

FINAL REPORT

**CRUDE MCHM**

**HAEL No.: 97-0216**

**EAN: 972790**

**PM No.: 18717-00**

**ACUTE ORAL TOXICITY STUDY IN THE RAT**

GUIDELINE

OECD: 401

EEC: Annex V., Test B.1

AUTHOR

Lisa G. Bernard, M.S.

TESTING FACILITY

Toxicological Sciences Laboratory  
Health and Environment Laboratories  
Eastman Kodak Company  
Rochester, New York 14652-6272  
USA

LABORATORY PROJECT ID

97-0216A8

STUDY SPONSOR

Eastman Chemical Company  
P.O. Box 431  
Kingsport, TN 37662-5280

STUDY COMPLETION DATE

December 1, 1999

QUALITY ASSURANCE INSPECTION STATEMENT  
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0216-1 STUDY DIRECTOR: BERNARD, L.G.

PAGE 1  
11/10/99

ACCESSION NUMBER: 972790

STUDY TYPE: ACUTE ORAL TOXICITY

M. James  
(AUDITOR, QUALITY ASSURANCE UNIT)

11/10/99  
DATE

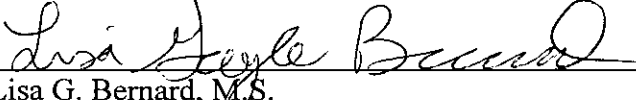
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THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY  
ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE  
FOLLOWING DATES.  
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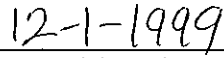
INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
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10/19/99	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
10/21/99	CLINICAL SIGNS AT 48 HRS.	11/10/99
11/10/99	FINAL REPORT REVIEW	11/10/99

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

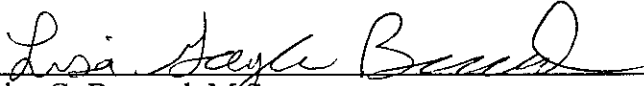
This study was conducted according to:

OECD Principles of Good Laboratory Practice (as revised in 1997)  
[C(97)186/Final].

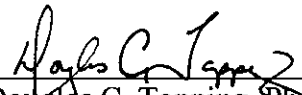
  
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Study Director

  
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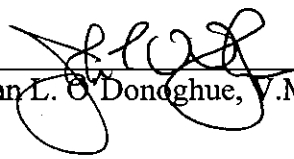
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Lisa G. Bernard, M.S.  
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Month/Day/Year

  
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John L. O'Donoghue, V.M.D., Ph.D. (pathology)

11/12/99  
Month/Day/Year

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ABSTRACT

**Crude MCHM**

**HAEL No.: 97-0216**

**EAN: 972790**

**PM No.: 18717-00**

**ACUTE ORAL TOXICITY STUDY IN THE RAT**

The purpose of the study was to evaluate the acute toxicity of the test substance in the female Sprague-Dawley rat (CrI:CD(SD)IGS BR) following a single oral dose. Of specific interest, was whether this strain of rat would exhibit hematuria.

A single dose of 500 mg/kg of the neat test substance was administered by gavage to female rats. Abnormal clinical signs were limited to transient reduced activity for all rats and transient stumbling for two rats on the day of dosing. No other abnormal clinical signs were noted at any time during the 14-day observation period. No mortality was observed, and all animals gained weight. No treatment-related changes were observed at necropsy, and no tissues were collected for histological examination.

A single oral dose of 500 mg/kg the test substance did not cause hematuria in female rats of this strain.

## STUDY AND TEST SUBSTANCE INFORMATION

### Testing Facility

Toxicological Sciences Laboratory  
Health and Environment Laboratories  
Eastman Kodak Company  
Rochester, New York 14652-6272  
USA

### Project Participants

Study Director: Lisa G. Bernard, M.S.  
Toxicologist: John W. Mosher, B.S.

### Sponsor

Eastman Chemical Company  
P.O. Box 431  
Kingsport, TN 37662-5280

Sponsor's Representative:  
Karen R. Miller, Ph.D.

### Test Substance Characterization

Test Substance Name: Crude MCHM  
HAEL No.: 97-0216  
EAN No.: 972790  
PM No.: 18717-00  
SRID No.: 6-97  
Physical State and Appearance: Clear, colorless liquid  
Source of Test Substance: Eastman Chemical Company, Kingsport, TN  
Laboratory Project ID: 97-0216A8

### Study Dates

Study Initiation Date: October 19, 1999  
Experimental Start Date: October 19, 1999  
Experimental Completion Date: November 2, 1999

## PURPOSE

The purpose of the study was to evaluate the acute toxicity of the test substance in the female Sprague-Dawley rat (CrI:CD(SD)IGS BR) following a single oral dose. Of specific interest, was whether this strain of rat would exhibit hematuria.

