

FINAL REPORT

**CRUDE MCHM**

**HAEL No.: 97-0216**

**EAN: 972790**

**PM No.: 18717-00**

**ACUTE DERMAL IRRITATION STUDY IN THE RABBIT**

GUIDELINE

OECD: 404

EEC: Annex V., Test B.4

AUTHOR

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TESTING FACILITY

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

LABORATORY PROJECT ID

97-0216A2

STUDY SPONSOR

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


STUDY COMPLETION DATE

November 10, 1997

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  
ACCESSION NUMBER: 972790 10/27/97

STUDY TYPE: ACUTE DERMAL IRRITATION TEST

  
(AUDITOR, QUALITY ASSURANCE UNIT)

11/3/97  
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
08/05/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
08/07/97	CLINICAL SIGNS AT 48 HRS.	10/27/97
10/27/97	FINAL REPORT REVIEW	10/27/97

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

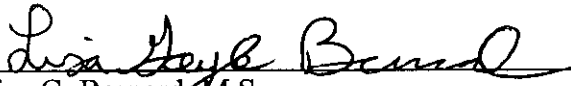
This study was conducted according to:

Annex 2, Organisation for Economic Cooperation and Development, Guidelines  
for Testing of Chemicals [C(81)30(Final)].

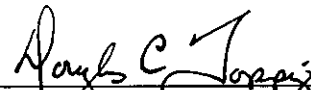
  
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Study Director

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**SIGNATURE PAGE**

  
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Nov 4, 1997  
Month/Day/Year

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ABSTRACT

**CRUDE MCHM**

**HAEL No.: 97-0216**

**EAN: 972790**

**PM No.: 18717-00**

**ACUTE DERMAL IRRITATION STUDY IN THE RABBIT**

A dermal irritation study was conducted by administering single topical doses of 0.5 mL of the test substance to rabbits. The test substance was left in contact with the skin under an occlusive wrap for four hours. Skin lesions were graded according to OECD Guideline 404 (Annex V., Test B.4).

Severity of irritant response either increased or remained the same during the 72-hour observation period. At the 1-hour observation, signs of irritation consisted of very slight (grade 1) to well-defined (grade 2) erythema and very slight (grade 1) to moderate (grade 3) edema. At the 72-hour observation, signs of irritation consisted of well-defined (grade 2) to moderate-severe (grade 3) erythema and slight (grade 2) to moderate (grade 3) edema. Due to the degree of irritation observed, the animals were euthanized following the 72-hour examination.

Based on the responses observed, the test substance is classified as "irritating to skin" as defined in the 18th Adaptation of the EC Classification, Packaging and Labelling of Dangerous Substances Directive.

## 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.
Principal Investigator:	John W. Mosher, B.S.
Veterinarian:	Milan S. Vlaovic, D.V.M., Ph.D.

### Sponsor

Eastman Chemical Company P.O. Box 431 Kingsport, TN 37662-5280	Sponsor's Representative: Karen R. Miller, Ph.D.
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### Test Substance Characterization

Test Substance Name:	Crude MCHM
HAEL No.:	97-0216
EAN:	972790
PM No.:	18717-00
SRID No.:	6-97
Physical State and Appearance:	Liquid, Clear and colorless
Source of Test Substance:	Eastman Chemical Company, Kingsport, TN
Laboratory Project ID:	97-0216A2

### Study Dates

Study Initiation Date:	August 5, 1997
Experimental Start Date:	August 5, 1997
Experimental Completion Date:	August 8, 1997

## PURPOSE

The purpose of the study was to determine the potential of the test substance to cause primary irritation of mammalian skin.

## MATERIALS AND METHODS

### Test System

Three albino rabbits (Hra:(NZW)SPF) obtained from Covance Research Products, Inc. (Denver, PA) were assigned to the study. The rabbits were young adults (at least three months old) and weighed at least 2000 grams at the start of the study. Rabbits were chosen for this study because they are a common representative species for dermal irritation studies. The rabbit is the preferred species recommended for use in the OECD Guideline.

### Husbandry

#### Housing

Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The rabbits were singly housed in suspended, stainless-steel mesh cages. Cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed every other day.

#### Environmental Conditions

The study room was maintained at 19-21°C and 56-67% 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.

#### Feed

Certified High Fiber Rabbit Diet (PMI #5325) 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.



