May 25, 1993

Ms. Lynn A. Kszos, Aquatic Toxicologist
Environmental Sciences Division
Oak Ridge National Laboratory
P. O. Box 2008, MS-6351
Oak Ridge, Tennessee 37831-6351

Dear Lynn:

Enclosed is a copy of the report presenting results of TVA toxicity testing during the Clinch River - Environmental Restoration Program (CR-ERP) Pilot Study, April 22-29, 1993. Attachments to the report include Chain of Custody Forms - Originals (I), Toxicity Test Bench Sheets and Statistical Analyses (II), Reference Toxicant Test Information (III), and Personnel Training Documentation (IV). Exposure of fathead minnows and daphnids to water column samples collected from Clinch River Mile 9.0 and Poplar Creek Kilometer 1.6 on April 21, 23, and 26 resulted in no toxicity to test organisms.

Please call Don Wade (205-386-2068) or me (205-729-3340) if you have questions or comments regarding the report or conductance of the study.

Sincerely,

Jennifer Moses, Unit Supervisor
Aquatic Research Laboratory

Prepared by Jennifer Moses
PLARC501-1530

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OFC. OF CHIEF COUNSEL, DOE/ORD
By Date 11/21/93
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1.0 EXECUTIVE SUMMARY

Clinch River - Environmental Restoration Program (CR-ERP) personnel and Tennessee Valley Authority (TVA) personnel conducted a pilot study during the week of April 22-29, 1993, prior to initiation of CR-ERP Phase II Sampling and Analysis activities as described in the Statement of Work (SOW) document. The organisms specified for testing were larval fathead minnows, Pimephales promelas, and the daphnid, Ceriodaphnia dubia. Surface water samples were collected by TVA Field Engineering personnel from Clinch River Mile 9.0 and Poplar Creek Kilometer 1.6 on April 21, 23, and 26. Samples were split and provided to the CR-ERP and TVA toxicology laboratories for testing.

Exposure of test organisms to these samples resulted in no toxicity (survival, growth, or reproduction) to either species in testing conducted by TVA.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): Samples were collected for biomonitoring at the following two sites: Clinch River Mile 9.0, downstream of the mouth of Poplar Creek and upstream of Brashean Island, and Poplar Creek Kilometer 1.6, downstream of the K-25 powerhouse. Clinch River samples were collected approximately 10 yards from the right bank (facing upstream), and Poplar Creek samples were collected approximately 10 feet from the right bank (facing upstream).
2.2 Control and/or Dilution Water: Laboratory culture medium consisting of moderately hard reconstituted water was used as control and dilution water for toxicity tests. The water used for daphnid testing was enriched with 10 percent filtered Tennessee River water and selenium as described in Section 3.2.2.

2.3 Sample Dates and Times: Test samples were collected on three days: April 21, 23, and 26, 1993. Collection times were between 1000 and 1130 CDT.

2.4 Sampling Method: All samples were collected using an Isco peristaltic pump with the inlet tubing lowered to approximately 1.0-1.5 meters below the surface. Pumping rate was approximately two liters per minute. A 20 L composite was collected at each station and partitioned (split) according to the workplan for shipping to the TVA Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site or direct transfer to ORNL (DOE) personnel in the field.

2.5 Pertinent Site Conditions: Rainfall (light/steady) occurred during sampling on April 21. April 23 and 26 sample days were clear and sunny.

2.6 Sample Storage/Handling: All samples were placed on ice in ice chests after partitioning. Zero headspace was established in each container by filling full and expressing any air bubbles before sealing. Samples remained on ice until shipped or transferred directly to ORNL (DOE) personnel. Excess composite sample was discarded. Samples were stored in refrigerators at 4°C ± 1°C after arrival at the Aquatic Research Laboratory (ARL).

2.7 Sample Transport: Samples collected on April 21 and 26 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Personnel from ARL picked up samples from the mailroom and transported them to ARL. Samples collected on April 23, 1993, were shipped to ARL by Federal Express "next day service."

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to near 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 μm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Samples from Clinch River Mile 9.0 and Poplar Creek Kilometer 1.6 were tested at 100 percent (undiluted) and diluted to 50 percent using the appropriate culture media.
3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: Pimephales promelas, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as CaCO₃.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns is reared to adults for replacement spawners.

3.1.5 Spawn Date: April 17, 1993

3.1.6 Hatch Date/Time: April 21, 1993/1200 CDT - April 22, 1993/0700 CDT

3.1.7 Culture/Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.07 mg

3.1.10 Diseases and Treatment: None.

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.
3.2 Species: *Ceriodaphnia dubia*, daphnids

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Moderately hard reconstituted water containing 10 percent filtered Tennessee River water and 1.0 ppb selenium. [2] Total hardness was approximately 95 mg CaCO₃/L.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are selected as neonates from broods as described below on 2 or 3 consecutive days 6-10 days prior to test initiation (Adults up to 14 days old may be used for neonate production.) These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups), and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the earliest brood used for test initiation.

Mass cultures may also be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: *Ceriodaphnia* are fed food made according to methods modified from EPA/600/4-89/013 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [3]

In addition to the yeast/alfalfa/fish food recipe, the alga *Selenastrum capricornutum* concentrated to 50 x 10⁶ cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [3]

4.1.1 Modification/Amendments to Method 1000.0:

4.1.1.1 A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.
Temperatures measured in Clinch River and Poplar Creek samples upon receipt on 4/24 were 6.6°C and 6.2°C, respectively. Although ice was still present in the cooler, the samples had not cooled to the prescribed 4°C ± 1°C storage temperature. The samples were placed in a refrigerator following removal of the sample volumes needed that day and stored at the proper temperature the remaining two days the samples were used.

Date/Time Test Initiated: April 22, 1993/1050 CDT

Date/Time Test Terminated: April 29, 1993/0950 CDT

Test Chamber: 600-mL borosilicate glass beakers
Volume per chamber: 350 mL

Number of Test Organisms per Chamber: 10

Number of Replicates per Treatment: 4

Dilution Water/Control Water: Moderately hard reconstituted water

Renewal Period: 24-h

Test Temperature: 25.0°C ± 1°C

Feeding Regime During Test: Fathead minnow larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

Physical and Chemical Parameters Measured: Parameters measured daily (initially) on test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH and conductivity. In addition, alkalinity, hardness, and total residual chlorine were measured on each new sample at the time of first use (4/22, 4/24 and 4/27).

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in section 6.3.

Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. [3] Decision processes followed for testing survival and sublethal (growth) effects are shown in sections 6.1.2.2 and 6.1.3.1. Based on tests for normal distribution and homogenous variance of data, the statistical test used for endpoint determination of survival data was Steel's Many-one Rank Test and for growth data was Dunnett's Test.
4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [3]

4.2.1 Modifications/Deviations to Method 1002.0:

4.2.1.1 Enriched culture medium (see item 3.2.2.).

4.2.1.2 Prepared food recipe (see item 3.2.5).

4.2.1.3 Algal cell density (see item 3.2.5).

4.2.1.4 Feeding regime (see item 4.2.10)

4.2.1.5 A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.2.1.6 Temperatures measured in Clinch River and Poplar Creek samples upon receipt on 4/24 were 6.6°C and 6.2°C, respectively. Although ice was still present in the cooler, the samples had not cooled to the prescribed 4°C ± 1°C storage temperature. The samples were placed in a refrigerator following removal of the sample volumes needed that day and stored at the proper temperature the remaining two days the samples were used.

