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Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients

Relationship to repeated open application test with a product containing formaldehyde releaser

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Our purpose was to investigate the eliciting threshold concentration of formaldehyde in formaldehyde-sensitive individuals in the occluded and non-occluded patch test, and to evaluate the relationship to repeated open application test (ROAT) with a product containing a formaldehyde releaser. 20 formaldehyde-sensitive patients and a control group of 20 healthy volunteers were included in the study. Occluded and non-occluded patch tests with formaldehyde solutions from 25 to 10,000 ppm, and ROAT for 1 week with a leave-on cosmetic product containing on average 300 ppm formaldehyde, were carried out simultaneously on each subject. In the occluded patch test, 1/2 of the 20 patients only reacted to 10,000 ppm formaldehyde, 9 reacted to 5,000 ppm, 3 reacted to 1,000 ppm, 2 reacted to 500 ppm and 1 reacted to 250 ppm. No definite positive reactions were observed in the non-occluded patch test or in the ROAT. No positive reactions were observed in the control group to any of the test procedures. We concluded that the threshold concentration for occluded patch test to formaldehyde in formaldehyde-sensitive patients was 250 ppm. The threshold in occluded patch test corresponded to the degree of sensitivity. Definite positive reactions in the ROAT were not seen, either indicating that they are unlikely to happen with the type of product used or that the exposure time was too short.

Key words: allergic contact dermatitis; formaldehyde; occluded path test; non-occluded patch test; repeated open application test; ROAT; eliciting threshold concentration. © Munksgaard, 1997.

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Formaldehyde is a common contact allergen. In a Swedish study of hand eczema in a *normal population* (1983–84), 1.6% of the individuals reporting hand eczema had a positive patch test to formaldehyde (2% aq.=20,000 ppm) (1, 2). In 1988–89, the prevalence of formaldehyde sensitivity (1% aq.= 10,000 ppm) in *consecutive patch tested patients* was 2.3% (range 0.7–3.1%) in Denmark and 2.6% (range 0–9.3%) in Europe (3). In 1984–85, the prevalence of sensitization to formaldehyde (2% aq.) in North American *consecutive patch tested patients* was 8.4% (4) and in 1992-94 the prevalence of sensitisation to formaldehyde (1% aq.) was 7.8% (5). Patch testing with 1% and 2% formaldehyde aq. in about 3000 consecutively patch tested patients showed no major differences in the number and degree of positive reactions (Torkil Menné, unpublished data).

Contact dermatitis caused by formaldehyde is often chronic, probably because it is difficult for the individual consumer to avoid exposure to formaldehyde-containing products. An investigation in Denmark showed that 1/3 of all cosmetic products contained formaldehyde or formaldehyde-releasing preservatives (6). Formaldehyde and formaldehyde-releasing preservatives were frequently registered in industrial and household products used in Denmark (7, 8). A study of washing and cleaning agents showed that formaldehyde-releasing compounds are among the most common reported preservatives in cleaning agents (9).

Formaldehyde-sensitive patients may suffer from long-lasting hand eczema (10). Investigations in London (11), Copenhagen (12) and in Portland, Oregon (13), have indicated that for patients with formaldehyde dermatitis, avoidance of the allergen

can have a profound impact on the medical prognosis.

There is a lack of eliciting threshold data based on systematic investigations. Clinical investigations by Jordan et al. (14), made as 1-week continuous closed patch testing with a dilution series, produced a response down to 30 ppm formaldehyde. In a provocative use test (open application over a period of a week or more) with a "formalin-containing" antiperspirant, the threshold for *no response* was 80 ppm (Maibach & Franz, unpublished data referred to in (15)). De Groot et al. (16) observed positive reactions to the lowest concentration of 1000 ppm formaldehyde in 8 of 35 subjects, when patch testing formaldehyde-sensitive patients with serial dilutions of formaldehyde (0.1%, 0.3%, 1.0% aq.). Fischer et al. (17) observed reactions to formaldehyde concentrations below 630 ppm in 5 of 22 formaldehyde-sensitive patients patch tested with serial dilutions of formaldehyde (0.015%, 0.032%, 0.063%, 0.13%, 0.25%, 0.5% and 1.0% aq.). These concentrations are significantly lower than the 2000 ppm presently permitted in cosmetic products in the EU, and it is also lower than the limit for declaration of free formaldehyde in cosmetics, which is 500 ppm (18).

