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C O N F I D E N T I A L

FORMALDEHYDE

STUDY OF SKIN SENSITIZATION EFFECT

ON GUINEA PIGS

by

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1. SUMMARY

Formaldehyde, a known contact allergen, was used in a skin sensitization test on male guinea pigs (Maximization Test of MAGNUSSON and KLIGMAN) in order to document that the guinea pig strain employed is suitable for the test system. The following test compound concentrations to be applied over the course of the study were obtained from previous studies:

Intradermal induction:	5%
Topical induction:	5%
1st Challenge:	2%
2nd Challenge:	0.5%

According to the method of evaluation, after the 1st challenge (test compound concentration: 2%), 7 animals in the test compound group exhibited a positive reaction, compared with 0 in the control group. After the 2nd challenge, which was conducted using a lower test compound concentration (0.5%), 7 animals in the test compound group exhibited a positive reaction, compared with 0 in the control group.

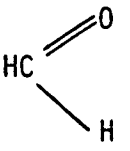
The results of the 2nd challenge thus confirmed the results of the 1st challenge. The observed reaction on the right flanks (application of formulation vehicle) indicates a hypersensitivity of the animals. Nevertheless, the difference in reactions between the treated group and each of the control groups on the whole is so distinct that it can be

regarded as an indication of a skin sensitization effect of the test compound in the case of guinea pigs.

It is thus clear that the employed strain of guinea pig exhibits an adequate sensitivity for the test system.

2. INTRODUCTION

The test compound was formaldehyde; its structural formula is as follows:



A test of the skin sensitization properties of the compound, a known contact allergen, was performed on guinea pigs in order to document the suitability of the employed guinea pig strain for the test system. The test system used was the maximization test of B. MAGNUSSON and A. M. KLIGMAN [J. Invest. Dermat., 52:268 (1969); and "Identification of Contact Allergens," Allergic Contact Dermatitis in the Guinea Pig, ed. C. C. THOMAS (1970), pp. 102-103].

The study was conducted from August to September 1984 at the Institute of Toxicology, BAYER AG, Wuppertal-Elberfeld, Federal Republic of Germany (FRG). The study documentation is also archived at the Institute.

Study No.: T 1017968

3. MATERIALS AND METHODS

3.1 Test Compound Sample

Formaldehyde solution, batch No.: Z 69099

The stability in the formulations was corroborated.

3.2 Experimental Animals

Forty male guinea pigs (strain: DHPW, bred by WINKELMANN, Borchen, FRG) weighing from 270 to 360 g were housed in Type IV Makrolon cages [SPIEGEL and GOENNERT, Z. Versuchstierkunde, 1:38 (1961)], 5 animals to a cage, on litter of low-dust wood shavings. The conditions in the animal quarters were as follows: temperature, 22-25°C; relative humidity, approx. 55-80%; 12-hour light/dark cycle.

The animals received Altromin 3022 feed for guinea pigs and drinking water from bottles for ad libitum consumption.

3.3 Experimental Animal Groups

Three groups of guinea pigs were formed (1 test compound group and 2 control groups). The test compound group consisted of 20 males and the 2 control groups consisted of 10 male guinea pigs each. The guinea pigs were classified by weight and then randomly apportioned to the groups by

means of random numbers. The animals were identified by cage number and by individual markings with an aqueous solution of picric acid.

The 2 control groups consisted of 10 animals each for a 1st and a 2nd challenge. The 2nd challenge is only conducted when the 1st challenge provides no clear result or when a confirmation of the results appears appropriate.

3.4 Experimental Procedure

3.4.1 Induction of Sensitization

3.4.1.1 Intradermal Injections

Starting behind the nape of the neck, 3 intradermal injections were made on each side, in two parallel rows. The distance between the injection sites was 1-2 cm. The volume injected per site was 0.1 ml. Twenty-four hours before injection, the skin was clipped free of hair.

The animals of the three groups were treated as follows:

a) Test Compound Group

1st Injection Site Pair (cranial)
Freund's Complete Adjuvant (Difco Laboratories), diluted 1:1 with deionized water.

2nd Injection Site Pair (medial)
5% Formaldehyde solution, formulated with physiological saline solution.

3rd Injection Site Pair (caudal)

5% Formaldehyde solution, formulated with equal parts of physiological saline solution and Freund's Complete Adjuvant.

b) Control Groups

The animals of the control groups were treated as were the guinea pigs in the test compound group, except that the formulations for the 2nd and 3rd injection site pairs contained no test compound.

3.4.1.2 Topical Induction (1 week after intradermal injection)

Hypoallergenic patches (2 x 4 cm) were placed over the injection sites, covered with aluminum foil, and held securely in place for 48 hours with a Fermoflex adhesive bandage. Twenty-four hours before application, the application sites were clipped free of hair.

The hypoallergenic patches were treated (saturated) as follows:

a) Test Compound Group

5% Formaldehyde solution, formulated with physiological saline solution.

b) Control Groups

Formulation vehicle as in a) above, but containing no test compound.

3.4.2 Challenge Tests (3 and 5 weeks after intradermal induction or 2 and 4 weeks after topical induction)

3.4.2.1 Challenge Procedure

Twenty-four hours prior to treatment, the guinea pigs were clipped and depilated with Pilca-Creme [depilatory cream]. Hypoallergenic patches saturated with a 2% test compound formulation were placed on the left flank of animals of the test compound group and control group 1, and held securely in place for 24 hours by a Saniplast elastic adhesive bandage wound around the torso of the animal.

A corresponding control patch saturated with only the formulation vehicles was applied on the right flank for comparison.

Fourteen days later, control group 2 and the test compound group were treated as described above using a 0.5% concentration.

