

Finding the optimal patch test material and test concentration to detect contact allergy to geraniol

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Summary

Background. Geraniol is a commonly used fragrance terpene, and is tested in the baseline series in fragrance mix I. Geraniol is a pro-hapten and a pre-hapten, and sensitizers are formed in the autoxidation and skin metabolism of geraniol. Previous patch testing with air-exposed (oxidized) geraniol has suggested that oxidized geraniol could be a better marker for contact allergy to geraniol than pure geraniol.

Objectives. To find the optimal patch test substance and concentration for detecting contact allergy to geraniol.

Patients and methods. Six hundred and fifty-five patients were patch tested with pure and oxidized geraniol at 4.0%, 6.0% and 11.0% in petrolatum. Before patch testing, the irritant properties of pure and oxidized geraniol were studied in 27 patients at 2.5%, 5.0%, 10.0% and 20.0% pet.

Results. Pure geraniol detected positive reactions in 0.15–1.1% of the patients, and oxidized geraniol detected positive reactions in 0.92–4.6% of the patients. Reactions to pure geraniol in patients not reacting to oxidized geraniol indicated metabolic activation of geraniol. Neither pure nor oxidized geraniol gave significant irritant reactions.

Conclusions. Increasing the test concentrations of pure and oxidized geraniol enables the detection of more cases of contact allergy. Oxidized geraniol detects more patients than pure geraniol, but patch testing with only oxidized geraniol does not detect all cases of contact allergy to geraniol.

Key words: allergic contact dermatitis; autoxidation; CAS 106-24-1; CAS 106-26-3; CAS 141-27-5; CAS 5392-40-5; fragrances; geraniol; hydroperoxides; metabolism; oxidation products; patch test; patch test concentration; terpenes.

Geraniol is a widely used fragrance terpene that is appreciated for its fresh, flowery odour. It occurs naturally in many flowers and plants, and is present in high concentrations in essential oils of rose and geranium,

among others. Geraniol is present in many different product types. In studies of consumer products, it was declared or detected in 40–60% of the studied products (1–3). Geraniol was found to have one of the highest maximum daily exposures among the studied fragrance chemicals in a recent assessment of consumer exposure to terpene alcohols used as fragrance ingredients (4). This was attributed to exposure from many different sources in everyday life.

Fragrance allergy is considered to be the second most common contact allergy in the western world, and is diagnosed by use of the fragrance markers *Myroxylon pereirae*, fragrance mix I (FM I), fragrance mix II (FM

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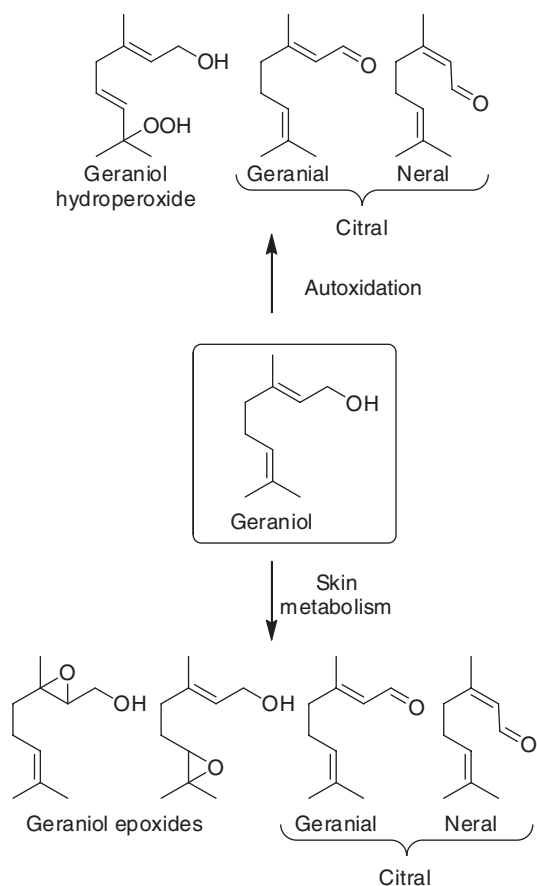


Fig. 1. Chemical structures of geraniol and compounds detected in experiments on the autoxidation and cutaneous metabolism of geraniol (6, 7).

