

Elutriate Toxicity Evaluation:
Ceriodaphnia dubia and Pimephales promelas

Final Report In Accordance With:

**Kingston Fossil Plant
Fly Ash Recovery Project
Sampling Plan For Phase 1 Dredging Operations**

Prepared For:

**Tennessee Valley Authority
1101 Market Street, PSC 1X-C
Chattanooga, TN 37402
Contract No. 61887**

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EXECUTIVE SUMMARY

A dike failed on December 22, 2008, at the Kingston Fossil Plant releasing approximately 5.4 million cubic yards of fly ash that ultimately reached the Emory River. As a result, TVA developed a Kingston Fossil Plant Fly Ash Recovery Plan for Phase I Dredging Operations which included a number of toxicological tests. The University of Maryland Wye Research and Education Center (WREC) was tasked to conduct 96-hour acute survival tests with *Ceriodaphnia dubia* and *Pimephales promelas* exposed to elutriates of ash samples prepared from cores collected immediately upstream and downstream of the most impacted area of the Emory River. In addition, WREC was tasked to send samples of the elutriates to Test America for chemical analysis.

Upstream and downstream ash samples were obtained by TVA personnel from the Emory River on March 17, 2009. Unaffected Emory River water (ERM 12.2), which was received from TVA on March 31, 2009, was used to prepare the ash elutriates and also served as the dilution water for the various elutriate toxicity treatments. Both the *C. dubia* and *P. promelas* 96-hour acute toxicity tests were initiated on April 1, 2009. Aliquots taken from the ash elutriates used to initiate the toxicity tests were shipped to Test America on April 2, 2009, for chemical analyses.

The elutriates (including the centrifuged elutriates) prepared from the Emory River upstream and downstream ash samples were not acutely toxic ($p > 0.05$) to *C. dubia* (see Table).

Summary of the Results of the *C. dubia* 96-Hour Survival Ash Elutriate Toxicity Test

Treatment	No. of Replicates	No. of Animals per Replicate	Mean Upstream Survival (%)	Mean Downstream Survival (%)
MHSW Negative Control	5	5	100	100
Emory River Control	5	5	100	100
6.25 % elutriate	5	5	100	100
12.5 % elutriate	5	5	100	100
25 % elutriate	5	5	100	100
50 % elutriate	5	5	100	100
100 % elutriate	5	5	100	92
100 % elutriate (Centrifuged)	5	5	100	100

Likewise, the non-centrifuged and centrifuged elutriates prepared from the Emory River upstream and downstream ash samples were not acutely toxic ($p > 0.05$) to *P. promelas* (see Table).

**Summary of the Results of the *P. promelas* 96-Hour
Survival Ash Elutriate Toxicity Test**

Treatment	No. of Replicates	No. of Animals per Replicate	Mean Upstream Survival (%)	Mean Downstream Survival (%)
MHSW Negative Control	5	10	98	98
Emory River Control	5	10	98	98
6.25 % elutriate	5	10	96	96
12.5 % elutriate	5	10	96	92
25 % elutriate	5	10	94	96
50 % elutriate	5	10	92	94
100 % elutriate	5	10	84	96
100 % elutriate (Centrifuged)	5	10	96	98

In summary, *C. dubia* and *P. promelas* No Observed Adverse Effect Concentrations (NOAECs) for survival in both the upstream and downstream elutriates are 100% elutriate by volume. The 96-hour LC50s for both *C. dubia* and *P. promelas* exposed to upstream and downstream elutriates are >100% elutriate by volume.

1.0 INTRODUCTION

A dike failed on December 22, 2008, at the Kingston Fossil Plant releasing approximately 5.4 million cubic yards of fly ash that ultimately reached the Emory River. As a result, TVA developed a Kingston Fossil Plant Fly Ash Recovery Plan for Phase I Dredging Operations which included a number of toxicological tests (TVA, 2009). The University of Maryland Wye Research and Education Center (WREC) was tasked to conduct 96-hour acute survival tests with *C. dubia* and *P. promelas* exposed to elutriates of ash samples prepared from cores collected immediately upstream and downstream of the most impacted area of the Emory River. In addition, WREC was tasked to send samples of the elutriates to Test America (Nashville, Tennessee) for chemical analysis.

Upstream and downstream ash samples were obtained by TVA personnel from the Emory River on March 17, 2009. Thirteen composite samples of both upstream and downstream ash were shipped from TVA to WREC via a refrigerated carrier and received at WREC on March 24, 2009. Upon receipt, the 26 ash samples were immediately placed in the dark in a 4 °C refrigerator. Unaffected Emory River water, which was received from TVA on March 31, 2009, was used to prepare the ash elutriates and also served as the dilution water for the various elutriate toxicity treatments. Both the *C. dubia* and *P. promelas* 96-hour toxicity tests were initiated on April 1, 2009, from ash taken from 1 of the 13 upstream and 1 of the 13 downstream containers. Aliquots taken from the ash elutriates used to initiate the toxicity tests were shipped to TestAmerica on April 2, 2009, for chemical analyses.

2.0 MATERIALS AND METHODS

2.1 Preparation of Ash Elutriates

Prior to preparing the elutriates, both the upstream and downstream Emory River ash samples were stirred in their respective 5-gallon shipping containers with a stainless steel auger attached to an electric drill to make certain the consolidated ash and overlying water were homogeneously mixed before the ash samples were used to prepare the elutriates. The auger was cleaned prior to use by soaking overnight in 10% hydrochloric acid followed by an acetone and well water rinse. The auger was then rinsed three times with distilled water. Both the upstream and downstream elutriates were prepared by generally following the standard elutriate preparation procedure given in Section 10.1.2.1 of USEPA *Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing Manual Inland Testing Manual* (USEPA, 1998).

After mixing of the overlying water and ash in the 5-gallon containers as described above, the elutriates were prepared by combining 1.5 L of ash with 6 L of unaffected Emory River water in 10 L glass jars that had been cleaned as described above. The ash:water mixture was vigorously stirred for 30 minutes using a magnetic stir plate and large magnetic stir bar at 25 °C. Prior to placing the ash:water mixture on the stir plate, the mixture was stirred again using a long-handled stainless steel spoon cleaned as described above to ensure complete mixing. The ash:water mixture was also stirred at time 10 and 20 minutes during the 30-minute stirring period to assure complete mixing.

