

FINAL REPORT

CRUDE MCHM

HAEL No.: 97-0216

EAN: 972790

PM No.: 18717-00

SKIN SENSITIZATION STUDY (FOOTPAD METHOD) IN THE GUINEA PIG

GUIDELINE

OECD: 406

AUTHOR

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TESTING FACILITY

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Health and Environment Laboratories
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USA

LABORATORY PROJECT ID

97-0216A5

STUDY SPONSOR

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

STUDY COMPLETION DATE

December 12, 1997

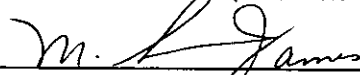
QUALITY ASSURANCE INSPECTION STATEMENT

(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.
ACCESSION NUMBER: 972790

PAGE 1
11/20/97

STUDY TYPE: SENSITIZATION (FOOTPAD METHOD)



(AUDITOR, QUALITY ASSURANCE UNIT)

11/21/97
DATE


THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
08/12/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
08/14/97	CLINICAL SIGNS AT 48 HRS. PRIMARY IRRITATION	11/20/97
11/20/97	FINAL REPORT REVIEW	11/20/97

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

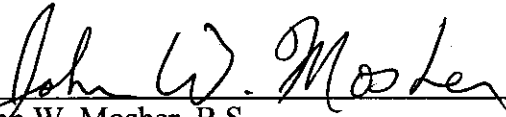
Annex 2, Organisation for Economic Cooperation and Development, Guidelines
for Testing of Chemicals [C(81)30(Final)].



Lisa G. Bernard, M.S.
Study Director

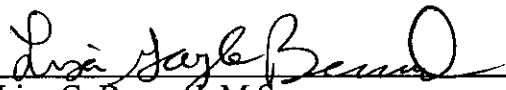
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John W. Mosher, B.S.
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December 12, 1997
Month/Day/Year



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12-12-97
Month/Day/Year



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Nov 21, 1997
Month/Day/Year

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ABSTRACT

CRUDE MCHM

HAEL No.: 97-0216

EAN: 972790

PM No.: 18717-00

SKIN SENSITIZATION STUDY (FOOTPAD METHOD) IN THE GUINEA PIG

A dermal sensitization study was conducted with this test substance in guinea pigs using the footpad method. In a primary irritation screen, three animals were administered 10% of the test substance in a vehicle of acetone, dioxane, and guinea pig fat (7:2:1) topically. No signs of irritation were noted at 24 or 48 hours after administration of the test substance; therefore, the concentration used in the challenge dose of the sensitization study was set at 10% of the test substance in the vehicle.

In the sensitization study, no dermal responses were noted for the ten animals previously induced with Freund's Complete Adjuvant (FCA) (control animals) or for the ten animals previously induced with 1% of the test substance in FCA (test animals). All animals in both groups were graded as having no response to the test substance after the challenge application.

Based on these results, the test substance was not considered to be a dermal sensitizer in guinea pigs. The lack of any response at challenge indicates that the test substance has a low potential for human dermal sensitization. The test substance requires no label for sensitization by skin contact, 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.
Report Author:	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.
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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:	97-0216A5

Study Dates

Study Initiation Date:	August 12, 1997
Experimental Start Date:	August 12, 1997
Experimental Completion Date:	August 28, 1997

PURPOSE

The purpose of the study was to determine whether the test substance has the ability to produce delayed contact hypersensitivity (skin sensitization).

MATERIALS AND METHODS

Test System

Male guinea pigs (CrI:(HA)BR VAF/Plus[®]) obtained from Charles River Laboratories, Portage, MI were assigned to this study. For the primary irritation portion of the study, three male guinea pigs, previously exposed to Freund's Complete Adjuvant (FCA), were assigned to each dose level to be tested for signs of irritation; these animals were 9 to 10 weeks of age. For the induction and challenge portion of the sensitization study, 20 male guinea pigs were randomly assigned to one of two groups (Control Group or Test Group). These guinea pigs were 5 to 6 weeks of age and weighed 334 to 410 grams at the start of the study. Guinea pigs were chosen for this study because they are the animal of choice for predictive sensitization studies and are 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 guinea pigs 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 18-22°C and 54-69% 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

PMI® Certified Guinea Pig Diet (5026) 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. Semi-annual analyses of water are maintained on file within the testing facility.

Identification

Upon arrival, all guinea pigs 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 406 (Annex), Skin Sensitisation, Dated 12 May, 1981.

Randomization

A clinical examination was performed on each animal to ensure that only healthy animals were utilized. The procedure for including animals in the sensitization study was to randomly select and assign animals from the same shipment to each group (test and control). Randomization was done by a computer-generated list. After assignment of animals to individual groups, the body weights were determined to ensure that weights were no greater than 500 grams at the initiation of the induction phase.

Experimental Design, continued

Distribution of Animals

Animals were distributed into groups as described in Table 1.

TABLE 1
Distribution Of Animals Into Groups

Study Phase	Group	Number Of Animals	Animal Numbers
Primary Irritation Screen	10%	3	941 - 943
Sensitization Study	Test	10	961 - 970
	Control	10	951 - 960

Primary Irritation Screen

Animals that were previously exposed to FCA were tested for primary skin irritation. Hair was removed from the backs of the animals with an electric clipper and 0.3 mL of a 10% solution of the test compound in a vehicle of acetone, dioxane, and guinea pig fat (7:2:1) was applied to the clipped area. Twenty-four hours later, the animals were depilated and scored for edema and erythema. The skin reaction was also scored at 48 hours. The highest average score for either day determined the concentration to be used in the challenge dose of the main study.

The challenge dose was based on the highest concentration of the test substance (up to 10%) that produced no irritation for any of the three animals of a dose group in the primary irritation screen.