The purpose of the present study was to investigate the eliciting threshold of formaldehyde concentration in formaldehyde-sensitive individuals (occluded and non-occluded patch test) and the reaction under experimental *use* conditions (repeated open application test, ROAT) with a leave-on cosmetic product. Free formaldehyde was measured in all formaldehyde-containing test materials.

Materials and Methods

Patients and control group

All consecutive patch-tested patients seen between September 1993 and May 1995 at the Department of Dermatology, Gentofte Hospital, who had a positive patch test to formaldehyde (1% aq.) and negative patch tests to paraben mix, Germall 115 and rubber from finger cots used in the ROAT, were invited to participate in the study. Additional exclusion criteria were: dermatitis or other skin diseases at or near the skin sites to be used for testing, and diseases, exposure or use of medication which could be expected to interfere with the testing.

A total of 36 patients suitable for the study were recruited. 20 formaldehyde-sensitive patients (14 women, 6 men; age 32–71) agreed to participate in the study. 16 patients (12 women, 4 men; age 32–68) refused to participate for various reasons (44% of the total relevant patients). In addition, 4 patients were excluded as their sensitivity to formaldehyde could not be confirmed and 3 were excluded because of positive reactions to Germall 115.

A control group of 20 healthy volunteers (12 women, 8 men; age 22–54), with negative patch tests to formaldehyde, parabens, Germall 115 and rubber from finger cots, were tested with the same procedures and test materials.

Table 1 shows the sex-ratio, mean age, age range and strength of patch test reaction to 1% formaldehyde aq. for participating patients, patients who refused to participate and the control group.

The test procedures, occluded and non-occluded patch tests, and ROAT were carried out simultaneously on each subject. During the whole test procedure, patients and controls were instructed not to expose the test areas to washing. Patients and controls were investigated in the period from September 1993 to May 1995. All readings were made blind. The study was approved by the Ethical Committee of the Copenhagen Municipality. All patients gave a written consent to participation after having received oral and written information.

Table 1. Sex-ratio, mean age, age range and patch test reactions to 1% formaldehyde for participating patients, patients who refused to participate and the control group

	n	F/M	Mean age (years)	Age range (years)	Reaction to patch test with 1% formaldehyde
Patients included	20	14/6	48	32–71	19 positive[a]: 9 with + 8 with ++ 2 with +++
Patients refused	16	12/4	44	32–68	15 positive[b]: 10 with + 5 with ++
Control group	20	12/8	39	22–54	All negative

[a] 1 patient with negative reaction to 1% formaldehyde had positive reaction to 0.5% formaldehyde.
[b] 1 patient with negative reaction to 1% formaldehyde had positive reaction to 2% formaldehyde.

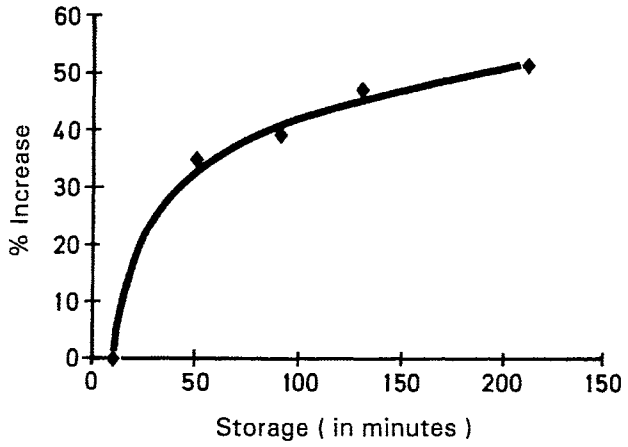

Fig. 1. Stability of Germall 115 (formaldehyde releaser) in an aqueous solution. Increase in formaldehyde content, expressed as percentage of the level originally present.

Test materials

The occluded patch testing included formaldehyde solutions of 0, 25, 50, 250, 500, 1,000, 5,000 and 10,000 ppm. The non-occluded patch testing included formaldehyde solutions of 0, 25, 50, 100, 250, 500, 1,000, 5,000 and 10,000 ppm. The formaldehyde solutions were coded and placed on the back in a randomized way.

In addition to the above mentioned formaldehyde solutions, occluded patch testing was made with formaldehyde (1% aq.) and paraben mix from the European standard series (15% pet.), Germall 115 (imidazolidinyl urea) (2% pet.) (Hermal, Germany) and rubber from finger cots used for ROAT.