After the 30-minute mixing period, the mixtures were allowed to settle for one hour. The supernatant (elutriate) was then siphoned off without disturbing the settled material. The turbidity of each elutriate was measured immediately after being siphoned. An aliquot from each elutriate was also centrifuged at 2000 rpm for 30 minutes at 25 °C for a centrifuged elutriate toxicity treatment for each species. The turbidity of the centrifuged samples was measured again following centrifugation. The non-centrifuged and centrifuged elutriate samples were then used to prepare the test solutions for the toxicity tests and samples for chemical analyses.

Immediately after the toxicity tests were initiated, an aliquot of each of the filtered and centrifuged elutriates and non-filtered, non-centrifuged elutriates from the upstream and downstream sites were placed in chemical containers containing acid supplied by Test America. Upon filling, the chemical containers were refrigerated at 4 °C in the dark. The samples were shipped on ice to Test America the following day (April 2, 2009) for chemical analyses.

2.2 Toxicity Tests

2.2.1 *Ceriodaphnia dubia*

The 96-hour survival toxicity test with *C. dubia* exposed to the elutriates generally followed USEPA Test Method 2002.0 given in *Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms*, 5th ed. (USEPA, 2002). A summary of the test conditions for *C. dubia* is given in Table 1.

C. dubia were cultured at 25 ± 0.5 °C in 600 mL glass beakers filled with 400 mL of MHSW. The diet consisted of a mixture of Cerophyl[®] (Cerophyl[®] Laboratories, Inc., Kansas City, Missouri) and the green alga, *Selenastrum capricornutum*, added to the *C. dubia* culture to achieve final concentrations of approximately 120 µg Cerophyl[®]/mL and $\sim 6.7 \times 10^5$ *S. capricornutum* cells/mL.

All neonates used in the 96-h survival test were produced by *C. dubia* in a brood board that had released at least three broods and were <24 hours old at the start of the exposure. The exposures were conducted in 50 mL glass beakers containing 25 mL of test solution. The test was conducted in an environmental chamber at 25 ± 0.5 °C under a 16-hours light:8-hours dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the exposure vessels). All test organisms were fed prior to the exposure and at 48-hours.

Temperature, dissolved oxygen (DO), and pH were measured randomly in one replicate in all upstream and downstream treatments at time 0 hours and every 24 hours during the 96-hour exposure. Conductivity, alkalinity, and hardness were measured randomly in one replicate at time 0 hours and at 96 hours in all treatments. Ammonia was measured randomly in one replicate at time 0 hours and at 96 hours. Turbidity was measured in the upstream and downstream non-centrifuged elutriate samples immediately after the elutriate was prepared; during test setup; and at the end of the test. No aeration was needed during the exposures because the DO in the overlying water was ≥ 7.1 mg/L at all times.

Table 1. Summary of Test Conditions and Test Acceptability Criteria for the *Ceriodaphnia dubia* 96-Hour Acute Toxicity Test with Emory River Fly Ash Elutriate

Test method: 2002.0	In: EPA-821-R-02-012 (USEPA, 2002)
Test type:	Static, non-renewal
Test duration:	96 hours
Temperature:	25 ± 1 °C
Light quality	Ambient laboratory illumination
Light intensity:	60-85 foot candles at surface of water
Photoperiod:	16 hours light, 8 hours dark
Test chamber size:	50 mL
Test solution volume:	25 mL
Renewal of elutriate:	None
Age of test organisms:	<24-hours old at start of test
No. organisms per test chamber:	5
No. replicate chambers per concentration:	5
No. organisms per concentration:	25
Feeding regime:	Fed YCT and <i>Selenastrum capricornutum</i> while holding prior to test and at 48 hours; newly-released young had food available a minimum of 2 hours prior to use in the test and at 48 hours; 0.1 mL each of YCT and <i>S. capricornutum</i> added at 48 hours
Test chamber cleaning:	Cleaning not required
Test chamber aeration:	None
Dilution water:	Unaffected Emory River water
Test concentrations:	<u>Non-Centrifuged</u> : 100, 50, 25, 12.5, and 6.25 % elutriate by volume, 100% unaffected Emory River (river control), and 100% MHSW (negative control) <u>Centrifuged</u> : 100% elutriate
Supernatant holding time:	<4 hours
Endpoint:	Survival
Test acceptability criterion:	90% or greater survival in Emory River water and MHSW negative controls

2.2.2 *Pimephales promelas*

The 96-hour survival toxicity test with *P. promelas* exposed to the elutriates generally followed USEPA Test Method 2000.0 given in *Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms*, 5th ed. (USEPA, 2002). A summary of the test conditions for *P. promelas* is given in Table 2.

All larvae used in the 96-hour survival test were <24 hours old at the start of the test. The tests were conducted in 500 mL glass beakers containing 210 mL of test solution. All test organisms were fed brine shrimp (*Artemia* sp.) nauplii <24 hours old prior to the exposure and at 48 hours. The test was conducted at 25 ± 0.5 °C under a 16-hours light:8-hours dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the exposure vessels). The *P. promelas* larvae were obtained from Chesapeake Cultures, Inc. (Hayes, Virginia).

Temperature, DO, pH, and ammonia were measured randomly in one replicate in all upstream and downstream treatments at time 0 hours and every 24 hours during the 96-hour exposure. Conductivity, alkalinity, and hardness were measured randomly in one replicate at time 0 hours and at 96 hours in all treatments. Turbidity was measured in the upstream and downstream non-centrifuged elutriate sample immediately after the elutriate was prepared; during test setup; and at the end of the test. No aeration was needed during the exposures because the DO in the overlying water was ≥ 6.7 mg/L at all times.

2.3 Test Endpoints, Test Acceptability Criteria, and Data Analyses

The endpoint for the 96-hour tests was survival. The test acceptability criteria for the toxicity tests were 90% or greater survival in the Emory River controls and MHSW negative controls. No statistics were run on the upstream *C. dubia* data since no mortality occurred at any treatment including the Emory River controls, MHSW negative controls, and 100% non-centrifuged or centrifuged elutriate. The remaining data were analyzed as follows. All percent survival data were arc sine square root transformed before any statistical analyses were conducted. The null hypothesis that all elutriate treatments were equal was tested using Steel's Many-One Rank Test for non-centrifuged treatments (multi-concentration series requirement per USEPA 2002) and Wilcoxon Rank Sum Test for the centrifuged treatment (single-concentration series requirement per USEPA 2002). All statistical tests were performed using ToxCalc (TSS, 2006).

