

Contact allergy to air-exposed geraniol: clinical observations and report of 14 cases

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Summary

Background. The fragrance terpene geraniol forms sensitizing compounds via autoxidation and skin metabolism. Geranial and neral, the two isomers of citral, are the major haptens formed in both of these activation pathways.

Objectives. To investigate whether testing with oxidized geraniol detects more cases of contact allergy than testing with pure geraniol.

Patients and methods. The pattern of reactions to pure and oxidized geraniol, and metabolites/autoxidation products, was studied to investigate the importance of autoxidation or cutaneous metabolism in contact allergy to geraniol. Pure and oxidized geraniol were tested at 2.0% petrolatum in 2227 and 2179 consecutive patients, respectively. In parallel, geranial, neral and citral were tested in 2152, 1626 and 1055 consecutive patients, respectively.

Results. Pure and oxidized geraniol gave positive patch test reactions in 0.13% and 0.55% of the patients, respectively. Eight of 11 patients with positive patch test reactions to oxidized geraniol also reacted to citral or its components. Relevance for the positive patch test reactions in relation to the patients' dermatitis was found in 11 of 14 cases.

Conclusions. Testing with oxidized geraniol could detect more cases of contact allergy to geraniol. The reaction pattern of the 14 cases presented indicates that both autoxidation and metabolism could be important in sensitization 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 concentration; patch test; terpenes.

Fragrance allergy is currently estimated to be the second most common contact allergy in the western world, on the basis of positive patch test reactions in consecutive dermatitis patients to the fragrance markers in the baseline series (1). One of these markers, fragrance mix I (FMI), contains the fragrance chemical geraniol at a concentration of 1%. In large clinical studies, 9–13% of tested

consecutive dermatitis patients react to FMI; however, geraniol is responsible for only 5% of positive patch test reactions to the individual components of FMI (2). Geraniol 1% in petrolatum gave positive patch test reactions in 0.4% of 2063 dermatitis patients tested in a German multi-centre study performed during the years 2003–2004 (3).

Geraniol is a widely used fragrance terpene, appreciated for its fresh, flowery odour. It occurs naturally in large amounts in various plant materials, and is present in high concentrations in the essential oils of rose, citronella, and palmarosa. According to several studies, geraniol is among the most common fragrance ingredients in consumer products, and was detected in 76% of investigated deodorants on the European market (4), in 41% of domestic and household products (5), and in

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33% of cosmetics based on natural ingredients (6). In exposure assessments, geraniol was found to have one of the highest maximum daily exposures among the studied chemicals, owing to exposure from many different sources in everyday life (7).

The fragrance and flavour substance citral is a mixture of the two isomeric aldehydes geranial and neral (Fig. 1), in the proportion 2:1. Citral is the major component of lemongrass (*Cymbopogon citratus*) and *Litsea cubeba* oil (8, 9), and one of the major components in lemon and lime leaf oils (10). It has been identified in 25% of domestic and occupational products (5), indicating that skin exposure to citral is frequent. Citral has been used for patch testing in several studies, showing positive patch test reactions in 0.6–4.7% of tested dermatitis patients when tested at 2% pet. (3, 11, 12). This led to the inclusion of citral in fragrance mix II (FM II) in the European baseline series for patch testing (13, 14). It was shown to be one of the most important fragrance allergens in this mix in an evaluation after 4 years of testing (15). Interestingly, in the German multicentre screening study from 2003 to 2004, a high frequency of concomitant reactions to geraniol and citral was seen, as 11 of the 13 patients reacting to citral also reacted to geraniol (3).

We have shown in experimental studies (16) that geraniol can autoxidize in contact with air, thus forming sensitizing oxidation products (Fig. 1). Previous studies have shown that the fragrance terpenes limonene, linalool and linalyl acetate autoxidize on air exposure at room temperature, forming hydroperoxides as primary oxidation products (18–21). The hydroperoxides were shown to be strong sensitizers and major contributors to the allergenic activity of the oxidation mixtures (8–11). Subsequent clinical studies identified the oxidation mixtures

of limonene and linalool, as well as their hydroperoxides, as frequent causes of contact allergy in dermatitis patients (22–26).