## MATERIALS AND METHODS

### Test System

Five female Sprague-Dawley rats (CrI:CD(SD)IGS BR) obtained from Charles River Laboratories, Stone Ridge (Kingston), NY were randomly assigned to the dose group. The rats were 7 weeks of age and weighed 134 to 158 grams at the start of the study. Rats were chosen for this study because they are a common representative species for toxicity studies. The rat is the preferred rodent species recommended for use in the Organisation for Economic Cooperation and Development (OECD) and European Economic Community (EEC) Test Guidelines.

### Husbandry

#### Housing

Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The rats were singly housed in suspended, stainless-steel, wire mesh cages. Cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed at least three times a week.

#### Environmental Conditions

The study room was maintained at 18.5-24.6°C and 38.7-67.2% relative humidity. A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained.

#### Acclimation Period

The animals were isolated upon arrival and allowed to acclimate for a period of 5 days. Animals were judged to be healthy prior to testing.



## **Husbandry, continued**

### Feed

Certified Rodent Diet (PMI #5002, pelleted) was available *ad libitum*. Feed containers were cleaned and refilled at least once a week. No known contaminants which would interfere with the outcome of this study were present in the feed. Analyses of feed are maintained on file within the testing laboratory.

### Water

Water was available *ad libitum* through an automatic watering system. The source of the water was the local public water system. There have been no contaminants identified in periodic water analyses that would be expected to interfere with the conduct of the study. Semiannual analyses of water are maintained on file within the testing laboratory.

### Identification

Upon arrival, all rats were identified by uniquely-numbered metal ear tags. During randomization, study-specific animal numbers were assigned to each animal. Cage cards contained the study-specific animal number and the ear tag number.

## **Experimental Design**

### Test Procedures

This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals: Guideline 401, Acute Oral Toxicity; and European Economic Community (EEC): Annex V., Test B.1, Acute Toxicity (Oral).

### Randomization

The procedure for including animals in the study was to randomly select and assign animals from the same shipment to the study. Randomization was done by computer-generated lists. After assignment of animals to the study, the body weights were determined to ensure that individual body weights were within 20% of the mean weight.

## Experimental Design, continued

### Determination of Dose Levels

A dose of 500 mg of the test substance/kg body weight was selected as the dose level for this study.

### Test Substance Exposure

A single dose of the test substance was administered by gavage to animals that had been fasted overnight.

### Preparation of Test Substance in the Vehicle

The test substance was administered as received.

### Distribution of Animals

**TABLE 1**

<b>Dose Level</b>	<b>Number Of Animals</b>	<b>Animal Numbers</b>
500 mg/kg	5 Females	551 - 555

### Body Weights

Body weights were collected on Days 0 (prior to treatment), 7, and 14.

### Clinical Observations

Animals were observed three times on the day of dosing (Day 0), and once each day thereafter for the duration of the experiment. Observations included, but were not limited to, examination of the hair, skin, eyes, mucous membranes, motor activity, feces, urine, respiratory system, circulatory system, autonomic nervous system, central nervous system, and behavior patterns.

### Necropsy

All animals were euthanatized and necropsied at the completion of the 14-day observation period.

### **Data Storage**

The final report, data sheets, all nonperishable raw data, and an aliquot of the test substance have been stored in the testing facility archive managed under GLP-mandated conditions.

### **Data Analysis**

No statistical procedures were required during the study. No dose/mortality curve was constructed since graphs become statistically useful only with the use of large numbers of animals and dose groups.

### **Protocol and Standard Operating Procedure Deviations**

There were no SOP or protocol deviations during the study.

**RESULTS**

Mortality

The dose level, the number of animals administered the test substance at each dose level, the number of deaths, and the Day of death are listed in Table 2.

**TABLE 2**  
**Mortality Table**

<b>Dose (mg/kg)</b>	<b>Number Of Female Rats Exposed</b>	<b>Number Of Deaths</b>	<b>Time Of Death</b>
500	5	0	-----

Clinical Signs

Abnormal clinical signs were limited to reduced activity and stumbling on the day of dosing. The time of each observation and the number of animals involved at each dose level are listed in Table 3.

**TABLE 3**  
**Table Of Clinical Observations**

<b>Dose (mg/kg)</b>	<b>Time</b>	<b>Clinical Signs</b>	<b>Number Of Animal Affected</b>
500	Day 0: Immediately and 1 hour after dosing	Appeared Clinically Normal	5/5 Females
500	Day 0: 4 hours after dosing	Reduced Activity Stumbling	5/5 Females 2/5 Females
500	Days 1-14	Appeared Clinically Normal	5/5 Females

Body Weights

All animals gained weight during both weeks of the study. The individual body weights are listed in Table 4.