2.4 Quality Assurance/Quality Control

As part of WREC's ongoing quality assurance/quality control program, *C. dubia* <24 hours old and *P. promelas* <24 hours old at the start of the exposures were exposed to the reference toxicant potassium chloride (KCl) using MHSW. The 48-h LC50s were determined using a dilution series consisting of five concentrations. The results were compared to in-house cumulative control chart limits (20 most recent data points).

Table 2. Summary of Test Conditions and Test Acceptability Criteria for the *Pimephales promelas* 96-Hour Acute Toxicity Test with Emory River Fly Ash Elutriate

Test method: 2000.0	In: EPA-821-R-02-012 (USEPA, 2002)
Test type:	Static, non-renewal
Test duration:	96 hours
Temperature:	25 ± 1 °C
Light quality	Ambient laboratory illumination
Light intensity:	60-85 foot candles at surface of water
Photoperiod:	16 hours light, 8 hours dark
Test chamber size:	500 mL
Test solution volume:	210 mL
Renewal of elutriate:	None
Age of test organisms:	<24 hours at start of test
No. organisms per test chamber:	10
No. replicate chambers per concentration:	5
No. organisms per concentration:	50
Feeding regime:	<i>Artemia</i> sp. nauplii were made available while holding prior to tests and at 48 hours; 0.2 mL <i>Artemia</i> sp. nauplii concentrate were added at 48 hours
Test chamber cleaning:	Cleaning not required
Test chamber aeration:	None
Dilution water:	Unaffected Emory River water
Test concentrations:	<u>Non-Centrifuged</u> : 100, 50, 25, 12.5, and 6.25 % elutriate by volume, 100% unaffected Emory River (river control), and 100% MHSW (negative control) <u>Centrifuged</u> : 100% elutriate
Supernatant holding time:	<4 hours
Endpoint:	Survival
Test acceptability criterion:	90% or greater survival in Emory River water and MHSW negative controls

2.5 Chain of Custody Records

The biomonitoring chain of custody record for the upstream and downstream ash samples were received and signed when the shipment arrived at WREC on March 24, 2009. The chain of custody seals on the 26 five-gallon containers (13 upstream and 13 downstream containers) were all intact upon receipt of the samples. The chain of custody records for the upstream and downstream ash samples and Emory River samples are given in Attachment 1. The chain of custody record for the elutriate samples sent to Test America is also presented in Attachment 1.

3.0 RESULTS AND DISCUSSION

The 96-hour upstream and downstream Emory River and MHSW negative controls for both *C. dubia* and *P. promelas* met the test acceptability criteria of 90% or greater survival. One hundred percent survival occurred in the *C. dubia* Emory River controls and MHSW negative controls. Ninety-eight percent survival occurred in the *P. promelas* Emory River controls and MHSW negative controls.

The results of *C. dubia* exposed to the upstream and downstream ash elutriates and 100% centrifuged elutriates are summarized in Table 3. The laboratory bench sheets for the overlying elutriate chemistry and survival data for *C. dubia* are given in Attachment 2. One hundred percent survival occurred in all upstream treatments; thus, no statistical analyses were performed because the upstream elutriates were not acutely toxic to *C. dubia* (Table 3). No significant difference ($p = 1.00$) was found between the downstream Emory River controls and MHSW negative controls; thus, the elutriate dilutions were tested against the Emory River controls. The non-centrifuged elutriates prepared from the Emory River downstream ash were not acutely toxic to *C. dubia* (Table 3). No mortality occurred in the 100% centrifuged elutriate; thus, no statistics were applied for this single treatment.

No 96-hour LC50 point estimate was attempted since 50% mortality did not occur in these tests. The statistical analyses for the downstream *C. dubia* are given in Attachment 2.

The results of the *P. promelas* exposed to the upstream and downstream ash elutriates and 100% centrifuged elutriates are summarized in Table 4. The *P. promelas* laboratory bench sheets for the overlying elutriate chemistry and survival data are given in Attachment 3. No significant difference was found between the upstream Emory River controls and MHSW negative controls ($p = 1.00$) or between the downstream Emory River controls and MHSW negative controls ($p = 0.35$); thus, the five elutriate treatments in both data sets were tested against the Emory River controls. The non-centrifuged elutriates prepared from the Emory River upstream and downstream ash were not acutely toxic ($p > 0.05$) to *P. promelas* (Table 4). No difference ($p > 0.05$) occurred between the Emory River controls and *P. promelas* larvae exposed to 100% centrifuged elutriate.

No 96-hour LC50 could be estimated for the upstream or downstream *P. promelas* data since 50% mortality did not occur. The statistical analyses for the downstream *P. promelas* are given in Attachment 3.

Table 3. Summary of the Results of the *C. dubia* 96-Hour Survival Ash Elutriate Toxicity Test

Treatment	No. of Replicates	No. of Animals per Replicate	Mean Upstream Survival (%)	Mean Downstream Survival (%)
MHSW Negative Control	5	5	100	100
Emory River Control	5	5	100	100
6.25 % elutriate	5	5	100	100
12.5 % elutriate	5	5	100	100
25 % elutriate	5	5	100	100
50 % elutriate	5	5	100	100
100 % elutriate	5	5	100	92
100 % elutriate (Centrifuged)	5	5	100	100

Table 4. Summary of the Results of the *P. promelas* 96-Hour Survival Ash Elutriate Toxicity Test

Treatment	No. of Replicates	No. of Animals per Replicate	Mean Upstream Survival (%)	Mean Downstream Survival (%)
MHSW Negative Control	5	10	98	98
Emory River Control	5	10	98	98
6.25 % elutriate	5	10	96	96
12.5 % elutriate	5	10	96	92
25 % elutriate	5	10	94	96
50 % elutriate	5	10	92	94
100 % elutriate	5	10	84	96
100 % elutriate (Centrifuged)	5	10	96	98

In summary, the *C. dubia* and *P. promelas* NOAECs for survival in both the upstream and downstream elutriates are 100% elutriate by volume. Likewise, the 96-hour LC50s for both *C. dubia* and *P. promelas* exposed to upstream and downstream elutriates are >100% elutriate by volume.

The KCl reference toxicant 48-h LC50 (and its 95% confidence limits) for *C. dubia* was 330 (277-385) mg/L which fell within WREC's acceptable cumulative control chart limits (Attachment 4). The KCl reference toxicant 48-h LC50 (and its 95% confidence limits) for *P.*

promelas was 1320 (1318-1458) mg/L which fell within WREC's acceptable cumulative control chart limits (Attachment 4).