3.4.2.2 Evaluation of the Reaction

Twenty-four and 48 hours after removal of the adhesive bandages, the treated skin areas were grossly evaluated (for scoring criteria, see Attachment I).

3.4.2.3 Assessment of Possible Sensitization

The results are evaluated by subtracting the number of animals with an irritant reaction on the control side from the number of animals with an irritant reaction on the test compound side (in so doing, the time at which the reaction is observed, at 24 or 48 hours, is not important). This procedure is followed for the animals of both the test compound group and the control group.

The group results, corrected as described above, are then compared (= corrected value).

4. RESULTS AND ASSESSMENT

The results are presented in Attachment II. The results of the range-finding test to determine concentrations are given in Attachment I. The data on body weights (start of the study, 1st and 2nd challenge) are presented in Attachment III.

The results for the groups, corrected as described in section 3.4.2.3, are as follows:

1st Challenge

Animals with Positive Reaction

Test Compound Group (20 animals)		Control Group 1 (10 animals)	
Test compound patch	Control patch	Test compound patch	Control patch
15	8	4	4
Corrected Value: 7		0	

2nd Challenge

Animals with Positive Reaction

Test Compound Group (20 animals)		Control Group 2 (10 animals)	
Test compound patch	Control patch	Test compound patch	Control patch
16	9	3	4
Corrected Value: 7		0	

In the evaluation of the 1st challenge (2% concentration), internal correction resulted in 7 animals with a positive [sensitization] reaction in the test compound group, compared with 0 in the control group.

In the evaluation of the 2nd challenge (0.5% concentration), internal correction resulted in 7 animals with a positive [sensitization] reaction in the test compound group, compared with 0 in the control group.

The results of the 2nd challenge thus confirmed the results of the 1st challenge, even though a markedly lower concentration was used. The observed reactions on the right flanks (application of formulation vehicle) indicate a hypersensitivity of the animals. Nevertheless, the difference in the reactions between the treated group and each of the control groups is so distinct that it can be regarded as an indication of a skin sensitization effect of the test compound in the case of guinea pigs.

The guinea pig strain employed thus exhibits an adequate sensitivity for the test system.

[signed]	[signed]
Dr. K.-G. Heimann Study Director	Dr. L. Machemer Section Director

APPENDIX

Attachment I:

Scoring Criteria

0 = no reaction

1 = scattered mild erythema

2 = confluent, moderate erythema

3 = intense erythema and/or edema

Attachment II: Individual Findings

Sensitization Test on Guinea Pigs

Skin Findings/Sensitization Reaction

-- 3 Weeks after 1st Induction (= 3 Weeks after Start of Experiment)

Animal No.	Test Compound Group				Animal No.	Control Group 1			
	Test compound patch		Control patch			Test compound patch		Control patch	
	24h	48h	24h	48h		24h	48h	24h	48h
21	1	1	0	0	1	0	0	0	0
22	0	0	0	0	2	0	0	0	0
23	1	0	0	0	3	1	1	1	1
24	1	1	1	1	4	0	0	0	0
25	2	1	1	1	5	0	0	0	1
26	1	0	1	0	6	0	0	0	0
27	0	1	0	1	7	1	0	1	0
28	0	0	0	0	8	0	0	0	0
29	2	1	0	0	9	1	1	1	1
30	0	0	0	0	10	1	0	0	0
31	0	0	0	0					
32	1	2	0	1					
33	1	1	1	0					
34	0	0	0	0					
35	0	1	0	0					
36	0	1	0	0					
37	2	2	0	0					
38	0	1	0	1					
39	1	2	1	1					
40	0	1	0	0					

Attachment II: Individual Findings

Sensitization Test on Guinea Pigs

Skin Findings/Sensitization Reaction

-- 5 Weeks after 1st Induction (= 5 Weeks after Start of Experiment)

Animal No.	Test Compound Group				Animal No.	Control Group 2			
	Test compound patch		Control patch			Test compound patch		Control patch	
	24h	48h	24h	48h		24h	48h	24h	48h
21	2	1	1	0	11	0	0	0	0
22	1	1	0	1	12	0	0	0	0
23	1	1	1	0	13	0	0	0	0
24	0	1	0	0	14	0	0	0	0
25	1	2	0	0	15	1	1	1	1
26	1	0	0	0	16	0	0	1	0
27	1	0	1	0	17	1	1	1	1
28	0	0	0	0	18	0	-	0	-
29	0	0	0	0	19	0	0	0	0
30	1	0	0	0	20	1	0	1	1
31	1	1	0	0					
32	1	1	1	1					
33	2	2	1	1					
34	0	0	0	0					
35	1	1	1	0					
36	1	1	0	0					
37	2	2	1	1					
38	0	0	0	0					
39	1	0	0	0					
40	1	0	1	0					

"-" = not amenable to evaluation

Attachment III: Body Weights in g

Animal No.	Start of Test	1st Challenge	2nd Challenge
1	300	446	
2	311	511	
3	314	464	
4	313	501	
5	357	525	
6	270	433	
7	323	522	
8	343	542	
9	339	518	
10	328	461	
11	308		542
12	327		550
13	352		581
14	328		542
15	340		557
16	292		631
17	313		593
18	333		712
19	305		604
20	329		564
(Control Groups)			
21	306	471	599
22	304	468	557
23	317	428	495
24	321	496	574
25	332	507	599
26	328	490	561
27	312	516	614
28	335	547	672
29	347	485	604
30	338	533	647
31	321	467	521
32	360	520	613
33	348	481	550
34	321	472	547
35	352	565	637
36	326	468	543
37	352	497	552
38	359	510	586
39	356	536	621
40	349	477	468
(Test Compound Group)			