4.2.2 Date/Time Test Initiated: April 22, 1993/1115 CDT

4.2.3 Date/Time Test Terminated: April 28, 1993/1115 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1) Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Enriched moderately hard reconstituted water

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25.0°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10⁶ cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH and conductivity. In addition, alkalinity, hardness, and total residual chlorine were measured on each new sample at the time of first use (4/22, 4/24, and 4/27).
"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in section 6.3.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [3] Fisher's Exact Test was used to evaluate Ceriodaphnia survival effects during the study. The decision process followed for testing sublethal (reproduction) effects is shown in section 6.2.3.1. Based on tests for normal distribution and homogeneous variance of data, the statistical test used for endpoint determination was Dunnett's Test.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [3] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, calibration, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using glass mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [4]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures S&F DS-43.6 and DS-42.4, respectively. [4]
5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [4]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [4]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H₂SO₄ to an end point of 4.5 according to TVA procedure DS-42.1. [4]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [4]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish – 7-day chronic (NOEC)

Daphnids – 7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution – fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water (enriched for daphnids) – fish and daphnid chronic.

5.3.4 Statistics: Chronic Test, FH Survival/Growth and Ceriodaphnia Reproduction, NOEC – Dunnett’s Test or Steel’s Many-one Rank Test, EPA Bootstrap Procedure – IC₂₅

Ceriodaphnia Survival – Fisher’s Exact Test

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results: No survival or growth effects were demonstrated in fathead minnows exposed to Clinch River Mile 9.0 or Poplar Creek Kilometer 1.6 samples during the CR-ERP Ambient Water Toxicity Pilot Study conducted from April 22-29, 1993.
6.1.2 Results, Survival Data:


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Daily % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>Medium</td>
<td>100 100 100 100 100 98 98</td>
</tr>
<tr>
<td>CRM 9.0-50%</td>
<td>100 100 100 100 100 100 100</td>
</tr>
<tr>
<td>CRM 9.0-100%</td>
<td>100 100 100 100 100 98 98</td>
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<tr>
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<td>100 100 100 100 100 100 95</td>
</tr>
<tr>
<td>PCK 1.6-100%</td>
<td>100 100 100 100 100 98 95</td>
</tr>
</tbody>
</table>


SURVIVAL EFFECTS


<table>
<thead>
<tr>
<th>Group</th>
<th>Replicate</th>
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<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Medium</td>
<td>90 100 100 100</td>
</tr>
<tr>
<td>CRM 9.0-50%</td>
<td>100 100 100 100</td>
</tr>
<tr>
<td>CRM 9.0-100%</td>
<td>100 90 100 100</td>
</tr>
<tr>
<td>PCK 1.6-50%</td>
<td>100 100 90 90</td>
</tr>
<tr>
<td>PCK 1.6-100%</td>
<td>100 90 90 100</td>
</tr>
</tbody>
</table>

IC25 >100% DSN001.
Steel's Many-one Rank Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicates</th>
<th>Critical Rank Sum</th>
<th>Rank Sum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRM 9.0-50%</td>
<td>4</td>
<td>10</td>
<td>20.0</td>
</tr>
<tr>
<td>CRM 9.0-100%</td>
<td>4</td>
<td>10</td>
<td>18.0</td>
</tr>
<tr>
<td>PCK 1.6-50%</td>
<td>4</td>
<td>10</td>
<td>16.0</td>
</tr>
<tr>
<td>PCK 1.6-100%</td>
<td>4</td>
<td>10</td>
<td>16.0</td>
</tr>
</tbody>
</table>

*Values less than or equal to the Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:


GROWTH AND REPRODUCTION EFFECTS

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, CR-ERP Pilot Study, April 22-29, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.07.

This represents an 12.51% decrease in dry weight, mg.

\[ T = 2.36 \quad \alpha = 0.05 \quad \text{(one-tailed test)} \]

Growth Data (mg)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate</th>
<th>Mean</th>
<th>S*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
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<td>0.47</td>
<td>0.59</td>
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<tr>
<td></td>
<td>2</td>
<td>0.52</td>
<td>0.60</td>
</tr>
<tr>
<td>CRM 9.0-50%</td>
<td>3</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>CRM 9.0-100%</td>
<td>4</td>
<td>0.57</td>
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<td>5</td>
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<td>PCK 1.6-50%</td>
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<tr>
<td>PCK 1.6-100%</td>
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<tr>
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<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.54</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*Values significantly less than the control (Medium). \( \text{EMS} = 0.002 \).

IC25 >100% DSN001.
6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No survival or reproduction effects were demonstrated in daphnids exposed to Clinch River Mile 9.0 or Poplar Creek Kilometer 1.6 samples during the CR-ERP Ambient Water Toxicity Pilot Study conducted from April 22-28, 1993.

6.2.2 Results, Survival Data:


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Daily % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Medium</td>
<td>100</td>
</tr>
<tr>
<td>CRM 9.0-50%</td>
<td>100</td>
</tr>
<tr>
<td>CRM 9.0-100%</td>
<td>100</td>
</tr>
<tr>
<td>PCK 1.6-50%</td>
<td>100</td>
</tr>
<tr>
<td>PCK 1.6-100%</td>
<td>100</td>
</tr>
</tbody>
</table>

6.2.3 Results, Reproduction Data:


- Test requires 4 replicates/treatment

No transformation applied before data analysis. For this set of data, the minimum significant difference is 2.17.

This represents a 10.81% reduction in Reproduction (# young/female/6 days). $T = 2.22$ $\alpha = 0.05$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate</th>
<th>Mean</th>
<th>S*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>19 17 22 19 18 20 23 22</td>
<td>20.10</td>
<td></td>
</tr>
<tr>
<td>CRM 6.0-50%</td>
<td>20 23 22 21 22 20 23 22</td>
<td>21.60</td>
<td></td>
</tr>
<tr>
<td>CRM 6.0-100%</td>
<td>21 27 27 29 24 23 28 29</td>
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<tr>
<td>FCK 1.6-50%</td>
<td>23 24 20 24 23 20 25 23 26.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCK 1.6-100%</td>
<td>20 26 27 28 30 24 24 28 25</td>
<td>25.70</td>
<td></td>
</tr>
</tbody>
</table>

*Values significantly less than the control (Medium). $EMS = 4.79$. $IC_{25} >100%$.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: 25.1°C (24.6°-25.4°C)

6.3.1.2 Ceriodaphnia: 25.4°C (25.3°-25.7°C)


See: Appendix A Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, CR-ERP Pilot Study, April 22-29, 1993

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

Reference toxicant tests conducted prior to CR-ERP Pilot Study showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.4.2 Fathead Minnows

6.4.2.1 Date/Time of Most Recent Test: March 23-30, 1993/1010 CDT

6.4.2.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
$IC_{25}$: 0.006 mg Cu/L
6.4.2.3 Control Chart Information:

Number of standard tests completed by laboratory: 42

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC25 Range: 0.004-0.011 mg Cu/L

6.4.3 Ceriodaphnia

6.4.3.1 Date/Time of most recent test:

March 23-30, 1993/0930 CDT

6.4.3.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC25: 0.012 mg Cu/L

6.4.3.3 Control Chart Information:

Number of standard tests completed by laboratory: 40

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC25 Range: 0.011-0.028 mg Cu/L

7.0 CONCLUSION

Tests conducted using Clinch River Mile 9.0 and Poplar Creek Kilometer 1.6 samples collected on April 21, 23, and 26 showed no toxicity (survival, growth, or reproduction effects) to fathead minnows or Ceriodaphnia.