**TABLE 4**  
**Table Of Individual Body Weights (grams)**

<b>Dose (mg/kg)</b>	<b>Animal Number</b>	<b>Day 0</b>	<b>Day 7</b>	<b>Day 14</b>
<b>FEMALE RATS</b>				
500	551	134	172	191
500	552	158	199	219
500	553	149	189	211
500	554	144	189	207
500	555	150	178	213

Necropsy Findings

No treatment-related changes were observed at necropsy, and no tissue was collected for microscopic examination. A record of the incidence and severity of all gross abnormalities is presented in computer-generated tables which are included in the Appendix.

## DISCUSSION

For the female Sprague-Dawley rats (CrI:CD(SD)IGS BR) used in this study, abnormal clinical signs were limited to transient reduced activity and stumbling on the day of dosing.. No red urine or hematuria were observed following treatment in this study.

In a previous study conducted using a different strain of Sprague-Dawley rat [SAS:VAF(SD)], female rats administered a comparable dose of the test substance exhibited similar signs of transient slight weakness and stumbling on the day of dosing. However, these animals also exhibited red urine and/or hematuria (TX-97-306, 1998).

## CONCLUSION

A single oral dose of 500 mg/kg the test substance did not cause hematuria in female rats of this strain.

## REFERENCES

- National Research Council (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press. Washington, D.C.
- TX-97-306 (1998). Crude MCHM: Acute Oral Toxicity Study In The Rat. Unpublished report, Health and Environment Laboratories, Eastman Kodak Company.

## **APPENDIX**

SUMMARY GROSS PATHOLOGY INCIDENCE TABLE - FEMALE RATS

GROUP	500 MG/KG
TRACHEA	5
LUNGS	5
THYMUS	5
HEART	5
ESOPHAGUS	5
STOMACH	5
DUODENUM	5
JEJUNUM	5
ILEUM	5
CECUM	5
COLON	5
RECTUM	5
LIVER	5
KIDNEYS	5
URINARY BLADDER	5
PITUITARY GLAND	5
ADRENALS	5
PANCREAS, NOS	5
THYROID GLANDS	5
PARATHYROID GLANDS	5
SPLEEN	5
MESENTERIC LYMPH NODES	5
BONE MARROW	5
BRAIN	5
EYES	5
SALIVARY GLANDS	5
ADIPOSE TISSUE	5
SKIN, NOS	5
HAIR	5
FALLOPIAN TUBES	5
VAGINA	5
UTERUS	5
HYDROMETRA	1
OVARIES	5
CERVIX UTERI	5

NUMBERS REPRESENT NUMBER OF TISSUES EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF TISSUES WITH EACH ABNORMALITY



## INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE - FEMALE RATS

ANIMAL #	500 MG/KG				
	551	552	553	554	555
DAYS ON TEST	14	14	14	14	14
TRACHEA	X	X	X	X	X
LUNGS	X	X	X	X	X
THYMUS	X	X	X	X	X
HEART	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X
STOMACH	X	X	X	X	X
DUODENUM	X	X	X	X	X
JEJUNUM	X	X	X	X	X
ILEUM	X	X	X	X	X
CECUM	X	X	X	X	X
COLON	X	X	X	X	X
RECTUM	X	X	X	X	X
LIVER	X	X	X	X	X
KIDNEYS	X	X	X	X	X
URINARY BLADDER	X	X	X	X	X
PITUITARY GLAND	X	X	X	X	X
ADRENALS	X	X	X	X	X
PANCREAS, NOS	X	X	X	X	X
THYROID GLANDS	X	X	X	X	X
PARATHYROID GLANDS	X	X	X	X	X
SPLEEN	X	X	X	X	X
MESENTERIC LYMPH NODES	X	X	X	X	X
BONE MARROW	X	X	X	X	X
BRAIN	X	X	X	X	X
EYES	X	X	X	X	X
SALIVARY GLANDS	X	X	X	X	X
ADIPOSE TISSUE	X	X	X	X	X
SKIN, NOS	X	X	X	X	X
HAIR	X	X	X	X	X
FALLOPIAN TUBES	X	X	X	X	X
VAGINA	X	X	X	X	X
UTERUS	X	X	X	X	
HYDROMETRA					2
OVARIES	X	X	X	X	X
CERVIX UTERI	X	X	X	X	X

KEY: N-NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, X-NORMAL BUT NOT COLLECTED, 1-MINIMAL, 2-MINOR, 3-MODERATE, 4-SEVERE, P-PRESENT, A-ABSENT, \* -SEE COMMENT REPORT