The ROAT was performed with a leave-on cosmetic product preserved with parabens (methyl paraben 0.1%, propyl paraben 0.1%) and Germall 115 (imidazolidinyl urea) (0.3%). The product was a oil-in-water emulsion containing common cosmetic ingredients.

Analytical methods

Reagents used were of analytical grade or pharmaceutical quality. The concentration of the formaldehyde solutions were assayed by the iodine titration method (19). The product used for ROAT was analyzed for free formaldehyde and total formaldehyde by an HPLC method as described below.

Measurement of total formaldehyde by post column derivatisation HPLC. The official EU method (20) for the determination of free formaldehyde in

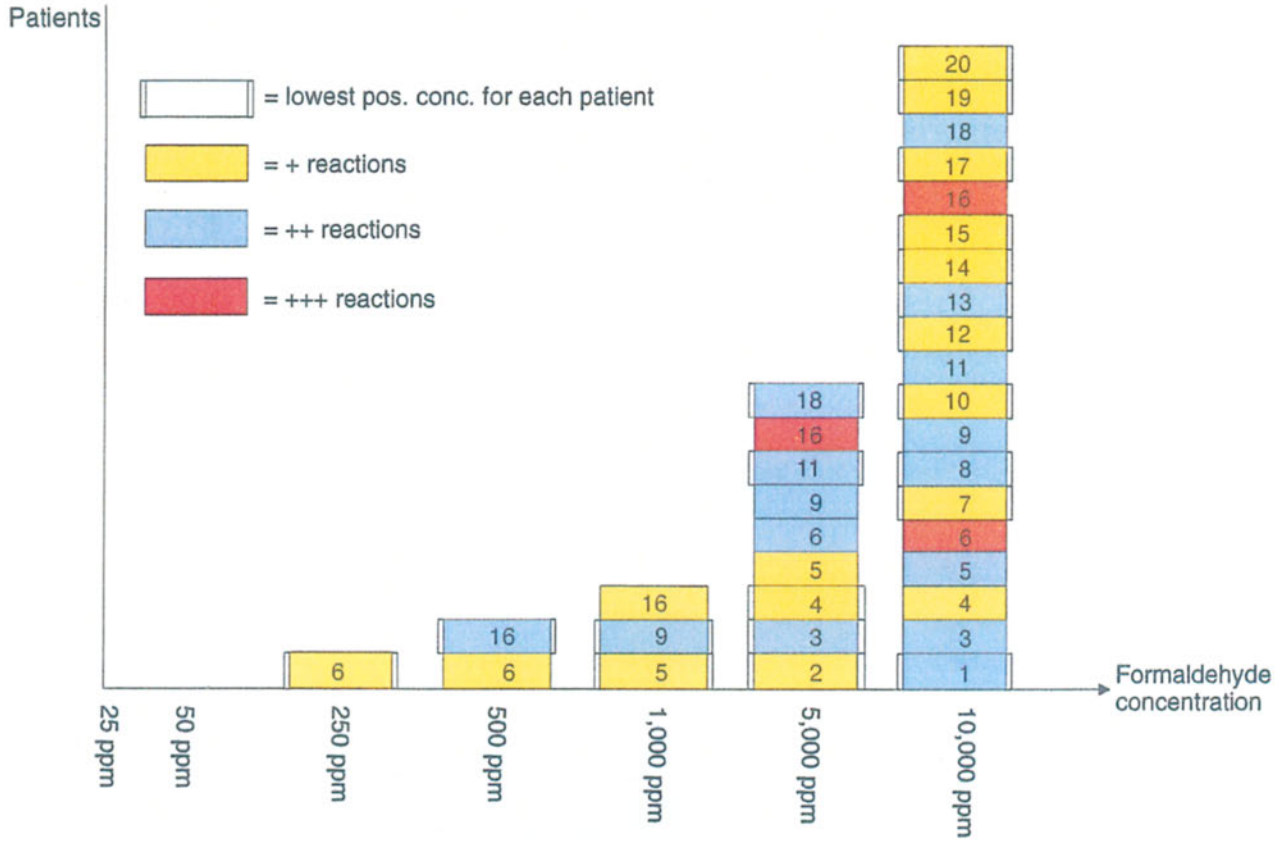

Fig. 2. Positive patch test reactions to formaldehyde in occluded patch testing or in diagnostic patch testing (10,000 ppm) among 20 formaldehyde-sensitive eczema patients. N.B. The number in each box is the patient number.

