12	12	12	10	10	8	9	100	105	DW	JG
	6	0	0	0	0	0	0	0	0	0	0	100	0	DW	DW
	7	18	16	16	17	18	14	14	21	16	15	100	165	DW	NA
	3rd Brood Reproduction Per Replicate											Mean	CV %	///////	
		33	33	32	34	36	30	29	37	29	29	32.2	9.12		

Comments:

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

C. dubia Daily Survival & Reproduction Data Cont.

Treatment: 100% ERM1.0 w ERW.

DAY	REP	91	92	93	94	95	96	97	98	99	100	% Sur.	No. of Neonates Per Day	Tech CD	Tech H2O	Time
	0	0	0	0	0	0	0	0	0	0	0	///	///	DWD	DWD	1300
	1	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD	0945
	2	0	0	0	0	0	0	0	0	0	0	100	0	DWD	DWD	1145
	3	0	0	0	0	0	0	0	0	0	0	100	0	DWD	JG	1135
	4	6	6	6	5	5	6	6	5	5	6	100	56	DWD	JG	1430
	5	10	13	9	10	13	10	12	6	10	12	100	105	DWD	JG	1150
	6	0	0	0	0	0	0	0	*3	0	0	100	3	DWD	DWD	1100
	7	15	17	14	15	20	14	17	16	18	18	100	164	DWD	N/A	1210
	3rd Brood Reproduction Per Replicate											Mean	CV %	//////		
		31	36	29	30	38	30	35	30	33	36	32.8	9.83			

Calculations by: [signature] QA/QC by: Jennifer Griffith

Data Entry by: [signature]

QA/QC Officer: Mark A. O'Neill (verify data entry!)

Comments: * split brood DWD 6/24/11

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

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 0	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/18/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO I	7.9	7.3	7.5	4.8	5.3	5.0	5.2	5.2	5.6	5.3	S7
Temp I	25.2	25.0	25.0	25.3	25.1	25.0	25.4	25.3	25.2	25.0	A46
Tech. Initials	Finals:						Initials: DW				
Times	Final Time: N/A						Initial Time: 1500				

Day 1	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/19/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	7.4	7.1	7.0	6.9	5.9	5.8	5.6	6.1	5.9	5.2	S7
I	7.8	7.6	7.5	6.4	7.0	6.6	6.8	7.1	7.3	7.1	~~A~~ DW S7 6/19/11
Temp F	25.1	25.2	25.2	25.1	25.1	25.0	25.0	24.9	24.9	24.9	A46
I	24.4	24.6	24.7	24.6	24.6	24.6	24.5	24.6	24.6	24.6	A46
Tech. Initials	Finals: DW						Initials: DW				
Times	Final Time: 0730						Initial Time: 1230				

Day 2	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/20/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	8.2	8.1	7.9	6.4	5.9	6.0	6.2	6.1	6.2	6.0	S7
I	7.5	7.5	7.4	6.6	6.5	6.7	6.6	6.7	6.8	6.7	S7
Temp F	24.7	24.8	24.8	24.7	24.7	24.6	24.6	24.5	24.5	24.5	A46
I	23.7	24.0	24.0	24.0	24.1	24.1	24.1	24.1	24.1	24.0	A46
Tech. Initials	Finals: ME						Initials: ME				
Times	Final Time: 0858						Initial Time: 1307				

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

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 3	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/21/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	7.6	7.3	6.6	5.8	6.1	5.8	5.8	5.9	5.9	5.7	57
I	7.4	7.1	6.7	5.9	5.9	6.0	6.2	6.0	5.9	5.9	57
Temp F	24.5	24.7	24.8	24.8	24.8	24.9	24.9	24.8	24.7	24.8	A46
I	24.0	24.0	24.1	24.1	24.1	24.1	24.1	24.0	24.0	24.1	A46
Tech. Initials	Finals: ME						Initials: ME				
Times	Final Time: 0938						Initial Time: 1423				

Day 4	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/22/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	6.9	6.4	5.6	5.4	5.6	5.2	5.3	5.8	5.5	5.2	57
I	7.7	7.3	6.9	6.0	6.1	6.1	6.2	6.1	5.5	5.1	57
Temp F	24.4	24.6	24.7	24.7	24.7	24.7	24.8	24.7	24.7	24.7	A46
I	24.1	24.1	24.1	24.1	24.0	24.0	24.0	24.0	24.1	24.1	A46
Tech. Initials	Finals: ME						Initials: ME				
Times	Final Time: 0951						Initial Time: 1527				

Comments:

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

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 5	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/23/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	7.7	7.8	6.6	5.5	5.6	5.6	5.2	5.2	5.1	5.1	57
I	7.6	7.4	6.4	6.0	6.0	5.9	5.8	6.0	5.8	5.7	57
Temp F	24.5	24.2	24.3	24.3	24.3	24.2	24.2	24.2	24.2	24.1	A46
I	24.4	24.6	24.4	24.4	24.5	24.6	24.5	24.5	24.5	24.5	A46
Tech. Initials	Finals: ME					Initials: DLD					
Times	Final Time: 0926					Initial Time: 1602					

Day 6	Controls				Treatment % ERM1.0						Meter #
	MHSW		Emory River Water								
06/24/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	6.9	6.6	6.0	5.3	5.4	5.7	5.6	5.5	5.6	5.4	57
I	7.5	7.1	6.6	5.9	5.7	6.0	5.9	6.0	6.0	6.1	57
Temp F	24.5	24.5	24.4	24.6	24.5	24.5	24.4	24.4	24.4	24.4	A46
I	24.7	24.8	24.9	24.9	24.9	24.8	24.9	24.9	24.8	24.9	A46
Tech. Initials	Finals: DLD					Initials: DLD					
Times	Final Time: 0900					Initial Time: 1525					

Comments:

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

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 7	Controls				Treatment % ERM1.0						Meter #
	MHSW		TVA Supplied Emory River Water								
06/25/11	MHSW	LCS + MHSW	LCS + ERW	ERS	10.0%	20.0%	40.0%	60.0%	80.0%	100.0%	
DO F	6.3	6.5	6.4	5.0	5.4	5.3	5.2	4.3	4.6	5.1	S7
Temp F	24.9	24.8	24.8	24.9	24.8	24.7	24.6	24.8	24.9	24.7	A46
Tech. Initials	Finals: TK						Initials: N/A				
Times	Final Time: 1000						Initial Time:				

Comments:

RAW DATA QA/QC: Veronica McKeen

TVA, ERM1.0, Site Sediment
TVA, ERS, Reference Sediment
TVA, ERW, River Water

E-390-11
E-382-11
E-440-11

APPENDIX B

Ceriodaphnia Survival and Reproduction Test-7 Day Survival

Start Date:	6/18/2011	Test ID:	cd39011	Sample ID:	ERM1.0
End Date:	6/25/2011	Lab ID:	EE USA	Sample Type:	WHOLE SEDIMENT
Sample Date:	5/23/2011	Protocol:	ASTM E1706-05 Annex A2	Test Species:	CD-Ceriodaphnia dubia

Comments: LCS=Lab control Sediment; ERW=Emory River Water; ERS=Emory Reference Sediment

