14/01/01

(b) (4) (b) (4)

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SKIN PHOTOTOXICITY STUDY IN THE MOUSE OF (b) (4)

Sponsor: (b) (4)

Test material received: 21.02.80

Validity: not determined

Study initiated: 24.03.80

Study completed: 28.03.80

Summary and Conclusion

Under the experimental conditions employed, (b) (4) was found to cause neither erythema nor edema in hairless mice when applied topically followed by UV irradiation.

(b) (4) was found to be devoid of a phototoxic potency in hairless mice.

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1. Materials

Test material: (b) (4)

Identification: EN EP 102.

Concentrations: 0.3 %, 1 % and 3 %.

Positive control: 0.1 % 8-methoxypsoralene (Sigma), 8-MOP.

Test vehicle: Acetone / Ethanole (50/50).

2. Animals

Strain: Mouse, SC: hairless HR/HR.

Breeder: Bomholtgard.

Animals received: 13.03.80.

Acclimatisation period: 10 days.

3. Light source characteristics

Solar Ultraviolet Simulator (Solar Light Company, 6655 Lawnton Ave, Philadelphia, Pennsylvania 19126).

Burner: 150 W Xenon.

Wavelength range for irradiation: 320 - 400 nm.

Irradiation intensity at focused distance: 35 mW/cm^2.

4. Method

The test was performed on 3 male and 3 female mice per group (SC: hairless HR/HR outbred mice) weighing 16 - 25 g (2 to 2½ months old). They were housed individually in wire cages (size 1), assigned to the different cages by means of random numbers generated by the random number generator incorporated in the Hewlett-Packard desk computer, kept at a constant room temperature of 24 $\pm$ 1° C, at a relative humidity of 50 $\pm$ 5 % and on a 14 hours light cycle day. The animals received ad libitum standard mouse pellets No. 890 - Nafag Gossau SG - and water.

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The original suspension was diluted to concentrations of 0.3 %, 1 % and 3 % using a mixture of 50 % acetone and 50 % ethanole. Aliquots of 0.05 ml of each concentration were applied evenly with a pipet onto the skin of both flanks of the mice. For each concentration level a separate group of animals was employed. Negative and positive control groups treated either with the vehicle or a solution of 0.1 % 8-methoxypsoralene respectively were included in the test system.

Half an hour after application the animal was fixed in front of the light source in an animal holder at a distance of 5.6 cm from the lamp box and a circular skin area of 1 cm in diameter of one side of the mouse was exposed to UV-light for a duration of 2 minutes. The contralateral side, not exposed to light, served as the control.

The skin reactions were evaluated 24, 48 and 72 h after the irradiation according to the Draize Scoring system for primary irritation reactions (Appendix).

5. Results

No adverse skin reaction was observed when TK 10'665 was applied topically to hairless mice followed by UV-irradiation.

Moderate oedema reactions were seen 24 h after application of 0.1 % 8-methoxypsoralene topically to hairless mice followed by UV-irradiation. Fresh necrosis surrounded by erythema was observed 48 and 72 h after irradiation in the 0.1 % 8-methoxypsoralene group.

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Table 1: Number of positive animals per group

	No. of positive animals/ No. of treated animals irradiated flank	No. of positive animals No. of treated animals non irradiated flank
24 h after irradiation		
vehicle control	0/6	0/6
0.1 % 8-MOP	6/6	0/6
0.3 % (b) (4)	0/6	0/6
1.0 %	0/6	0/6
3.0 %	0/6	0/6
48 h after irradiation		
vehicle control	0/6	0/6
0.1 % 8-MOP	6/6	0/6
0.3 % (b) (4)	0/6	0/6
1.0 %	0/6	0/6
3.0 %	0/6	0/6
72 h after irradiation		
vehicle control	0/6	0/6
0.1 % 8-MOP	6/6	0/6
0.3 % (b) (4)	0/6	0/6
1.0 %	0/6	0/6
3.0 %	0/6	0/6

Every animal with a defined erythema or edema was evaluated as a positive animal.

Table 2: Evaluation of skin reactions

(b) (4)

Preparation		Skin reaction after 24 h							48 h						72 h					
	Sex		♂			♀			♂			♀			♂			♀		
50% Acetone / 50% Ethanole	Animal No.		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	Erythema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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	Sex		♂			♀			♂			♀			♂			♀		
0.1 % 8-MOP	Animal No.		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	Erythema	i	0	0	0	0	0	0	3	0	1	0	0	0	3	0	0	0	3	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	i	2	3	2	2	3	2	2	2	2	2	2	1	2	2	2	2	6	1
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	i	2	3	2	2	3	2	5	2	3	2	2	1	5	2	2	2	6	1
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

i = irradiated flank ni = non irradiated flank

Table 3: Evaluation of skin reactions

Preparation		Skin reaction after 24 h							48 h						72 h					
0.3 % [redacted] (b) (4)	Sex		♂			♀			♂			♀			♂			♀		
	Animal No.		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	Erythema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0 % [redacted] (b) (4)	Sex		♂			♀			♂			♀			♂			♀		
	Animal No.		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	Erythema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted] (b) (4)

[redacted] (b) (4)

i = irradiated flank ni = non irradiated flank

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Table 4 : Evaluation of skin reactions

Preparation		Skin reaction after 24 h							48 h						72 h					
3.0 % (b) (4)	Sex		♂			♀			♂			♀			♂			♀		
	Animal No.		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	Erythema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Edema	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(b) (4)

Sex		♂			♀			♂			♀			♂			♀		
Animal No.		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Erythema	i																		
	ni																		
Edema	i																		
	ni																		
Total	i																		
	ni																		

(b) (4)

i = irradiated flank ni = non irradiated flank

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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
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 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure	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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Verteiler:

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