J. Soc. Cosmet. Chem., 28, 53–64 (February 1977)

Screening of fragrance materials for allergenicity in the guinea pig
I. Comparison of four testing methods

G. KLECAK, H. GELEICK, *and* J. R. FREY.* *Pharmaceutical Research Department,*
F. Hoffmann-La-Roche & Co. Ltd., CH 4002 Basel, Switzerland.

Received November 10, 1975

Synopsis

An OPEN EPICUTANEOUS TEST (OET) is proposed for the detection of SKIN IRRITATION and CONTACT HYPERSENSITIVITY induced in GUINEA PIGS. Thirty-two compounds described in the literature as being ALLERGENIC for man were tested in the guinea pig by the OET technique, and for the purpose of comparison, by three other techniques, namely the DRAIZE TEST (DT), the MAXIMIZATION TEST (MT) and a test with FREUND'S COMPLETE ADJUVANT (FCAT). In the OET, a high degree of correlation was found between the allergenicity of the tested compounds for the guinea pig and for man.

I. INTRODUCTION

A considerable amount of work has been done over many years developing procedures for detecting skin-irritating and/or sensitizing effects of chemicals on laboratory animals, in order to preselect compounds likely to be well tolerated by man. As in general toxicology and pharmacology, the results of such tests on animals cannot be fully valid for humans because of interspecies differences in the absorption, metabolism, and excretion of the compounds concerned. Furthermore, in the case of topical medication and of cosmetics, the conditions of exposure, such as concentration, frequency of application, and site of contact, can never be identical in human use and in animal experiments.

The problem of identifying contact allergens, in humans as well as in laboratory animals, was first approached critically and on a scientific basis by Kligman (1, 2, 3) and by Magnusson and Kligman (4, 5). These authors carried out comparative tests of numerous drugs, industrial contactants and cosmetics with well-known sensitizing properties, by various so-called predictive procedures commonly used on humans (6–21), and have shown conclusively that these procedures often fail to identify even known sensitizers. These procedures are, therefore, also likely to be inadequate for the recognition of the allergenicity of new synthetic compounds. By carefully analyzing all

*Deceased February 8, 1974.

the factors influencing contact sensitization in man and in the guinea pig, and by including all these factors in the same experiment, these authors developed a testing technique which they called the maximization test (MT). With this technique, they found a close correlation between clinical experience and experimental results. The authors conclude that by testing compounds on laboratory animals under extreme experimental conditions it should be possible to detect the whole sensitizing potentiality of a compound.

This statement should permit the assumption that the probability of a compound causing sensitization in humans could be estimated. The authors emphasize, however, that it is impossible, on the basis of animal experiments alone, to formulate fully reliable predictions valid for humans.

The purpose of our work, taking into account the knowledge acquired by Kligman and Magnusson, was to develop a testing procedure for guinea pigs convenient for testing new synthetic chemical compounds intended for use in fragrances and cosmetics. Special attention was paid to developing a test which would be simple to perform, would simulate the conditions in human use, would yield quantitative data, and would minimize the effects of subjective factors in evaluating the results. Like Kligman, we checked the reliability of our test by performing concurrently 3 other tests commonly used in this field. We tested a series of compounds with a well-known sensitizing capacity for man, in order to establish whether or not a correlation exists between clinical experience and the results of our animal experiments.

For the first step in these investigations, we chose 32 compounds described in the literature as being allergenic for man. These compounds were tested concurrently, on groups of 6 to 8 guinea pigs each, by the open epicutaneous test (OET), the Draize test (DT), the maximization test (MT) and an intradermal test with Freund's complete adjuvant (FCAT), all described below.

II. MATERIALS AND METHODS

CHEMICAL COMPOUNDS

Thirty-two compounds used in the perfume industry were tested. All these compounds were tested under code numbers, their nature and potential allergenicity being unknown to the experimenters prior to testing.

ANIMALS

The animals used were male and female outbred Himalayan white-spotted guinea pigs bred at the Institute of Biomedical Research, Füllinsdorf, Switzerland. The guinea pigs weighed 400 to 500 g. They were fed on pelleted feed supplemented with green vegetables, carrots and vitamin C in the drinking water, all available *ad libitum*.