In the oxidation mixture of geraniol, a geraniol hydroperoxide (Fig. 1), with a sensitization potency similar to that of the other hydroperoxides, was detected (18, 20). However, geraniol hydroperoxide was detected at a lower concentration than the hydroperoxides of linalool and limonene. Other major compounds detected in the oxidation mixture were the two isomeric aldehydes geranial and neral, which are stable secondary oxidation products from a very unstable hydroxyhydroperoxide (16). The sensitization potency of the 10-week autoxidation mixture of geraniol was considerably increased according to the local lymph node assay (LLNA) (EC3 4.4%) as compared with that of pure geraniol (EC3 22.4%) (16). This indicates that autoxidation could be important in contact allergy to geraniol, and, based on the individual LLNA results, that geraniol hydroperoxide, geranial and neral all make a major contribution to the allergenic activity of the oxidation mixture of geraniol (16). In addition, we have shown that geraniol can act as a prohaptens and be bioactivated via cutaneous metabolism in the skin (17). The same aldehydes, geranial and neral, that were formed by autoxidation were also identified as main metabolites of geraniol by use of a cytochrome P-450 skin-like cocktail (17).

The impact of autoxidation and/or metabolism on contact allergy to geraniol has not been evaluated in a clinical setting. The aim of the present study was to investigate whether air-exposed geraniol would detect more cases of contact allergy than pure geraniol in patch testing of consecutive dermatitis patients. Furthermore, the pattern of reactions to pure and oxidized geraniol, in relation to the major metabolites and autoxidation products of geraniol (geranial, neral, and their isomeric mixture citral), was studied in order to investigate the importance of autoxidation or cutaneous metabolism in contact allergy to geraniol.

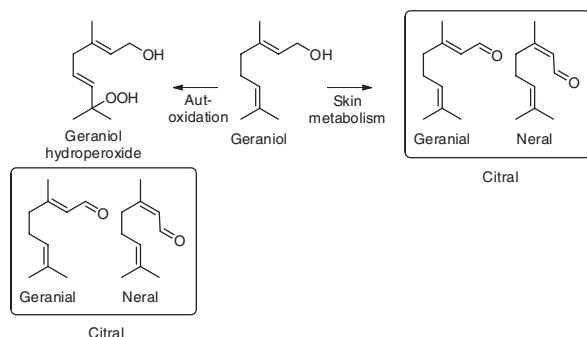


Fig. 1. Activation of geraniol through autoxidation and cutaneous metabolism. Geraniol hydroperoxide, geranial and neral were identified as sensitizing products of the autoxidation of geraniol (16). Geranial and neral were identified as sensitizing metabolites in cytochrome P450-mediated metabolism of geraniol (17).

Materials and Methods

Chemicals

Geraniol (98%) was purchased from Sigma Aldrich (St Louis, MO, USA). Citral (66% geranial + 34% neral), 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 $\times$ 150 mm; Agilent Technologies, Santa Clara, CA, USA) was used. The sample rack was kept at a constant temperature of 20°C, and the column oven temperature was set to 35°C. A mobile phase starting at 70% water and 30% acetonitrile at 0 min, and then with a linear gradient to 22% water and 78% acetonitrile at 12 min, was used. The flow rate was 1 ml/min. Quantification was performed with external calibration curves of geraniol, geraniol hydroperoxide, geranial, and neral.

Synthesis

Geraniol hydroperoxide was synthesized as previously described (16), and used as the 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, as previously described (16). Samples were taken on a regular basis, and stored in the freezer under nitrogen until being analysed with HPLC–ultraviolet to determine the concentrations of geraniol and its major oxidation products. The oxidation was interrupted after 10 weeks, and thereafter the oxidation mixture was stored at –20°C during the patch test study. The oxidation mixture of geraniol was analysed, and found to contain 48% geraniol, 3.9% geranial, 3.5% geraniol hydroperoxide, and 1.0% neral. Samples were taken 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 (27), non-stabilized pet. (Prolabo, USA) was used for all patch test preparations. Patch test preparations were stored at –20°C until use. Each preparation was used for a test period of 6 months, during which time the preparation was stored in the refrigerator. Geraniol and oxidized geraniol were tested at 2.0% pet. throughout the study. The patch test concentrations of geranial and neral were adjusted during the study to determine whether this would detect more cases of contact allergy. Thus, geranial and neral were tested at 1.0% pet. during the years 2006 and 2007, and at 1.5% during the years 2008–2010. Citral was tested at 1.5% pet. during the years 2006–2008. During this period, patch testing was performed with oxidized limonene at 3.0% pet. and oxidized linalool at four different concentrations (2.0%, 4.0%, 6.0% and 11.0% pet.), owing to parallel studies. The standard allergens