II) and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) in the European baseline series for patch testing of dermatitis patients (5). Geraniol is part of FM I, and is thus included in the European baseline series. In a recent large screening study, the frequency of positive reactions to FM I was found to be 6.6% (5).

We have previously shown that geraniol can be activated by autoxidation, and thus form sensitizers when in contact with air (6) (Fig. 1). In our study of autoxidation of geraniol, the aldehydes geranial and neral were the main oxidation products detected in the oxidation mixture. A geraniol hydroperoxide was also detected. Autoxidation was shown to increase the sensitization potency of geraniol in the local lymph node assay, indicating that autoxidation can be important in contact allergy to geraniol (6).

Previous studies have shown that the fragrance terpenes *R*-limonene, linalool and linalyl acetate also autoxidize on air exposure at room temperature,

forming hydroperoxides as the primary oxidation products (8–11). These hydroperoxides were shown to be strong sensitizers. Subsequent clinical studies identified the oxidation mixtures and hydroperoxides of *R*-limonene and linalool as frequent causes of contact allergy in dermatitis patients (9, 12–16).

Many compounds, so called pro-haptens, are activated to become sensitizers via cutaneous metabolism in the skin (17). In our studies of the cutaneous metabolism of geraniol, the aldehydes geranial and neral were identified as metabolites of geraniol by use of a skin-like cytochrome 450 cocktail (7). No hydroperoxides of geraniol were detected in the metabolic experiments. However, in the metabolic experiments, we identified epoxides of geraniol, which were not found in the autoxidation mixture (Fig. 1).

In a clinical study of contact allergy to geraniol, a frequency of 0.39% positive reactions to geraniol, pure or not intentionally oxidized, tested at 1% in petrolatum was observed when the reactions to the individual components of FM I were studied (5). In another large screening study of fragrance allergy, 0.4% of the patients showed reactions to geraniol 1% pet. (18). We have previously found a frequency of 0.13% positive reactions to pure geraniol 2.0% pet. in 2227 patients. However, patch testing with oxidized geraniol 2.0% pet. detected significantly more cases of contact allergy – 0.55% when tested in 2179 patients (19). In most of these cases, the reaction was found to be clinically relevant. Therefore, oxidized geraniol might be a better diagnostic tool than pure geraniol for detecting contact allergy to fragrances.

In previous clinical studies of contact allergy to oxidized linalool, we found that increasing the patch test concentration could be important for detecting more cases of contact allergy among the tested patients (12, 16, 20). Similar studies have not been performed for geraniol or oxidized geraniol.

The aim of the present study was to investigate whether increasing the patch test concentrations of geraniol and oxidized geraniol would detect more cases of contact allergy without causing adverse effects such as irritant reactions or sensitization. The aim was also to determine whether oxidized geraniol is a better marker of contact allergy to the pre-hapten and pro-hapten geraniol, and to determine the optimal test concentration for this fragrance marker.

Materials and Methods

Chemicals

Geraniol (98%) was purchased from Sigma Aldrich (St Louis, MO, USA), and geranial (98%) and neral (>99%)

were obtained from Bedoukian Research Inc. (Danbury, CT, USA). The chemicals were distilled prior to use.

Instrumentation and mode of analysis

High-performance liquid chromatography (HPLC) analyses were conducted on an Agilent 1260 HPLC system with a 1260 DAD VL+ detector. An Eclipse Plus C8 column (3.5 μ m, 4.6 $\times$ 50 mm; Agilent Technologies, Santa Clara, CA, USA) was used. Analysis of pure and oxidized geraniol was conducted with external standards, as previously described (19).

Synthesis

Geraniol hydroperoxide was synthesized as previously described (6), and used as a reference compound in the analysis of oxidized geraniol.

