TRADE SECRET

Study Title

H-25134: Acute Dermal Irritation/Corrosion Study in Rabbits

Laboratory Project ID: DuPont-8778

TEST GUIDELINES: OECD Guidelines for Testing of Chemicals
Section 4: Health Effects, No. 404 (1992)

Commission Directive 92/69/EEC
EEC Method B.4 (1992)

AUTHOR: Carol Finlay, B.A.

STUDY COMPLETED ON: December 10, 2001

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
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[redacted]

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of the data obtained from this study.

Reviewed by: James Mackay II 5-Dec-2001
James C. Mackay II
Associate Scientist
Date

Issued by Study Director: Carol Finlay 10-Dec-2001
Carol Finlay, B.A.
Staff Scientist
Date

Company Sanitized. Does not contain TSCA CBI

STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: [redacted]
H-25134
[redacted]

Submitter's Notebook Number(s): [redacted]

Haskell Number: 25134

CAS Registry Number: [redacted]

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Dark brown solid

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: November 6, 2001/ (see report cover page)

In-Life Initiated/Completed: November 7, 2001 / November 10, 2001

STUDY PERSONNEL

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SUMMARY

H-25134 was evaluated for acute skin irritation potential in 3 male New Zealand White rabbits. Approximately 0.5 g of the test substance was administered to a localized test site on the back of each animal. The test sites were covered with a semi-occlusive dressing to ensure contact between the skin and test substance. The animals were exposed to the test substance for approximately 4 hours. The test sites were evaluated and scored according to a numerical scale approximately 1, 24, 48, and 72 hours after the end of the 4-hour exposure period.

No dermal irritation was observed during the study. No clinical signs were observed, and no body weight loss occurred.

Mean values were calculated for each animal separately from numerical scores obtained from the quantitative evaluation of the 2 dermal responses (erythema and edema) observed in the rabbits at 24, 48, and 72 hours following test substance removal. These values are as follows:

Rabbit Number	Erythema	Edema
35313	0	0
35323	0	0
35336	0	0

On the basis of the mean values for each rabbit and according to the guide for the labeling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), H-25134 is not classified as irritant.

INTRODUCTION

A. Objective

The objective of this study was to evaluate the skin irritation/corrosive potential and the reversibility of dermal effects of H-25134 following a 4-hour dermal exposure in albino rabbits. The results of the study were used to determine the appropriate toxicity classification and labeling requirements in accordance with EEC (Directive 93/21).

B. Principles of the Methodology

Dermal erythema and edema are evaluated and scored at 1, 24, 48, and 72 hours following the removal of the test substance at the end of the 4-hour exposure in each of 3 rabbits. The reversibility of any dermal effects is assessed for up to 14 days, if necessary. Dermal effects are quantified according to the Draize Scale (Table 1).

For each animal, scores obtained at the 24-, 48-, and 72-hour evaluations are summed and a mean value for each lesion is calculated. Classification of dermal irritancy potential according to the EEC is based on the mean values calculated for each animal for the lesions observed as well as the persistence of the lesions observed.

MATERIALS AND METHODS

A. Test Guidelines

The study design complies with the following testing guidelines:

- Organisation for Economic Co-Operation and Development (OECD) (1992). 404 Acute Dermal Irritation/Corrosion. *Guideline for Testing of Chemicals.*
- European Economic Communities (EEC) (1992). Directive 92/69/EEC Annex V, Part B.4, Acute Toxicity (Dermal Irritation). *Methods for the Determination of Toxicity.*

B. Test Substance

The test substance, H-25134, was supplied by the sponsor as a dark brown solid. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Animal Husbandry

Adult HM:(NZW)fBR New Zealand White rabbits were received from Covance Research Products, Denver, Pennsylvania. The rabbits were housed singly in stainless steel, wire-mesh cages suspended above cage boards. Each rabbit was assigned a unique identification number which was recorded on a card affixed to the cage. The last 3 digits of the identification number were written on the inside of each rabbit's ear with a water insoluble marker. The rabbits were offered approximately 125 grams of PMI Nutrition International, Inc. Certified High Fiber Rabbit LabDiet® 5325 daily during the study. Water was available *ad libitum*.