8.0 REFERENCES


## Appendix A

**Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, CR-ERP Pilot Study, April 22-29, 1993**

<table>
<thead>
<tr>
<th>Source</th>
<th>Temperature</th>
<th>Dissolved Oxygen</th>
<th>pH</th>
<th>Conductivity</th>
<th>Alk</th>
<th>Hardness</th>
<th>Chlorine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(°C)</td>
<td>(°C)</td>
<td>(mg/L)</td>
<td>(mg/L)</td>
<td>(S.U.)</td>
<td>(S.U.)</td>
<td>(μS/cm)</td>
</tr>
<tr>
<td>CRM 9.0-50% w/F. Medium†</td>
<td>25.1</td>
<td>25.1</td>
<td>8.2</td>
<td>5.7</td>
<td>8.2</td>
<td>7.8</td>
<td>304</td>
</tr>
<tr>
<td></td>
<td>(24.9-25.4)</td>
<td>(8.1-8.3) (4.5-6.5)</td>
<td>(8.1-8.3) (7.7-8.1)</td>
<td>(298-310)</td>
<td>(303-316)</td>
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<td>(7.2-7.6)</td>
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<td>(8.2-8.4)</td>
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<td>(265-279)</td>
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*mg/L as CaCO3  †Fathead Minnow Test  ‡Ceriodaphnia Test
ATTACHMENT I

CR-ERP AMBIENT WATER TOXICITY PILOT STUDY
Chain-of-Custody Forms – Originals
# CLINCH RIVER RFI FIELD CHAIN-OF-CUSTODY FORM FOR SURFACE WATER SAMPLES

## Site: PCK1.6, CRM9.0

### Field Custodian:

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<thead>
<tr>
<th>Time</th>
<th>hh:mm (military)</th>
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</thead>
<tbody>
<tr>
<td>11:55</td>
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### Sampling Team Signatures:

- [Signature]

## Date: 21 Apr 93

## Collection

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<th>Sample Identification</th>
<th>Collection Bottle Type</th>
<th>Container Type</th>
<th>Preservative Action (init compl.)</th>
<th>Requested Analyses</th>
<th>LABORATORY (INITIALS) Sample transferred to:</th>
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</thead>
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<tr>
<td>1279Q</td>
<td>portable pump</td>
<td>4L amber bottle</td>
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<td></td>
<td></td>
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<td>1284Q</td>
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<td>4L amber bottle</td>
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<td></td>
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## Sample custody transfer

1) Sample custody transfer DATE: 31 Oct 93 TIME: 6:07 Relinquished by: [Signature] of TVA (org.)

2) Sample custody transfer DATE: \dd mm yy TIME: \hh \mm Relinquished by: [Signature] of [Organization] (org.)

Received by: [Signature] of [Organization] (org.)

---

V.3.4. 2/24/92

FCN: COCFW-1044 28OCT92
### Clinch River RFI Field Chain of Custody Form for Surface Water Samples

**Site:** PCK 1.6, CEM 9.9

**Field Custodian:** [Signature]

**Sampling Team Signatures:** [Signature]

**Date:** 23 Apr 93

**Time:** (Military) hh:mm

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<th>Sample Identification</th>
<th>Collection Bottle Type</th>
<th>Container Type</th>
<th>Preservative Action (init compl.)</th>
<th>Requested Analyses</th>
<th>Laboratory (Initials)</th>
<th>Sample transferred to:</th>
<th>C-of-C Form No.</th>
<th>Sample to Analysis</th>
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<td>1285Q</td>
<td>peri-static pump</td>
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<td>chill</td>
<td></td>
<td>FTA, medaka</td>
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<tr>
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1) Sample custody transfer DATE: 23 Apr 93 TIME: 3:40

Relinquished by: [Signature] of [Org.]

Received by: [Signature] of [Org.]

2) Sample custody transfer DATE: [dd mm yyyy] TIME: [hh:mm]

Relinquished by: [Signature] of [Org.]

Received by: [Signature] of [Org.]
**CLINCH RIVER RFT FIELD CHAIN-OF-CUSTODY FORM FOR SURFACE WATER SAMPLES**

**Site:** PCK1.6, CMA 08

**Field Custodian:** [Name]

**Sampling Team Signatures:** [Signature]

**AIRBILL**

**PACKAGE TRACKING NUMBER:** 566566327

**RETURN TO:** C. J. Ford  
P.O. Box 2008, M.S. 6036  
Oak Ridge, TN 37831-6036

**RECIPIENT'S COPY**

**INITIALS**

**REFERENCE:** 

**C-of-C Form No.:** 0423933

**Page 1 of 1**

**From (Your Name) Please Print**

**Date**

**Your Phone Number (Very Important)**

**To (Recipient's Name) Please Print**

**Recipient's Phone Number (Very Important)**

**Company**

**Department/Section No.**

**Exact Street Address**

**City**

**State**

**ZIP Required**

**YOUR INTERNAL BILLING REFERENCE INFORMATION (optional) (First 24 characters will appear on invoice)**

**IP HOLD FOR PICK-UP**

**Print FedEx Address Here**

**Street Address**

**City**

**State**

**ZIP Required**

**PAYMENT**

**Bill Sender**

**Bill Recipient's FedEx Acct. No.**

**Bill 3rd Party FedEx Acct. No.**

**Bill Credit Card**

**SERVICES**

**(Check only one box)**

- [ ] Standard Overnight (Delivery by next day)
- [ ] Express Overnight (Delivery by next day)
- [ ] FedEx Two-Day (Delivery by 12:00 noon the second day after pick-up)
- [ ] Economy Two-Day (Delivery by 5:00 p.m. the second day after pick-up)
- [ ] Economy Three-Day (Delivery by 5:00 p.m. the third day after pick-up)
- [ ] Two-Day Freight (Delivery by 5:00 p.m. the second day after pick-up)
- [ ] Economy Three-Day Freight (Delivery by 5:00 p.m. the third day after pick-up)
- [ ] Oversize (Delivery by 5:00 p.m. on the second day after pick-up)

**PAYMENT**

**Due On Receipt**

**Due On Delivery**

**CASHIER**

**Cashier's Name**

**Cashier's Phone Number**

**Cashier's Fax Number**

**CASHIER**

**Cashier's Name**

**Cashier's Phone Number**

**Cashier's Fax Number**

**TOTAL CHARGES**

**Total Amount of Charge**

**Sample to be Delivered:**

**Sample to be Analyzed:**

**Emp. No.**

**Date**

**Frequent Shipment Use**

- [ ] Cash Received
- [ ] Return Shipment
- [ ] Third Party
- [ ] Chg. To Del.
- [ ] Chg. To Hold

**Street Address**

**City**

**State**

**ZIP Required**

**X**

**DATE/TIME RECEIVED**

**FedEx Employee Number**

**REVISION DATE 2002**

**PART # 137005 GBPE FORMAT 4086**

** dd mm yy hh mm**

**TIME:**

**Received by:** [Signature]

**V.3.4. 2/24/92**

**There were no specimens sent on ice cooler or cubitainers 4-24-93 DN**

**FNC: COCFW-1047 28OCT92**
CLINCH RIVER RFT FIELD CHAIN-OF-CUSTODY FORM FOR SURFACE WATER SAMPLES

<table>
<thead>
<tr>
<th>Sample Identification</th>
<th>Collection Bottle Type</th>
<th>Container Type</th>
<th>Preservative Action (init compl.)</th>
<th>Requested Analyses</th>
<th>LABORATORY (INITIALS)</th>
<th>Sample transferred to:</th>
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<td>4L cub + 1L bottle</td>
<td>chill</td>
<td>CNS, metal na Cl</td>
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<td>1296Q</td>
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<td>&quot;</td>
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<td>&quot;</td>
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1) Sample custody transfer DATE: 26 APR 93 TIME: 3:15
Relinquished by: [Signature] of TVA (org.)
Received by: [Signature] of [Org.] (org.)