Air exposure procedure

A distilled sample of geraniol (>98%) was air-exposed in an Erlenmeyer flask, covered with aluminium foil to prevent contamination. It was stirred at room temperature for 1 hr, four times a day, and exposed to light for 12 hr a day, with a daylight fluorescent lamp (Philips; 18 W, Eindhoven, The Netherlands), as previously described (6, 11). Samples were taken on a regular basis, and stored at -20°C under nitrogen until being analysed by HPLC with ultraviolet detection to determine the concentrations of geraniol and its major oxidation products. The oxidation was interrupted after 10 weeks, whereafter the oxidation mixture was stored at -20°C under nitrogen during the patch test study. The oxidation mixture of geraniol was analysed, and found to contain geraniol 48% (wt/wt), geranial 3.9%, neral 1.0%, and geraniol hydroperoxide 3.2%. The non-quantified material consists of other previously identified oxidation products (e.g. geraniol formate and 3,7-dimethyl-octa-2,5-diene-1,7-diol) that do not contribute to the sensitization potency of oxidized geraniol (6) and of polymers formed over time. Samples of the oxidation mixture were taken out and used for patch test preparations.

Patch test materials

All chemicals were analysed prior to preparation of the patch test materials. On the basis of previous experience (21), non-stabilized pet. (Prolabo, Chester, PA, USA) was used for all patch test preparations. Patch test preparations were stored at -20°C when not in use. Each preparation was used for a test period of 6 months,

and during this time the preparation was stored in the refrigerator.

For the study of irritancy, patch test preparations of geraniol and oxidized geraniol were prepared at 2.5%, 5.0%, 10.0% and 20.0% pet. These test concentrations were chosen on the basis of previous experience of studies of irritancy of oxidized fragrance terpenes (22). The results from the study of irritancy allowed patch testing with geraniol and oxidized geraniol for the subsequent concentration study in accordance with our previous experience of patch testing with oxidized linalool, a structurally related fragrance terpene (12). Thus, geraniol and oxidized geraniol were patch tested at 4.0%, 6.0% and 11.0% pet. in the present study.

Patients: study of irritancy

Dermatitis patients ($n = 27$, 19 women and 8 men, mean age 45.0 years) were asked to participate in the study in addition to their regular patch testing. The study of irritancy was performed during the period December 2009 to April 2010. The same preparations were used throughout the study. Patients 1–5 were tested in December 2009, patients 6–11 were tested in February 2010, and patients 12–27 were tested in April 2010. Exclusion criteria were previously diagnosed contact allergy to fragrances or previous reactions to fragranced products. The test substances were applied together with the ordinary patch test to the upper back of the patient for 48 hr, and removed by the patients. The irritant reactions were evaluated on D3–D4. The reactions were evaluated visually, with a scale described by Basketter et al. (23). Ethical permission was obtained from the local ethics committee. Informed consent was obtained.

Patients: study of contact allergy

Consecutive patients patch tested with the Swedish baseline series at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, during the period September 2010 to December 2011, were included in the subsequent screening study. In all, 655 patients participated in the study (200 men and 455 women; mean age 45.2 years; standard deviation 17.8). Patch test preparations of ~ 20 mg were applied in small Finn Chambers[®] (diameter 8 mm, inner area of 0.5 cm²; Epitest Ltd Oy, Tuusula, Finland) on Scanpor[®] tape (Norgesplaster A/S, Vennsela, Norway) to the back of the patient, left under occlusion for 2 days, and then removed by the patient. Readings were performed according to

Table 1. Results of the study of irritancy of geraniol and oxidized geraniol; numeric readings of irritant reactions to geraniol and oxidized geraniol on a visual scale from 0 to 9^a (23) at D3–D4 readings are presented with mean score and standard deviation, as well as the cumulative score for 27 patients

Substance	Concentration (% pet.)	Median	Mean	SD	Cumulative score (n = 27)
Geraniol	2.5	0	0.074	0.27	2
Geraniol	5.0	0	0.037	0.19	1
Geraniol	10.0	0	0.11	0.42	3
Geraniol	20.0	0	0.30	0.91	8
Oxidized geraniol	2.5	0	0.0	0.0	0
Oxidized geraniol	5.0	0	0.0	0.0	0
Oxidized geraniol	10.0	0	0.22	0.42	6
Oxidized geraniol	20.0	0	0.48	0.70	13

SD, standard deviation.