Conc-%	1	2	3	4	5	6	7	8	9	10
LCS+ERW	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
ERS+ERW	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 Exact P	1-Tailed Critical	Isotonic Mean	Isotonic N-Mean
LCS+ERW	1.0000	1.0000	0	10	10	10	0.6238			
ERS+ERW	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 ERS+ERW				

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:	6/18/2011	Test ID:	cd39011	Sample ID:	ERM1.0
End Date:	6/25/2011	Lab ID:	EE USA	Sample Type:	WHOLE SEDIMENT
Sample Date:	5/23/2011	Protocol:	ASTM E1706-05 Annex A2	Test Species:	CD-Ceriodaphnia dubia
Comments:	LCS=Lab control Sediment; ERW=Emory River Water; ERS=Emory Reference Sediment				

Conc-%	1	2	3	4	5	6	7	8	9	10
LCS+ERW	31.000	27.000	31.000	31.000	27.000	27.000	26.000	31.000	30.000	26.000
ERS+ERW	33.000	29.000	32.000	30.000	32.000	33.000	33.000	32.000	31.000	33.000
10	34.000	29.000	34.000	34.000	36.000	28.000	33.000	31.000	33.000	34.000
20	34.000	36.000	30.000	30.000	33.000	33.000	29.000	36.000	26.000	34.000
40	34.000	34.000	28.000	32.000	33.000	26.000	32.000	26.000	29.000	35.000
60	34.000	31.000	31.000	30.000	31.000	28.000	33.000	28.000	31.000	37.000
80	33.000	33.000	32.000	34.000	36.000	30.000	29.000	37.000	29.000	29.000
100	31.000	36.000	29.000	30.000	38.000	30.000	35.000	30.000	33.000	36.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
LCS+ERW	28.700	0.9025	28.700	26.000	31.000	7.886	10					
ERS+ERW	31.800	1.0000	31.800	29.000	33.000	4.398	10	*			32.200	1.0000
10	32.600	1.0252	32.600	28.000	36.000	7.679	10	-0.629	2.347	2.985	32.200	1.0000
20	32.100	1.0094	32.100	26.000	36.000	10.116	10	-0.236	2.347	2.985	32.100	0.9969
40	30.900	0.9717	30.900	26.000	35.000	10.943	10	0.708	2.347	2.985	31.825	0.9884
60	31.400	0.9874	31.400	28.000	37.000	8.650	10	0.315	2.347	2.985	31.825	0.9884
80	32.200	1.0126	32.200	29.000	37.000	9.119	10	-0.315	2.347	2.985	31.825	0.9884
100	32.800	1.0314	32.800	29.000	38.000	9.832	10	-0.786	2.347	2.985	31.825	0.9884

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.77369	0.895	-0.08729	-0.71361
Bartlett's Test indicates equal variances (p = 0.30)	7.235895	16.81189		
The control means are significantly different (p = 1.70E-03)	3.684794	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	2.98479	0.093861	4.42381	8.085714	0.770342	6, 63
Treatments vs ERS+ERW										

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:	6/18/2011	Test ID:	cd39011c	Sample ID:	ERM1.0
End Date:	6/25/2011	Lab ID:	EE USA	Sample Type:	WHOLE SEDIMENT
Sample Date:	5/23/2011	Protocol:	ASTM E1706-05 Annex A2	Test Species:	CD-Ceriodaphnia dubia

Comments: MHSW=Mod Hard Synthetic Water; LCS=Lab Control Sediment; ERW=EMORY 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+ERW	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 Exact P	1-Tailed Critical
MHSW	1.0000	1.0000	0	10	10	10	0.6238	
LCS+MHSW	1.0000	1.0000	0	10	10	10	*	
LCS+ERW	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:	6/18/2011	Test ID:	cd39011c	Sample ID:	ERM1.0
End Date:	6/25/2011	Lab ID:	EE USA	Sample Type:	WHOLE SEDIMENT
Sample Date:	5/23/2011	Protocol:	ASTM E1706-05 Annex A2	Test Species:	CD-Ceriodaphnia dubia
Comments:	MHSW=Mod Hard Synthetic Water; LCS=Lab Control Sediment; ERW=EMORY River Water				

Conc-%	1	2	3	4	5	6	7	8	9	10
MHSW	30.000	32.000	31.000	31.000	27.000	34.000	31.000	30.000	26.000	33.000
LCS+MHSW	29.000	29.000	27.000	31.000	27.000	30.000	31.000	29.000	30.000	27.000
LCS+ERW	31.000	27.000	31.000	31.000	27.000	27.000	26.000	31.000	30.000	26.000

			Transform: Untransformed					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW	30.500	1.0517	30.500	26.000	34.000	8.068	10		
LCS+MHSW	29.000	1.0000	29.000	27.000	31.000	5.391	10	*	
LCS+ERW	28.700	0.9897	28.700	26.000	31.000	7.886	10	102.50	82.00

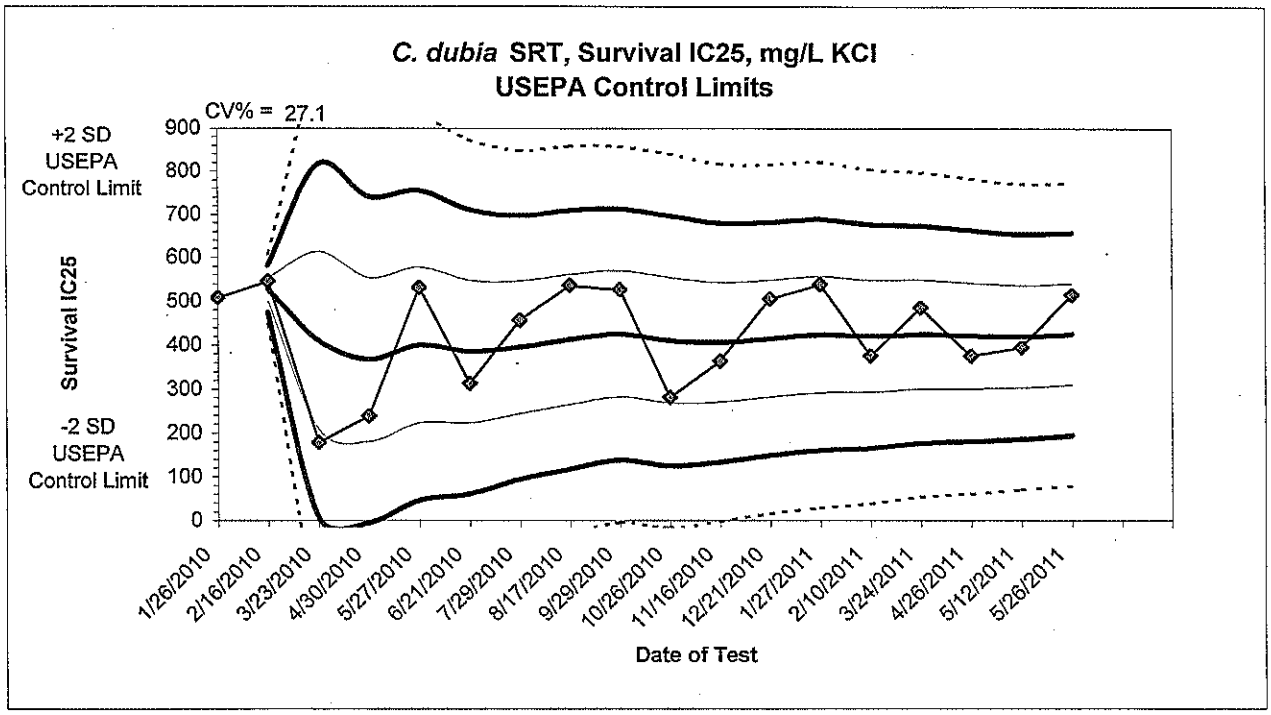
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.865675	0.905	-0.07963	-1.69402
F-Test indicates equal variances (p = 0.29)	2.095454	6.54109		
The control means are not significantly different (p = 0.12)	1.626978	2.100922		

Hypothesis Test (1-tail, 0.05)

Wilcoxon Two-Sample Test indicates no significant differences
Treatments vs LCS+MHSW

Environmental Enterprises USA, Inc.