4.0 REFERENCES

- TSS (Tidepool Scientific Software). 2006. ToxCalc Toxicity Data Analysis Software. Version 5.0.26. Tidepool Scientific Software, McKinleyville, CA.
- TVA. 2009. Kingston Fossil Plant Fly Ash Recovery Project Sampling Plan for Phase I Dredging Operations. March 2009. Tennessee Valley Authority, Kingston, TN.
- USEPA. 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing Manual Inland Testing Manual. EPA 823-B-98-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC.
- USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. EPA-821-R-02-012. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

ATTACHMENT 1
CHAIN OF CUSTODY RECORDS
(5 Pages)

BIOMONITORING CHAIN OF CUSTODY RECORD

Client: TVA
 Project Name: KIF Ash Toxicity Study
 P.O. Number: N/A
 Facility Sampled: N/A
 NPDES Number: N/A
 Collected By: See Comment Section for Sample team

Delivered By (Circle One):
 FedEx UPS Bus Client

Other (specify): TVA CHASE TRAINER CO. *3/23/09

General Comments: Sample Team members:
 TWA
 Jonathan Spolker
 Paul Smith
 Larry Steffen
 Tim Steffen
 Bobby Niditer
 Cynthia Russell

JACOBS
 Misha Martin
 Jeff Bale
 Adam Johnson
 Eric Standards
 Stephen Brower

University Of Maryland/ WREC.
 124 Wye Narrows Drive
 Queenstown, MD
 21658
 Phone: 410-827-8056

Field Identification / Sample Description	Grab/Comp	Collection Date/Time		Number of Containers & Volume Collected	Flow (MGD)	Rain Event? (Mark as Appropriate)		UMD Log Number	Arrival Temp. (°C)	By	Time	Appearance
		Date	Time			Yes, Inches	No					
UPSTREAM VB.1	C	03-17-09	1431	13 / ~ 58.5 gal	N/A							
DOWNSTREAM VB.2	C	03-17-09	1810	13 / ~ 58.5 gal	N/A							
					N/A							
					3-23-09							

Sample Custody - Fill In From Top Down

Relinquished By (Signature): JEFF BALE / MBL
 Date/Time: 3-23-09 @ 1030

Received By (Signature): Steve Turley
 Date/Time: 3-24-09 12:40

*All 26 buckets sealed with tape and all custody seals intact

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 6°C and shipping them in-ice. The hold-time for each sample is 36 hours from the time-of-collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that time frame. Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday.

MAR 24 2009

JEFF BALE

865-719-9706

COURIER RELEASE/CUSTODY DOCUMENTATION

Project: TVA Kingston Ash Toxicity Study

Initial Delivery Location (From): TVA Kingston KIF Facility
Shipment Date: March 23, 2009

Final Delivery Location: University of Maryland/WREC
124 Wye Narrows Drive
Queenstown, MD 21658
PHONE: 410-827-8056

ATTN: DENNIS BURTON

Delivery Description: Twenty-six (26) five-gallon buckets with lids. Five-gallon bucket lids taped with grey duct tape and then Custody Sealed in two places with Custody Tamper Seals. Buckets secured in front of chilled tractor trailer on wood pallets with shrink wrap and blocking. Entire load covered with blanket. Load stabilizer bar placed on back end of load to prevent movement during shipment.

Shipment Relinquished By: JEFF BALE / [Signature] (RSI) 3-23-09 @ 1030 RSI
Name Printed/Signature Date/Time Company

Shipment Accepted By: Anthony Burt / [Signature] 3-23-09 @ 1030 TVA
Name Printed/Signature Date/Time Company

ATTACHMENT 2

**LABORATORY BENCHSHEETS AND SUPPORTING STUDY DOCUMENTS FOR
THE *Ceriodaphnia dubia* ELUTRIATE TOXICITY TESTS**

(8 Pages)

TVA Emory River Project

Elutriate Tests

Test Species: Ceriodaphnia dubia

Age at Start of Test: <24-h-old

Test Dates: 4/1/09 - 4/5/09

Concentration	Temperature					Dissolved Oxygen				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	25.0	25.0	25.1	25.0	25.0	8.4	8.1	7.9	7.8	7.6
Emory River - Control	25.0	25.0	25.1	25.0	25.0	8.5	8.1	7.9	7.7	7.6
6.25% ASH-UP	25.0	25.0	25.1	25.0	25.0	8.4	8.0	7.8	7.6	7.6
12.5% ASH-UP	25.0	25.0	25.1	25.0	25.0	8.4	8.0	7.7	7.6	7.4
25% ASH-UP	25.0	25.0	25.1	25.0	25.0	8.4	8.0	7.8	7.7	7.5
50% ASH-UP	25.0	25.0	25.1	25.0	25.0	8.5	8.1	7.9	7.6	7.4
100% ASH-UP	25.0	25.0	25.1	25.0	25.0	8.6	8.1	7.5	7.3	7.2
Centrifuged → *100% ASH-UP	25.0	25.0	25.1	25.0	25.0	8.7	8.0	7.7	7.6	7.4

Concentration	pH					Conductivity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	7.75	7.47	7.52	7.66	7.61	270				320
Emory River - Control	6.80	6.51	6.64	6.70	6.68	120				110
6.25% ASH UP	6.67	6.39	6.44	6.49	6.65	110				110
12.5% ASH UP	6.75	6.42	6.46	6.44	6.57	110				110
25% ASH UP	6.81	6.46	6.49	6.45	6.57	100				110
50% ASH UP	7.23	6.63	6.55	6.48	6.66	110				140
100% ASH UP	7.66	7.02	6.37	6.39	7.04	200				230
Centrifuged → *100% ASH UP	7.61	6.96	6.48	6.47	6.86	130				175

Concentration	Alkalinity					Hardness				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	60				60	80				84
Emory River - Control	20				20	32				32
6.25% ASH UP	25				25	36				36
12.5% ASH UP	25				25	36				36
25% ASH UP	25				25	40				44
50% ASH UP	30				25	44				44
100% ASH UP	35				30	52				52
Centrifuged → *100% ASH UP	35				35	48				52

TVA Emory River Project

Elutriate Tests

Test Species: Ceriodaphnia dubia Age at Start of Test: < 24 h old

Test Dates: 4/11/09 - 4/15/09

Concentration	Ammonia (Total)					Turbidity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	0.12				0.35					
Emory River - Control	0.15				0.38					
6.25% ASH - UP	0.27				0.30					
12.5% ASH UP	0.30				0.29					
25% ASH UP	0.21				0.31					
50% ASH UP	0.37				0.39					
100% ASH UP	0.43				0.49	64				15
Centrifuged → * 100% ASH UP	0.33				0.48	31				10