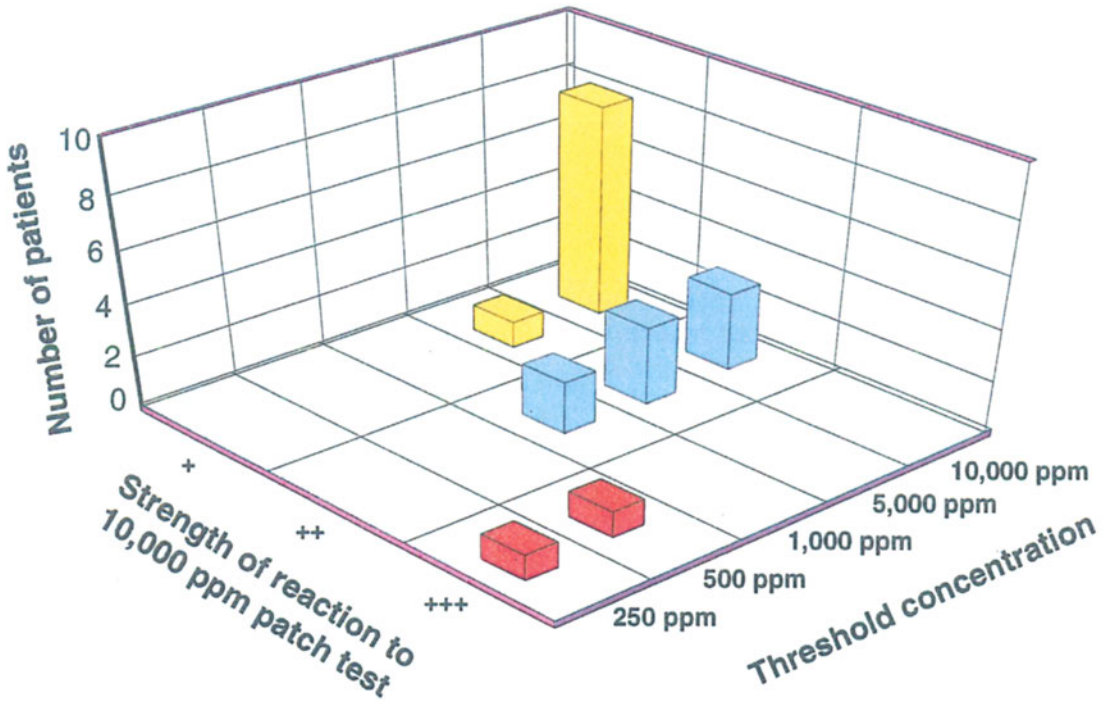

Fig. 3. Lowest formaldehyde concentrations giving positive reactions in occluded patch testing, compared to the strength of the reactions in the diagnostic patch testing (10,000 ppm) among 19 formaldehyde-sensitive eczema patients.

cosmetic products has been modified to measure total formaldehyde. To ensure that all the formaldehyde is released, the aqueous sample solutions are heated for 30 min at approximately 100°C prior to analysis. The total formaldehyde level is determined by HPLC, utilizing a high level of acetonitrile (90%) in the eluent to ensure a sharp peak, with post-column derivatization with acetyl acetone. The absorbance of the yellow 1,4-dihydrolutidine derivative formed is measured at 410 nm and quantitation is by external calibration.

Measurement of free formaldehyde by pre-column derivatization HPLC. A new pre-column derivatization approach for the determination of formaldehyde, using 2,4-dinitrophenylhydrazine (DNPH) as the reagent, was used. It replaced the EU acetyl acetone derivatization previously recommended. Less degradation of formaldehyde-releasing chemicals is observed, which makes the technique more suitable for detection of free formaldehyde. However, the release of formaldehyde from formaldehyde-releasing chemicals during analysis still remains a problem, as it has been shown that aqueous solutions of these chemicals degrade quickly, for example see Fig. 1.

It is now proven that the stability of aqueous solutions of formaldehyde-releasing chemicals is poor. Upon storage of formaldehyde-releasing chemical solutions, increased levels of formaldehyde will be observed, regardless of the technique used (pre- or post-column derivatisation). In future, samples containing formaldehyde-releasing components should be analyzed without any delay, to avoid excessive degradation.