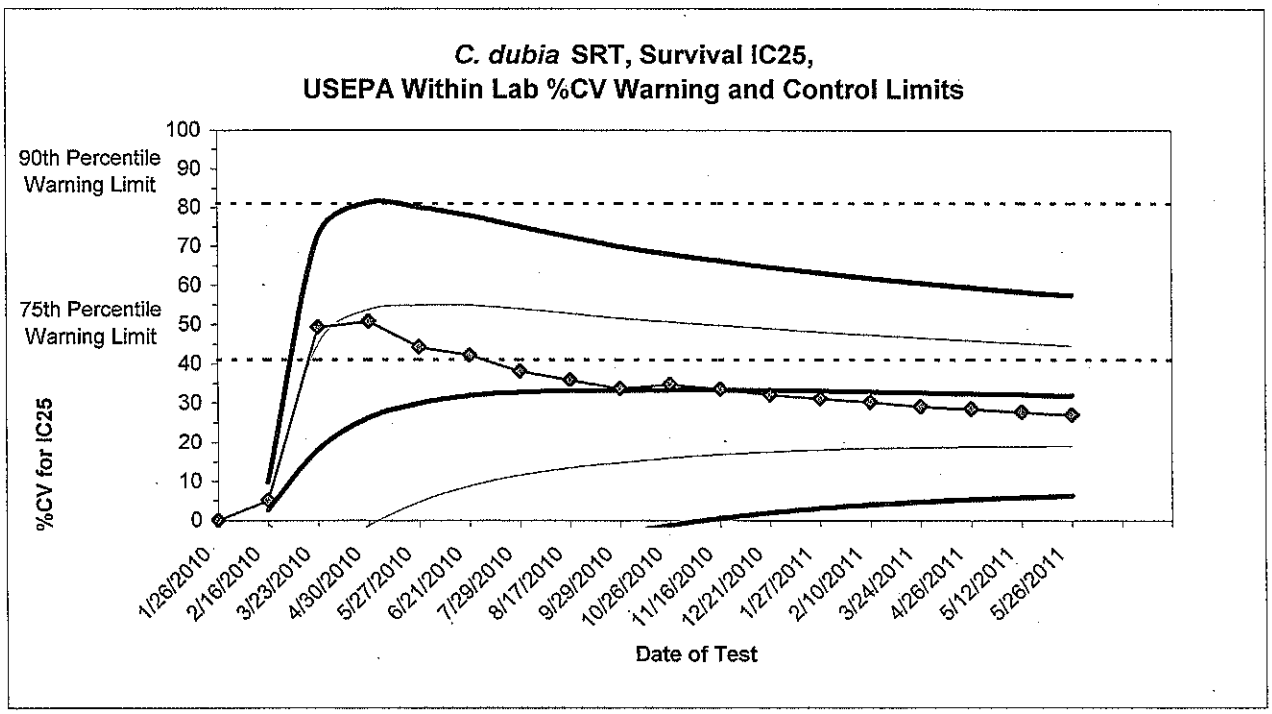
APPENDIX C



Test #	Test Date	Survival IC25	Mean IC25	-1 SD	-2 SD	+1 SD	+2 SD	-3 SD	+3 SD	Toxicant Lot #
CD1002	1/26/2010	508								079K0011
CD1003	2/16/2010	546	527	500	473	554	581	446	608	079K0011
CD1004	3/23/2010	178	411	208	6	613	815	-197	1018	049K0305
CD1006	4/30/2010	238	368	181	-5	554	740	-192	927	049K0305
CD1007	5/27/2010	531	400	223	46	577	755	-132	932	049K0305
CD1009	6/21/2010	312	386	223	60	548	711	-102	873	049K0305
CD1010	7/29/2010	456	396	245	94	546	697	-57	848	079K0011
CD1011	8/17/2010	536	413	265	117	561	709	-31	858	079K0011
CD1012	9/29/2010	527	426	282	138	569	713	-5	857	079K0011
CD1013	10/26/2010	281	411	268	125	554	697	-18	840	099K0202
CD1014	11/16/2010	364	407	271	134	543	680	-2	816	099K0202
CD1015	12/21/2010	506	415	282	149	548	682	16	815	099K0202
CD1101	1/27/2011	539	425	293	161	557	689	29	821	099K0202
CD1102	2/10/2011	376	421	294	166	549	676	39	804	099K0202
CD1103	3/24/2011	485	426	302	178	550	674	54	797	099K0202
CD1104	4/26/2011	376	422	302	182	543	663	61	784	099K0202
CD1105	5/12/2011	396	421	304	187	538	654	71	771	099K0202
CD1106	5/26/2011	515	426	311	195	542	657	80	772	060M0116V

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

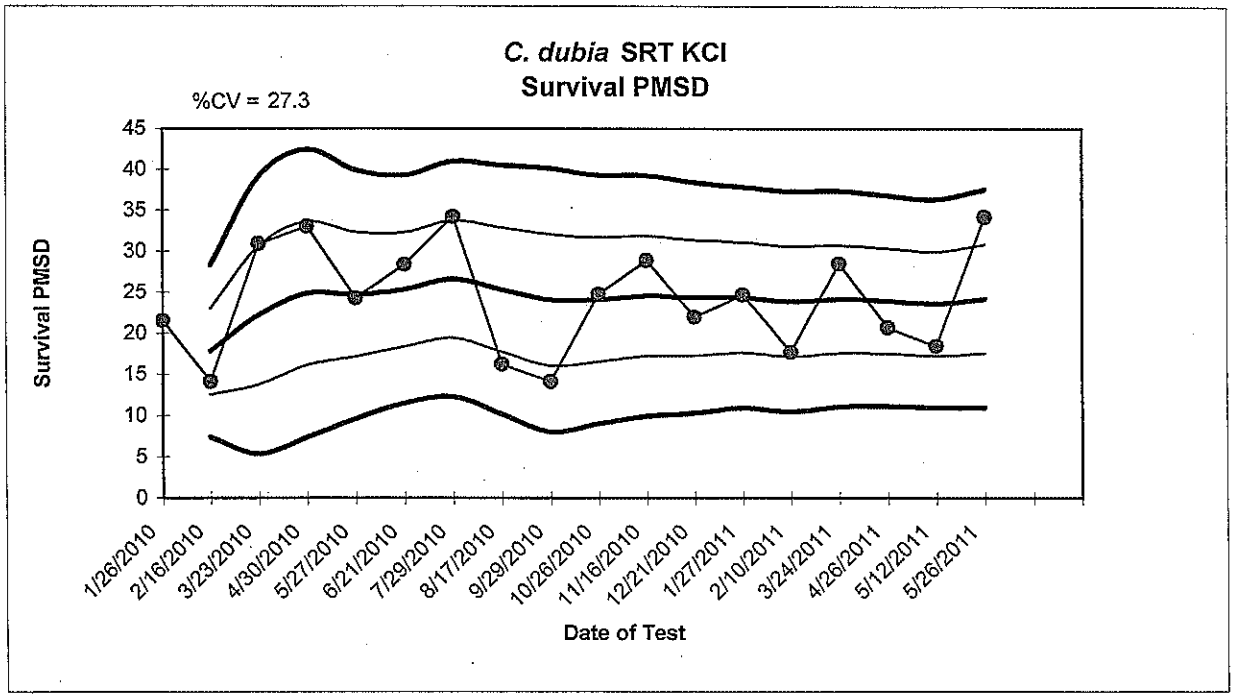
CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl



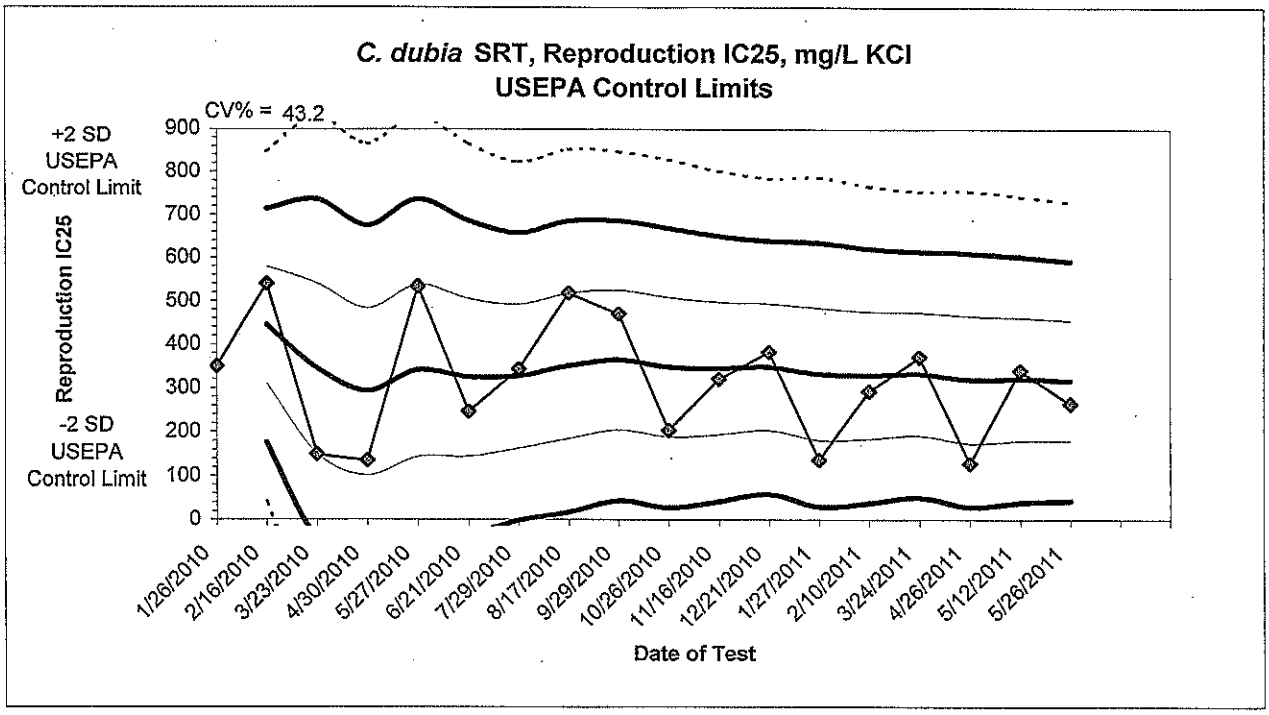
Test #	Test Date	%CV for IC25	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
CD1002	1/26/2010	0.0						41.0	81.0	079K0011
CD1003	2/16/2010	5.1	2.5	-1.1	-4.7	6.2	9.8	41.0	81.0	079K0011
CD1004	3/23/2010	49.3	18.1	-9.0	-36.1	45.2	72.3	41.0	81.0	049K0305
CD1006	4/30/2010	50.7	26.3	-1.2	-28.7	53.8	81.2	41.0	81.0	049K0305
CD1007	5/27/2010	44.3	29.9	4.8	-20.4	55.0	80.1	41.0	81.0	049K0305
CD1009	6/21/2010	42.2	31.9	8.9	-14.1	55.0	78.0	41.0	81.0	049K0305
CD1010	7/29/2010	38.1	32.8	11.7	-9.5	54.0	75.1	41.0	81.0	079K0011
CD1011	8/17/2010	35.9	33.2	13.6	-6.0	52.8	72.4	41.0	81.0	079K0011
CD1012	9/29/2010	33.7	33.3	14.9	-3.4	51.6	70.0	41.0	81.0	079K0011
CD1013	10/26/2010	34.8	33.4	16.1	-1.2	50.7	68.0	41.0	81.0	099K0202
CD1014	11/16/2010	33.5	33.4	17.0	0.6	49.8	66.3	41.0	81.0	099K0202
CD1015	12/21/2010	32.1	33.3	17.6	2.0	49.0	64.6	41.0	81.0	099K0202
CD1101	1/27/2011	31.1	33.1	18.1	3.1	48.1	63.1	41.0	81.0	099K0202
CD1102	2/10/2011	30.3	32.9	18.5	4.1	47.4	61.8	41.0	81.0	099K0202
CD1103	3/24/2011	29.1	32.7	18.7	4.8	46.6	60.6	41.0	81.0	099K0202
CD1104	4/26/2011	28.5	32.4	18.9	5.4	45.9	59.4	41.0	81.0	099K0202
CD1105	5/12/2011	27.7	32.1	19.0	5.9	45.3	58.4	41.0	81.0	099K0202
CD1106	5/26/2011	27.1	31.9	19.1	6.3	44.7	57.5	41.0	81.0	060M0116V
								41.0	81.0	
								41.0	81.0	