Turbidity of 100% Ash up sample - immediately after elutriate process - 181

TVA Emory River Project

Elutriate Tests

Test Species: Ceriodaphnia dubia Age at Start of Test: <24h old

Test Dates: 4/6/09 - 4/15/09

Concentration		Number of Organisms Alive				
		0 H	24 H	48 H	72 H	96 H
MHSW Control	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y
Emory River Control	01	5	5	5	5	5 Y
	Water 02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	UP 05	5	5	5	5	5 Y
6.25% Ash 01-05	01 ST	5	5	5	5	5 Y
	02 ST	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	UP 05	5	5	5	5	5 Y
12.5% Ash 01-05	01	5	5	5	5	5 Y
	02 ST	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	UP 05	5	5	5	5	5 Y
25% Ash 01-05	01	5	5	5	5	5 Y
	02 ST	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	UP 05	5	5	5	5	5 Y
50% Ash 01-05	01	5	5	5	5	5 Y
	02 ST	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	UP 05	5	5	5	5	5 Y
100% Ash 01-05	01	5	5	5	5	5 Y
	02 ST	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y

Y = young neonates produced

TVA Emory River Project

Elutriate Tests

Test Species: *Ceriodaphnia dubia* Age at Start of Test: < 24 h old

Test Dates: 4/1/09 - 4/5/09

Concentration	Temperature					Dissolved Oxygen				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	25.0	25.0	25.1	25.0	25.0	8.4	8.1	7.9	7.7	7.6
Emory River Control	25.0	25.0	25.1	25.0	25.0	8.5	8.1	7.9	7.8	7.6
6.25% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.5	8.0	7.8	7.7	7.5
12.5% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.5	8.1	7.8	7.6	7.5
25% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.5	8.1	7.7	7.6	7.4
50% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.6	8.2	7.6	7.5	7.4
100% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.7	8.0	7.3	7.2	7.1
*100% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.6	8.1	7.5	7.4	7.3

Centrifuged →

Concentration	pH					Conductivity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	7.75	7.47	7.52	7.66	7.61	270				320
Emory River - Control	6.30	6.51	6.64	6.70	6.68	120				110
6.25% ASH DOWN	6.03	6.73	6.69	6.74	6.67	70				105
12.5% ASH DOWN	6.06	6.71	6.61	6.70	6.61	70				105
25% ASH DOWN	6.12	6.68	6.59	6.64	6.58	70				110
50% ASH DOWN	6.69	6.72	6.56	6.62	6.65	80				120
100% ASH DOWN	7.05	6.83	6.50	6.52	6.72	105				140
*100% ASH DOWN	6.87	6.77	6.61	6.65	6.79	105				145

Centrifuged →

Concentration	Alkalinity					Hardness				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	60				60	80				84
Emory River - Control	20				20	32				32
6.25% ASH DOWN	20				20	36				36
12.5% ASH DOWN	25				20	36				36
25% ASH DOWN	25				25	40				44
50% ASH DOWN	25				25	44				48
100% ASH DOWN	30				30	54				54
*100% ASH DOWN	30				30	56				58

Centrifuged →

TVA Emory River Project

Elutriate Tests

Test Species: Ceriodaphnia dubia Age at Start of Test: < 24 h old

Test Dates: 4/1/09 - 4/5/09

Concentration	Ammonia (Total)					Turbidity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW Control	0.12				0.35					
Emory River - Control	0.15				0.38					
6.25% ASH DOWN	0.46				0.39					
12.5% ASH DOWN	0.50				0.41					
25% ASH DOWN	0.76				0.44					
50% ASH DOWN	0.97				0.47					
100% ASH DOWN	1.01				0.61	930				67
Centrifuge → 100% ASH DOWN	0.99				0.54	20				15

TVA Emory River Project

Elutriate Tests

Test Species: *Ceriodaphnia dubia* Age at Start of Test: <24 h old

Test Dates: 4/4/09 - 4/5/09

Concentration		Number of Organisms Alive				
		0 H	24 H	48 H	72 H	96 H
100% ASH UP Centrifuged	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y
6.25% ASH DOWN	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y
12.5% ASH DOWN	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y
25% ASH DOWN	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y
50% ASH DOWN	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y
100% ASH DOWN	01	5	A	A	A	5 Y
	02	5	A	A	A	5 Y
	03	5	A	A	A	4 ^u Y
	04	5	A	A	A	4 ^u Y
	05	5	A	A	A	5 Y
100% ASH DOWN Centrifuged	01	5	5	5	5	5 Y
	02	5	5	5	5	5 Y
	03	5	5	5	5	5 Y
	04	5	5	5	5	5 Y
	05	5	5	5	5	5 Y

* Had to count with light shining through side of beakers due to flocculent.

A - Some alive - too difficult to count

Y - Young neonates produced

Acute Invertebrate Test-96 Hr Survival

Start Date: 04/01/2009	Test ID: KIF Elutr.	Sample ID: Kingston Ash Downstream
End Date: 04/05/2009	Lab ID: UMD-WREC	Sample Type: UPPER 10'
Sample Date: 03/17/2009	Protocol: EPA.-821-R-02-012	Test Species: CD-Ceriodaphnia dubia
Comments: Emory River Mile 12.2 Control & Dilution Water Collected 03/30/2009		

Conc-%	1	2	3	4	5
MHS-Control	1.0000	1.0000	1.0000	1.0000	1.0000
Emory River	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	0.8000	0.8000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
MHS-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5		
Emory River	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
6.25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
12.5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
50	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
100	0.9200	0.9200	1.2500	1.1071	1.3453	10.434	5	22.50	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.55935	0.9	-1.0534	4.98677
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.306		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Emory River				

ATTACHMENT 3

**LABORATORY BENCHSHEETS AND SUPPORTING STUDY DOCUMENTS FOR
THE *Pimephales promelas* ELUTRIATE TOXICITY TESTS**

(11 Pages)