^a0, no reaction; 1, marginal reaction; 2, slight, perceptible erythema; 3, a greater than slight reaction that is not sufficient to be classified as distinct; 4, distinct erythema; 5, a greater than distinct reaction that is insufficient to be classed as well developed; 6, well-developed, possibly spreading erythema; 7, a greater reaction that is not sufficient to be classed as strong; 8, strong, deep erythema that may extend beyond the treatment site; 9, a more intense reaction than above.

the International Contact Dermatitis Research Group recommendations (24) on D3–D4 and D7.

Statistical analysis

Analysis was carried out with SPSSTM 15.0 for WindowsTM (SPSS Inc., Chicago, IL, USA). The visual readings of rising concentrations of geraniol and oxidized geraniol were compared as paired values by use of the Wilcoxon signed rank test at the level of $p < 0.05$. The McNemar test was used to evaluate differences in frequencies of positive reactions in the patch testing of geraniol and oxidized geraniol. Fisher's exact test was used to evaluate differences in concomitant reactions to fragrance markers of the baseline series.

Results

Study of irritancy

The median and mean values from the visual readings of irritation by geraniol and oxidized geraniol are shown in Table 1. The irritation effect of both geraniol and oxidized geraniol was low, ranging from 0 to 4 on the visual evaluation grading scale (23). The distribution of the readings is shown in Fig. 2.

Among the 27 patients in the study of irritancy, *M. pereirae* caused 2 positive reactions and 1 doubtful reaction, FM I caused 2 positive and 3 doubtful reactions,

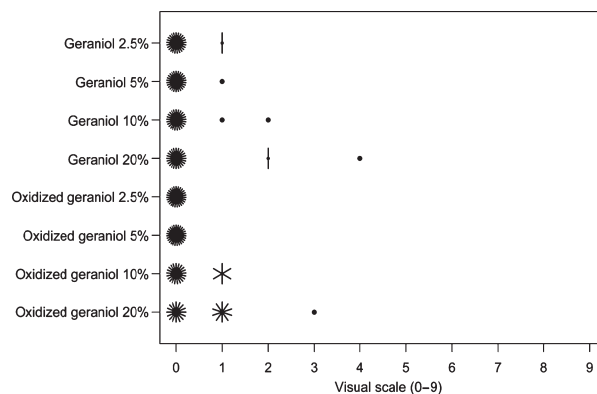


Fig. 2. Sunflower plot of visual readings of irritation (scale 0–9) of geraniol and oxidized geraniol according to the erythema grading scale (23). A dot represents 1 patient, and each petal of the flower represents 1 additional patient showing the same result. Oxidized geraniol did not give rise to significantly stronger irritant reactions than geraniol.

and none of the patients reacted to FM II or HICC. No allergic reactions were seen to the test materials of the irritation study.

There was no statistically significant difference in irritation between geraniol and oxidized geraniol at equal concentrations. Also, there was no statistically significant increase in irritation with increasing concentrations of geraniol and oxidized geraniol, except for the difference in irritation between oxidized geraniol 5.0% and oxidized geraniol 10.0% ($p = 0.031$).

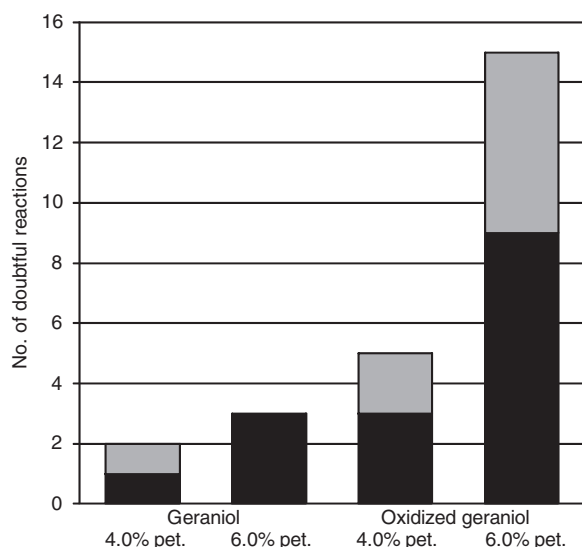
Study of contact allergy

The results of the patch testing in terms of total number of tested patients, and the frequency of positive and doubtful reactions, are shown in Table 2. No irritant reactions were recorded, and no late patch test reactions were recorded, indicating no cases of active sensitization. Oxidized geraniol gave a higher frequency of positive reactions than geraniol at all tested concentrations.