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for total bacterial, spore, and fungal counts.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

Rabbits were quarantined, weighed, and observed for general health for 16 days. Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted for a temperature of 20°C ± 1°C and relative humidity of 50% ± 10%. Excursions outside these ranges were judged to have been of insufficient magnitude and/or duration to have adversely affected the validity of the study.

D. Protocol

Approximately 24 hours prior to treatment, the hair of 3 male New Zealand White rabbits was closely shaved to expose the skin from the scapular to the lumbar region of the back. The rabbits weighed from 2258 to 2415 grams on the day of treatment.

The area to be treated (approximately 6 cm^2) was marked on each rabbit's back with a water-insoluble marker. Approximately 0.5 g of H-25134 was applied to the test site and covered with

a 2-ply, 1-inch square gauze pad. The pad was held in place with non-irritating tape. The trunk of each rabbit was wrapped with porous tape. The tape was further secured with waterproof tape. The rabbits were returned to their cages after treatment. One other substance was tested concurrently on separate localized test sites on these rabbits.

Approximately 4 hours after application of the test substance, the rabbits were removed from their cages and the wrappings and gauze pads were removed. The test sites were gently washed with warm water to remove excess test substance and gently patted dry. The rabbits were then returned to their cages.

Approximately 1 hour after removal of the test patches, the test sites were evaluated for erythema, edema, and other evidence of dermal effects and were scored according to the Draize Scale (Table 1). A glossary of dermal effects and abbreviations is presented in Table 2. Additional evaluations were made at approximately 24, 48, and 72 hours after removal of the patches. The adjacent areas of untreated skin were used for comparison. Additionally, the rabbits were examined for clinical signs of toxicity at each observation period. The rabbits were weighed on the day of treatment and at the last dermal evaluation.

E. Data Analysis and Interpretation of Results

The numerical values corresponding to each animal for erythema and edema were recorded at each observation period. Mean values for each lesion (erythema and edema) were calculated for each animal separately from numerical scores obtained at the 24-, 48-, and 72-hour observations. The results were interpreted according to EEC Commission Directive 93/21 relative to the general classification and labeling requirements for dangerous substances.

Interpretation according to Annex VI Skin Corrosion or Irritation Criterion:

Corrosive

The test substance will be considered to be "CORROSIVE" and will require the symbol "C" and the indication of danger "CORROSIVE" if it produces full thickness destruction of the skin tissue on at least 1 animal during the skin irritation test.

Risk phrases will be assigned in accordance with the following criteria.

R35 "CAUSES SEVERE BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 3 minutes exposure or if this result can be predicted.

R34 "CAUSES BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 4 hours exposure or if this can be predicted.

Irritant

The test substance will be classified as "IRRITANT" and will require the symbol "Xi" and the indication of danger "IRRITANT" in accordance with the criteria given below.

In addition, the following risk (R) phrase will be assigned to substances, if appropriate, according to the criteria indicated below:

R38 "IRRITATING TO SKIN"

If, when applied to healthy intact animal skin for up to 4 hours, significant inflammation is caused and is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean values of the scores for either erythema and eschar formation or edema formation corresponds to one or more of the following mean values calculated for each animal separately and has been observed in 2 or more animals:

- Erythema and eschar formation 2.0 or more

- Edema 2.0 or more

All scores at each of the reading times (24, 48, and 72 hours) for an effect should be used in calculating the respective mean values.

An R38 "IRRITATING TO SKIN" phrase should also be assigned if:

The inflammation persists in at least 2 animals at the end of the observation time. Particular effects such as hyperplasia, scaling, discoloration, fissures, scabs, and alopecia should be taken into account.

RESULTS AND DISCUSSION

No dermal irritation was observed during the study. No clinical signs were observed, and no body weight loss occurred.