2) Sample custody transfer DATE: [dd mm YY] TIME: [hh mm]
Relinquished by: [Signature] of [Org.] (org.)
Received by: [Signature] of [Org.] (org.)

Page 1 of 1

Return to: C. J. Ford
P.O. Box 2008, M.S. 6036
Oak Ridge, TN 37831-6036
ATTACHMENT II

CR-ERP AMBIENT WATER TOXICITY PILOT STUDY
Toxicity Test Bench Sheets and Statistical Analyses
**ARL CHRONIC TOXICITY TEST MASTER CHECKLIST**

```
Date Issued: 3-26-93  To:  DAH  From:  FJK

1. Test organism availability: FH Minnows - T.L.*  C.U.†  Date 3-26
   Daphnids - T.L.  C.U.  Date 3-24

2. Sample Collection Coordination: Date 4-6-93 By FM With ORNL/Eng.


4. Glassware prep. complete: Date 4-23-93 By GKK

5. Data Sheet prep. complete: Date 4-23-93 By CPM

6. Test organism availability verification: FH-OK? Daphnids-OK? 

7. FH Minnow acclimation:
   Day 1 2
   By N/A

8. Test Initiation: FH-Date/Time 4-23-93/1:52  Daphnid-Date/Time 4-23-93/1:52
   FH Sample, By: 

9. Test Renewal: Day #: 0 1 2 3 4 5 6 7

10. Test Termination: FH-Date/Time 4-29-93/1:52  Daphnid-Date/Time 4-29-93/1:52
    Clean-up, By: 

11. Weigh Fish: Date 4-29-93 By DKH

12. Run Stats: Date 4-30-93 By C.L.K

13. Report Prep.: Date 5-13-93 By FM

14. Report Final: Date 5-21-93 By FM (EM) Approved: JJK (FM)

*Team Leader  †Culture Unit  #Team Member
```

---

1. Test organism availability:
   - FH Minnows: T.L.*  C.U.†  Date 3-26
   - Daphnids: T.L.  C.U.  Date 3-24

2. Sample Collection Coordination:
   - Date 4-6-93
   - By FM
   - With ORNL/Eng.

3. Glassware availability:
   - T.L.  C.U.  Tech: Date 4-20-93

4. Glassware prep. complete:
   - Date 4-23-93
   - By GKK

5. Data Sheet prep. complete:
   - Date 4-23-93
   - By CPM

6. Test organism availability verification:
   - FH-OK?
   - Daphnids-OK?

7. FH Minnow acclimation:
   - Day 1 2
   - By N/A

8. Test Initiation:
   - FH-Date/Time 4-23-93/1:52
   - Daphnid-Date/Time 4-23-93/1:52
   - FH Sample, By:

9. Test Renewal:
   - Days 0 1 2 3 4 5 6 7

10. Test Termination:
    - FH-Date/Time 4-29-93/1:52
    - Daphnid-Date/Time 4-29-93/1:52
    - Clean-up, By:

11. Weigh Fish:
    - Date 4-29-93
    - By DKH

12. Run Stats:
    - Date 4-30-93
    - By C.L.K

13. Report Prep.:
    - Date 5-13-93
    - By FM

14. Report Final:
    - Date 5-21-93
    - By FM (EM)
    - Approved: JJK (FM)

---

*Team Leader  †Culture Unit  #Team Member
Chronic Study Fathead Record Sheet

Study Date: Study I (CREP Pilot Study)
Test Organism: Pimephales promelas
Beginning Date/Time: 4-22-93 / 1050
Ending Date/Time: 04-29-93 / 04-29-93
Personnel: [Signatures]

Control/Dilution Water: Fish (H3 on chem. sheet)

Test Treatment Identification
1. **F-med (Control)**
2. CRM 4.0 50%
3. CRM 4.0 100%
4. PCK 1.0 50%
5. PCK 1.0 100%
6. 
7. 
8. 

Spawn Date | Tile # | Hatch Date
---|---|---
4/21/93 | 16, 11, 11, 14 | 4/21 A 1200
| 21, 23, 27, 27 | 4/22 B 0100

Test # 1 (Pilot Study)
NOEC: > 1000 mg/L
LOEC: > 100 mg/L

Notes: CRM 4.0

Sample No. | Dates Used
---|---
1. 1284 | 4/22, 04/12
2. 1290 | 04/24, 04/25, 04/26
3. 1318 | 04/27, 04/28

PCK 1.0

1. 1279 | 4/22, 04/12
2. 1283 | 04/24, 04/25, 04/26
3. 1278 | 04/27, 04/28
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Notes: Used only 300 mL per rep on Day 0. Returned to 500 mL per rep on Day 1.
Analysis of Survival

DOE #1
Starting Date: 04/22/93

Survival (%) Data

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<td>0.98</td>
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<td>CRM9.0 50%</td>
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Steel’s Many-One Rank Test

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<td>RM9 100%</td>
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<td>1(U)</td>
<td>18</td>
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<tr>
<td>CK1.6 50%</td>
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<td>1(U)</td>
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<tr>
<td>CK1.6 100%</td>
<td>4</td>
<td>1(U)</td>
<td>16</td>
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* Values less than Critical Rank Sum significantly less than the control (MEDIUM ).
Non-parametric analysis of toxicity data

12:26 Thursday, July 2, 1992

1 DOE #1 042293 MINS 2 0 10 4 4 4 4 4 4 4 4 STEEL 1 CRM9.0 50% 16
2 DOE #1 042293 MINS 2 0 10 4 4 4 4 4 4 4 4 STEEL 2 CRM9.0 50% 20
3 DOE #1 042293 MINS 3 0 10 4 4 4 4 4 4 4 4 STEEL 1 CRM9 100% 18
4 DOE #1 042293 MINS 3 0 10 4 4 4 4 4 4 4 4 STEEL 3 CRM9 100% 18
5 DOE #1 042293 MINS 4 0 10 4 4 4 4 4 4 4 4 STEEL 1 PCK1.6 50% 20
6 DOE #1 042293 MINS 4 0 10 4 4 4 4 4 4 4 4 STEEL 4 PCK1.6 50% 16
7 DOE #1 042293 MINS 5 0 10 4 4 4 4 4 4 4 4 STEEL 1 PCK1.6 100% 20
8 DOE #1 042293 MINS 5 0 10 4 4 4 4 4 4 4 4 STEEL 5 PCK1.6 100% 16
Non-parametric analysis of toxicity data

12:26 Thursday, July 2, 1992

<table>
<thead>
<tr>
<th>OBS</th>
<th>TREATMENT</th>
<th>N CONTROL</th>
<th>N EFFLUENT</th>
<th>RANK SUM</th>
<th>CRITICAL RANK SUM</th>
<th>SIGNIFICANT DIFFERENCE</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>CRM9.0 50%</td>
<td>4</td>
<td>4</td>
<td>20</td>
<td>10</td>
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<tr>
<td>2</td>
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<td>4</td>
<td>18</td>
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</tr>
<tr>
<td>3</td>
<td>PCK1.6 50%</td>
<td>4</td>
<td>4</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PCK1.6 100%</td>
<td>4</td>
<td>4</td>
<td>16</td>
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</table>
Tests of assumptions for survival data (arcsine transformed)