## **Husbandry, continued**

### 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 previous 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 rabbits were identified by uniquely-numbered ear tags. 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) Guideline for Testing of Chemicals: Guideline 404, Acute Dermal Irritation/Corrosion; and European Economic Community (EEC): Annex V., Test B.4, Acute Toxicity (Skin Irritation).

### Identification Numbers of Animals Used

Animal numbers 328, 329, and 330 were used in this study.

### Preparation of Test Substance

The test substance, a liquid, was administered as received.

### Test Substance Exposure

The hair was removed from an area of the dorsal skin with an electric clipper. A single dose of 0.5 mL of the test substance was applied topically to each animal using a fiber pad and an occlusive wrap to hold the test substance in place for four hours. At the end of the exposure period, the application site was rinsed with running water.

## **Experimental Design, continued**

### Control Substance

No control substance was used. Adjacent areas of untreated skin of each animal served as control sites for the test areas.

### Vehicle

No vehicle was used.

### Clinical Observations

The site of application was examined at 1, 24, 48, and 72 hours after removal of the occlusive patch. Observations included estimation of erythema, edema, necrosis, eschar formation, scarring, erosion, and staining caused by the test substance as well as general systemic effects.

### Grading the Irritant Response

The most severely affected area within the site of application of the test substance will be scored for erythema and edema at each observation period. Skin reactions were graded and scored as described in Table 1.

**TABLE 1**  
**Grading Of Skin Reaction<sup>α</sup>**

<b>Erythema and Eschar Formation</b>	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4
<b>Edema Formation</b>	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (edges raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm and extending beyond area of exposure)	4

<sup>α</sup> Graded as described in OECD Guideline 405 (Annex V., Test B.4) (Grading of Skin Reaction)

## Experimental Design, continued

### Grading Other Clinical Observations

In addition to the observations of irritation (erythema and edema), other serious skin lesions, abnormal clinical signs, or toxic effects were graded and scored as described in Table 2.

**TABLE 2**  
**Grading Of Other Clinical Observations**

<b>Degree of Severity</b>	
Very Slight	1
Slight	2
Moderate	3
Severe	4

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### Body Weight

Body weights were measured on the day of initiation of the study.

### Necropsy

No necropsies were conducted at the conclusion of the 72-hour 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.

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

There were no SOP or protocol deviations during the study.

## RESULTS

### Observations for Irritation

The application site of each animal was examined for signs of irritation at 1, 24, 48, and 72 hours after termination of exposure to the test substance. Observations for irritation (erythema and edema) are presented in Table 3.

**TABLE 3**  
**Response At Application Site**

ANIMAL NUMBER	Erythema, Edema			
	1 HOUR	24 HOURS	48 HOURS	72 HOURS
328	2,2	2,1	2,1	2,2
329	1,1	2,1	2,2	3,3
330	2,3	3,3	3,3	2,3

<sup>a</sup> Graded as described in OECD Guideline 404 (Annex V., Test B.4) (Grading of Skin Reaction)

### Description of Serious Lesions and Irritation Other Than Erythema and Edema

No other lesions were noted during the 72-hour observation period. Due to the degree of irritation observed, the animals were euthanatized following the 72-hour examination.

### Toxic Effects

No toxic effects were noted during the study.

### Body Weights

At initiation of the study, Rabbit Numbers 328, 329, and 330 weighed 3058, 3280, and 3259 grams, respectively.

## DISCUSSION

Prior to initiation of the dermal irritation study, the pH of the test substance was measured and the value obtained (6.4) demonstrated that the test substance was neither strongly acid ( $\text{pH} \leq 2.0$ ) nor strongly alkaline ( $\text{pH} \geq 11.5$ ). Therefore, an acute dermal irritation study was conducted.

The test substance was left in contact with the skin under an occlusive wrap for four hours. Severity of the irritant response either increased or remained the same during the 72-hour observation period. At the 1-hour observation, signs of irritation consisted of very slight (grade 1) to well-defined (grade 2) erythema and very slight (grade 1) to moderate (grade 3) edema. At the 72-hour observation, signs of irritation consisted of well-defined (grade 2) to moderate-severe (grade 3) erythema and slight (grade 2) to moderate (grade 3) edema. Due to the degree of irritation observed, the animals were euthanized following the 72-hour examination.

## CONCLUSION

Based on the responses observed, the test substance is classified as "irritating to skin" as defined in the 18th Adaptation of the EC Classification, Packaging and Labelling of Dangerous Substances Directive.

## REFERENCES

National Research Council (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press. Washington, D.C.