TESTING METHODS

OET: All the compounds were tested undiluted as well as dissolved in acetone, ethanol, diethyl phthalate, etc., at concentrations of 30, 10, 3, 1, 0.3, 0.1, and 0.03 per cent (or less when necessary) in order to establish a dose-response curve making it possible to determine the minimal irritating and the maximal tolerated concentrations on an "all or

none" basis. This contrasts with another method still in use (11), where only one arbitrarily fixed concentration is used, and its activity is evaluated subjectively according to the intensity of the lesions observed.

Before the induction procedure (see below), we determined the skin irritation caused by a single application. For this purpose, we applied 0.025 ml of each undiluted compound and of its progressively diluted solutions to an area measuring 2 cm^2 previously marked with a circular stamp on the clipped flank skin of 6 to 8 animals per group. In each case, the liquid tested was applied uniformly with a pipette. After evaporation of the solvent, the application site was left uncovered. The reactions were read after 24 h using an "all or none" criterion, i.e., the dose-response curve was established by end-point determination. The minimal irritating concentration was defined as the lowest concentration causing mild erythema in at least 25 per cent of the animals of the group concerned, and the maximal nonirritant concentration as the highest concentration causing no macroscopically discernible reactions in any of the animals of the group. The highest concentration of a compound used in this local application test was determined by its solubility and skin irritating capacity. Most of the substances used could be applied undiluted. However, high concentrations causing strong reactions, e.g., swelling, necrosis, or ulceration, were not used for evaluation because the end-point determination was considered a more sensitive index of activity.

The determination of the tolerance threshold on the guinea pig in the OET is mainly done for methodical reasons, in order to quantitatively realize an eventual sensitization, and besides it gives information on the concentration-dependent skin tolerance of substances. Carrying over these results onto man is possible under restriction.

Induction procedure: On day 0 we applied 0.1 ml of each undiluted compound and of its progressively diluted solutions to an area measuring 8 cm^2 on the clipped flank skin of 6 to 8 guinea pigs per concentration group, using 4 to 6 such groups for each compound. The applications were repeated daily for 21 days, always using the same skin site. The application site was left uncovered and the reactions were read 24 h after each application. The maximum nonirritant and the minimal irritating concentrations were determined by the same "all or none" criterion as described above. When necrotic or ulcerating reactions were provoked, the application site was changed.

Generally speaking, the degree of the topically irritant effect after one single or after repeated application is characterized by the intensity of the skin reaction and by the magnitude of the minimal irritating concentration. For evaluation of the irritations, we applied the following scale:

0 = no skin reaction	2 = redness plus swelling
0.5 = red spots	3 = redness, swelling, crusts
1 = confluent redness	4 = necrotic skin alterations

Challenge procedure: To determine whether or not allergic contact dermatitis was induced, all the groups of guinea pigs previously treated for 21 days as described above, as well as 6 to 8 untreated controls for each compound, were tested on days 21 and 35 on the contralateral flank with the same compound at the minimal irritating concentration and at some lower nonirritant concentrations. We used the minimal irritating

concentration of each compound in order to confirm the biological activity determined after the first application and to exclude false results. These tests were performed by applying with a pipette 0.025 ml of each concentration to skin areas measuring 2 cm^2, the reactions being read after 24, 48 and/or 72 h. This procedure enabled us to determine the minimal sensitizing concentration necessary to induce contact hypersensitivity and the minimal eliciting concentration necessary to cause a positive reaction. A concentration was considered allergenic when at least 2 out of the 8 animals of the concentration group concerned showed positive reactions with nonirritant concentrations used for challenge, based on practical experience.

The total dose of compound administered in the OET ranged from 2100 to 63 mg or less, depending on the concentrations used.

Solvents, like water, ethanol and acetone, even when applied repeatedly, yielded no macroscopically detectable alteration on the treated skin. When other bases were applied, like vaseline, diethyl phthalate or polyethylene glycol, additional controls for the vehicles were set in.

DT (11): A dose of 0.05 ml of a 0.1 per cent solution of the compound tested in isotonic saline was injected intradermally on day 0 and further doses of 0.1 ml each were injected on 9 alternate days (total dose = 0.95 mg). The treated animals and untreated controls were challenged intradermally with 0.05 ml of a 0.1 per cent solution on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions.