used for patch testing were obtained from Chemotechnique Diagnostics AB (Vellinge, Sweden).

Patients and patch testing

Consecutive patients patch tested with the Swedish baseline series at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, during the period January 2006 to August 2010, were included in the study. Patch test preparations of approximately 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 the International Contact Dermatitis Research Group recommendations (28) on D3–4 and D7.

Results

The total numbers of tested patients, positive reactions and doubtful reactions are shown in Table 1. No irritant reactions to any of the test materials were seen during the study. A total of 14 of 2227 tested patients showed positive reactions to at least one of the test materials during the study (Table 2). Three patients reacted to geraniol, 11 to oxidized geraniol, 11 to geranial, 5 to neral, and 7 to citral. The reaction pattern and further details of these cases are shown in Tables 2 and 3. No difference in the frequency of positive reactions to geranial and neral after increases of the test concentrations from 1% to 1.5% was observed.

The pattern of concomitant reactions to the fragrance markers of the baseline series is also shown in Table 2. All 3 patients reacting to pure geraniol also showed positive patch test reactions to FMI. Ten of 11 patients reacting to

Table 1. Summary of tested patients, and frequencies of positive and doubtful reactions to geraniol, oxidized geraniol, geranial, neral, and citral

Test material	Total tested	Positive reactions (%)	Doubtful reactions (%)
Oxidized geraniol 2%	2179	12 (0.55)	16 (0.73)
Geraniol 2%	2227	3 (0.13)	12 (0.54)
Geranial 1%	948	6 (0.63)	1 (0.11)
Geranial 1.5% ^a	1204	5 (0.42)	3 (0.25)
Neral 1%	946	4 (0.42)	1 (0.11)
Neral 1.5% ^a	680	1 (0.15)	1 (0.14)
Citral ^b 1.5%	1055	7 (0.66)	3 (0.28)

^aThe concentrations of geranial and neral were adjusted during the study to determine whether this would detect more cases of contact allergy.

^bIsomeric mixture of geranial and neral (2:1).

Table 2. Summary of the 14 cases showing positive reactions to one or more of the test materials of the study

	Patient number																
	1	2 ^c	3	4	5	6	7	8	9	10	11	12	13	14 ^c			
Age (years)	20	15	40	44	51	12	76	54	49	54	31	23	55	52			
Sex	F	M	F	M	F	M	F	F	F	F	F	M	M	F			
Geraniol	—	++	—	++	—	+	—	—	—	—	—	—	—	—			
Oxidized geraniol	++	++	—	++	NT	+	++	+	++	—	+	++	++	+			
Citral ^a	—	++	—	++	+	+	++	—	++	+	NT	—	NT	NT			
Geraniol	—	++	+	++	+	+	++	—	NT	NT	NT	NT	NT	NT			
Geraniol	NT	NT	NT	NT	—	NT	NT	NT	++	++	+	—	++	+			
Neral	—	++	—	++	+	?	++	—	NT	NT	NT	NT	NT	NT			
Neral	NT	NT	NT	NT	NT	NT	NT	NT	—	—	+	NT	NT	NT			
Oxidized linalool	—	NT	—	—	—	—	—	—	—	NT	NT	NT	NT	NT			
4.0% ^b	NT	—	NT	NT	NT	NT	NT	+	NT	NT	NT	NT	NT	NT			
6.0% ^b	NT	NT	—	—	NT	—	+	+	?	NT	—	++	++	—			
Oxidized limonene	+	—	—	—	++	—	—	—	—	—	—	++	—	?			
FM I	+	+++	—	++	+	+	++	+	++	++	+	++	+	—			
FM II	NT	NT	—	++	NT	—	—	+	++	++	+	++	+	+			
MP	+	—	—	+	—	+	+	—	—	—	—	++	+	—			
HICC	—	—	—	—	—	—	—	+	—	++	—	—	—	—			
Colophonium	+	—	—	—	—	—	—	—	—	++	?	—	—	—			
Dermatitis location	Legs, face	Axillae, perioral	Axillae, face	Hands	Face	General	Legs	Hands	Hands, face	Hands	Hands, feet	Neck, arms	General	Hands			