For pure geraniol, the increase in positive reactions with increasing test concentration was not statistically significant as compared with the next lowest test concentration. The only statistically significant increase was seen for geraniol 11.0% (7 of 655, 1.2%) as compared with geraniol 4.0% (1 of 655, 0.15%) ($p = 0.031$). For oxidized geraniol, the increase in positive reactions with increasing concentrations was statistically significant as compared with the next lowest concentration: oxidized geraniol 4.0% (6 of 655, 1.1%) versus 6.0% (15 of 655,

Table 2. Total number of patients tested in the screening study with geraniol and oxidized geraniol at each patch test concentration, also showing the number and frequency of positive and doubtful reactions

Substance	Concentration (% pet.)	Tested n	Positive		Doubtful	
			n	%	n	%
Geraniol	4.0	655	1	0.15	3	0.46
Geraniol	6.0	649	3	0.46	3	0.46
Geraniol	11.0	655	7	1.1	5	0.76
Oxidized geraniol	4.0	655	6	0.92	5	0.76
Oxidized geraniol	6.0	655	15	2.3	16	2.4
Oxidized geraniol	11.0	653	30	4.6	32	4.9

**Fig. 3.** Number of patients with doubtful reactions to geraniol and oxidized geraniol 4.0% pet. and 6.0% pet., respectively, with marked (black) subgroups representing patients with a concomitant positive patch test reaction to the next higher test concentration of geraniol or oxidized geraniol.

2.4%), $p = 0.0015$; oxidized geraniol 6.0% (15 of 655, 2.4%) versus 11.0% (30 of 655, 4.6%), $p = 0.0026$.

The higher frequencies of positive reactions to oxidized geraniol than to pure geraniol at equal test concentrations were statistically significant at all test concentrations except 4.0%, with $p = 0.0018$ at 6.0%, and $p = 3.4 \times 10^{-5}$ at 11.0%.

A higher proportion of the patients with doubtful reactions to oxidized geraniol than of the patients with doubtful reactions to pure geraniol showed positive reactions at the next higher test concentration (Fig. 3). Two patients with doubtful reactions to geraniol 4.0% were not tested with geraniol 6.0%, owing to a temporary shortage of test material, but both showed positive

reactions to geraniol 11.0%. One patient with a doubtful reaction to oxidized geraniol 6.0% was not tested with oxidized geraniol 11.0%, for the same reason. These 3 patients are not included in the analysis presented in Fig. 3.

The distribution of strength of the positive patch test reactions to pure and oxidized geraniol, respectively, is shown in Table 3.

The pattern of concomitant reactions between geraniol and oxidized geraniol is shown in Table 4. Of the 33 patients showing positive reactions to oxidized geraniol at any test concentration, 28 reacted only to oxidized geraniol. Of the 8 patients showing positive reactions to geraniol, 4 reacted only to geraniol.

Eight of 32 patients (25%) with reactions to oxidized geraniol showed concomitant reactions to more than one of the fragrance markers of the baseline series, as compared with 12 of 618 patients (1.9%) not reacting to any test material of the study ($p = 1.17 \times 10^{-6}$). One of 7 patients (14%) with reactions to pure geraniol showed concomitant reactions to more than one of the fragrance markers of the baseline series, as compared with 12 of 618 patients not reacting to any test material of the study. This difference was not found to be statistically significant ($p = 0.14$).

During the study period, the overall frequencies of positive reactions to the fragrance markers in the baseline series were 6.5% for FM I, 4.5% for *M. pereirae*, 1.4% for FM II, and 0.7% for HICC.

Discussion

In the present study, a high frequency of positive patch test reactions to oxidized geraniol was observed, giving 0.92–4.5% positive patch test reactions at test concentrations of 4.0–11.0%. Only 0.15–1% of the patients reacted to pure geraniol at the same concentrations. Thus, testing with oxidized geraniol detects more cases of contact allergy than testing with pure geraniol, and oxidized geraniol may be an important tool for the diagnosis of contact allergy in dermatitis patients.

