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SKIN PHOTOALLERGENICITY STUDY IN THE GUINEA PIG OF (b) (4)

Sponsor: (b) (4)

Test material received: 21.02.1980

Validity: Determined by sponsor

Study initiated: 14.04.1980

Study completed: 13.06.1980.

Summary and conclusion

Under the experimental condition employed, no differences between the test group and the vehicle-treated controls were seen, after epidermal challenge application either followed by UV-B and UV-A irradiation or followed by UV-A irradiation alone.

(b) (4) was found to be devoid of a photoallergenic potency in albino guinea-pigs.

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1. Materials

Test material: (b) (4)

Identification: EN EP 102

Physical appearance: white powder

Concentration: Epidermal application of freshly prepared 0.1 % solution of (b) (4) in 80 % DAE433

Vehicles:
- 80 % DAE433 / 20 % physiological saline
  (DAE433 = 40 % Dimethylacetamide,
  30 % Acetone,
  30 % Ethanol)
- Adjuvant complete Freund (Difco).

2. Animals

Strain: Guinea pigs of the Pirbright white strain bred on our premises were used.

Animals received: 08.04.1980

Acclimatisation period: 7 days.

The test was performed on 10 male and 10 female guinea pigs per group weighing between 280 - 380 g (∿ 10 weeks old). The animals were housed individually in Macrolon cages (type 3), assigned to the different groups by means of random numbers generated by the random number generator incorporated in the Hewlett-Packard desk computer, identified with individual ear tags, kept at a constant room temperature of 20 ± 1° C, at a relative humidity of 50 ± 5 % and on a 14 hours light cycle day. The animals received ad libitum standard guinea pig pellets - NAFAG No. 830, Gossau SG and fresh water, supplemented with fresh carrots.

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3. Light Source Characteristics

Burner: Special irradiation chamber with a water cooled 6000 W Xenon burner (Osram).

Distance between burner and animals: 80 cm

Maximal irradiation intensity used:

280 - 320 nm	(UV-B)	785 µW/cm2
320 - 400 nm	(UV-A)	4.55 mW/cm2
400 - 700 nm	(visible)	25.64 mW/cm2

Energy output controlling system:

Spectroradiometer EG & G, Model No. 585.

4. Method

Induction: 4 days before starting the induction, the animals were shaven on the neck and chemically depilated (Veet^{R}). During the whole induction period the animals were only shaven (Aesculap^{R}, ¼20 mm).

4 times a week for three weeks the animals were treated by epidermal open exposure with a 0.1 % solution of (b) (4) in 80 % DAE433 (0.1 ml per application; 2 x 2 cm application area on the animals' neck).

One hour after application the animals were irradiated for 10 minutes during the first induction week (UV-A, visible) 10 minutes during the second and third week (UV-B, UV-A, visible).

During the irradiation process the animals were immobilized in a special animal holder. In order to protect the skin surrounding of the irradiated area, this part was covered with a lightproof bandage.

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24 hours after each of the first four induction applications, the skin reactions were evaluated according the Draize Scoring Scale (see appendix). The reactions during the second and third induction week were not recorded.

A total of 4 adjuvant injections were made to the four corners of the application site on Monday and Wednesday of the second and third induction week (0.1 ml suspension of adjuvant complete Freund and physiological saline per injection).

Challenge I: 12 days after the last induction irradiation, the animals were shaven on the back and the skin was chemically depilated (Veet^{R}, 5 min.). 4 days later the animals were treated by epidermal open exposure for three days as during the induction period. One hour after each application the animals were irradiated for 3 minutes with a sub-erythematogenic dose of UV-B, UV-A and visible light (lamp filtered with a Schott filter WG 280, 3 mm).

Challenge II: A second challenge was done after a rest period of 14 days. The substance was applied again three times on the shaven skin of the animals' back: the test site was irradiated after each application for 10 minutes with a suberythematogenic dose of UV-A and visible light (lamp filtered with a Schott filter WG 320, 3 mm).

24 hours after each application of both challenges, the skin reactions were evaluated according the Draize Scoring Scale.

Every animal with one or more score values higher at the end of the two challenge periods than that obtained at the end of the first induction week was termed positiv. The number of

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positive animals in the test group was compared with the number of animals in the control group treated with the vehicle alone*.

5. Results

Table I summarizes the incidence of positive animals per group after challenge I and II.

In Table II, the individual erythema reactions are listed as evaluated 24 hours after the last irradiation of either the induction, challenge I or challenge II.

Only one animal of the test as well as of the control group reacted during challenge I. The same animal of the test group reacted during challenge II. All other animals were negativ. There is no statistical difference between the test and control group.

* The exact Fisher test for comparison of the basic probability of the binomial distributions; L. Sachs, Statistische Auswertungsmethoden, Thieme Verlag, Stuttgart, 1971. A probability of $P \leq 0.01$ was considered to indicate a significant difference.

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T A B L E I

Incidence of positive animals after challenge I and challenge II

	No. of positive animals / No. of treated animals
Challenge I	
DAE433	1/20
(b) (4)	1/20
Challenge II	
DAE433	0/20
(b) (4)	1/20

T A B L E I I

Individual erythema reaction score 24 hours after the last irradiation of the induction, challenge I and challenge II

Control group DAE433	Animal number																			
	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591
Induction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Challenge I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Challenge II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Test group [redacted]	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611
Induction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Challenge I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Challenge II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0

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Evaluation of skin reactions according to Draize[1)]

	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

1) in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1959), the US Association of Food and Drug Officials (AFDO).

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