TEST FOR NORMAL DISTRIBUTION

12:22 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MINS

UNIVARIATE PROCEDURE

Variable=CENTER

Moments

<table>
<thead>
<tr>
<th>N</th>
<th>20</th>
<th>Sum Wgts</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
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<td>Sum</td>
<td>0</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.069946</td>
<td>Variance</td>
<td>0.004893</td>
</tr>
<tr>
<td>Skewness</td>
<td>-0.55475</td>
<td>Kurtosis</td>
<td>-1.07773</td>
</tr>
<tr>
<td>USS</td>
<td>0.092958</td>
<td>CSS</td>
<td>0.092958</td>
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<tr>
<td>CV</td>
<td>.</td>
<td>Std Mean</td>
<td>0.015641</td>
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<td>T:Mean=0</td>
<td>0</td>
<td>Prob&gt;</td>
<td>T</td>
</tr>
<tr>
<td>Sgn Rank</td>
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<td>Prob&gt;</td>
<td>S</td>
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<tr>
<td>Num ^= 0</td>
<td>16</td>
<td></td>
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</tr>
<tr>
<td>W:Normal</td>
<td>0.865298</td>
<td>Prob&lt;W</td>
<td>0.0089</td>
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</tbody>
</table>

Quantiles(Def=5)

| 100% Max | 0.081485 | 99% | 0.081485 |
| 75% Q3 | 0.040743 | 95% | 0.081485 |
| 50% Med | 0.020371 | 90% | 0.081485 |
| 25% Q1 | -0.08149 | 10% | -0.10186 |
| 0% Min | -0.12223 | 5% | -0.12223 |
|         |         | 1% | -0.12223 |
| Range | 0.203713 |
| Q3-Q1 | 0.122228 |
| Mode | 0.040743 |

Extremes

<table>
<thead>
<tr>
<th>Lowest</th>
<th>Obs</th>
<th>Highest</th>
<th>Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.12223</td>
<td>22</td>
<td>0.040743</td>
<td>24</td>
</tr>
<tr>
<td>-0.12223</td>
<td>1</td>
<td>0.081485</td>
<td>31</td>
</tr>
<tr>
<td>-0.08149</td>
<td>43</td>
<td>0.081485</td>
<td>32</td>
</tr>
<tr>
<td>-0.08149</td>
<td>42</td>
<td>0.081485</td>
<td>41</td>
</tr>
<tr>
<td>-0.08149</td>
<td>34</td>
<td>0.081485</td>
<td>44</td>
</tr>
</tbody>
</table>

Missing Value

| Count | 30 |
| % Count/Nobs | 60.00 |
Tests of assumptions for survival data (arcsine transformed)

TEST FOR NORMAL DISTRIBUTION

12:22 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MINS

UNIVARIATE PROCEDURE

Variable=CENTER

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<th>Stem Leaf</th>
<th>#</th>
<th>Boxplot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 8888</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>0 0000444444</td>
<td>10</td>
<td>+++++++</td>
</tr>
<tr>
<td>-0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0 8888</td>
<td>4</td>
<td>+++++++</td>
</tr>
<tr>
<td>-1 22</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Multiply Stem.Leaf by 10**-1

Normal Probability Plot

-2  -1    0    +1    +2
Tests of assumptions for survival data (arcsine transformed)

TEST FOR NORMAL DISTRIBUTION
12:22 Thursday, July 2, 1992

UNIVARIATE PROCEDURE
Schematic Plots

Variable - CENTER

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  0.08 +
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Tests of assumptions for survival data (arcsine transformed)

TEST FOR NORMAL DISTRIBUTION

12:22 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MINS

Plot of CENTER*RANKIT. Symbol is value of TRT_NO.

<table>
<thead>
<tr>
<th>CENTER</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.081</td>
<td>+</td>
</tr>
<tr>
<td>0.041</td>
<td>+</td>
</tr>
<tr>
<td>0.000</td>
<td>+</td>
</tr>
<tr>
<td>-0.041</td>
<td>+</td>
</tr>
<tr>
<td>-0.081</td>
<td>+</td>
</tr>
<tr>
<td>-0.122</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE: 30 obs had missing values. 15 obs hidden.
Tests of assumptions for survival data (arcsine transformed)

TEST FOR NORMAL DISTRIBUTION

12:22 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MINS

FREQUENCY OF CENTER

<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>CENTER MIDPOINT</th>
</tr>
</thead>
<tbody>
<tr>
<td>6+</td>
<td>-0.100 -0.075 -0.050 -0.025 0.000 0.025 0.050 0.075</td>
</tr>
<tr>
<td>5+</td>
<td></td>
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<tr>
<td>4+</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td></td>
</tr>
</tbody>
</table>
Tests of assumptions for survival data (arcsine transformed)  
TEST FOR NORMAL DISTRIBUTION  
12:22 Thursday, July 2, 1992  

VARIANCE EQUAL TO ZERO FOR ONE OR MORE TREATMENTS  
BARTLETT'S TEST CAN NOT BE CALCULATED  
LEVENE'S TEST WILL BE USED
Tests of assumptions for survival data (arcsine transformed)
LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE

12:22 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MINS

General Linear Models Procedure
Class Level Information

Class Levels Values

TRT  5  CRM9 100% CRM9.0 50% MEDIUM PCK1.6 100% PCK1.6 50%

Number of observations in by group = 50

NOTE: Due to missing values, only 20 observations can be used in this analysis.
Tests of assumptions for survival data (arcsine transformed)

LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE

12:22 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MINS

General Linear Models Procedure

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>4</td>
<td>0.01792755</td>
<td>0.00448189</td>
<td>6.75</td>
<td>0.0026</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>0.00995975</td>
<td>0.00066398</td>
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</tr>
<tr>
<td>Corrected Total</td>
<td>19</td>
<td>0.02788730</td>
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</tr>
</tbody>
</table>

R-Square: 0.642857
C.V.: 45.17540
Root MSE: 0.025768
ABS_VAL Mean: 0.05703962

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>4</td>
<td>0.01792755</td>
<td>0.00448189</td>
<td>6.75</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>4</td>
<td>0.01792755</td>
<td>0.00448189</td>
<td>6.75</td>
<td>0.0026</td>
</tr>
<tr>
<td>Sample ID</td>
<td>Tare Wt.</td>
<td>Tare &lt; Fish Wt.</td>
<td>Fish Wt.</td>
<td># Fish</td>
<td>Mean Wt.</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------------</td>
<td>----------</td>
<td>--------</td>
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<tr>
<td>1.</td>
<td>1.09207</td>
<td>1.09271</td>
<td>0.00064</td>
<td>10</td>
<td>0.000064</td>
</tr>
<tr>
<td>Initial  2.</td>
<td>1.02658</td>
<td>1.02723</td>
<td>0.00067</td>
<td>10</td>
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</tr>
<tr>
<td>3.</td>
<td>1.04235</td>
<td>1.04300</td>
<td>0.00065</td>
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<td>0.000065</td>
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<tr>
<td>4.</td>
<td>1.03851</td>
<td>1.04003</td>
<td>0.00072</td>
<td>10</td>
<td>0.000072</td>
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<table>
<thead>
<tr>
<th>Initial</th>
<th>Mean Wt.</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>1.</td>
<td>1.05356</td>
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<tr>
<td>MEDIUM  2.</td>
<td>1.02615</td>
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<tr>
<td>3.</td>
<td>1.05522</td>
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<tr>
<td>4.</td>
<td>1.05162</td>
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<table>
<thead>
<tr>
<th>CRM 9.0</th>
<th>Mean Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>1.10342</td>
</tr>
<tr>
<td>3.</td>
<td>1.12903</td>
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<tr>
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<td>0.98808</td>
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</table>