Among the patients with positive reactions to oxidized geraniol, the majority did not react to geraniol, and most of the cases (28 of 33) of contact allergy to oxidized geraniol would have been missed if only pure geraniol was tested. As the main haptens in oxidized geraniol are the oxidation products geranial, neral, and geraniol hydroperoxide, which are all moderate or strong allergens (6), this pattern was expected, on the basis of the studies on oxidized linalool and limonene (9, 12–16).

For geraniol, sensitization and elicitation can most probably be induced by cutaneous metabolism via

Table 4. Pattern of concomitant reactions to geraniol and oxidized geraniol at any test concentration

	Geraniol-positive (n = 8)	Geraniol-negative (n = 647)	Total
Oxidized geraniol-positive (n = 33)	4	29	33
Oxidized geraniol-negative (n = 622)	4	618	622
Total	8	647	655

Total number of tested patients, n = 655.

enzymes in the skin, such as cytochrome 450, whereby geranial and neral can be formed (7). However, in patch testing, only small amounts of geranial and neral may be formed in the skin when geraniol is applied in a single exposure, and the patch test reaction to pure geraniol may thus remain negative or be only very weak.

In our study, not all cases of contact allergy to geraniol were detected by testing with oxidized geraniol, as 4 of 8 patients with positive reactions to geraniol did not react to oxidized geraniol at any concentration (Table 4). No doubtful reactions to oxidized geraniol were seen in any of these 4 patients. These patients could be sensitized to metabolites of geraniol other than geranial or neral. In experimental studies of the cutaneous metabolism of geraniol, sensitizing epoxides were detected (7), which could explain these reactions. The 4 patients reacted only to the higher test concentrations of geraniol, indicating that a high concentration of geraniol might be needed to detect contact allergy to metabolites, in order for the metabolites to be formed in sufficient amounts to cause an allergic reaction. When test preparations of geraniol and oxidized geraniol at equal test concentrations are compared, the concentration of geraniol remaining in the oxidation mixture could be too low for the formation of enough metabolite to induce a positive patch test reaction. Thus, our results indicate that, in most cases, a high test concentration of the pro-hapten geraniol is probably necessary to detect contact allergy to the metabolites formed.

In skin metabolism, there is also the possibility of induction of enzymes in certain skin areas after repeated exposure. If the patient has been avoiding perfumed products, or has not been exposed to geraniol on the patch test area (upper back), the formation of allergenic metabolites may be low, and the reaction when pure geraniol is applied may not be detectable. Conversely, in everyday life with repeated exposures, skin enzymes may be induced locally by repeated exposure to the substrate geraniol, thus increasing susceptibility to the development of allergic contact dermatitis caused by the allergenic metabolites of geraniol if geraniol-containing products are used (25).

According to the present data, air exposure did not significantly affect the irritant effect of geraniol, as only a weak correlation between air oxidation of geraniol and skin irritation could be seen. Although the results indicate an increase in irritation with increasing test concentrations of oxidized geraniol, both objective readings and statistical significance were weak. This indicates that patch testing with oxidized geraniol can be performed with up to 6.0% pet. without risking high frequencies of irritant reactions.

Geranial and neral are the main autooxidation products formed when geraniol autoxidizes. Geranial and neral are also the constituents of citral, a widely used fragrance ingredient. Although citral has been shown to be an irritant (26), it is used in FM II at a concentration of 1% pet. without causing irritant reactions. Irritant reactions are strongly dependent on the concentration at exposure, and the irritant response is expected to decrease rapidly with decreasing concentration. In the test preparations of oxidized geraniol used in this irritation study, the concentrations of citral (geranial and neral together) range from 0.12% to 1%. Thus, the concentrations of geranial and neral in the test preparations should be too low to cause irritant reactions, as they are lower than or equal to the current patch test concentration of citral in FM II.