FM, fragrance mix; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; MP, *Myroxylon pereirae*; NT, not tested.^aIsomeric mixture of geraniol and neral (2:1).^bPatch-tested as part of a parallel study.^cCase described in more detail in the Results section.

Table 3. Exposure and relevance assessments of the 14 patients showing positive reactions to 1 or more of the test materials of the study

Patient	Age (years)	Sex	Year tested	Dermatitis location	Occupation	Reaction to patch test material in the study	Relevance	Comments
1	20	F	2006	Legs, face	Student	Oxidized geraniol, FM I, MP	Unclear	No declaration of fragrances on products
2	15	M	2006	Axillae, perioral	Student	Geraniol, oxidized geraniol, citral, geraniol, neral, FM	Present	Citral and geraniol declared in deodorant. Positive reaction to aftershave, but fragrances not declared
3	40	F	2006	Axillae, face	Office	Geraniol	Probable	Use test with deodorant positive, fragrances not declared
4	44	M	2006	Hands	Truck driver	Geraniol, oxidized geraniol, citral, geraniol, neral, FM I, FM II, MP	Probable	Fragranced soap used at work, fragrances not declared
5	51	F	2006	Face	Office	Citral, geraniol, neral, FM I, oxidized limonene	Probable	Use test with day cream positive, fragrances not declared
6	12	M	2006	General	Student	Geraniol, oxidized geraniol, citral, geraniol, neral	Unclear	Used Aloe vera gel on temporary tattoo, fragrances not declared
7	76	F	2006	Legs	Retired	Oxidized geraniol, citral, geraniol, neral, FM, MP	Probable	Positive reaction to Arnica cream, containing many essential oils and plant extracts. Used many plant-based creams
8	54	F	2007	Hands	Housewife	Oxidized geraniol, oxidized linalool, FM I, FM II	Unclear	Positive reaction to detergent tested semi-open, no declaration of fragrances
9	49	F	2007	Hands, face	Masseuse	Oxidized geraniol, citral, geraniol, FM I, FM II	Present	Eczema since 2003 when using massage oils with rose, lavender, and bergamot. Geraniol and citral in essential oil of rose. Eczema when using fragranced oils. Negative patch test reaction to massage oil, use test positive
10	54	F	2008	Hands	Nurse	Citral, geraniol, FM I, FM II	Probable	The eczema healed after removal of fragranced products
11	31	F	2009	Hands, feet	Masseuse	Oxidized geraniol, citral, geraniol, neral, FM I, FM II	Present	Contact with many essential oils for massage, eczema improves when fragrance-free oil is used
12	23	M	2010	Neck, arms	Gardener	Oxidized geraniol, FM I, FM II, MP, oxidized linalool, oxidized limonene	Present	Eczema when using sunscreen. Positive reaction to sun lotion, fragrances not declared, no reaction to declared sunscreens
13	55	M	2010	General	Masseuse	Oxidized geraniol, geraniol, FM I, FM II, MP, oxidized linalool	Present	Used essential oils on eczema, which worsened nummular eczema. Used many products with essential oils. Citral declared in essential oil
14	52	F	2010	Hands	Masseuse	Oxidized geraniol, geraniol, FM II	Present	Positive reaction to massage oil containing citral. Improvement of eczema when fragrance-free oils are used

FM, fragrance mix; MP, *Myroxylon pereirae*.

oxidized geraniol also reacted to FM I. Nine of 11 patients with positive reactions to geranial, neral or citral were also tested with FM II, and 6 of these 9 patients reacted to FM II. Among the 8 patients reacting to FM II, 2 reacted to hydroxyisohexyl 3-cyclohexene carboxaldehyde. Six of 14 patients reacted to *Myroxylon pereirae*, whereas only 2 of 14 reacted to colophonium, which is associated with fragrance allergy. Four of 14 patients also showed positive reactions to oxidized linalool at 2.0%, 4.0%, or 6.0% pet., and 3 of 14 patients showed reactions to oxidized limonene 3.0% pet.