<table>
<thead>
<tr>
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<th>Mean Wt.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.05123</td>
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</table>

<table>
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<th>Mean Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>1.02230</td>
</tr>
<tr>
<td></td>
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<tr>
<td>-----</td>
<td>---</td>
</tr>
<tr>
<td>3.</td>
<td>1.08410</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>1.08981</td>
</tr>
</tbody>
</table>

| 1.  | 1.03557 | 1.04093 | 0.00536 | 10 | 0.000536 | 0.000469 | 0.536 |

| PCK 1.6 | 2. | 1.08165 | 1.08693 | 0.00530 | 10 | 0.000530 | 0.000463 | 0.530 |
| 50%     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3.  | 0.97829 | 0.98298 | 0.00469 | 9 | 0.000521 | 0.000454 | 0.521 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4.  | 1.05432 | 1.05932 | 0.00500 | 9 | 0.000556 | 0.000489 | 0.556 |

| 1.  | 1.04275 | 1.04795 | 0.00520 | 10 | 0.000520 | 0.000453 | 0.520 |

| PCK 1.6 | 2. | 1.07345 | 1.07671 | 0.00426 | 9 | 0.000473 | 0.000406 | 0.473 |
| 100%    |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3.  | 1.05678 | 1.06180 | 0.00502 | 9 | 0.000558 | 0.000491 | 0.550 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4.  | 1.08747 | 1.09259 | 0.00510 | 10 | 0.000510 | 0.000443 | 0.510 |

Reviewed By: Russell
Analysis of 7-day Larval Growth Test

DOE #1
Starting Date: 04/22/93

Analysis of Dry Weight (mg) Data Dunnett's Test

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.07

This represents a 12.51% reduction in Dry Weight (mg).

T = 2.36 ALPHA = 0.05

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>MEAN</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIUM</td>
<td>0.47</td>
<td>0.59</td>
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<tr>
<td>CRM9 50%</td>
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<td>CRM9 100%</td>
<td>0.53</td>
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<tr>
<td>PCK1.6 50%</td>
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Asterisk (*) indicates values significantly less than control (MEDIUM).

Analysis of Variance

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<td>0.002</td>
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<td>0.300</td>
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<tr>
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Analysis of untransformed data

15:09 Sunday, July 5, 1992

STUDY=DOE #1
ST_DATE=042293
PARAM=MING
DUNNETT'S TEST
T = 2.36 ALPHA = 0.05

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<td>CRM9 100%</td>
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<td>PCK1.6 50%</td>
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<td>0.54</td>
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MINIMUM SIGNIFICANT DIFFERENCE = 0.07
THIS REPRESENTS A 12.51 % DECREASE IN SURVIVAL FROM THE CONTROL
Analysis of untransformed data

STUDY=DOE #1
ST_DATE=042293
PARM=MINING

DUNNETT'S TEST
T = 2.36 ALPHA = 0.05

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MINIMUM SIGNIFICANT DIFFERENCE = 0.07
THIS REPRESENTS A 12.51% DECREASE IN SURVIVAL FROM THE CONTROL
Analysis of untransformed data

General Linear Models Procedure
Class Level Information

Class Levels Values
TRT 5 CRM9 100% CRM9 50% MEDIUM PCK1.6 100% PCK1.6 50%

Number of observations in data set = 50

NOTE: Due to missing values, only 20 observations can be used in this analysis.
Analysis of untransformed data

General Linear Models Procedure

Dependent Variable: RESP

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<tr>
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<th>Mean Square</th>
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<th>C.V.</th>
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<td>0.8706</td>
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Tests of assumptions for untransformed data
Test for Normal Distribution
15:07 Sunday, July 5, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=MING

UNIVARIATE PROCEDURE

Variable=CENTRER

Moments

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<td>Sum</td>
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<td>Std Dev</td>
<td>0.036292</td>
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<td>T</td>
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<td>Num ^= 0</td>
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</table>

Quantiles(Def=5)

| 100% Max | 0.055 | 99% | 0.055 |
| 75% Q3   | 0.035 | 95% | 0.05 |
| 50% Med  | -0.00375 | 90% | 0.045 |
| 25% Q1   | -0.02125 | 10% | -0.05125 |
| 0% Min   | -0.075 | 5%  | -0.06625 |
| Range    | 0.13 |
| Q3-Q1    | 0.05625 |
| Mode     | -0.0125 |

Extremes

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<th>Highest</th>
<th>Obs</th>
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Missing Value .
Count 30
% Count/Nobs 60.00
Tests of assumptions for untransformed data
Test for Normal Distribution
15:07 Sunday, July 5, 1992

UNIVARIATE PROCEDURE

Variable=CENTER

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<th>Stem Leaf</th>
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<td>2 28</td>
<td>2</td>
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<td>0 822852</td>
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<tr>
<td>-2 85</td>
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<tr>
<td>-4 85</td>
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<tr>
<td>-6 5</td>
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Multiply Stem Leaf by 10**-2

Normal Probability Plot

```
0.05+
| * * *++++   *
| * *++++    
-0.01+      
| *++***    
| +++**    
-0.07+      
| +++**    

---2 -1 0 +1 +2
```
Tests of assumptions for untransformed data.
Test for Normal Distribution
15:07 Sunday, July 5, 1992

UNIVARIATE PROCEDURE
Schematic Plots

Variable=CENTER

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Tests of assumptions for untransformed data
15:07 Sunday, July 5, 1992

Plot of CENTER*RANKIT. Symbol is value of TRT_NO.

NOTE: 30 obs had missing values. 2 obs hidden.
Bartlett’s Test for Homogeneous Variance

15:07 Sunday, July 5, 1992

STUDY=DOE #1
ST_DATE=042293
PARAM=MING

<table>
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<th>N</th>
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<tr>
<td>MEDIUM</td>
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<td>0.000</td>
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<tr>
<td>PCK1.6 50</td>
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BARTLETT’S TEST STATISTIC = 5.928
PROB>B = 0.205
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<td>0.47</td>
<td>0.56</td>
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</tbody>
</table>
Chronic Study Zooplankton Record Sheet

Study DOE Study I (CREP Pilot Study)
Test Organism Ceriodaphnia dubia
Beginning Date/Time 4-22-93 11:15
Ending Date/Time 4-28-93 11:15
Personnel ON

Control/Dilution Water C-MED (#5 on chem & count sheets)

Test Treatment Identification
1. C-MED (Control)  
2. CRM 9.0 50%
3. CRM 9.0 100%
4. PCK 1.6 50%
5. PCK 1.6 100%
6. 
7. 
8. 

Released From 

Date: 4-21-93  
Time: 12:00 noon  
To 
Date: 4-21-93  
Time: 8:00 am

Test # 1 (Pilot Study) 
NOEC > 100 %
LOEC

Notes:

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Reviewed By: PLARCS01-310
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Analysis of 6-day Reproduction Test

DOE #1
Starting Date: 04/22/93

Analysis of Reproduction (# young/female/6 days) Data Dunnett's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 2.17

This represents a 10.81% reduction in Reproduction (# young/female/6 days).

\[ T = 2.22 \text{ ALPHA} = 0.05 \]

Reproduction (# young/female/6 days) Data

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Asterisk (*) indicates values significantly less than control (MEDIUM).