The dermal scores from individual animals with respect to observation time are presented in Table 3. Summary of mean scores calculated in accordance with EEC Directive 93/21 are presented in Table 4. Incidences of dermal irritation responses (scores) are summarized for each observation period in Table 5. Individual rabbit body weights and clinical signs of toxicity are presented in Table 6.

CONCLUSIONS

On the basis of the mean values for each rabbit and according to the guide for the labeling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), H-25134 is not classified as irritant.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

TABLES

TABLE 1

DRAIZE[a] SCALE FOR SCORING SKIN IRRITATION

Evaluation of Skin Reactions	**Score**
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

a Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

TABLE 2

GLOSSARY OF DERMAL EFFECTS

Blanching	white appearance to skin
Eschar	scab on the skin that is more superficial than necrosis. This is considered to be severe irritation.
Desquamation	dry, flaking of the skin
Fissuring	a split or cleft in the top layer of skin without bleeding
Fissuring with Bleeding	a split or cleft in the skin with bleeding
Hyperkeratosis	thick, dry discoloration (usually but not limited to brown or white in color) of the top layer of skin. This is considered to be severe irritation.
Sloughing	peeling of the top layer of skin, and epidermal scaling that has detached
Necrosis	area of rough/hard/dry black or dark colored skin that may crater. This is considered to be corrosion and an irreversible effect.
Epidermal Scaling	platelike areas of the top layer of skin that have separated from but are still attached to viable skin. This may progress to sloughing.
Thickening	skin is firm and/or dense to the touch.
Ulceration	open sore representing loss of superficial tissue

ABBREVIATIONS OF OTHER DERMAL EFFECTS

- = No Effect
A = Abraded
B = Blanching
C = Eschar
D = Desquamation
F = Fissuring
G = Fissuring with Bleeding
H = Hyperkeratosis
I = Intact
L = Sloughing
N = Necrosis
R = Raw Areas
S = Epidermal Scaling
T = Thickening
X = Compound Adhered to Skin

TABLE 3

DERMAL RESPONSES OBSERVED IN INDIVIDUAL RABBITS

ERYTHEMA

RABBIT NUMBER	HOURS AFTER REMOVAL OF TEST SUBSTANCE			
	1	24	48	72
35313	0	0	0	0
35323	0	0	0	0
35336	0	0	0	0

EDEMA

RABBIT NUMBER	HOURS AFTER REMOVAL OF TEST SUBSTANCE			
	1	24	48	72
35313	0	0	0	0
35323	0	0	0	0
35336	0	0	0	0

TABLE 4

SUMMARY OF MEAN SCORES FOR DERMAL RESPONSES

RABBIT NUMBER	ERYTHEMA[a]	EDEMA[a]
35313	0	0
35323	0	0
35336	0	0

a Calculated from the 24-, 48-, and 72-hour dermal responses (3 rabbit test).

TABLE 5

INCIDENCES OF DERMAL RESPONSES (SCORES)

ERYTHEMA

SCORE	1 HOUR	24 HOURS	48 HOURS	72 HOURS
0	3/3	3/3	3/3	3/3
1	0/3	0/3	0/3	0/3
2	0/3	0/3	0/3	0/3
3	0/3	0/3	0/3	0/3
4	0/3	0/3	0/3	0/3

EDEMA

SCORE	1 HOUR	24 HOURS	48 HOURS	72 HOURS
0	3/3	3/3	3/3	3/3
1	0/3	0/3	0/3	0/3
2	0/3	0/3	0/3	0/3
3	0/3	0/3	0/3	0/3
4	0/3	0/3	0/3	0/3

TABLE 6

INDIVIDUAL BODY WEIGHTS (g) OF RABBITS

RABBIT NUMBER	SEX	INITIAL WEIGHT	FINAL WEIGHT (72 hours)
35313	Male	2415	2596
35323	Male	2402	2597
35336	Male	2258	2470

CLINICAL SIGNS OF TOXICITY

No clinical signs of toxicity were observed in any of the rabbits during the study.