

TRADE SECRET

Study Title

H-22994: SKIN IRRITATION TEST IN RABBITS

LABORATORY PROJECT ID: HL-1997-01205

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STUDY COMPLETED ON: December 5, 1997

PERFORMING LABORATORY: E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

WORK REQUEST NUMBER: [REDACTED]

CERTIFICATION

Study Director:

Issued by: Tracy A. Filliben
Tracy A. Filliben
Toxicology Associate

12/5/97
Date

STUDY INFORMATION

Substance Tested [REDACTED]

Synonyms/Codes: • H-22994
[REDACTED]

Haskell Number(s): 22994

Known Impurities: [REDACTED]

Physical Characteristics: Light tan liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

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

Study Initiated / Completed: November 18, 1997 / (see report cover page)

In-Life Initiated / Completed: November 19, 1997/November 22, 1997

STUDY PERSONNEL

Manager(s): Judith C. Stadler, Ph.D.

Study Director: Tracy A. Filliben

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SUMMARY

H-22994 was evaluated for acute skin irritation potential in 6 female New Zealand White rabbits. Approximately 0.5 mL 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.

H-22994 produced slight or mild erythema by 1 hour after test substance removal. By 24 hours, 2 rabbits were clear of all dermal irritation, and the remaining rabbits exhibited slight or mild erythema. Two additional rabbits exhibited no dermal irritation by 48 hours after test substance removal. The remaining 2 rabbits exhibited mild erythema at 48 hours and slight erythema by 72 hours. No edema was observed 1, 24, or 48 hours after test substance removal; however, slight edema was observed in 1 rabbit at 72 hours. No clinical signs of toxicity were observed during the study.

Under the conditions of this study, H-22994 was a mild skin irritant.

INTRODUCTION

The purpose of this study was to evaluate the skin irritation potential of H-22994 when applied to the shaved, intact skin of New Zealand White rabbits.

MATERIALS AND METHODS

A. Animal Husbandry

Young adult female HM:(NZW)fBR New Zealand White rabbits were received from Hare Marland. 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 Purina® Certified High Fiber Rabbit Chow® #5325 daily during the study. Water was available *ad libitum* except during the exposure period while the animals were in stocks.

Haskell Laboratory has an animal health monitoring program. This program is monitored and administered by the Laboratory Veterinarian. Water samples are periodically analyzed for total bacterial counts and for the presence of coliforms, lead, and other contaminants. Additionally, samples from freshly washed cages and cage racks are periodically analyzed to ensure adequate sanitation by the cagewashers. Data from this program are maintained separately from study records. Animal feed is certified by the manufacturer to meet specified nutritional requirements and to be free of a list of specified contaminants. On the basis of these analyses, there is no evidence suggesting that contaminants were present in the feed or water in amounts which may have interfered with the results of this study.

Rabbits were quarantined, weighed, and observed for general health for approximately 2 weeks. 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 $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and relative humidity of $50\% \pm 10\%$. Excursions outside these ranges were judged to have been of insufficient magnitude and/or duration to have adversely affected the validity of the study.

B. Protocol

On the day prior to treatment, the hair of 6 female New Zealand White rabbits was closely shaved to expose the skin from the scapular to the lumbar region of the back. The body weights of the rabbits ranged from 2508 to 2785 grams on the day of treatment.

Each rabbit was placed into a stock which had been fitted with a piece of rubber sheeting, approximately 8" x 18". The rabbits remained in the stocks throughout the exposure period and during that time did not have access to food or water. Approximately 0.5 mL of H-22994 was

applied directly on the test site beneath a 1-inch, 2-ply gauze square that was held in place with non-irritating tape. Rubber sheeting was then wrapped around the animal and secured with clips to retard evaporation and to keep the test substance in contact with the skin without undue pressure. One other test substance was tested concurrently on a separate, localized test site on the backs of these rabbits.

Approximately 4 hours after application of the test substance, the rubber sheeting was loosened, and the skin at the corners of the gauze squares was marked with a waterproof pen; wrappings and gauze squares were then removed. The test sites were gently washed with warm water to remove excess test substance. The skin was gently patted dry, and the animals were 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). 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.

RESULTS AND DISCUSSION

H-22994 produced slight erythema in 4 rabbits and mild erythema in 2 rabbits by 1 hour after test substance removal. By 24 hours, 2 rabbits were clear of all dermal irritation. Of the remaining rabbits, 3 exhibited slight erythema, and 1 exhibited mild erythema. Two additional rabbits exhibited no dermal irritation by 48 hours after test substance removal. The remaining 2 rabbits exhibited mild erythema at 48 hours and slight erythema by 72 hours. No edema was observed 1, 24, or 48 hours after test substance removal; however, slight edema was observed in 1 rabbit at 72 hours. No clinical signs of toxicity were observed. Individual skin irritation scores are presented in Table 2. A summary of skin responses is presented in the following table.

SUMMARY OF SKIN RESPONSES TO H-22994

RESPONSE	ERYTHEMA				EDEMA			
	1 hour	24 hours	48 hours	72 hours	1 hour	24 hours	48 hours	72 hours
No Response	0/6	2/6	4/6	4/6	6/6	6/6	6/6	5/6
Slight	4/6	3/6	0/6	2/6	0/6	0/6	0/6	1/6
Mild	2/6	1/6	2/6	0/6	0/6	0/6	0/6	0/6

CONCLUSIONS

Under the conditions of this study, H-22994 was a mild 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-22994 probably should not be classified as an irritant unless further data proves otherwise.

RECORDS RETENTION

All study records, raw data, and the final report are retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain, 200 Todds Lane, Wilmington, Delaware 19802 (formerly known as the E. I. du Pont de Nemours and Company Records Management Center).

TABLE 1

DRAIZE^a SCALE FOR SCORING SKIN IRRITATION

<u>Evaluation of Skin Reactions</u>	<u>Score</u>	
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)	
Abbreviations of other dermal effects are:		
A = Abraded	F = Fissuring	L = Sloughing
I = Intact	N = Necrosis	R = Raw Areas
T = Thickening	G = Fissuring with	X = Compound Adhered
C = Eschar	Bleeding	to Skin
- = No Effect	S = Epidermal	SN = Superficial
B = Blanching	Scaling	Necrosis
D = Desquamation		

^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

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
32361	1	0	0	0	0	0	0	0
32313	1	0	0	0	0	0	0	0
32409	1	1	2	1	0	0	0	1
32403	1	1	2	1	0	0	0	0
32404	2	2	0	0	0	0	0	0
32405	2	1	0	0	0	0	0	0