Among the reactions to geraniol, its oxidation products, or its metabolites, there was present relevance for the dermatitis for which the patient had been referred to the clinic for 6 patients. In these cases, citral or geraniol, or essential oils expected to contain these terpenes, were found on the labelling of products used on the locations of the patients' dermatitis. Four of these patients were masseurs, all of whom had noticed worsening of their eczema when using fragranced massage oils containing essential oils. One had stopped working as a masseur (patient 13). In addition, there was probable relevance for the fragrance materials in the study for 5 patients. On products purchased before 2005, the fragrance terpenes were not declared according to the EU directive (29), but positive use test results for perfumed day creams and plant-based creams were found in 3 of 5 patients, and fragranced sunscreens or soaps were identified as having been used on the locations of the patients' dermatitis.

We present here representative case reports of two cases (patients 2 and 14 in Table 2), in which patient 14 had occupational exposure to fragrances and essential oils.

Patient 2

An atopic 20-year-old man was referred with eczema of the axillae and the perioral region following the use of fragranced hygiene products. In addition to the reactions shown in Table 2, the patient had positive patch test reactions to Amerchol™ L101 (+), an aftershave (+), and a deodorant stick (+). The deodorant stick contained citral and geraniol as declared by the manufacturer, whereas the fragrance contents of the aftershave could not be determined, as no fragrance materials were declared on the labelling. The reactions to oxidized geraniol, geranial, neral, and citral were thus found to be relevant.

Patient 14

A non-atopic 52-year-old woman presented with a 5-year history of hand eczema. She had also experienced skin reactions from using deodorants and facial creams. She was working as a masseuse, using an array of different

massage oils, some of which contained essential oils. She suspected one massage oil to be the main cause of her hand eczema, as her hands improved when she avoided this massage oil. She had also noticed that she tolerated a fragrance-free massage oil; however, she could not always use this, as customers often booked aromatherapy treatments, which specifically included the fragranced massage oils. In addition to the reactions shown in Table 2, the patient had positive patch test reactions to nickel (++) and to the massage oil suspected to be the cause of the eczema (++) . This massage oil contained high concentrations of citral as declared by the manufacturer. The reactions to oxidized geraniol, geranial and FM II were thus found to be relevant.

Discussion

The present study gives an overview of consecutive patch testing with geraniol and its oxidation products and metabolites, as well as a focus on 14 patients reacting to these patch test materials in a clinical setting. In the present clinical study, we observed a pattern of concomitant reactions, showing that both activation pathways are important in the development of contact allergy to geraniol, as discussed below. The relevance of the positive reactions detected in the patients was high, and many were attributed to exposure to essential oils in the workplace.

The overall low frequency of positive reactions to pure geraniol (0.13%, 3 patients) seen in our study is in line with results from previous studies, and even lower than the frequency of positive reactions to geraniol in the German multicentre study (0.4% positive patch test reactions detected at geraniol 1% pet.) (3). The majority of the patients (11 of 14) with a positive reaction to any of the test materials in the present investigation reacted to oxidized geraniol. This is in accordance with our experimental results, which show that oxidized geraniol is more sensitizing than pure geraniol in the murine LLNA (16). A number of doubtful reactions were observed, both to geraniol and to oxidized geraniol, indicating the possibility of very weak positive responses that may become positive at a higher patch test concentration. The importance of investigating the responses to different patch test concentrations has clearly been shown for oxidized linalool (23, 24). In one of these studies, it was shown that many doubtful reactions became positive at a higher test concentration (24).

