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RESULTS OF ACUTE TOXICITY TESTS
WITH *Ceriodaphnia dubia* AND *Pimephales promelas*
ON A SAMPLE OF BOTTOM ASH

Prepared for:

Nebraska Emergency Management Agency
1300 Military Road
Lincoln, Nebraska 68508-1090


Prepared by:

EA Engineering, Science and Technology, Inc.
15 Loveton Circle
Sparks, Maryland 21152

April 2001

EA Project 70004.10

Report Number 3714



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Date

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INTRODUCTION

At the request of Nebraska Emergency Management Agency (NEMA), EA Engineering, Science, and Technology performed 48-hour static, non-renewal acute toxicity tests on a sample of "Bottom Ash". The Bottom Ash sample was collected by NEMA personnel on 26 February 2001, and was received at EA on 13 April 2001. The test species were *Ceriodaphnia dubia* (water flea) and *Pimephales promelas* (fathead minnow). The test organisms were exposed for 48 hours to 10,000; 5,000; 1,000; 100; and 10 mg/L Bottom Ash and a laboratory dilution water control of moderately hard synthetic fresh water. The objective of the study was to assess the acute lethality of the Bottom Ash to each test species, expressed as 48-hour median lethal concentrations (LC50s). This toxicity testing was conducted under the biomonitoring requirements of NPDES permit number NE0132152.

Summaries of sample and test information including reference toxicant data and acute toxicity test results are presented on pages 3-4 for the *C. dubia* test and on pages 5-6 for the *P. promelas* test. Copies of toxicity test protocols, raw data sheets, and the Report Quality Assurance Record are included in Attachments I, II, and III, respectively.

SUMMARY OF RESULTS

The results of the acute toxicity tests indicated that the Bottom Ash sample was not acutely toxic to *Ceriodaphnia dubia* or *Pimephales promelas* at the highest concentration tested (48-hour LC50s were >10,000 mg/L Bottom Ash).

In the *C. dubia* acute toxicity test (page 4), there was a minimum of 95 percent survival in the 10 through 10,000 mg/L test concentrations after 48 hours of exposure. Survival in the control was also 95 percent. The resulting 48-hour LC50 for *C. dubia* was >10,000 mg/L Bottom Ash.

In the *P. promelas* acute toxicity test (page 6), there was 100 percent survival in the control and in all of the test concentrations at 48-hours. The 48-hour LC50 for *P. promelas* was >10,000 mg/L Bottom Ash.

In conformance with EA's quality assurance/quality control program, monthly reference toxicant tests were conducted on the in-house cultures of *C. dubia* and *P. promelas*. The 48-hour LC50 from the *C. dubia* reference toxicant test using sodium chloride (NaCl) was 1,473 mg/L NaCl which fell within EA's acceptable control chart limits of 1,164-2,541 mg/L NaCl (page 3). The results of the *P. promelas* reference toxicant test using potassium chloride (KCl) were also acceptable with a 48-hour LC50 of 724 mg/L KCl and acceptable control chart limits of 551-1,130 mg/L KCl (page 5).

SUMMARY OF SAMPLE/TEST INFORMATION

Test: *Ceriodaphnia dubia* 48-hour static non-renewal acute LC50 assay

Test Procedure: **EA Protocol ATS-SAI-CD-05**
Acute assay with *Ceriodaphnia dubia*

Client Name: **Nebraska Emergency Management Agency**

Sample Description: **Bottom Ash**

EA Accession Number: AT1-164

Collection Date: 26 February 2001

Receipt Time and Date: 1030, 13 April 2001

Dilution Water Description: **Moderately hard synthetic fresh water**

EA Test Number: **TN-01-175**

Test Initiation Time and Date: 0955, 18 April 2001

Test Completion Time and Date: 0948, 20 April 2001

Test Vessel: **30-ml cup**

Test Volume: **15 ml**

No. organisms per replicate: **4**

No. replicates per concentration: **5**

Organism Lot Information

Lot Number: Not Applicable
Source: EA's Culture Facility (Sparks, Maryland)
Organism Age: <24 hours old