TVA Emory River Project

Elutriate Tests

Test Species: Fathead Minnow Age at Start of Test: < 24 h old

Test Dates: 4/1/09 - 4/15/09

Concentration	Temperature					Dissolved Oxygen				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	25.0	25.0	25.1	25.0	25.0	8.4	7.8	7.7	7.6	7.5
Emory River - Control	25.0	25.0	25.1	25.0	25.0	8.5	7.9	7.6	7.6	7.5
6.25% ASH UP	25.0	25.0	25.1	25.0	25.0	8.4	7.9	7.5	7.5	7.5
12.5% ASH UP	25.0	25.0	25.1	25.0	25.0	8.4	8.0	7.6	7.4	7.3
25% ASH UP	25.0	25.0	25.1	25.0	25.0	8.4	7.9	7.8	7.5	7.3
50% ASH UP	25.0	25.0	25.1	25.0	25.0	8.5	7.9	7.8	7.5	7.2
100% ASH UP	25.0	25.0	25.1	25.0	25.0	8.6	7.8	6.9	6.8	6.7
*100% ASH UP	25.0	25.0	25.1	25.0	25.0	8.7	7.8	7.2	7.1	7.1

Centrifuged →

Concentration	pH					Conductivity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	7.75	7.37	7.43	7.71	7.75	270				320
Emory River - Control	6.30	6.42	6.58	6.66	6.51	120				110
6.25% ASH UP	6.67	6.27	6.39	6.43	6.55	110				100
12.5% ASH UP	6.75	6.26	6.37	6.39	6.39	110				100
25% ASH UP	6.81	6.32	6.37	6.38	6.35	100				110
50% ASH UP	7.23	6.52	6.47	6.52	6.50	110				115
100% ASH UP	7.66	6.86	6.21	6.35	6.68	200				220
*100% ASH UP	7.61	6.80	6.31	6.41	6.71	130				160

Centrifuged →

Concentration	Alkalinity					Hardness				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW - Control	60				60	80				80
Emory River - Control	20				20	32				32
6.25% ASH UP	25				25	36				36
12.5% ASH UP	25				25	36				36
25% ASH UP	25				25	40				40
50% ASH UP	30				25	44				44
100% ASH UP	35				30	52				52
*100% ASH UP	35				35	48				52

Centrifuged →

TVA Emory River Project

Elutriate Tests

Test Species: Fathead Minnow Age at Start of Test: < 24 h old

Test Dates: 4/1/09 - 4/15/09

Concentration	Ammonia (Total)					Turbidity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
M.H.S.W. Control	0.02	0.13	0.11	0.13	0.31					
Emory River - Control	0.05	0.17	0.13	0.27	0.33					
6.25% ASH - UP	0.27	0.29	0.31	0.35	0.41					
12.5% ASH - UP	0.30	0.31	0.34	0.35	0.47					
25% ASH - UP	0.21	0.27	0.30	0.37	0.51					
50% ASH - UP	0.37	0.39	0.36	0.39	0.58					
100% ASH UP	0.43	0.48	0.44	0.49	0.70	64				14
Centrifuged → 100% ASH UP	0.33	0.35	0.39	0.42	0.74	31				10

* Turbidity of Ash Upstream immediately after elutriate process - 181

TVA Emory River Project

Elutriate Tests

Test Species: Fathead Minnow

Age at Start of Test: <24-h-old

Test Dates: 4/1/09 - 4/15/09

Concentration		Number of Organisms Alive				
		0 H	24 H	48 H	72 H	96 H
MHSW-Control	01	10	10	10	10	10
	02	10	10	10	10	10
	03	10	10	10	10	10
	04	10	10	10	10	9 ^W
	05	10	10	10	10	10
Emory River Water Control	01	10	10	10	10	10
	02	10	10	10	10	10
	03	10	10	10	10	10
	04	10	10	10	10	10
	05	10	10	10	10	9 ^W
6.25% ASH-UP	01	10	10	10	10	10
	02	10	10	10	10	9 ^W
	03	10	10	10	10	10
	04	10	10	10	10	10
	05	10	10	10	10	9 ^W
12.5% ASH-UP	01	10	10	10	10	10
	02	10	10	10	10	10
	03	10	10	10	10	8 ^W
	04	10	10	10	10	10
	05	10	10	10	10	10
25% ASH-UP	01	10	10	10	10	10
	02	10	10	10	10	9 ^W
	03	10	10	10	10	10
	04	10	10	10	10	10
	05	10	10	10	10	8 ^W
50% ASH-UP	01	10	10	10	10	10
	02	10	10	10	10	8 ^W
	03	10	10	10	10	9 ^W
	04	10	10	10	10	9 ^W
	05	10	10	10	10	10
100% ASH-UP	01	10	10	9 ^W	9	8 ^W
	02	10	10	10	9 ^W	9
	03	10	10	10	9 ^W	9
	04	10	10	9 ^W	7 ^W	7
	05	10	10	10	9 ^W	9

TVA Emory River Project

Elutriate Tests

Test Species: Fathead Minnow

Age at Start of Test: < 24 h old

Test Dates: 4/1/09 - 4/5/09

Concentration	Temperature					Dissolved Oxygen				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW-Control	25.0	25.0	25.1	25.0	25.0	8.4	7.8	7.7	7.6	7.5
Emory River-Control	25.0	25.0	25.1	25.0	25.0	8.5	7.9	7.6	7.6	7.5
6.25% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.5	7.9	7.9	7.7	7.7
12.5% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.5	8.0	7.8	7.7	7.6
25% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.5	8.0	7.7	7.5	7.4
50% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.6	7.9	7.6	7.5	7.4
100% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.7	7.9	6.8	6.7	6.5
Centrifuged → *100% ASH DOWN	25.0	25.0	25.1	25.0	25.0	8.6	7.9	7.3	7.2	7.0

Concentration	pH					Conductivity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW-Control	7.75	7.37	7.43	7.71	7.75	270				320
Emory River-Control	6.30	6.42	6.58	6.66	6.51	120				110
6.25% ASH DOWN	6.03	6.68	6.58	6.59	6.80	70				105
12.5% ASH DOWN	6.06	6.61	6.49	6.47	6.63	70				105
25% ASH DOWN	6.12	6.61	6.49	6.46	6.63	70				110
50% ASH DOWN	6.69	6.65	6.51	6.58	6.57	80				100
100% ASH DOWN	7.05	6.68	6.29	6.34	6.71	105				155
Centrifuged → *100% ASH DOWN	6.87	6.65	6.41	6.44	6.78	105				170

Concentration	Alkalinity					Hardness				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW-Control	60			60	60	80				84
Emory River-Control	20			20	20	32				32
6.25% ASH DOWN	20			20	20	36				36
12.5% ASH DOWN	25			25	25	36				36
25% ASH DOWN	25			25	25	40				40
50% ASH DOWN	25			25	25	44				44
100% ASH DOWN	30			30	30	54				56
Centrifuged → *100% ASH DOWN	30			30	30	56				56