Analysis of Variance

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Analysis of untransformed data

12:40 Thursday, July 2, 1992

STUDY=DOE #1
ST_DATE=042293
PARAM=CERR
DUNNETT'S TEST
T = 2.22 ALPHA = 0.05

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MINIMUM SIGNIFICANT DIFFERENCE = 2.17
THIS REPRESENTS A 10.81% DECREASE IN SURVIVAL FROM THE CONTROL
STUDY-DOE #1
ST_DATE=042293
PARM=CERR
DUNNETT'S TEST
T = 2.22 ALPHA = 0.05

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MINIMUM SIGNIFICANT DIFFERENCE = 2.17
THIS REPRESENTS A 10.81% DECREASE IN SURVIVAL FROM THE CONTROL
Analysis of untransformed data
12:40 Thursday, July 2, 1992

General Linear Models Procedure
Class Level Information

Class  Levels  Values

TRT       5  CRM9.0 100% CRM9.0 50% MEDIUM PCK1.6 100% PCK1.6 50%

Number of observations in data set = 50
Analysis of untransformed data
12:40 Thursday, July 2, 1992

General Linear Models Procedure

Dependent Variable: RESP

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R-Square: 0.548426  C.V.: 9.375977  Root MSE: 2.1883530  RESP Mean: 23.34000000

Source | DF | Type I SS | Mean Square | F Value | Pr > F |
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Source | DF | Type III SS | Mean Square | F Value | Pr > F |
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Tests of assumptions for untransformed data
Test for Normal Distribution
12:33 Thursday, July 2, 1992

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Tests of assumptions for untransformed data
Test for Normal Distribution
12:33 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=CERR

UNIVARIATE PROCEDURE

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Tests of assumptions for untransformed data
Test for Normal Distribution

12:33 Thursday, July 2, 1992

STUDY=DOE #1 ST_DATE=042293 PARM=CERR

UNIVARIATE PROCEDURE

Variable=CENTER

Normal Probability Plot

4.25+  +++
|      +*
3.25+  +++
|      +*
2.25+  +++
|      +*
1.25+  +++
|      +*
0.25+  +++
|      +*
-0.75+  ++
|      *  
-1.75+  ++
|      *  
-2.75+  ++
|      *  
-3.75+  ++
|      *  
-4.75+  ++
|      *  
-5.75+  *    

-2 -1 0 +1 +2
Tests of assumptions for untransformed data
Test for Normal Distribution
12:33 Thursday, July 2, 1992

UNIVARIATE PROCEDURE
Schematic Plots

Variable=CENTER

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Tests of assumptions for untransformed data

12:33 Thursday, July 2, 1992

Plot of CENTER*RANKIT. Symbol is value of TRT_NO.

RANK FOR VARIABLE CENTER

NOTE: 21 obs hidden.
Tests of assumptions for untransformed data

Bartlett’s Test for Homogeneous Variance

12:33 Thursday, July 2, 1992

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PARM=CERR

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BARTLETT'S TEST STATISTIC = 8.009
PROB>B = 0.091
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# Ceriodaphnia Randomization

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**Treatment Identification:**

1. C Mep (Control)
2. C Mep 9.0 50%
3. C Mep 9.0 100%
4. Fck 1.0 50%
5. Fck 1.0 100%
6. Temperature
7. 
8. 
9. 
10. 

**Notes:**
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**Notes:**
- Date: 12/1/73
- Temperature: 72°F
- Room: 124
- Food: 
- Comments:
### Ceriodaphnia Brood Record Sheet

**Batch ID:** 1

**Date:** 4-14-43

**Tray:**

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**Day:**

| 11 | 10 | 9 | 8 |

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</table>

**Day:**

| 11 | 10 | 9 | 8 |

**Day:**

| 11 | 10 | 9 | 8 |

**Notes:**

+ = Young present in brood cup at 3 days or more than one brood present in individual cup at renewal.
## INITIAL CHEMISTRY

**Study Date**: 4-22-93  
**Date**: 4-22-93  
**Personnel**: Harvey

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<th>pH</th>
<th>Cond.</th>
<th>Hard</th>
<th>Alk</th>
<th>x10</th>
<th>TRC</th>
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**NOTES**: Before action

Reviewed By: [Signature]
### INITIAL CHEMISTRY

**Study** DOE Study I (CRERP P-1 of Study)  
**Personnel** Havens, Russell, Simbuck  
**Date** 4-22-93

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**Notes:** Before aeration

Reviewed By: EAS
### Initial Chemistry

**Study:** CRERP Pilot Study  
**Date:** 4-25-93  
**Personnel:** Stenback

**Beginning Date:** 4-22-93  
**Ending Date:** 4-24-93

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**Notes:**  
- X: Below sensor  
- Reviewed By: [Redacted]  

**File Number:** PLARC501-252
# Initial Chemistry

**Study:** Initial Study 2 (CREEP Pilot Study)  
**Date:** 4-27-93

**Beginning Date:** 04-22-93  
**Ending Date:** 04-24-93

### By

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<th>Cond.</th>
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<th>Cur</th>
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**Notes:**
- A: Calcium Aluminate
- A: specified

Reviewed By: DH/Car
### Initial Chemistry

**Study:** DOE Study 1 (KEEPE P.I. Study)

**Personnel:** Haarman

**Beginning Date:** 4-22-93

**Ending Date:** 4-28-93

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**Study:** DOE Study I (Creep Pilot Study)

**Beginning Date:** 4-22-93

**Ending Date:** 04-23-93

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Reviewed By: [Signature]

PLARC501-255
### FINAL CHEMISTRY

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**Beginning Date** 04-22-93  
**Ending Date** 04-28-93  

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**Reviewed By:**
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**NOTES:**
# Final Chemistry

**Study**: Doe Study I (CEEP P1+ Study)

**Beginning Date**: 04-22-22

**Ending Date**: 04-28-22

## Personnel
- **Halaway**: Russell
- **Simbeck**

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**NOTES:**

Reviewed By: [Signature]

PLARC501-255
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MAX 25.4 6.5 7.9 290

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Ending Date 4-27-93

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**Notes:**
- Method: Standardization
- Equipment: pH Meter
- Calibration: Using Buffer Standards
- Conditions: Temperature, Initial and Corrected Readings
- Accuracy: Measured with % Slope
- Results: Standard and Additional Readings
### pH Meter Standardization

**Meter ID:** #7

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<td>ON</td>
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PLAR501-980(C)
### pH Meter Standardization

**Meter ID:**

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PLARC501-980(C)
## Conductivity Meter Standardization

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**Notes:**

PLARC501-900(D)
## Conductivity Meter Standardization

**METER ID:** #7 #1

### Standard Temp

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**Notes:** Some migrants were observed on 05-03-93. 05-04-93...

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**Notes:**

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**Notes:**

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**Notes:**

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**PIARC501-980(D)**
## Sample Collection

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(PLARC501-95)
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<th>Requested Analyses</th>
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1) Sample custody transfer DATE: 21 April 93 TIME: 08:07
Relinquished by: [Signature]
Received by: [Signature]

2) Sample custody transfer DATE: [DD/MM/YY] TIME: [HH:MM]
Relinquished by: [Signature] of [Agency] (org.)
Received by: [Signature] of [Agency] (org.)
CLINCH RIVER RFT FIELD CHAIN-OF-CUSTODY FORM FOR SURFACE WATER SAMPLES

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2) Sample custody transfer DATE: dd mm yy TIME: hh mm Relinquished by: [Signature] of [Organ] (org.) Received by: [Signature] of [Organ] (org.)