MT (4, 5): On day 0 the animals were injected intradermally with 0.1 ml of a 5 per cent solution of the compound tested, with 0.1 ml of a 5 per cent emulsion of the same compound in Freund's complete adjuvant (FCA) and with 0.1 ml of FCA alone, each injection being given twice. In addition, 250 mg of the compound dissolved in petrolatum at a concentration of 25 per cent, which always causes mild to moderate skin irritation under occlusion, was applied on day 8 to a clipped skin area of the neck and was kept under occlusive bandage for 2 days (total dose 20 mg intradermally plus 250 mg epicutaneously). On day 21 an occlusive patch test with the compound at a subirritant concentration in petrolatum was applied to the flank for 24 h. The reactions were read 24 and 48 h after removing the patch.

FCAT: Doses of 0.05 ml of the undiluted compound mixed with the same volume of FCA were injected intradermally into the neck on days 0, 2, 4, 7, and 9 (total dose 250 mg). The control animals were similarly treated with 5 × 0.05 ml of FCA alone. All the animals were tested epicutaneously on days 21 and 35 as described in the section above.

III. RESULTS

The results obtained by testing the 32 compounds by the 4 procedures described above are presented in Table I. The compounds are subdivided into 4 groups according to their allergenicity in the 4 animal tests, namely: Group I, not sensitizing in any test; Group II, sensitizing in the OET and in one or more of the other tests; Group III, sensitizing exclusively in the OET; Group IV, not sensitizing in the OET but sensitizing in one or more of the other tests.

Table I
Skin Irritating and Sensitizing Properties of 32 Compounds in Guinea Pigs and in Humans

Compound	OET: Minimum Irritating Concn. in % After 1 Application	OET: Minimum Irritating Concn. in % After 21 Applications	OET: Minimum Sensitizing Concn. in %	OET: Minimum Eliciting Concn. in %	Allergenicity in Guinea Pigs: OET	DT	MT	FCAT	Allergenicity in Humans: Type	References
Group I										
Acetophenone	100	1			−	−	−	−	A	22
Benzophenone[a]	30	3			−	−	−	−	A	22
Diethyl phthalate	100	100			−	−	−	−	A	22
Dimethyl anthranilate	100	3			−	−	−	−	A	22
Dimethyl-benzyl carbinol	30	1			−	−	−	−	A	22
Hydroxycitronellal	30	10			−	−	−	−	A	22,50
Thymol[a]	3	3			−	−	−	−	B/E	24,29/22,23,30
Group II										
Benzyl alcohol	30	3	30	10	+	−	−	+	D	22,24,25,26,30,31,44,45
Benzyl cinnamate	3	3	3	0.3	+	+	+	+	D	22,24,25
Carvacrol	3	0.3	3	1	+	−	+	+	B/E	48/22,24
Cinnamic aldehyde	3	3	3	0.3	+	+	+	+	B/D	24,25,29,51/22,25,29,30
Citral	3	3	10	1	+	+	+	+	B/D	22,48,53/24,37
Citronellal	10	3	30	3	+	−	+	+	D	22,24,31,32,33,37,41
Cuminic aldehyde	30	3	3	3	+	+	+	+	A	22
Geraniol	30	3	10	3	+	−	+	+	D	22,24,25,29,31,32,33,37, 44,50
Heliotropin	30	3	30	1	+	−	+	−	D	22,25,30,32,37,40
Iso-eugenol	30	10	10	1	+	+	+	+	D	22,24,25,37
Limonene	3	3	100	1	+	−	+	+	D	24,25,30,42,43,44,48,52
Methyl cinnamate	30	3	30	3	+	+	+	+	D	22,24,25
Methyl heptine carbonate	10	0.3	3	0.3	+	−	+	+	B/C/E	22,29,34,36,50/22,35,1/22, 24,29,30,34,35,36,37,38
Methyl octine carbonate	10	3	10	3	+	−	+	+	C/E	35/22,24,35
Phenylacetaldehyde	0.3	0.3	0.3	0.03	+	+	+	+	D	22,25,27
Phenyl-ethyl salicylate	0.1	0.1	30	0.03	+	−	+	+	A	22
3-Phenyl-propionaldehyde	30	10	30	3	+	−	−	+	A	22
10-Undecenal	3	3	30	1	+	−	−	+	A	22
Group III										
Amyl salicylate	10	3	30	3	+	−	−	−	A	22
Benzyl salicylate	0.1	0.1	30	0.03	+	−	−	−	B/E	28/29,39,41,50
Bromostyrol	30	3	10	3	+	−	−	−	A	22
Methyl salicylate	3	3	30	1	+	−	−	−	B/E	23,30,37,49
Group IV										
Benzaldehyde	10	3			−	+	+	+	D	22,24,25,26,29,30,45
Cinnamic alcohol	10	3			−	−	+	+	D/E	25/32,44,50
Vanillin	30	3			−	−	+	+	D/B	22,24,25,29,30,44/22,48,52

[a] Benzophenone was not sensitizing at 60 per cent; thymol was not sensitizing at 10 per cent. Higher testing concentrations of these two compounds were not used because of systemic toxicity.