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



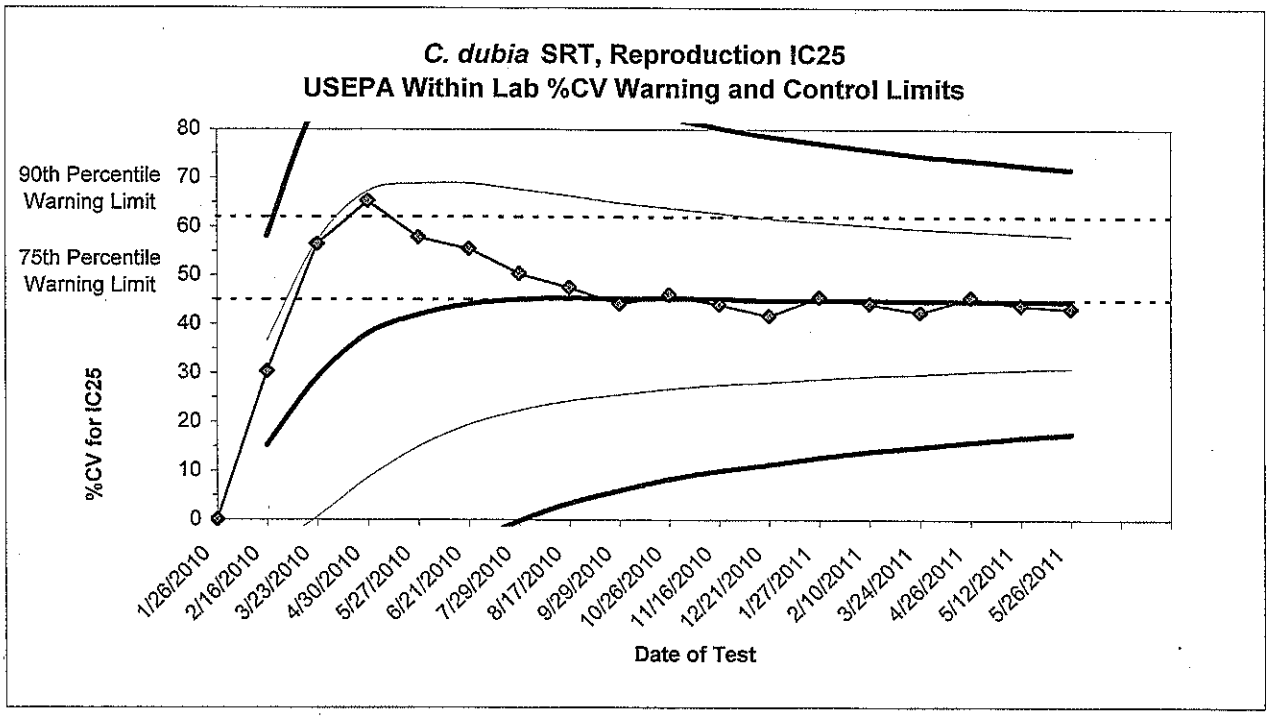
Test #	Test Date	Survival PMSD	Mean	-1 SD	-2 SD	+1 SD	+2 SD	Toxicant Lot #
CD1002	1/26/2010	21.5						079K0011
CD1003	2/16/2010	14.1	17.8000	12.5674	7.3348	23.0326	28.2652	079K0011
CD1004	3/23/2010	30.9	22.1667	13.7468	5.3270	30.5865	39.0063	049K0305
CD1006	4/30/2010	33.0	24.8750	16.1227	7.3704	33.6273	42.3796	049K0305
CD1007	5/27/2010	24.3	24.7600	17.1759	9.5919	32.3441	39.9281	049K0305
CD1009	6/21/2010	28.4	25.3667	18.4224	11.4782	32.3109	39.2552	049K0305
CD1010	7/29/2010	34.2	26.6286	19.4639	12.2992	33.7932	40.9579	079K0011
CD1011	8/17/2010	16.2	25.3250	17.7360	10.1469	32.9140	40.5031	079K0011
CD1012	9/29/2010	14.1	24.0778	16.0532	8.0285	32.1024	40.1270	079K0011
CD1013	10/26/2010	24.8	24.1500	16.5809	9.0117	31.7191	39.2883	099K0202
CD1014	11/16/2010	28.9	24.5818	17.2597	9.9376	31.9040	39.2261	099K0202
CD1015	12/21/2010	22.0	24.3667	17.3456	10.3246	31.3877	38.4088	099K0202
CD1101	1/27/2011	24.7	24.3923	17.6695	10.9467	31.1151	37.8379	099K0202
CD1102	2/10/2011	17.7	23.9143	17.2122	10.5101	30.6164	37.3185	099K0202
CD1103	3/24/2011	28.5	24.2200	17.6540	11.0881	30.7860	37.3519	099K0202
CD1104	4/26/2011	20.7	24.0000	17.5959	11.1919	30.4041	36.8081	099K0202
CD1105	5/12/2011	18.5	23.6765	17.3339	10.9913	30.0190	36.3616	099K0202
CD1106	5/26/2011	34.2	24.2611	17.6268	10.9925	30.8954	37.5298	060M0116V



Test #	Test Date	Repro. IC25	Mean IC25	-1 SD	-2 SD	+1 SD	+2 SD	-3 SD	+3 SD	Toxicant Lot #
CD1002	1/26/2010	349								079K0011
CD1003	2/16/2010	539	444	310	175	578	713	41	847	079K0011
CD1004	3/23/2010	149	346	151	-44	541	736	-239	931	049K0305
CD1006	4/30/2010	135	293	102	-89	484	675	-280	866	049K0305
CD1007	5/27/2010	533	341	144	-53	538	735	-250	932	049K0305
CD1009	6/21/2010	246	325	145	-36	506	686	-216	867	049K0305
CD1010	7/29/2010	343	328	163	-2	493	658	-167	823	079K0011
CD1011	8/17/2010	518	352	185	18	518	685	-149	852	079K0011
CD1012	9/29/2010	469	365	204	43	525	686	-118	847	079K0011
CD1013	10/26/2010	202	348	188	28	508	669	-132	829	099K0202
CD1014	11/16/2010	320	346	194	41	498	650	-111	802	099K0202
CD1015	12/21/2010	382	349	203	58	494	640	-88	785	099K0202
CD1101	1/27/2011	136	332	181	30	484	635	-121	786	099K0202
CD1102	2/10/2011	292	330	184	38	475	621	-108	767	099K0202
CD1103	3/24/2011	370	332	191	51	473	614	-90	755	099K0202
CD1104	4/26/2011	128	319	174	29	465	610	-117	755	099K0202
CD1105	5/12/2011	340	321	180	39	461	602	-102	743	099K0202
CD1106	5/26/2011	264	318	180	43	455	592	-94	729	060M0116V

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

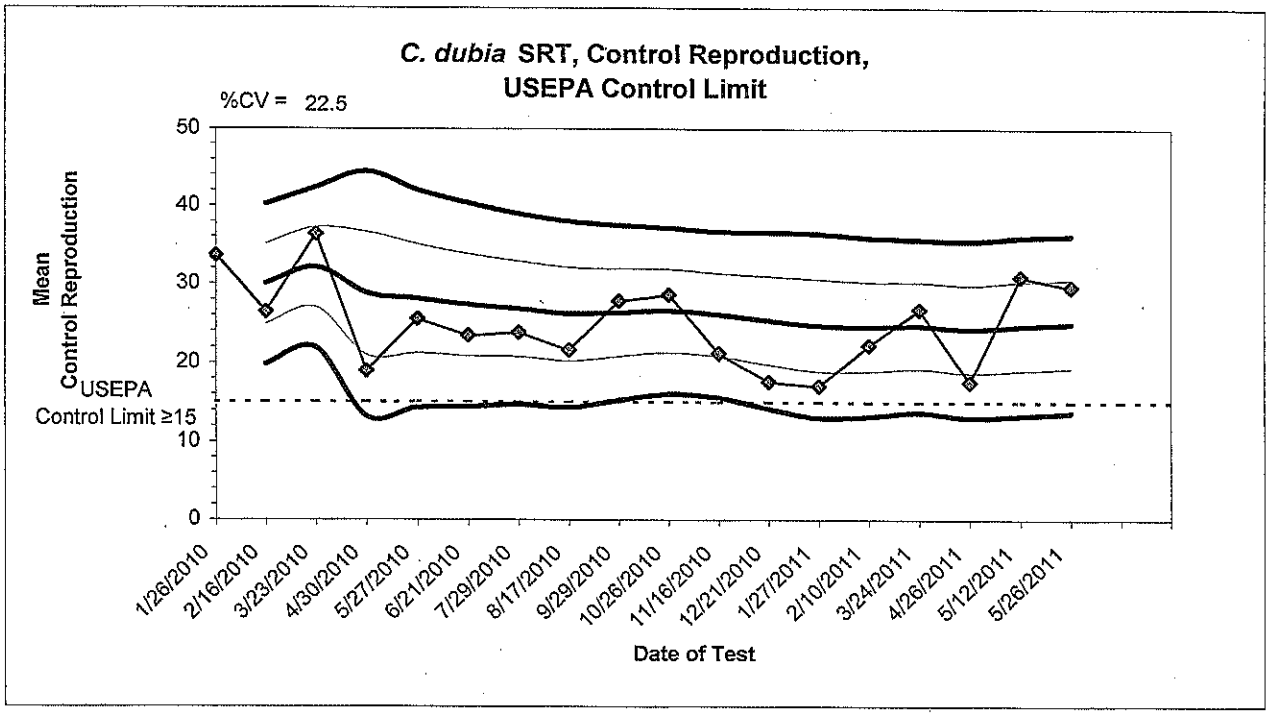
CD1002 - Widened dilution series to 140, 212, 316, 476, & 716 mg/L KCl