V.34. 2/24/92 There were no securing seats on ice cooler or cabinet.

4-24-93 Df

FCN: COCFW-1047 280CT92
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Sample to Laboratory Address:

Return to: C. J. Ford
P.O. Box 2008, M.S. 6036
Oak Ridge, TN 37831-6036

Received by: [Signature]

*Note: The form appears to be filled out with handwritten text and some unclear or incorrect entries.*
ATTACHMENT III

CR-ERP AMBIENT WATER TOXICITY PILOT STUDY
Reference Toxicant Test Information
Chronic Reference Toxicant Test

Fathead Minnows - ARL

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Means: 0.043 0.018  Means: 0.011 0.004 0.0068

*0.003 was significant

* started using less than 24hr old fish
**Chronic Reference Toxicant Record Sheet**

**Study** CuRef tox  
**Test Organism** Fathead  
**Beginning Date/Time** 3/23/93, 10:10  
**Ending Date/Time** 3/30/93, 09:30  
**Personnel**  

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**Stock Concentration(s)**  
**Test Concentrations** 0.001, 0.003, 0.008, 0.02, 0.05  
**Dilution Water** 1:10  

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(PLARC501-257) Reviewed By: TCS TCS TCS TCS TCS TCS TCS
### Analysis of Survival

**APRF 1040**

Starting date: 01/23/83

**Survival of Data**

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**Steel's Many-One Rank Test**

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*Values less than Critical Rank Sum significantly less than the control (Upstream).*
Analysis of 7-day Barotol Growth Test

APRIL 12, 1970
Starting Date: 04/02/70

Analysis of Dry Weight (mg) Data by Duanne's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 2.36.

This represents a 15.70% reduction in dry weight (mg).

T = 2.36  Alpha = 0.05

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*Erisk (*) indicates values significantly less than control mean.*

Analysis of Variance

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THE NUMBER OF RESAMPLES IS 80

*** LISTING OF GROUP CONCENTRATIONS (% EFF.) AND RESPONSE MEANS ***

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THE LINEAR INTERPOLATION ESTIMATE OF THE TOTAL IMPACT CONCENTRATION FROM THE INPUT SAMPLE IS .0056.

.050 .007 .007

THE LINEAR INTERPOLATION ESTIMATE OF THE TOTAL IMPACT CONCENTRATION FROM THE INPUT SAMPLE IS .0056.

******************************************************************************
* BOOTSTRAP PROCEDURE TO ESTIMATE VARIABILITY OF THE ESTIMATED ICp *
******************************************************************************

THE MEAN OF THE BOOTSTRAP ESTIMATES IS .0056.

THE STANDARD DEVIATION OF THE BOOTSTRAP ESTIMATES IS .0004.

AN EMPIRICAL 95.0% CONFIDENCE INTERVAL FOR THE BOOTSTRAP ESTIMATE IS (.0050, .0067).

C:\BOOTSTRP>
### Chronic Reference Toxicant Test

Cerio - ARL

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**Means**:

Survival: Mean = 0.05, Mean = 0.02

Reproduction: Mean = 0.01, Standard Deviation = 0.0195
Chronic Study Zooplankton Record Sheet

Study CuRef tox
Test Organism Cetio
Beginning Date/Time 3/23/93; 0930
Ending Date/Time 3/30/93; 0830
Personnel Posey, Sedlin

Control/Dilution Water C-med

Test Treatment Identification
1. Control
2. 0.001
3. 0.003
4. 0.008
5. 0.02
6. 0.05
7. Control with new food
8. 

Released From
Date 3/22/93
Time 1300
To
Date 3/22/93
Time 2200

Test # 40
NOEC 0.00x
LOEC 0.02
Notes: Icos
## Ceriodaphnia Survival and Reproduction Test

**Beginning Date:** 3-23-73  
**Ending Date:** 3-30-73  
**Personnel:**  
**Reviewed By:**

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**Notes:**
Analysis of 7-day Reproduction Test

APR#40 REPTOX
Starting Date: 03/23/93

Analysis of Reproduction (# young/female/7 days) Data Duncan's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 2.03
This represents a 22.48% reduction in Reproduction (# young/female/7 days)

\( T = 2.29 \)  \( \alpha = 0.05 \)

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Asterisk (*) indicates values significantly less than control (CONTROL).

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THE NUMBER OF RESAMPLES IS 80

*** LISTING OF GROUP CONCENTRATIONS (% EFF.) AND RESPONSE MEANS ***

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THE LINEAR INTERPOLATION ESTIMATE OF THE TOTAL IMPACT CONCENTRATION FROM THE INPUT SAMPLE IS .0123.

.050 1.800 1.800

THE LINEAR INTERPOLATION ESTIMATE OF THE TOTAL IMPACT CONCENTRATION FROM THE INPUT SAMPLE IS .0123.

******************************************************************************************
*BOOTSTRAPPROCEDURE TO ESTIMATE VARIABILITY OF THE ESTIMATED ICp*
******************************************************************************************

THE MEAN OF THE BOOTSTRAP ESTIMATES IS .0098.

THE STANDARD DEVIATION OF THE BOOTSTRAP ESTIMATES IS .0044.

AN EMPIRICAL 95.0% CONFIDENCE INTERVAL FOR THE BOOTSTRAP ESTIMATE IS (.0013, .0170).

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1. checked twice
2. checked 1/2 sample (50.0 ml) 2.5 reading

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* 0.0, meter + pH meter used from Watt's Lab #9 test.

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**Notes:**

Reviewed By: [Signature]
ATTACHMENT IV

CR-ERP AMBIENT WATER TOXICITY PILOT STUDY
Personnel Training Documentation
I, [Name], have read the "Referenced Chapters" of the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ column and will sign and date the TRAINEE SIGNATURE AND DATE column after each referenced chapter.

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The trainee will initial the READ and TRAINED columns for the referenced SOP after (1) each applicable Standard Operating Procedure has been read, and (2) after training has been received by the Unit Supervisor or approved trainer.

The Unit Supervisor or approved trainer will initial the PROFICIENT column after the calibrations, procedures, and calculations (if applicable) have been satisfactorily performed by the trainee.

After the READ, TRAINED, and PROFICIENT columns have been initialed, the trainee will sign and date the TRAINEE SIGNATURE AND DATE column.

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PLARS01-975
I, [Trainee's Name], have read the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual. The trainee will initial the READ and TRAINED columns for the referenced SOP after (1) each applicable Standard Operating Procedure has been read, and (2) after training has been received by the Unit Supervisor or approved trainer.

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PLAR0001-975(4)
I, Teresa C. Sesler, have read the "Referenced Chapters" of the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ column and will sign and date the TRAINEE SIGNATURE AND DATE column after each referenced chapter.

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PLARCS01-975
I, **Irene C. Leslie**, have read the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

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Training Checklist
ARL Toxicology Laboratory
October 30, 1992
Page 2 of 4

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<td>Algal Cell Enumeration</td>
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<td>Fathead Minnow, Pimephales promelas, Acute Toxicity Test</td>
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<td>Fathead Minnow, Pimephales promelas, Embryo Larval Survival and Teratogenicity Test</td>
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PLARCS01-975(3)