A compound shown as not sensitizing in the OET does not sensitize even when applied undiluted; otherwise, the minimal sensitizing concentration is given.

Data concerning the allergenicity of these 32 compounds for humans were collected from the literature and are summarized in the last 2 columns of Table I. Compounds considered as allergenic were classified into 5 types, as follows: Type A, compound simply described as "sensitizers," without any confirmatory data being presented; Type B, compounds with confirmed sensitizing capacity for man; Type C, compounds sensitizing experimental animals; Type D, compounds causing positive patch tests in eczematous patients hypersensitive to complex allergens, such as balsam of Peru and turpentine, which contain the incriminated compound or are known to cross-react with it; Type E, compounds causing positive patch tests in eczematous patients with no history of sensitization either to this particular compound or to the complex allergen known to contain it.

SKIN IRRITATION

The OET findings relating to skin irritation are summarized in Table II. It can be seen that, after one application, a few compounds caused mild erythema at a minimal concentration as low as 0.1 per cent, whereas a few others did so only when applied undiluted. In most cases, however, the minimal irritating concentration (after one application) was between 3 and 30 per cent.

After 21 applications, the minimal irritating concentration of most of the compounds tested was 3 per cent. Surprisingly, the minimal irritating concentration of 10 compounds was the same after 21 applications as after one application, whereas in the remaining 22 cases, it was lower after 21 applications, as expected. This may be explained by the accumulation of skin injuries after repeated applications or by the induction of contact hypersensitivity as demonstrated by subsequent contact testing. No relationship was found between the capacity to cause skin irritation and the capacity to induce contact hypersensitivity.

ALLERGENICITY AS DETERMINED BY THE OET

All the guinea pigs which had been treated for 21 days with the undiluted compounds or their various diluted solutions were challenged on the contralateral flank on day 21. By this procedure, the minimal concentration necessary for the induction of contact hypersensitivity and the minimal concentration for eliciting contact reactions at the time of challenge, which corresponds to the degree of hypersensitivity induced, could be determined and expressed in per cent. These data are presented in Table III. It can be seen that, out of the 32 compounds studied, 22 compounds (70 per cent) induced contact hypersensitivity at one or another of the applied dosages. It is also evident from Table III that there was no correlation between the minimal sensitizing concentration and the degree of hypersensitivity induced.

CORRELATION BETWEEN SKIN IRRITATION AND ALLERGENICITY

A scrutiny of the data presented in Tables IV and V shows that no relationship was found between skin irritation and the capacity to induce contact hypersensitivity, i.e.,

Table II
Minimal Skin Irritating Concentrations of 32 Compounds

Concentration %	100	30	10	3	1	0.3	0.1
Number of compounds causing irritation after one application	3	12	6	8		1	2
Number of compounds causing irritation after 21 applications	1		3	21	2	3	2

Table III
Correlation between the Minimal Sensitizing Concentration and the Degree of Contact Hypersensitivity

Minimal Sensitizing Concentration %	100	30	10	3	1	0.3
Number of sensitizing compounds	1	10	5	5		1
Minimal eliciting concentration %:						
10		1				
3		4	3	1		
1	1	3	2	1		
0.3				3		
0.1						
0.03		2				1

Table IV
Correlation between Skin Irritation and Allergenicity

Concentration %	100	30	10	3	1	0.3	0.1
Number of compounds causing sensitization after 21 applications	1	10	5	5		1	
Number of compounds causing skin irritation after 1 application		9	3	7		1	2

Table V
Correlation between the Minimal Sensitizing and the Minimal Irritating Concentrations

Minimal Sensitizing Concentration %	Number of Compounds	Minimal Irritating Concentration After 1 Application %	Number of Compounds
100	1	100	0
		30	0
		10	0
		3	1
30	10	30	4
		10	2
		3	2
		0.1	2
10	5	30	3
		10	1
		3	1
3	5	30	1
		10	1
		3	3
0.3	1	0.3	1

that the degree of hypersensitivity induced was independent of the irritating capacity of the compound concerned.

