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I. Study Type: Dermal Sensitization (guinea pigs)
(Guideline § 81-6)

Study Title: Closed-Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with IN V9360-35 (Granules) in Guinea Pigs

EPA Identification Numbers:

EPA Identification: 352-EUP-RLR
EPA MRID No.: 409242-12
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HED Project No.: 9-0536
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Sponsor: E. I. du Pont de Nemours and Company, Inc.

Testing Laboratory: E. I. du Pont de Nemours and Company, Inc.
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Elkton Road, P. O. Box 50
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Study Number: Laboratory Report No. 476-88

Study Date: August 8, 1988

Study Author: William J. Brock

II. Materials and Methods

A. Animal Husbandry

Young adult male and female Duncan Hartley albino guinea pigs were received from Charles River Breeding Laboratories, Stone Ridge, NY. The animals were singly housed, quarantined for about one week and allowed food and water ad libitum. Temperature, humidity and a 12 hour light/dark cycle were controlled.

B. Rangefinding Study

The backs of 2 male and 2 female guinea pigs (406-424 grams) were shaved and aliquots of 0.4 ml (0.2756 gm) of test material as received and 70%, 35% and 17.5% (w/v) suspensions in distilled water were applied to the intact skin under a 25 mm Hill Top Chamber Delivery System® (patch). A plastic wrap was placed over the patch and the animals wrapped with adhesive bandage. After 6 hours of exposure, the wrappings and patches were removed and the test sites washed with warm water. Irritation responses were scored about 24 hours post-treatment.

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C. Induction Phase

Five male and five female guinea pigs (473-603 grams) had 0.4 ml of test material as received (75% pure) applied to the shaved intact skin on the animal's back and covered with the patch, plastic wrap and adhesive bandage. This procedure was performed once/week for three consecutive weeks (three 6-hour treatments). Additionally, 3 males and 2 females (482-612 grams) were treated with 0.4 ml of 1-Chloro-2,4-dinitrobenzene (DNCB) as a 0.2% suspension in 80% ethanol in water (DNCB acted as the positive control). After 6 hours of exposure, the patch and wrappings were removed and the test sites washed. Irritation was scored after 24 and 48 hours.

D. Challenge Phase

The animals were challenged for sensitization two weeks after the last induction treatment by application of 0.4 ml of test material on the shaved intact skin of the back. The patches, wraps and bandages were applied and removed as described in the range-finding study. Additionally, 3 males and 2 females (483-628 grams) were treated with 0.4 ml of the test material and with 0.1% DNCB (80% ethanol in water) as the negative controls. Bandages were removed after 6 hours of exposure. About 22 hours post-treatment, the test sites were depilated (depilatory was applied to test sites and surrounding areas for approximately 30 minutes after which the skin was washed and dried). Irritation responses were scored about 2 hours post depilation and 48 hours post-treatment.

The incidence of sensitization is defined as the number of guinea pigs in each group sensitized to the test material divided by the total number of animals in the group. Severity of each response is the sum of the test scores in each group divided by the total number of animals in that group (for both the 24- and 48-hour evaluations).

III. Results

A. Rangefinding Study

Because no irritation was observed, the test material as received (75%) was used in the main study.

B. Body Weight Gains

The following are the mean body weight gains (and range) for the animals during the study:

Group	Sex	No. of Animals	Mean Body Weight Gain (g)	Range (g)
Test	M	5	118	97-153
	F	5	87	64-109
Neg. Control	M	2	105	90-170
	F	3	89	79-105
Pos. Control	M	3	177	146-177
	F	2	90	83-97

Calculations from data appearing in Appendix A, report pages 20-22.

There did not appear to be any adverse effect on body weight gains caused by the test material.

C. Induction Phase

No irritation was observed in the IN V9360-35 (granules) treated guinea pigs.