TVA Emory River Project

Elutriate Tests

Test Species: Fathead Minnow Age at Start of Test: <24 h

Test Dates: 4/1/09 - 4/15/09

Concentration	Ammonia (Total)					Turbidity				
	0 H	24 H	48 H	72 H	96 H	0 H	24 H	48 H	72 H	96 H
MHSW Control	0.12	0.13	0.11	0.13	0.31					
Emory River-Con	0.15	0.17	0.13	0.27	0.33					
6.25% Ash Down	0.46	0.49	0.39	0.43	0.55					
12.5% Ash Down	0.50	0.53	0.47	0.51	0.74					
25% Ash Down	0.76	0.81	0.77	0.67	0.73					
50% Ash Down	0.97	1.02	0.92	0.71	0.81					
100% Ash Down	1.01	1.09	0.93	0.79	0.91	930				69
Centrifuged → 100% Ash Down	0.99	1.01	0.97	0.71	0.67	20				18

* Turbidity of 100% Ash Down Sample immediately after elutriate process - 2390

TVA Emory River Project

Elutriate Tests

Test Species: Fathead Minnow Age at Start of Test: < 24-h-old

Test Dates: 4/11/09 - 4/15/09

Concentration		Number of Organisms Alive				
		0 H	24 H	48 H	72 H	96 H
100% ASH-UP Centrifuged	01	10	10	10	10	10
	02	10	10	10	10	10
	03	10	10	10	10	9 ^W
	04	10	10	10	10	9 ^W
	05	10	10	10	10	10
6.25% ASH DOWN	01	10	10	10	10	9 ^W
	02	10	10	10	10	10
	03	10	10	10	10	9 ^W
	04	10	10	10	10	10
	05	10	10	10	10	10
12.5% ASH DOWN	01	10	*	*	* ^E	9
	02	10	*	*	*	9 ^W
	03	10	*	*	*	10
	04	10	*	*	* ^E	9 ^W
	05	10	*	*	*	9 ^E
25% ASH DOWN	01	10	*	*	*	10
	02	10	*	*	*	10
	03	10	*	*	*	9 ^W
	04	10	*	*	* ^E	9
	05	10	*	*	*	10
50% ASH DOWN	01	10	*	*	*	10
	02	10	*	*	*	10
	03	10	*	*	*	10
	04	10	*	*	*	9 ^W
	05	10	*	*	*	8 ^W
100% ASH DOWN	01	10	*	*	*	10
	02	10	*	*	*	10
	03	10	*	*	*	9 ^W
	04	10	*	*	*	9 ^W
	05	10	*	*	*	10
100% ASH DOWN Centrifuged	01	10	10	10	10	10
	02	10	10	10	10	10
	03	10	10	10	10	9 ^W
	04	10	10	10	10	10
	05	10	10	10	10	10

* Multiple fish visible in water column, when shining light from bottom and side. Too difficult to get accurate count due to flocculents.

Acute Fish Test-96 Hr Survival

Start Date: 04/01/2009	Test ID: KIF Elutr.	Sample ID: Kingston Ash Upstream
End Date: 04/05/2009	Lab ID: UMD-WREC	Sample Type: UPPER 10'
Sample Date: 03/17/2009	Protocol: EPA.-821-R-02-012	Test Species: PP-Pimephales promelas
Comments: Emory River Mile 12.2 Control & Dilution Water Collected 03/30/2009		

Conc-%	1	2	3	4	5
MHS-Control	1.0000	1.0000	1.0000	0.9000	1.0000
Emory River	1.0000	1.0000	1.0000	1.0000	0.9000
6.25	1.0000	0.9000	1.0000	1.0000	0.9000
12.5	1.0000	1.0000	0.8000	1.0000	1.0000
25	1.0000	0.9000	1.0000	1.0000	0.8000
50	1.0000	0.8000	0.9000	0.9000	1.0000
100	0.8000	0.9000	0.9000	0.7000	0.9000

Conc-%	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical
	Mean	N-Mean	Mean	Min	Max	CV%		
MHS-Control	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5	
Emory River	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5	
6.25	0.9600	0.9796	1.3468	1.2490	1.4120	6.628	5	25.00
12.5	0.9600	0.9796	1.3510	1.1071	1.4120	10.092	5	27.00
25	0.9400	0.9592	1.3184	1.1071	1.4120	10.436	5	24.50
50	0.9200	0.9388	1.2859	1.1071	1.4120	10.026	5	22.00
100	0.8400	0.8571	1.1691	0.9912	1.2490	10.000	5	16.50

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.86454	0.9	-0.9419	-0.2718
Bartlett's Test indicates equal variances (p = 0.83)	2.14455	15.0863		
The control means are not significantly different (p = 1.00)	0	2.306		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs Emory River	100	>100		1

Acute Fish Test-96 Hr Survival

Start Date: 04/01/2009	Test ID: KIF Elutr.	Sample ID: Kingston Ash Upstream
End Date: 04/05/2009	Lab ID: UMD-WREC	Sample Type: UPPER 10'
Sample Date: 03/17/2009	Protocol: EPA.-821-R-02-012	Test Species: PP-Pimephales promelas
Comments: Emory River Mile 12.2 Control & Dilution Water Collected 03/30/2009		

Conc-%	1	2	3	4	5
MHS-Control	1.0000	1.0000	1.0000	0.9000	1.0000
Emory River	1.0000	1.0000	1.0000	1.0000	0.9000
100 Centr.	1.0000	1.0000	0.9000	0.9000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
MHS-Control	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5		
Emory River	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5		
100 Centr.	0.9600	0.9796	1.3468	1.2490	1.4120	6.628	5	25.00	19.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.75876	0.781	-0.9546	-1.0157
F-Test indicates equal variances ($p = 0.70$)	1.5	23.1545		
The control means are not significantly different ($p = 1.00$)	0	2.306		

Hypothesis Test (1-tail, 0.05)

Wilcoxon Two-Sample Test indicates no significant differences
Treatments vs Emory River

Acute Fish Test-96 Hr Survival

Start Date: 04/01/2009	Test ID: KIF Elutr.	Sample ID: Kingston Ash Downstream
End Date: 04/05/2009	Lab ID: UMD-WREC	Sample Type: UPPER 10'
Sample Date: 03/17/2009	Protocol: EPA.-821-R-02-012	Test Species: PP-Pimephales promelas
Comments: Emory River Mile 12.2 Control & Dilution Water Collected 03/30/2009		