COMPARISON OF THE OET WITH 3 OTHER TESTS USED FOR DETECTING ALLERGENICITY OF FRAGRANCES FOR THE GUINEA PIG

In order to compare the sensitivity of the OET with that of the DT, the MT, and the FCAT as far as the detection of allergenicity is concerned, the 32 compounds tested were all tested concurrently by these 4 methods on separate groups of guinea pigs. It can be seen from Table VI that, by using all four tests, 25 of the 32 compounds were found to be allergenic in one or more of these tests.

Referring to the detailed data shown in Table I, it can further be seen that, of these 25 allergenic compounds, 22 were detected by the OET and 21 by the other tests. In other words, 4 allergenic compounds were detected exclusively by the OET and 3 others exclusively by one or more of the three intradermal tests. These results suggest that some compounds can only be recognized as allergenic when applied epicutaneously, whereas others only when injected intradermally. These differences may be due to the following: differences in the amount of compound administered in each test; the use of FCA which has well-known adjuvant properties, and/or; the nature of the solvent.

Thus, considering only the dosage, the differences are as follows. In the OET, 21 individual doses are used. Each individual dose may be 100, 30, 10 or 3 mg, or less, depending on the concentration used. In the DT, 0.1 mg is injected intradermally 10 times. In the MT, the total dose is once 20 mg intradermally plus once 250 mg epicutaneously. In the FCAT 50 mg is injected intradermally 5 times.

COMPARISON BETWEEN CLINICAL ALLERGENICITY AND THE FINDINGS ON THE GUINEA PIG

The correlation between the allergenicity of 32 "incriminated" compounds for humans and their allergenicity for the guinea pig can be derived from the data presented in Ta-

Table VI
Compounds Described as Allergenic for Man and Detected as Allergenic[a] for the Guinea Pig by the Four Tests Used

Group	Total Number of Compounds	Tests							
		OET		DT		MT		FCAT	
		+	−	+	−	+	−	+	−
Group II	18	18	0	7	11	15	3	17	1
Group III	4	4	0	0	4	0	4	0	4
Group IV	3	0	3	1	2	3	0	3	0
Subtotal	25	22	3	8	17	18	7	20	5
Group I	7	0	7	0	7	0	7	0	7
Subtotal	32	22	10	8	24	18	14	20	12
Total	32	32		32		32		32	

[a]The group code is the same as that in Table I (see Results Section of this paper for data). Group I represents compounds not sensitizing guinea pigs in any of the 4 tests used.

bles I and VI. Of these 32 compounds, 25 were found to be allergenic for the guinea pig in 1 or more of the 4 tests used. This includes 4 compounds found to be allergenic only in the OET and 3 compounds found to be allergenic only in 1 or more of the other 3 tests. The remaining 7 compounds were found to be nonallergenic in all 4 tests on guinea pigs. These 7 compounds are described in the literature (22) as sensitizing for humans, but without confirmatory data such as a positive history, a positive patch test or re-exposition. In view of our negative findings on the guinea pig, we consider that the reported allergenicity of these compounds for humans might perhaps reflect a possible cross-sensitizing capacity.

It can be seen from Table I that all the compounds with a well-established sensitizing capacity for man were found to be allergenic for the guinea pig in one or more of the 4 tests used. This could be expected for compounds of the "incrimination" Types B, C, D, and E. By contrast, the test results for compounds of Type A were divergent. These compounds induced hypersensitivity in guinea pigs when the incriminating data were accurate, and failed to do so when their reported allergenicity was based on an assumption or on an unconfirmed observation. The nonallergenicity of the first 6 compounds of Type A listed in Table I, as well as that of thymol, all of which failed to induce hypersensitivity in the guinea pig in any of the 4 tests used, may be considered fairly well-established, because they were negative in the OET even when tested undiluted (benzophenone and thymol could not be tested undiluted because of systemic toxicity, see Table I) and were also negative in the intradermal tests. On the other hand, 4 other compounds of Type A were shown to be weak sensitizers because they were positive in the OET at a concentration of 30 per cent.