When a dose-response study is performed, it is important to determine whether doubtful reactions will become positive, become irritant or remain doubtful at a higher

Table 3. Distribution of positive patch test reactions for the three tested concentrations of geraniol and oxidized geraniol

Compound	Concentration (% pet.)	No. of positive reactions	No. of + reactions	% of + positive reactions	No. of ++++ reactions	% of ++++ reactions
Geraniol	4.0	1	0	0.0	1	100
Geraniol	6.0	3	2	67	1	33
Geraniol	11.0	7	4	57	3	43
Oxidized geraniol	4.0	6	3	50	3	50
Oxidized geraniol	6.0	15	9	60	6	40
Oxidized geraniol	11.0	30	21	70	9	30

test concentration. In the present study, moderate numbers of doubtful reactions were seen at all test concentrations. However, many of these doubtful reactions became positive at the next higher test concentration (Fig. 2). These results are in line with previous clinical studies of oxidized linalool, in which similar proportions of doubtful reactions were seen among patients tested with oxidized linalool 6.0% pet. and oxidized linalool 11.0% pet. (12). For oxidized linalool, a test concentration of 6.0% pet. was chosen as an optimal patch test concentration. On the basis of the results from the present study, we consider a test concentration of 6.0% pet. to be most favourable also for oxidized geraniol. A large number of doubtful reactions to oxidized geraniol 11.0% pet. was seen in the present study. The test material needs further evaluation in larger clinical studies in order to determine the usefulness of this test concentration.

In our previous studies of oxidized linalool, which is not part of the baseline series, concomitant reactions to more than one of the fragrance markers of the baseline series were found to be significantly more frequent than in patients not reacting to oxidized linalool, supporting a clinical relevance for the detected contact allergies to oxidized linalool (12, 20). In the present study, a significantly higher frequency of concomitant reactions to fragrance markers of the baseline series was found among patients with positive reactions to oxidized geraniol than among patients not reacting to the test materials of the study. This supports the clinical relevance of the findings.

Most cases of contact allergy to geraniol found in the present study would not have been detected with only the fragrance markers of the baseline series. With only FM I, 26 cases of contact allergy to geraniol would have been missed. In our previous studies of contact allergy to oxidized geraniol 2.0% pet., a high frequency of concomitant reactions was seen between oxidized geraniol and FM I, containing geraniol 1% pet., but not between pure geraniol and FM I (19). In the present study, 30–43% of patients reacting to oxidized geraniol also reacted to FM I, containing geraniol. In the present study, there was no specific correlation between oxidized geraniol and FM I when patch tests were performed with higher concentrations of pure and oxidized geraniol. This indicates that testing with FM I will not detect all cases of contact allergy to geraniol. Testing with FM II, containing citral, did not

detect additional cases of contact allergy to geraniol, as all 3 patients reacting to FM II also reacted to FM I. Patch testing with higher test concentrations of citral might be necessary to detect contact allergy to geraniol and citral.

In the present study, a frequency of positive reactions to oxidized geraniol of 2–4% was seen, showing that oxidized geraniol could be a common contact allergen among the patients referred to our test centre. We have, in previous clinical multicentre studies on contact allergy to autooxidized fragrance terpenes, found that oxidized linalool 6.0% pet. and oxidized *R*-limonene 3.0% pet. are common contact allergens, showing frequencies of positive reactions of 7% and 5%, respectively, in patch testing of consecutive dermatitis patients (12, 14, 15). Patch testing of large patient groups with non-oxidized linalool and limonene has shown only occasional reactions to these compounds (27, 28). In the present study, a frequency of positive reactions to geraniol of 0.5–1% was seen, which is in accordance with studies of contact allergy to non-oxidized linalool and limonene. Thus, the results from the present study further underline that well-monitored mixtures of oxidized fragrance terpenes can be valuable tools for detecting contact allergy to fragrances.

In conclusion, increasing the test concentrations for patch testing with both oxidized and pure geraniol enables the detection of more cases of contact allergy, many of which would have been missed with only FM I of the baseline series. Testing with oxidized geraniol does not detect all cases of contact allergy to geraniol. This indicates that metabolites other than those compounds that can be formed both metabolically and via autooxidation of geraniol may be important haptens. Patch testing with pure and oxidized geraniol with the increased test concentrations, preferably in a multicentre setting, should be performed to evaluate their usefulness as diagnostic tools.

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