Conc-%	1	2	3	4	5
MHS-Control	1.0000	1.0000	1.0000	0.9000	1.0000
Emory River	1.0000	1.0000	1.0000	1.0000	0.9000
6.25	0.9000	1.0000	0.9000	1.0000	1.0000
12.5	0.9000	0.9000	1.0000	0.9000	0.9000
25	1.0000	1.0000	0.9000	0.9000	1.0000
50	1.0000	1.0000	1.0000	0.9000	0.8000
100	1.0000	1.0000	0.9000	0.9000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
MHS-Control	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5		
Emory River	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5		
6.25	0.9600	0.9796	1.3468	1.2490	1.4120	6.628	5	25.00	16.00
12.5	0.9200	0.9388	1.2816	1.2490	1.4120	5.687	5	20.00	16.00
25	0.9600	0.9796	1.3468	1.2490	1.4120	6.628	5	25.00	16.00
50	0.9400	0.9592	1.3184	1.1071	1.4120	10.436	5	24.50	16.00
100	0.9600	0.9796	1.3468	1.2490	1.4120	6.628	5	25.00	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.89755	0.9	-0.6084	-0.572
Bartlett's Test indicates equal variances (p = 0.81)	2.24566	15.0863		
The control means are not significantly different (p = 1.00)	0	2.306		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs Emory River				

Acute Fish Test-96 Hr Survival

Start Date: 04/01/2009	Test ID: KIF Elutr.	Sample ID: Kingston Ash Downstream
End Date: 04/05/2009	Lab ID: UMD-WREC	Sample Type: UPPER 10'
Sample Date: 03/17/2009	Protocol: EPA.-821-R-02-012	Test Species: PP-Pimephales promelas
Comments: Emory River Mile 12.2 Control & Dilution Water Collected 03/30/2009		

Conc-%	1	2	3	4	5
MHS-Control	1.0000	1.0000	1.0000	0.9000	1.0000
Emory River	1.0000	1.0000	1.0000	1.0000	0.9000
100 Centr.	1.0000	1.0000	0.9000	1.0000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
MHS-Control	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5		
Emory River	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5		
100 Centr.	0.9800	1.0000	1.3794	1.2490	1.4120	5.284	5	27.50	19.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.50963	0.781	-1.7788	1.40625
F-Test indicates equal variances ($p = 1.00$)	1	23.1545		
The control means are not significantly different ($p = 1.00$)	0	2.306		

Hypothesis Test (1-tail, 0.05)

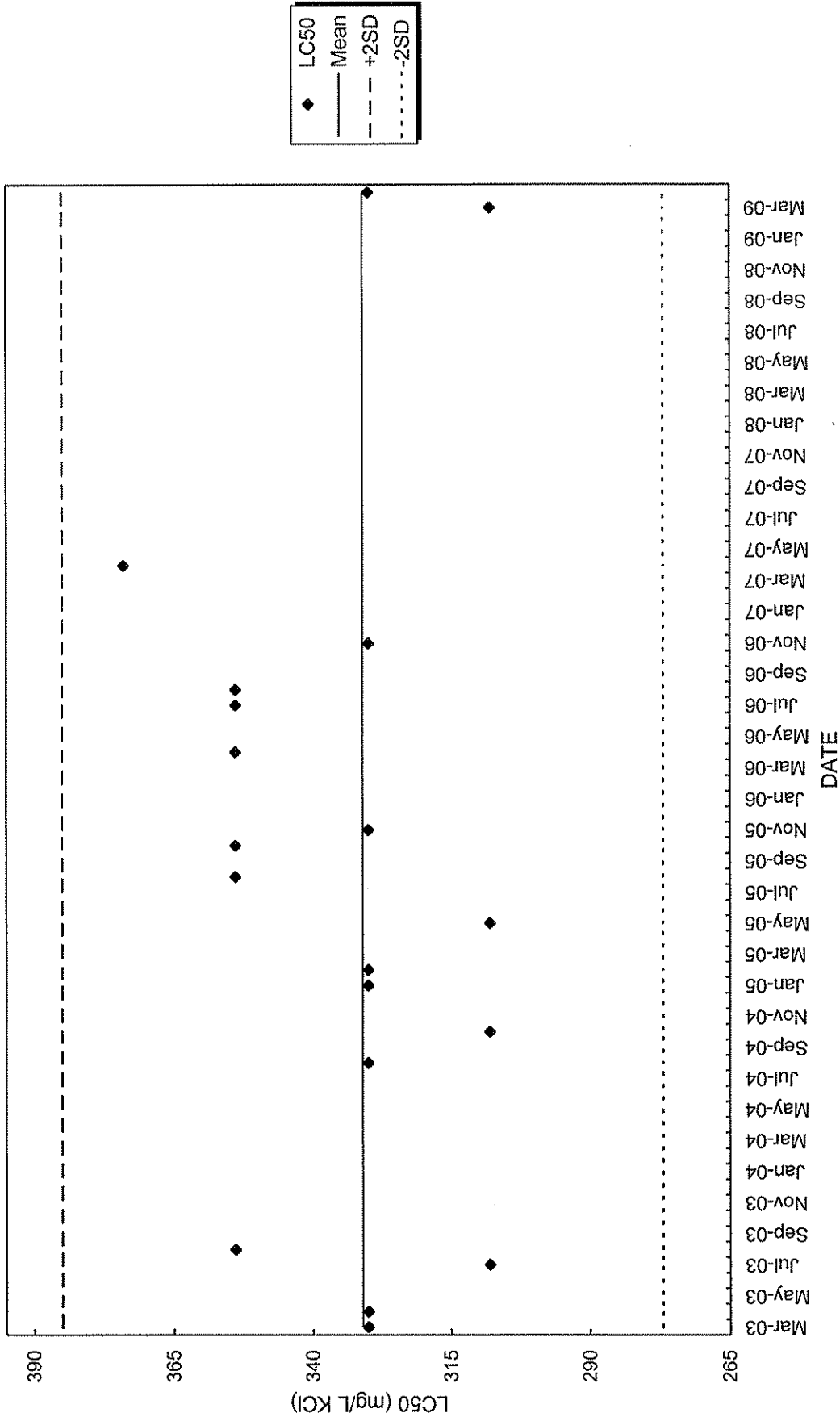
Wilcoxon Two-Sample Test indicates no significant differences
Treatments vs Emory River

ATTACHMENT 4

KCL REFERENCE TOXICANT CONTROL CHARTS

(3 Pages)

KCI 48-Hour LC50 Data - *Ceriodaphnia dubia*



KCI 48-Hour LC50 Data - *Pimephales promelas*