Test #	Test Date	%CV for IC25	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
CD1002	1/26/2010	0.0						45.0	62.0	079K0011
CD1003	2/16/2010	30.3	15.1	-6.3	-27.7	36.5	57.9	45.0	62.0	079K0011
CD1004	3/23/2010	56.4	28.9	0.7	-27.6	57.1	85.4	45.0	62.0	049K0305
CD1006	4/30/2010	65.2	38.0	8.6	-20.7	67.3	96.6	45.0	62.0	049K0305
CD1007	5/27/2010	57.8	41.9	15.0	-11.9	68.8	95.7	45.0	62.0	049K0305
CD1009	6/21/2010	55.5	44.2	19.5	-5.2	68.9	93.6	45.0	62.0	049K0305
CD1010	7/29/2010	50.3	45.1	22.4	-0.3	67.7	90.4	45.0	62.0	079K0011
CD1011	8/17/2010	47.5	45.4	24.4	3.4	66.4	87.4	45.0	62.0	079K0011
CD1012	9/29/2010	44.1	45.2	25.6	5.9	64.9	84.5	45.0	62.0	079K0011
CD1013	10/26/2010	46.0	45.3	26.8	8.3	63.8	82.4	45.0	62.0	099K0202
CD1014	11/16/2010	44.0	45.2	27.6	10.0	62.8	80.4	45.0	62.0	099K0202
CD1015	12/21/2010	41.7	44.9	28.1	11.3	61.7	78.5	45.0	62.0	099K0202
CD1101	1/27/2011	45.5	45.0	28.9	12.8	61.0	77.1	45.0	62.0	099K0202
CD1102	2/10/2011	44.2	44.9	29.5	14.0	60.3	75.8	45.0	62.0	099K0202
CD1103	3/24/2011	42.4	44.7	29.8	14.9	59.6	74.5	45.0	62.0	099K0202
CD1104	4/26/2011	45.5	44.8	30.4	16.0	59.2	73.6	45.0	62.0	099K0202
CD1105	5/12/2011	43.9	44.7	30.8	16.8	58.7	72.6	45.0	62.0	099K0202
CD1106	5/26/2011	43.2	44.6	31.1	17.6	58.2	71.7	45.0	62.0	060M0116V
								45.0	62.0	
								45.0	62.0	

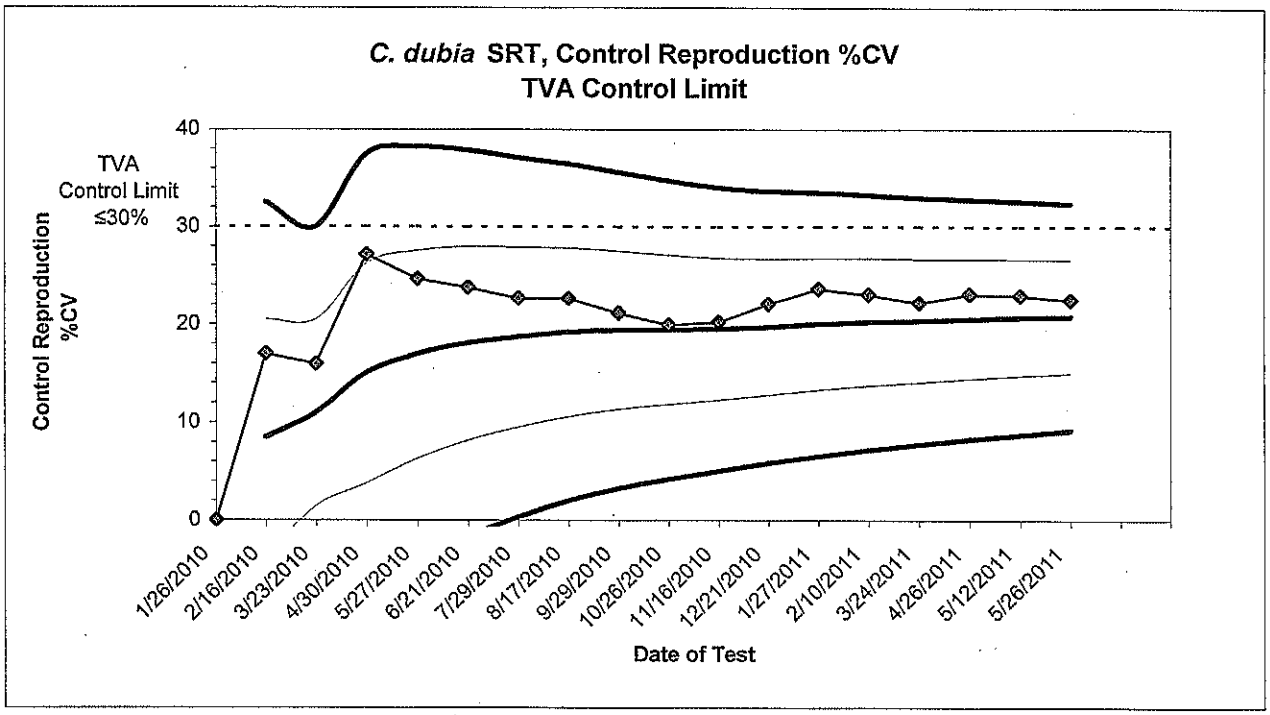
CD1005 - IC25 less than lowest concentration tested and could not be graphed

