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## COMPARISON OF ALLERGENICITY OF A PERFUME IN ANIMALS (GUINEA PIG MAXIMIZATION TEST) AND IN HUMANS (USAGE TEST)

By

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The aim of this investigation was to correlate the allergenic rating of a perfume, investigated by the guinea pig maximization (G.P.M.) test, with a sensitization rate in humans, after a usage test with the same perfume included in a soap.

It has been demonstrated that the agents which sensitize humans invariably do the same in guinea pigs (1). The G.P.M. test can, therefore, establish if a substance has the potentiality to cause contact sensitization in man. It cannot, however, indicate the prevalence of human sensitization during conditions of normal use in practice, as the antigenic stimuli may be quite different in the G.P.M.-test.

### MATERIAL AND METHODS

#### 1. *Compound Tested*

A perfume containing citrus oil and cinnamil acid (exact composition not known) was tested. The perfume was available for the usage test at a concentration of 0.3 per cent in a soap solution which contained potassium salts of oleic acid as the main ingredient; for the guinea pig maximization test, the perfume was used in different dilutions of: 50, 25, 10, 5 and 1 per cent to evaluate toxic effects and the sensitization rate.

## 2. Usage Test in Humans

300 families in a Swedish town (randomly selected from the Swedish Civil register ("Mantalslängderna")) received the perfumed soap, which was intended to be used daily for 2 to 3 months. Control checks were made to ensure that they actually used the soap. At the end of the test period, housewives from 75 randomly selected families were tested epicutaneously.

## 3. Maximization Test in Guinea Pigs

a. *Evaluation of the toxic limit:* In 20 guinea pigs, not included in the trial series, the toxic irritation threshold of the perfume solutions, was determined for intradermal sensitization in water in concentrations of: 25, 10, 5, 2 and 1 per cent. For topical sensitization, the concentrations were: 50, 25, 10, 5, 2 and 1 per cent. Toxic reactions were found for the highest concentrations, and therefore the following concentrations were chosen: for intradermal sensitization—20 per cent; for topical sensitization—20 per cent; for epicutaneous testing—10 per cent.

b. *Sensitization:* White female, non-pregnant guinea pigs of the Hartley strain, weight 450–500 g, were used. The animals were fed on pellets *ad libitum* and rutabaga, but were given no water. Twenty guinea pigs were clipped free of hair, and shaved clean with an electric razor in the area over the scapular region. Induction was performed with six intradermal injections: two with 0.1 ml of the perfume solution; two with 1 ml of Freund's adjuvans; and two with equal parts of the Freund's adjuvans and the perfume solution. Moderate skin reactions were observed on the site of injections, but no necrosis. One week after the intradermal injections, the guinea pigs were again shaved on the injection site, and 10 per cent sodium-lauryl sulphate in vaseline was rubbed into the area. After 24 h, a slight skin irritation was observed, and there was also erythema within the area. The allergen suspensions were applied on a Whatman filter paper, covered with overlapping impermeable plastic tape, and firmly held in position for 48 h by an elastic adhesive bandage, encircling the trunk. After 48 h, the bandage was removed, and the guinea pigs were housed in cages for fourteen d.

c. *Challenge:* The 20 perfume-sensitized animals and 20 unsensitized control animals were tested in exactly the same way. The area to be tested was clipped free of hair and then shaved clean with an electric razor. A Whatman filter paper, saturated with the perfume solution in a 10 per cent concentration, was applied to the skin, covered with impermeable plastic tape, and held in position for 24 h by an elastic bandage. Skin reactions were evaluated after 24 h, and graded according to the following scale: no visible change = 0; slight or discrete erythema = +; moderate or confluent erythema = ++; intense erythema and swelling = +++.

## 4. Epicutaneous Testing in Humans

The test concentration of the perfume was 2 per cent, in water. The epicutaneous patch test (according to Fregert) was used with an aluminium foil, and was

fixed with Dermicel plaster to the skin. All tests were performed on normal skin, on the back. The time of exposure was 48 h, and tests were read 3 h after removal of the test unit, and again the day after.

### 5. *Clinical Examination*

All patients were examined with respect to development of eczema in those areas which had been exposed to the perfume.

## RESULTS

### a. *Allergenic Rating in Guinea Pigs*

Two of the sensitized animals showed reactions towards the perfume, one strong (+++) and one weak (++). None of the animals in the control series reacted. The sensitization rate was thus 10 per cent of the exposed animals. This means that the test substance has the potentiality to cause contact sensitization on the border between weak and mild, according to the rules for the evaluation of the G.P.M.-test (1). The perfume was classified as a weak or mild allergen.

### b. *Usage Test on Humans*

One of the 75 women tested showed a papulous and erythematous skin reaction (++). None of the other women showed any reaction towards the perfume. None of the patients developed eczema on parts of the body exposed to the perfume.

## DISCUSSION

The skin reaction in one of the 75 women was, on morphological criteria, classified as allergic. It is thus apparent that, when the perfume is used in 0.3 per cent concentration in soap, and applied on human skin for 2-3 months, 1.3 per cent of those examined react positively to tests. The allergy in this woman could, of course, have been acquired earlier, due to contact with balsam of Peru (cross-reaction) or with other perfumes. It was not, however, possible to prove this. A test with, for example, balsam of Peru before the usage test could

have revealed a previous sensitization. On the other hand, the test itself might have sensitized the patients and would have given a false positive result regarding the sensitization risk of the perfume in the later usage test. The probability that this would have happened is, however, small (2). None of the individuals in the test group had any history of possible allergy towards balsam of Peru.

It must be stressed that a sensitization rate of 1.3 per cent is a maximum for sensitization frequency after usage of a perfume in a soap solution for 2–3 months. The sensitization rate could be lower, but not higher. The sensitization rate was 10 per cent, according to the G.P.M. test, which means a sensitization grade between I–II, i.e. weak or mild allergenicity. Using experimentally determined sensitization grades, it is difficult to predict the risk of sensitization from a substance, such as a perfume, when it is used together with other substances in, for example, soaps. Therefore it is important to compare the results in a usage test in daily life with the results of the experimental G.P.M. test with respect to sensitization rate. Not until experience has accumulated in this respect will it be possible to judge the practical application of the results of the G.P.M. test.

#### SUMMARY

Using the Guinea Pig Maximization Test, the sensitization rate of a perfume was shown to be 10 per cent, which means a mild allergenicity rate. A usage sensitization trial was performed with the same perfume included in a soap, on 300 persons for 2–3 months. Seventy-five of these, all house-wives, were randomly selected and tested epicutaneously, after the trial. One woman reacted allergically towards the perfume. This means a sensitization rate of 1.3 per cent, which is a maximum value, as she could, theoretically, have been sensitized before.

## REFERENCES

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