Reference Toxicant Test Information

Reference Toxicant: Sodium chloride (NaCl)
EA Test Number: RT-01-037
48-hour LC50: 1,473 mg/L NaCl
Laboratory control chart acceptability range for 48-hour LC50: 1,164-2,541 mg/L NaCl

SUMMARY OF SAMPLE/TEST INFORMATION (continued)

Test Species: *Ceriodaphnia dubia* (water flea)
 Client Name: Nebraska Emergency Management Agency
 Sample Description: Bottom Ash
 EA Test Number: TN-01-175

<u>Test Concentration (mg/L)</u>	<u>24-Hour Survival (percent)</u>	<u>48-Hour Survival (percent)</u>
Control	100	95
10	100	95
100	100	100
1,000	100	100
5,000	100	100
10,000	100	95
24-Hour LC50:	>10,000 mg/L	--
48-Hour LC50:	--	>10,000 mg/L

<u>Test Solution Water Quality Parameters</u>	<u>Range</u>
Temperature (°C):	19.9 - 20.6
pH:	7.9 - 8.2
Dissolved Oxygen (mg/L):	8.7 - 9.1
Conductivity (µS/cm):	310 - 363

<u>Additional Water Quality Parameters</u>	<u>Control</u>	<u>10,000 mg/L</u>
Alkalinity (mg/L as CaCO ₃):	56	70
Hardness (mg/L as CaCO ₃):	88	92

SUMMARY OF SAMPLE/TEST INFORMATION

Test: *Pimephales promelas* 48-hour static non-renewal acute LC50 assay

Test Procedure: **EA Protocol ATS-SAF-FM-05**

Acute assay with fathead minnows (*Pimephales promelas*)

Client Name: **Nebraska Emergency Management Agency**

Sample Description: **Bottom Ash**

EA Accession Number: AT1-164

Collection Date: 26 February 2001

Receipt Time and Date: 1030, 13 April 2001

Dilution Water Description: **Moderately hard synthetic fresh water**

EA Test Number: **TN-01-176**

Test Initiation Time and Date: 1050, 18 April 2001

Test Completion Time and Date: 1037, 20 April 2001

Test Vessel: **1-L beaker**

Test Volume: **250 ml**

No. organisms per replicate: **10**

No. replicates per concentration: **2**

Organism Lot Information

Lot Number: FH1-4/4-5

Source: EA's Culture Facility (Sparks, Maryland)

Organism Age: 13-14 days old (hatched within a single 24-hour period)

Reference Toxicant Test Information

Reference Toxicant: Potassium chloride (KCl)

EA Test Number: RT-01-041

48-hour LC50: 724 mg/L KCl

Laboratory control chart acceptability range for 48-hour LC50: 551-1,130 mg/L KCl

SUMMARY OF SAMPLE/TEST INFORMATION (continued)

Test Species: *Pimephales promelas* (fathead minnow)
 Client Name: Nebraska Emergency Management Agency
 Sample Description: Bottom Ash
 EA Test Number: TN-01-176

<u>Test Concentration (mg/L)</u>	<u>24-Hour Survival (percent)</u>	<u>48-Hour Survival (percent)</u>
Control	100	100
10	100	100
100	100	100
1,000	100	100
5,000	100	100
10,000	100	100

24-Hour LC50: >10,000 mg/L
 48-Hour LC50: -- >10,000 mg/L

<u>Test Solution Water Quality Parameters</u>	<u>Range</u>
Temperature (°C):	19.9 - 20.8
pH:	8.0 - 8.2
Dissolved Oxygen (mg/L):	8.4 - 9.1
Conductivity (µS/cm):	310 - 333

<u>Additional Water Quality Parameters</u>	<u>Control</u>	<u>10,000 mg/L</u>
Alkalinity (mg/L as CaCO ₃):	56	70
Hardness (mg/L as CaCO ₃):	88	92