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



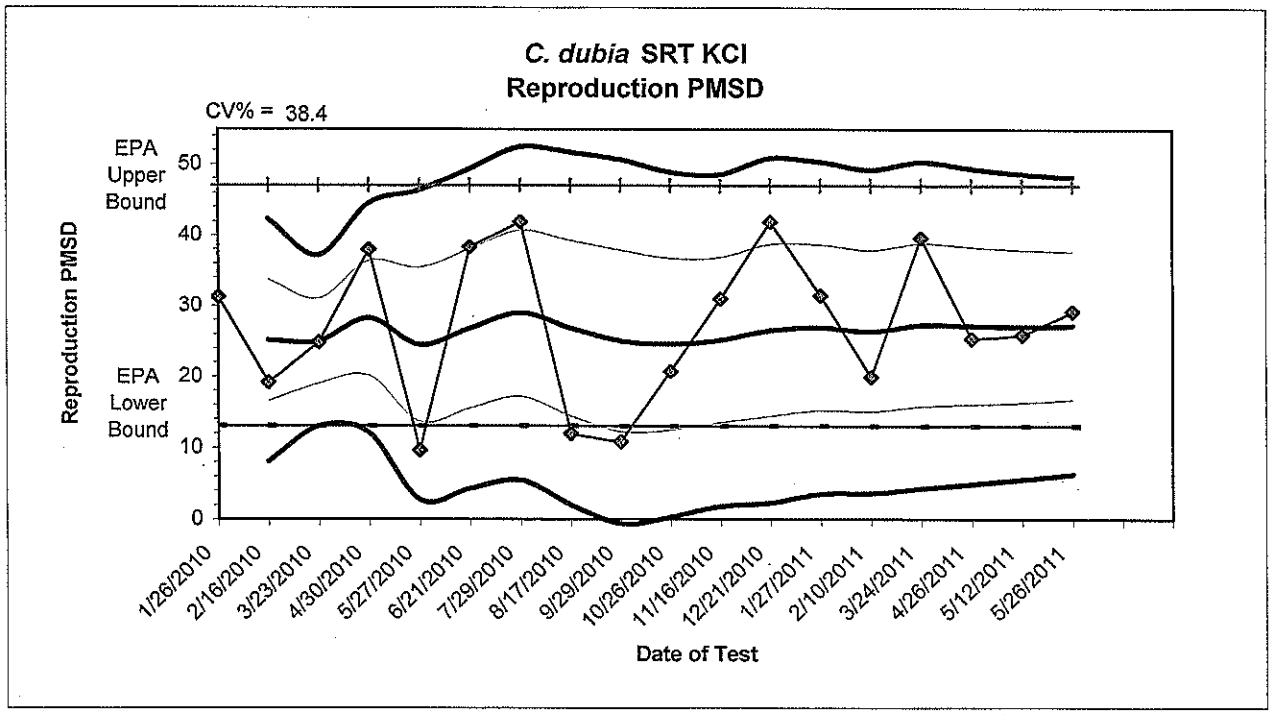
Test #	Test Date	Mean Control Repro.	Mean	-1 SD	-2 SD	+1 SD	+2 SD		Control Limit	Toxicant Lot #
CD1002	1/26/2010	33.6							15.0	079K0011
CD1003	2/16/2010	26.4	30.0	24.9	19.8	35.1	40.2		15.0	079K0011
CD1004	3/23/2010	36.3	32.1	27.0	21.9	37.2	42.3		15.0	049K0305
CD1006	4/30/2010	18.9	28.8	21.0	13.2	36.6	44.4		15.0	049K0305
CD1007	5/27/2010	25.5	28.1	21.2	14.3	35.1	42.0		15.0	049K0305
CD1009	6/21/2010	23.4	27.4	20.9	14.4	33.8	40.3		15.0	049K0305
CD1010	7/29/2010	23.8	26.8	20.8	14.7	32.9	39.0		15.0	079K0011
CD1011	8/17/2010	21.6	26.2	20.3	14.3	32.1	38.0		15.0	079K0011
CD1012	9/29/2010	27.8	26.4	20.8	15.2	31.9	37.5		15.0	079K0011
CD1013	10/26/2010	28.6	26.6	21.3	16.0	31.9	37.2		15.0	099K0202
CD1014	11/16/2010	21.2	26.1	20.8	15.5	31.4	36.7		15.0	099K0202
CD1015	12/21/2010	17.6	25.4	19.8	14.2	31.0	36.6		15.0	099K0202
CD1101	1/27/2011	17.0	24.7	18.9	13.1	30.6	36.4		15.0	099K0202
CD1102	2/10/2011	22.2	24.6	18.9	13.3	30.2	35.9		15.0	099K0202
CD1103	3/24/2011	26.7	24.7	19.2	13.8	30.2	35.7		15.0	099K0202
CD1104	4/26/2011	17.5	24.3	18.7	13.1	29.8	35.4		15.0	099K0202
CD1105	5/12/2011	30.9	24.6	19.0	13.4	30.3	35.9		15.0	099K0202
CD1106	5/26/2011	29.6	24.9	19.3	13.7	30.5	36.1		15.0	060M0116V
									15.0	
									15.0	

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



Test #	Test Date	Control Repro. %CV	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD		Control Limit	Toxicant Lot #
CD1002	1/26/2010	0.0							30.0	079K0011
CD1003	2/16/2010	17.0	8.5	-3.5	-15.5	20.5	32.5		30.0	079K0011
CD1004	3/23/2010	15.9	11.0	1.5	-8.1	20.5	30.0		30.0	049K0305
CD1006	4/30/2010	27.1	15.0	3.8	-7.4	26.2	37.4		30.0	049K0305
CD1007	5/27/2010	24.6	16.9	6.3	-4.3	27.5	38.2		30.0	049K0305
CD1009	6/21/2010	23.7	18.1	8.2	-1.7	27.9	37.8		30.0	049K0305
CD1010	7/29/2010	22.6	18.7	9.5	0.3	27.9	37.1		30.0	079K0011
CD1011	8/17/2010	22.6	19.2	10.6	2.0	27.8	36.4		30.0	079K0011
CD1012	9/29/2010	21.1	19.4	11.3	3.2	27.5	35.6		30.0	079K0011
CD1013	10/26/2010	19.9	19.5	11.8	4.2	27.1	34.7		30.0	099K0202
CD1014	11/16/2010	20.2	19.5	12.3	5.1	26.8	34.0		30.0	099K0202
CD1015	12/21/2010	22.0	19.7	12.8	5.9	26.7	33.6		30.0	099K0202
CD1101	1/27/2011	23.6	20.0	13.3	6.6	26.8	33.5		30.0	099K0202
CD1102	2/10/2011	23.0	20.3	13.7	7.2	26.8	33.3		30.0	099K0202
CD1103	3/24/2011	22.2	20.4	14.1	7.8	26.7	33.0		30.0	099K0202
CD1104	4/26/2011	23.0	20.5	14.4	8.3	26.7	32.8		30.0	099K0202
CD1105	5/12/2011	22.9	20.7	14.7	8.8	26.6	32.6		30.0	099K0202
CD1106	5/26/2011	22.5	20.8	15.0	9.2	26.6	32.4		30.0	060M0116V
									30.0	
									30.0	