Sensitization Study

Ten animals (control group) were injected in the footpad with 0.05 mL of Freund's Complete Adjuvant (FCA), Lot # 12K2269. At the same time, an additional 10 animals (test group) were injected in the same manner with 0.05 mL of FCA containing 1% test substance. Seven days later, the hair was removed from the backs of the animals with an electric clipper. The animals were then challenged with 0.3 mL of a solution of the test substance (at the concentration determined in the previous step) in the vehicle. The animals were depilated 24 hours after the challenge dose and the reaction to the topical challenge was scored. The next day (48 hours after challenge) the reaction was scored again.

Experimental Design, continued

Grading Sensitization Response

At both observation times, the challenged skin areas were graded for erythema and edema using numerical ratings as follows:

Erythema

- 0 - none
- 1 - just discernible - slight
- 2 - easily determined - moderate
- 3 - dark red-strong

Edema

- 0 - none
- 1 - just discernible to touch - slight
- 2 - easily determined - moderate
- 3 - difficult to pick up a fold of skin - strong

Body Weight Determinations

Body weights were collected on the day of the footpad induction and 48 hours after the challenge dose.

Necropsy

Animals were not necropsied at the conclusion of the test.

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

Evaluation of data was not done statistically, but rather by the following method. The response of each animal (erythema, edema) is interpreted as outlined below:

none:	0,0
slight:	1,0; 0,1; 1,1; 2,0; or 2,1
moderate:	2,2; 1,3; 3,0; or 3,1
strong:	2,3; 3,2; or 3,3

The numbers of animals which were graded as having either a negative (none), slight, moderate, or strong sensitization response, using the above criteria, were multiplied by the numerical values shown below:

<u>Response Degree</u>	<u>Numerical Value Assigned</u>
None	0
Slight	1
Moderate	5
Strong	10

The products of the multiplication were added together to obtain a total score. The estimated human risk potential for dermal sensitization is based on the highest total score of the test group at either 24 or 48 hours. A total score of 0-9 is rated "low potential", 10-49 is rated "moderate potential", and 50-100 is rated "high potential".

Protocol and Standard Operating Procedure Deviations

There were no SOP or protocol deviations during the study.

RESULTS

Mortality

No mortality was noted during the study.

Primary Irritation Screen

For the primary irritation screen, the animal numbers dosed, the dose level, and the irritation scores at the 24-hour and 48-hour examinations are listed in Table 2.

TABLE 2

Primary Irritation Screen: Irritation Following Topical Applications

Dose Group	Animal Number	Score (Erythema, Edema)	
		24 Hours	48 Hours
10%	941	0,0	0,0
10%	942	0,0	0,0
10%	943	0,0	0,0

Sensitization Study

Based on the maximum non-irritating dose in the primary irritation screen, the concentration used in the challenge dose of the sensitization study was set at 10%.

The scores of the dermal responses noted for each animal at the 24-hour and 48-hour examinations following the challenge application are listed in Table 3.

TABLE 3

Sensitization Study: Irritation Observed Following The Challenge Applications

Animal Number	Score (Erythema, Edema)		Animal Number	Score (Erythema, Edema)	
	24 Hours	48 Hours		24 Hours	48 Hours
CONTROL GROUP					
951	0,0	0,0	956	0,0	0,0
952	0,0	0,0	957	0,0	0,0
953	0,0	0,0	958	0,0	0,0
954	0,0	0,0	959	0,0	0,0
955	0,0	0,0	960	0,0	0,0

TABLE 3, continued

Sensitization Study: Irritation Observed Following The Challenge Applications

Animal Number	Score (Erythema, Edema)		Animal Number	Score (Erythema, Edema)	
	24 Hours	48 Hours		24 Hours	48 Hours
TREATED GROUP					
961	0, 0	0, 0	966	0, 0	0, 0
962	0, 0	0, 0	967	0, 0	0, 0
963	0, 0	0, 0	968	0, 0	0, 0
964	0, 0	0, 0	969	0, 0	0, 0
965	0, 0	0, 0	970	0, 0	0, 0

Description of Serious Lesions

No serious lesion was noted during the study.

Degree and Nature of Irritation

In the primary irritation screen no signs of irritation were noted. In the sensitization study, no dermal responses were noted for the ten animals previously induced with FCA (control animals) or for the ten animals previously induced with 1% of the test substance in FCA (test animals). Based on these results, the test animals were graded as having no sensitization response to the test substance after the challenge application.

Toxic Effects Other Than Irritation

No toxic effects or systemic clinical signs were noted during the study.

Body Weights

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

TABLE 4
Individual Animal Body Weights

Animal Number	Body Weights (Grams)		Animal Number	Body Weights (Grams)	
	Start	End		Start	End
CONTROL GROUP					
951	384	438	956	388	456
952	355	400	957	371	427
953	408	458	958	370	419
954	387	449	959	410	456
955	381	434	960	368	431
TREATED GROUP					
961	350	400	966	350	400
962	366	418	967	370	407
963	394	418	968	361	421
964	404	476	969	334	378
965	343	410	970	371	437

DISCUSSION

When animals were challenged with a concentration of 10% test substance in the vehicle, no dermal responses were noted for control or test animals. Based on the criteria described under the heading Data Analysis, the total score for controls following challenge was "0". For the test animals, the total score was also "0" (low potential). All animals in both groups (test and control) were graded as having no sensitization response to the test substance after the challenge application.

CONCLUSION

Based on these results, the test substance was not considered to be a dermal sensitizer in guinea pigs and has a low potential for human dermal sensitization. Since none of the animals responded positively, the test substance requires no label for sensitization by skin contact, 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.