Occluded patch test

Occluded patch testing was made with 15 μl of the formaldehyde solutions, formaldehyde, paraben mix, Germall 115 (imidazolidinyl urea) and rubber from finger cots used for ROAT. The test materials were applied to the upper back by Finn Chambers (diameter 0.8 cm) on Scanpor tape (Norgesplaster, Oslo). The tests were applied for 2 days and readings were performed after 2 days, 3 days and 6-9 days. Readings were made according to the ICDRG recommendations (21).

Non-occluded patch test

Non-occluded patch testing was made with 15 μl of formaldehyde solutions, as specified above, applied to a 1 cm^2 area of the forearm and allowed to dry at room temperature. Readings were made as described for the occluded patch testing.

Repeated open application test (ROAT)

The ROAT was carried out by the patients who applied the test material to a 5×5 cm area of the

flexor mid-aspect of the left upper arm. The patients were instructed to apply approximately 0.1 ml of the test material by means of the finger cots 2× daily for a maximum period of 1 week. Reading of the test site was done after 1 week (22) unless a positive reaction was observed beforehand. If a positive reaction was observed before the end of 1 week, the patients were instructed to come to the laboratory for evaluation.

For grading of the ROAT, any skin changes in the test area were described according to the following terms: (i) slight dryness and scaling in the test area without redness; (ii) slight uneven redness without infiltration (oedema) in the test area; (iii) papular, follicular reaction in the test area; (iv) even redness, infiltration (oedema) and scaling in the test area. In addition, papules and vesicles may also be observed.

In the reading of the ROAT, only term (iv) was defined as a positive outcome of the test.

Results

20 (56%) out of 36 consecutive formaldehyde-sensitive patients suitable for the study agreed to participate. Patients who refused to participate did not differ from the participating group with respect to sex, age and degree of reactivity to patch test with formaldehyde 1% aq. (see Table 1). No positive or irritant reactions were observed in the control group to any of the test procedures.

Occluded patch test

All included patients had positive patch test reactions to formaldehyde (standard patch test series, 10,000 ppm) before recruitment to the study. At retest with formaldehyde from the standard patch test series and 10,000 ppm in the dilution series, 19 out of 20 patients had positive patch test to 10,000 ppm formaldehyde. 1 patient with negative patch test to 10,000 ppm formaldehyde in the standard series and in the dilution series was positive to 5,000 ppm. Fig. 2 shows the grading of reactions to each patch test concentration and the lowest positive concentration for the 19 patients with positive patch test to 10,000 ppm. 9/20 reacted to a concentration of 5,000 ppm formaldehyde or lower. 3/20 reacted to 1,000 ppm, 2/20 to 500 ppm and 1/20 to 250 ppm. Retesting of the patient reacting to 250 ppm after 1 year with 50 ppm, 100 ppm and 250 ppm was negative.

Fig. 3 shows the lowest formaldehyde concentrations giving positive patch test reactions in the dilution series, compared to the strength (+, ++, +++) of the reactions to 10,000 ppm formaldehyde. Only 1 patient with a + reaction to 10,000 ppm formaldehyde and the patient who did not react to 10,000 ppm formaldehyde reacted to lower formaldehyde concentrations. All the other patients reacting to lower formaldehyde concentrations had ++ or +++ reactions to 10,000 ppm formaldehyde.

Table 2. Patch test reactions for 4 patients reacting to low formaldehyde concentrations (1,000 ppm and lower) in the occluded patch test

Patient number	Dilution series						Standard series 1% 10,000 ppm
	25 ppm	250 ppm	500 ppm	1,000 ppm	5,000 ppm	10,000 ppm	
#5	2 foll.	–	–	+	+	++	++
#6[a]	–	+	+	foll.[b]	++	++	+++
#9	–	3 foll.	foll.	++	++	++	++
#16	–	foll./+?[c]	++	+	+++	+++	+?

[a] Retest 1 year later with 50 ppm, 100 ppm and 250 ppm was negative.
[b] Follicular reactions: more than 3 follicular papules. All follicular reactions were considered negative.
[c] Even redness and follicular papules in the test area.