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



Test #	Test Date	Reprod. PMSD	Mean PMSD	-1 SD	-2 SD	+1 SD	+2 SD	Upper PMSD Bound	Lower PMSD Bound	Toxicant Lot #
CD1002	1/26/2010	31.2						47	13	079K0011
CD1003	2/16/2010	19.1	25.1500	16.5940	8.0380	33.7060	42.2620	47	13	079K0011
CD1004	3/23/2010	24.8	25.0333	18.9800	12.9266	31.0867	37.1401	47	13	049K0305
CD1006	4/30/2010	37.9	28.2500	20.1373	12.0245	36.3627	44.4755	47	13	049K0305
CD1007	5/27/2010	9.6	24.5200	13.6146	2.7093	35.4254	46.3307	47	13	049K0305
CD1009	6/21/2010	38.3	26.8167	15.5566	4.2965	38.0768	49.3369	47	13	049K0305
CD1010	7/29/2010	41.9	28.9714	17.2173	5.4632	40.7255	52.4796	47	13	079K0011
CD1011	8/17/2010	11.9	26.8375	14.3936	1.9497	39.2814	51.7253	47	13	079K0011
CD1012	9/29/2010	10.8	25.0556	12.2465	-0.5626	37.8646	50.6737	47	13	079K0011
CD1013	10/26/2010	20.7	24.6200	12.4652	0.3104	36.7748	48.9296	47	13	099K0202
CD1014	11/16/2010	31.0	25.2000	13.5096	1.8192	36.8904	48.5808	47	13	099K0202
CD1015	12/21/2010	41.9	26.5917	14.4474	2.3032	38.7359	50.8802	47	13	099K0202
CD1101	1/27/2011	31.5	26.9692	15.2626	3.5559	38.6759	50.3825	47	13	099K0202
CD1102	2/10/2011	19.9	26.4643	15.0593	3.6544	37.8693	49.2742	47	13	099K0202
CD1103	3/24/2011	39.6	27.3400	15.8385	4.3369	38.8415	50.3431	47	13	099K0202
CD1104	4/26/2011	25.4	27.2188	16.0966	4.9745	38.3409	49.4630	47	13	099K0202
CD1105	5/12/2011	25.9	27.1412	16.3675	5.5938	37.9149	48.6886	47	13	099K0202
CD1106	5/26/2011	29.2	27.2556	16.7923	6.3290	37.7188	48.1821	47	13	060M0116V

APPENDIX D

RECORD COPY

BIOMONITORING CHAIN OF CUSTODY RECORD

COC No. BULKSED-052711-EEUSA

Client: TVA

Project Name: KIF Ash Toxicity Study

Dates of Sample Collection: 05231,052411, 052511

Location: Emory River (ERM0.5, ERM0.8, ERM1.0, ERM2.5, ERM3.0, ERM3.5, ERM4.0, ERM5.5, ERM8.0, ERM10.0)

Collected By: R. Josefczyk/RSI, L. Burton/RSI, D. Mathis/RSI, A. Johnson/RSI, T. Walls/RSI: G. Frye/RSI

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):
FedEx UPS Bus Client Courier
Other (specify): Access America

General Comments:
Homogenized sediment from the Emory River.

"EMORYREFERENCE" is a composite sample of ERM8.0 and ERM10.0.

Logbook:TVA-KIF-NTC-TOX-001,002,004

Field Identification / Sample Description	Grab/ Comp	Collection Date/Time		Number of Containers &	Depth (ft)	Rain Event? (Mark as Appropriate)				Laboratory Use (as applicable)				
Example: BULKSED-ERM0.0-EEUSA-052411	—	Date	Time	—	—	Yes	If Yes, Inches	No	Trace	Log #	Arrival Temp. (°C)	By	Time	Appearance
BULKSED-ERM0.5-EEUSA- 052311	C	05/23/2011	0950	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-389-11	0.5	VL	0819	*
BULKSED-ERM0.8-EEUSA-052311	C	05/23/2011	~~1042~~ 1145	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-387-11	0.7	VL	0810	*
BULKSED-ERM1.0-EEUSA-052311	C	05/23/2011	0850	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-390-11	0.5	VL	0819	*
BULKSED-ERM2.5-EEUSA-052311	C	05/23/2011	~~1145~~ 1042	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-386-11	0.7	VL	0810	*
BULKSED-ERM3.0-EEUSA-052411	C	05/24/2011	0813	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-388-11	0.5	VL	0819	*
BULKSED-ERM3.5-EEUSA-052411	C	05/24/2011	1111	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-385-11	0.7	VL	0810	*
BULKSED-ERM4.0-EEUSA-052311	C	05/23/2011	1440	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-383-11	1.6	VL	0802	*
BULKSED-ERM5.5-EEUSA-052411	C	05/24/2011	0955	(4) 1000 mL	0.0-0.5	NA	NA	X	NA	E-384-11	1.6	VL	0802	*
BULKSED-EMORYREFERENCE-EEUSA-052511	C	05/25/2011	0855	(32) 1000 mL	0.0-0.5	NA	NA	X	NA	E-382-11	1.6	VL	0802	*

Sample Custody – Fill In From Top Down

Relinquished By (Signature)/Affiliation:	Date/Time	Received By (Signature)/ Affiliation:	Date/Time
Ramona Josefczyk /RSI	053111/0810	Jesse Morris/RSI	053111/0810
Jesse Morris/RSI	060611/1333	Veronica McNew/EEUSA	06/07/11 / 0740

Associated UPS Tracking #s (if applicable): NA

*Sample received with custody seals intact, on ice; and in fine condition. 06/07/11 VL

COURIER TRANSPORT DOCUMENTATION

DATE: 06/06/2011

COURIER COMPANY:

Access America Transport

From:	To:
TVA c/o Jesse Morris 189 Lakeshore Drive Harriman, TN 37748 865-685-8364	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:
12	Cooler(s) taped and custody sealed (Batched 6 of 6, 1 of 1, and 5 empty EEUSA coolers).

Shippers Name/Company: Jesse Morris / RSI

Date / Time: 060611/1539

Courier Signature/Company: F. Salvan / Express-1

Date / Time: 060611 / 1539

Receipt Signature/Company: Veronica McNew / EE USA

Date / Time: 06/07/11 0740

Corresponding Chains of Custody:

BULKSED-052711-EEUSA page 1 of 1	
BULKSW-060611-EEUSA page 1 of 1	

CHAIN OF CUSTODY RECORD

COC No. BULKSW-061511-EEUSA

Client: TVA

Project Name: KIF Ash Toxicity Study

Date of Sample Collection: 06/15/2011

Location: ERM9.0

Collected By: R. Josefczyk (RSI), L. Jackson (TVA), J. Kerr (RSI)

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):

FedEx UPS Bus Client Courier

Other (specify):

General Comments:

Bulk Emory River reference water for sediment toxicity study collected in 2.5 gallon (10L) cubitainers.

Turbidity 4.7 NTU

TVA-KIF-NTC-TOX-004

Batch B

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	Log #	Arrival Temp. (°C)	By	Time	Appearance
BULKSW-ERM9.0-EEUSA-061511	G	06/15/2011	1021	(3) 10L cubitainers	8.06	NA	NA	X	NA	E-440-1	0.7	VM	0830	*
											0.9	VM	0830	*
											0.5	VM	0831	*

Cg: 061511

Sample Custody – Fill In From Top Down

Relinquished By (Signature)/Affiliation:	Date/Time	Received By (Signature)/Affiliation:	Date/Time
Lynne Jackson (TVA)	061511 1115	RSI	061511 / 1115
RSI	061511 / 1526	Veronica McNew / EEUSA	061611 / 0825

* Samples received with custody seals intact, on ice, and in fine condition. 06/16/11 VM

Associated UPS Tracking #s (if applicable): UPS Tracking No: 1Z 939 EX 2 1592645306