TRADE SECRET

Study Title

H-24516: Skin Irritation Test in Rabbits

Laboratory Project ID: DuPont-6087

AUTHOR: Carol Finlay, B.A.

STUDY COMPLETED ON: April 9, 2001

PERFORMING LABORATORY: E. I. du Pont de Nemours and Company
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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 — 9-April-2001
James C. Mackay II
Associate Scientist
Date

Issued by Study Director: Carol Finlay — 9-Apr-2001
Carol Finlay, B.A.
Staff Scientist
Date

Company Sanitized. Does not contain TSCA CBI

STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: [redacted]
H-24516

Haskell Number: 24516

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Pale to medium tan waxy solid

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

STUDY INFORMATION (Continued)

Study Initiated/Completed: March 8, 2001 / (see report cover page)

In-Life Initiated/Completed: March 13, 2001 / March 16, 2001

STUDY PERSONNEL

Study Director: Carol Finlay, B.A.
Management: Judith C. Stadler, Ph.D., D.A.B.T.
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Toxicology Report Preparation: Wanda F. Dinbokowitz

Laboratory Veterinarian: Wanda L. West, D.V.M., A.C.L.A.M.

SUMMARY

H-24516 was evaluated for acute skin irritation potential in 6 male New Zealand White rabbits. Approximately 0.5 g of the test substance was applied to the shaved, intact skin of each rabbit and covered with a semi-occlusive dressing for a 4-hour exposure. Approximately 1, 24, 48, and 72 hours after removal of the test substance, the test sites were evaluated for erythema, edema, and other evidence of dermal effects and were scored according to the Draize Scale. The adjacent areas of untreated skin were used for comparison.

No dermal irritation was observed in 4 rabbits throughout the study. The remaining 2 rabbits exhibited slight erythema and no or slight edema one hour after test substance removal. No dermal irritation was observed at 24, 48, or 72 hours. No clinical signs of toxicity were observed, and no significant body weight loss occurred during the study.

Under the conditions of this study, H-24516 was a slight skin irritant.

INTRODUCTION

The purpose of this study was to evaluate the skin irritation potential of H-24516 when applied to the shaved, intact skin of New Zealand White rabbits.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-24516, was supplied by the sponsor as a pale to medium tan waxy solid. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color, was observed.

B. Animal Husbandry

Young adult HM:(NZW)fBR New Zealand White rabbits were received from Covance Research Products, Denver, Pennsylvania. The rabbits were housed singly in suspended, stainless steel, wire-mesh cages. 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 7 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.

C. Protocol

Approximately 24 hours prior to treatment, the hair of 6 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 2049 to 2591 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. The test substance was liquefied by heating it in an approximately 90°C water bath. For each animal, approximately 0.5 g of the liquefied test substance was applied evenly to a 2-ply, 1 inch square gauze pad. The gauze pad was then placed on the test site test substance side down. 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. Three other test substances were tested concurrently on separate, localized test sites on the backs of 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, 24, 48, and 72 hours after removal of the test substance, 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. The adjacent areas of the untreated skin were used for comparison. The rabbits were also examined for clinical signs of toxicity at every dermal evaluation. The rabbits were weighed on the day of treatment and at the last dermal evaluation.

RESULTS AND DISCUSSION

No dermal irritation was observed in 4 rabbits throughout the study. The remaining 2 rabbits exhibited slight erythema and no or slight edema one hour after test substance removal. No dermal irritation was observed at 24, 48, or 72 hours. No clinical signs of toxicity were observed, and no significant body weight loss occurred during the study. Individual skin

irritation scores are presented in Table 3. A summary of skin responses is presented in the following table.

SUMMARY OF SKIN RESPONSES TO H-24516

	HOURS AFTER TEST SUBSTANCE REMOVAL							
	ERYTHEMA				EDEMA			
RESPONSE	1	24	48	72	1	24	48	72
No Response	4/6	6/6	6/6	6/6	5/6	6/6	6/6	6/6
Slight	2/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6
Mild	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Moderate	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Severe	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

CONCLUSIONS

Under the conditions of this study, H-24516 was a slight skin irritant.

If these test scores are to be used for EEC/OECD classification, according to the guide to the labeling of dangerous substances published in the Official Journal of European Communities (EEC Directive 93/21, Annex VI), H-24516 probably should not be classified as an irritant unless additional data proves otherwise.

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 (formerly known as the E. I. du Pont de Nemours and Company Records Management Center).

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

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	I = Intact
A = Abraded	L = Sloughing
B = Blanching	N = Necrosis
C = Eschar	R = Raw Areas
D = Desquamation	S = Epidermal Scaling
F = Fissuring	T = Thickening
G = Fissuring with Bleeding	X = Compound Adhered to Skin
H = Hyperkeratosis	

TABLE 3

INDIVIDUAL SKIN IRRITATION SCORES AND
SKIN RESPONSES OBSERVED IN RABBITS

RABBIT NUMBER	HOURS AFTER TEST SUBSTANCE REMOVAL							
	ERYTHEMA				EDEMA			
	1	24	48	72	1	24	48	72
34923	0	0	0	0	0	0	0	0
34925	1	0	0	0	1	0	0	0
34927	0	0	0	0	0	0	0	0
34922	0	0	0	0	0	0	0	0
34928	1	0	0	0	0	0	0	0
34931	0	0	0	0	0	0	0	0

RABBIT NUMBER	HOURS AFTER TEST SUBSTANCE REMOVAL			
	OTHER DERMAL EFFECTS			
	1	24	48	72
34923	-	-	-	-
34925	-	-	-	-
34927	-	-	-	-
34922	-	-	-	-
34928	-	-	-	-
34931	-	-	-	-

Symbols are defined in Table 2.