IV. DISCUSSION

The OET is a procedure proposed for testing on guinea pigs the skin irritating and allergenic capacities of chemical compounds intended for use in perfumes, cosmetics, and dermatics. In the OET, the compounds to be tested are applied undiluted and in a descending series of concentrations. By establishing a dose-response curve, the minimal irritating and minimal sensitizing concentrations of a compound can be determined quantitatively. The end-point reactions are read on an "all or none basis," thus largely excluding subjective bias in the evaluation of the results.

A total of 32 compounds described in the literature as allergenic for man were tested by the OET. For purposes of comparison they were also tested by the DT, the test with FCAT, and the MT described by Magnusson and Kligman (4, 5). The highest number of allergenic compounds were detected by the OET, somewhat fewer by the FCAT and the MT, and a few by the DT. However, certain compounds were detected exclusively by the OET and others exclusively by one or more of the intradermal tests (DT, MT and/or FCAT). The reliability of the OET, i.e., its predictive value for man, was investigated by us, as had been done by Magnusson and Kligman, testing on animals the sensitizing properties of fragrances known from clinical experience to be allergenic for man and some known to be innocuous. Of the compounds tested, all those with well-established allergenicity for man were detected by the OET (see Tables I and VI). On the other hand, compounds with an unconfirmed clinical allergenicity yielded divergent results in the animal tests, as expected.

Table VII
Allergenicity of Compounds Tested in Humans by the Maximization Test (47) and in Guinea Pigs by 4 Different Procedures

	Humans		Guinea Pigs				
	MT[a]		OET		DT[b]	MT[b]	FCAT[b]
Compounds	Test conc. %	Results	Test conc. %	Results	Results	Results	Results
Amyl-cinnamic aldehyde	3	–	100	–	–	–	–
Diethyl phthalate	5	–	100	–	–	–	–
Methyl-ionone	5	–	100	–	–	–	–
Ionone	4	–	100	–	–	–	–
Hydroxycitronellal	6	–	100	–	–	–	–
Vanillin	1	–	100	+	+	+	+
Cinnamic alcohol	2	–	100	–	–	+	+
Coumarin	4	–	10	+	–	–	–
			3	–			
Eugenol	4	–	10	+	+	+	+
			3	–			
Geraniol	3	–	10	+	–	+	+
			3	–			
Heliotropin	3	–	30	+	–	+	+
			10	–			
d-Limonene	4	–	100	+	–	+	+
			30	–			

[a]The occlusive eliciting concentration application was only at the user concentration × 2.
[b]DT, MT, FCAT: concentrations used see Section II of this paper.

Table VII shows a comparison of the results of our animal tests with those obtained by Greif (47) who used the MT on human subjects for testing several fragrance compounds "which had successfully weathered the test of time," i.e., the innocuousness of which for humans had been demonstrated by many years of practical use. This comparison confirms the high predictive reliability of the guinea pig OET for humans. Table VII shows that some compounds, all negative in the human MT, were not allergenic for the guinea pig in the OET even when tested in the undiluted form. Others did not sensitize guinea pigs at concentrations 2 to 100 times as high as the conventional concentrations used on humans. This shows that the guinea pig OET can be used to determine a quantitatively precise risk of sensitization for humans. Table VII also shows that the results of the intradermal tests on the guinea pig seem to be less predictive for man (see comments on Table VII) and that a better correlation was found between the human MT and the guinea pig OET.

In a later paper, the correlation between the results of Kligman's Human MT and the OET, especially, will be discussed.

V. SUMMARY

An OET is proposed for the detection of skin irritation and contact hypersensitivity induced in guinea pigs by various compounds intended for use in perfumes and cos-

metics. A dose-response curve was plotted for each compound tested, and the irritant and/or allergenic activity of each compound was established in terms of concentration in percent. The proposed procedure is simple, yields quantitative data, minimizes subjective factors when evaluating the results, and simulates the conditions in human use.

A total of 32 compounds described in the literature as being allergenic for man were tested by the OET technique and, for the purpose of comparison, by three intradermal techniques, namely, the DT, the MT and the FCAT. Most of the compounds described as being allergenic for man were found to be allergenic for the guinea pig in the OET and in 1 or more of the 3 intradermal tests. A few of the compounds tested, however, were found to be allergenic only in the OET, and a few others only in the DT, MT and/or FCAT. In the OET, a high degree of correlation was found between the allergenicity of the tested compounds for the guinea pig and their allergenicity for man.

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