Table 3. Reactions in the ROAT test and lowest positive formaldehyde concentrations in the occluded patch test for patients with follicular reactions in the ROAT

Patient number	Occluded patch test		Repeated open application test (ROAT)		
	Lowest positive concentrations (ppm)	$\mu g/cm^2$	Reactions to the ROAT-cream	conc.[a] (ppm)	dose ($\mu g/cm^2$)
#7	10,000 (+)	0.30	1 foll. (day 5, 6)	291	0.71
#9	1,000 (++)	0.03	foll. (day. 7)	280	2.92
#10	10,000 (+)	0.30	2 foll. (day. 2)	258	0.84
#13	10,000 (++)	0.30	2 foll. (day 3)	289	1.12
#18	5,000 (++)	0.15	5 foll. (day 7)	367	1.81

[a] The cream used by the individual patients was analyzed for free formaldehyde.

Table 2 specifies the patch test reactions for 4 patients reacting to low formaldehyde concentrations (1,000 ppm and lower). Clear dose-response with negative and/or follicular reactions to the lowest concentrations and ++ or +++ to the highest concentrations was observed for these patients.

Non-occluded patch test

No positive reactions were observed in the non-occluded patch test. In 6 out of 20 patients we observed weak reactions showing erythema without infiltration or follicular reactions (not meeting the criteria for positive reactions).

Repeated open application test (ROAT)

No positive reactions were observed in the ROAT. A few follicular papules were observed in the test area in 5 out of 20 patients (Table 3). The concentration of free formaldehyde in the ROAT cream was approximately 300 ppm. The actual dose applied in the ROAT varied from 0.71 $\mu g/cm^2$ to 2.91 $\mu g/cm^2$, due to variations in the amount of cream the patients used for each application. Thus, the ROAT dose causing follicular reactions varied from 2 to 4× the dose causing positive reactions in the occluded patch test for patients with a threshold of 10,000 ppm formaldehyde, to 12× for the patient with a threshold of 5,000 ppm and 97× for the patient with a threshold of 1,000 ppm (this patient used about 3× as much cream).

Discussion

Occluded patch test

There were no major differences in the degree of sensitivity to formaldehyde among participants and non-participants. 2 extreme formaldehyde-sensitive patients were included in the study. The main outcome of the occluded patch test is summarized in Figs. 2, 3. 1/2 of the patients only reacted to 10,000 ppm formaldehyde. A relation was found between the degree of patch test reactivity and threshold concentration, as 7 out of 9 patients reacting to formaldehyde concentrations below 10,000 ppm had ++ or +++ reactions to 10,000 ppm formaldehyde. Only 1 patient reacted to 250 ppm formaldehyde. This patient was negative to this concentration and lower concentrations at retest 1 year later. Our results are comparable to the results of de Groot et al. (16), who observed reactions to 1,000 ppm formaldehyde in 8 out of 35 formaldehyde-sensitive patients, and Fischer et al. (17), who observed reactions below 10,000 ppm formaldehyde in 19 of 22 formaldehyde-sensitive patients, with 1 reacting to 150 ppm. The tendency towards a lower threshold in the latter study, compared to the de Groot et al. and present studies, may be explained by simultaneous testing with both formaldehyde and a formaldehyde-releaser. Thus, in the present study the threshold concentration for reactions to formaldehyde in formaldehyde-sensitive patients was 250 ppm. As only 4 out of 20 patients reacted to 1,000 ppm formaldehyde and lower concentrations (Fig. 2), it can be expected that extremely few individuals will react to less than 250 ppm formaldehyde in an occluded patch test.

Non-occluded patch test

The non-occluded application of 1% formaldehyde aq. and lower concentrations did not cause any positive reactions, as defined by ICDRG criteria (21). The explanation is probably that formaldehyde evaporates from the skin and the actual concentration therefore quickly decreases. Positive reactions with non-occluded patch testing can be obtained with certain substances, for example nickel, where even dose-response can be demonstrated without occlusion (23, 24).

Repeated open application test (ROAT)

Data on experimental exposure to a cosmetic product containing a defined concentration of free formaldehyde in formaldehyde-contact-allergic subjects are not available in the literature. Hannuksela (22) systematically examined the experimental product exposure test in contact allergic individuals and termed it repeated open application test (ROAT). With many different substances in both use test and exaggerated concentrations, 60 out of 86 patients gave a positive ROAT within 1 week, with most reacting after 4 to 5 days.

Bruze (25) summarized the outcome of 10 ROAT studies with commercial leave-on products preserved with Kathon® CG in a concentration range from 7.7 ppm to 15 ppm. Generally, 50% reacted after 1 week, and in the study that continued for 2 weeks 100% reacted. In a similar larger multicenter study, 31 out of 101 reacted within 1 week to a lotion containing 15 ppm Kathon® CG (3).