Theoretically, in patients reacting to pure geraniol and neral and/or geranial, metabolic conversion of geraniol to the allergenic metabolites geranial and neral can be suspected. In the present study, all 3 patients reacting to pure geraniol also had positive reactions to geranial

and citral, and 2 of 3 also reacted to neral. This pattern of concomitant reactions is most likely attributable to metabolism of geraniol to geranial and neral in the skin. In agreement with this, in the German multicentre study a high frequency of concomitant reactions to pure (or not intentionally oxidized) geraniol and citral was seen, as 11 of the 13 patients reacting to citral also reacted to geraniol, implying the possibility of metabolic conversion of geraniol in the skin to the components of citral, that is, geranial and neral (3).

On a similar note, in patients reacting only to oxidized geraniol and geranial and/or neral, the main route of activation is probably autooxidation. Among the 11 patients reacting to oxidized geraniol, 8 also reacted to geranial and 4 to neral. This indicates that geranial and neral are of importance in the sensitization to oxidized geraniol. Furthermore, 3 patients reacted to oxidized geraniol but not to geranial or neral. These patients may well be sensitized to geraniol hydroperoxide, formed in the autooxidation process but not metabolically. Geraniol hydroperoxide is a strong sensitizer according to the LLNA.

The observation that oxidized geraniol detected more cases than pure geraniol indicates that activation outside the skin is more important than metabolic bioactivation, but this may also be an effect of concentration, as small amounts of metabolites will be formed from geraniol 2.0% pet. at the location of the patch test, but not enough to give a positive test reaction. As oxidized geraniol contains the major haptens formed via both activation pathways, this patch test preparation could also detect cases that were originally sensitized because of bioactivation of geraniol. An alternative is to test with the metabolites suspected to be the main haptens. To evaluate this, further screening studies with increased test concentrations of pure and oxidized geraniol, and citral together with geranial and neral, should be performed.

On comparison of the reactions to geraniol and oxidized geraniol with the reactions to the fragrance markers of the baseline series, 11 of 12 patients reacting to oxidized geraniol also reacted to FM I, containing geraniol. However, only 3 of the 12 patients reacting to FM I also reacted to pure geraniol, whereas 10 of 12 reacted to oxidized geraniol (1 patient was not tested with oxidized geraniol). This supports the theory that oxidized geraniol might be a better marker for contact allergy to fragrances than pure geraniol. Although the purity of the commercial test material is well monitored during preparation, it is possible that the more frequent concomitant reactions

between FM I and oxidized geraniol are attributable to interactions between the other components of FM I and geraniol, or to the formation of oxidation products during use of the commercial test material. Of the patients reacting to geranial, 9 were tested with FM II (containing citral at 1% pet.), and 6 of 9 had positive reactions to FM II. This indicates that testing with citral in FM II does not detect all cases of contact allergy to citral.

It should be noted that no specific cross-reactivity was seen between oxidized geraniol and the other oxidized terpenes, that is, oxidized linalool and oxidized limonene. This is in agreement with our studies, in which we showed that the hydroperoxides, which are expected to be the main allergens in oxidized linalool and oxidized limonene, and also important allergen(s) in oxidized geraniol, behave as specific haptens (30, 31).

In the investigation of the 14 patients with positive reactions to the test substances of the study, it was found that 4 of these patients had exposure to essential oils at the workplace. These 4 patients were working as masseurs and had severe hand eczema, which, in some cases, had developed into chronic eczema. All of these patients found that they tolerated fragrance-free massage oils (Table 3). Although only patient 14 had a positive reaction to the massage oil that she used at work, the hand eczema of all the masseurs improved when they used massage oils without the addition of essential oils. Interestingly, in studies of contact allergy to essential oils, it has been shown that lemongrass oil, containing high concentrations of citral, is one of the most frequent sensitizers among the studied essential oils (32, 33).

The present study shows, for the first time, results from testing with the autooxidation mixture and metabolites of the prehaptens and prohaptens geraniol. The reaction pattern of the 14 cases presented indicates that both autooxidation and metabolism could be important in sensitization to geraniol. Screening with oxidized geraniol in higher concentrations in parallel with testing with the major autooxidation products/metabolites geranial and neral (i.e. citral) could provide more reliable tools for the detection of contact sensitization and cross-reactivity resulting from metabolic conversion to these fragrance haptens. It could provide an alternative to the present screening of contact allergy to geraniol with FM I. It is thus important that these alternative tools for the diagnosis of contact allergy are further investigated.

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