In the positive control (DNCB) animals, mild to severe erythema was observed following the 2nd induction treatment. At 24 hours after the 3rd induction treatment, severe erythema with necrosis was observed in 4 animals with severe erythema and superficial necrosis present in one animal. At 48 hours, all positive controls showed severe erythema with necrosis. See Tables I and II.

D. Challenge Phase (Tables III, IV and V)

No dermal irritation was observed in the animals treated with test material.

In the negative controls treated with test material, no dermal irritation was observed. In the negative controls treated with DNCB, no or slight erythema was observed, but one treated site exhibited blanching of the skin.

For positive controls, there was mild to severe erythema. In addition, blanching of the skin was also observed in 2 positive controls.

Table III

INCIDENCE AND SEVERITY OF RESPONSES AT CHALLENGE [a]

Group	24 hr		48 hr	
	Incidence	Severity	Incidence	Severity
Test-IN V9360-35	0/10	0	0/10	0
Neg. Con. IN V9360-35	0/5	0	0/5	0
Neg. Con. DNCB	0/5	0.6	0/5	0.4
Pos. Con. DNCB	4/5	3.2	2/5	2.6

a = The indicence of sensitization is defined as the number of animals in each group sensitized to the test material divided by the total number of animals tested in that group. Severity of the irritation response is the sum of the test scores in each group divided by the total number of animals tested in that group for both the 24- and 48-hour evaluations.

Data extracted from Table IV, page 18 of the report.

IV. Discussion and Conclusion

Dermal administration of IN V9360-35 (granules) in both induction and challenge phases did not appear to produce delayed hypersensitivity or allergic reactions in the guinea pig. Positive controls were observed to have erythema and necrosis.

V. Recommendation: This study is classified Core Minimum.

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Table I

SCORING SYSTEM USED TO EVALUATE SKIN RESPONSES

Skin Reaction	Score
No Reaction	0
Slight (barely perceptible erythema, usually nonconfluent)	1
Mild (well defined) erythema, usually confluent	2
Moderate erythema	3
Severe erythema (beet redness), with or without edema, necrosis or eschar formation	4

Table II

SKIN RESPONSES OBSERVED DURING THE INDUCTION PHASE IN POSITIVE CONTROL GUINEA PIGS FOLLOWING CLOSED-PATCH TOPICAL EXPOSURE TO 0.2% DNCB

Animal No.	Induction # 1		Induction # 2		Induction # 3	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
62463	0	0	3	4N	4N	4N
62464	0	0	3	4N	4N	4N
62465	0	0	2	3	4N	4N
62508	0	0	2	3	4N	4N
62509	0	0	3	3	4SN	4N

N = Necrosis SN = Superficial Necrosis

Table IV

CHALLENGE PHASE - SUMMARY OF SKIN RESPONSES

Erythema	IN V9360-35		Neg. Con. IN V9360-35		Neg. Con. DNCB		Pos. Con.	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
None	10/10	10/10	5/5	4/5a	2/5	3/5	-	-
Slight	0/10	0/10	0/5	0/5	3/5	2/5	-	-
Mild	-	-	-	-	-	-	1/5	3/5
Moderate	-	-	-	-	-	-	2/5	1/5
Severe	-	-	-	-	-	-	2/5	1/5

a = The dermal irritation in one test site was inadvertently not recorded.

Table V

CHALLENGE PHASE - INDIVIDUAL SKIN RESPONSES

Animal #	Neg. Con. IN V9360-35		Animal #	Neg. Con. DNCB		Animal #	Pos. Con. DNCB	
	24 hr	48 hr		24 hr	48 hr		24 hr	48 hr
62466	0	0	62466	0	0	62463	3	2
62467	0	0	62467	0	0	62464	4	4
62510	0	0	62510	1	0	62465	3B	3B
62511	0	0	62511	1	1	62508	2	2
62512	0	a	62512	1B	1R	62509	4B	2B

a=Dermal irritation in this site inadvertently not recorded. B=Blanching R=Raw Area

Data on this page were extracted from Tables I, II, III & IV, report pages 11-17.