In a ROAT study including patients contact allergic to neomycin, 9 out of 12 reacted with a positive reaction within 1 week (26). In a recent ROAT study including patients sensitive to cinnamic aldehyde (exposure over 6 weeks), 6 out of 13 patients reacted later than 1 week (27). In none of these studies were severe local reactions or spread of dermatitis seen. Systematic studies examining the time

and concentration relationship in the ROAT have not been performed. Based on the available literature, a 1 week exposure time seemed reasonable at the time of planning the study. Healthy skin on the upper arm was chosen as the exposure area, as this is the most sensitive next to the upper back (28).

An international reading scale does not exist for the ROAT. We decided upon careful clinical description of the individual test outcome, with the terminology described in the Materials and Methods section points (i) to (iv), and, when practical, with photographic documentation. The ICDRG terminology used for diagnostic patch testing was used to decide upon positive ROAT outcome.

None of the formaldehyde-contact-allergic patients or controls gave a positive reaction to a product containing approximately 300 ppm free formaldehyde* in the present study. The explanation for the negative ROAT may be too low an exposure concentration or too short an exposure time. The tolerable concentration of formaldehyde in a cosmetic product naturally also depends on the permeation ability and occlusive effect of the product. Probably most free formaldehyde evaporates from the product when placed on the skin without occlusion, and only a minor part will permeate into the skin. It is possible that products, like the one evaluated in the present study, do not cause allergic contact reactions in formaldehyde-sensitive individuals because the concentration of free formaldehyde is below the reaction threshold. This point can only be further evaluated by prolonged exposure studies. It should be kept in mind that the outcome might differ if exposure concerned facial skin or damaged skin.

Outside the protocol, 3 patients sensitive to both formaldehyde and Germall 115 were exposed to the same procedure. 1 of the 3 developed a positive ROAT reaction, meeting the criteria defined in term (iv) in the *Materials and Methods* section. This observation confirms that the methodology employed is sufficiently sensitive to study experimental allergic contact dermatitis. Probably individuals sensitive to both formaldehyde and a formaldehyde-releaser run an increased risk of developing overt clinical dermatitis, as simultaneous exposure to both haptens is not infrequent. Studies have shown that allergic responses to individual haptens tend to summate (29).

Little is known about elicitation symptoms of experimental allergic contact dermatitis in humans. The first objective changes are often papular reactions followed by even redness, infiltration and eventually vesicles. The initial papules in experimental contact dermatitis probably start in the hair follicles or in the sweat-duct orifices. This might differ from one hapten to another. Systematic studies, including histology and immunohistology, need to be done. In the present study, papular reactions were seen to subthreshold concentrations in the occluded patch test, in a few patients in the non-occluded patch test, and a few isolated papules (1 to 5) were seen in 5 of 20 patients in the ROAT. Similar papules were not seen in the controls, either in the patch test or in the ROAT, indicating that they probably represent a weak allergic reaction to formaldehyde. The eventual progress in these clinical symptoms can only be decided in more prolonged studies.

Conclusions

- Dose response can be illustrated in formaldehyde-sensitive individuals by 2 days of occluded patch testing.
- Approximately 1/2 of the formaldehyde-sensitive individuals only react to the diagnostic patch test concentration (10,000 ppm).
- Concentration threshold depends upon degree of reactivity to 10,000 ppm (diagnostic patch testing).
- Concentration threshold in occluded patch testing was demonstrated at 250 ppm.
- No definite positive reaction was seen in the non-occluded patch test or ROAT with a cosmetic product containing 300 ppm free formaldehyde in a 1 week trial.
- Isolated papular reaction pattern were seen in some formaldehyde-sensitive individuals, but not in controls, suggesting a weak allergic reaction to formaldehyde. Only prolonged exposure can decide whether such papular reactions eventually develop into dermatitis or whether the concentration in the product was below the reaction threshold in formaldehyde-sensitive individuals.
- 1 patient, tested outside the protocol, sensitive to both formaldehyde and Germall 115, gave a positive ROAT, confirming the general sensitivity of the methodology employed.

* It appears that release of free formaldeyde from Germall 115 depende on the nature of the cosmetic formulation and connot be extrapolated from one formulation ot another. For example, a marketed moisturizer formulation preserved with 0.3% Germall 115 was found (by the same analytical method) to contain on average 150 ppm free formaldehyde (Peter Greenhill, personal communication).

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