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Contact allergy to citral and its constituents geranial and neral, coupled with reactions to the prehapten and prohaptens geraniol

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Abstract

Background: Citral is commonly used as a fragrance and flavor material and consists of the aldehydes geranial and neral. Citral is included in fragrance mix (FM) II. Geranial and neral have also been identified in autoxidation of geraniol, a fragrance compound present in FM I.

Objectives: To study contact allergy to citral, geranial, and neral, and concomitant reactivity to oxidized geraniol and fragrance markers of the baseline series.

Methods: A total of 1476 dermatitis patients with suspected allergic contact dermatitis were patch tested using geranial, neral, and citral, all 3.5% petrolatum (pet.) as well as geraniol 6.0% and oxidized geraniol 11% pet. in addition to the Swedish baseline series.

Results: Frequencies of positive reactions to citral, geranial, and neral were 2.9%, 3.4% and 1.9%, respectively. Together, citral and geranial gave 4.2% positive patch test reactions in consecutive dermatitis patients. In patients with positive reactions to citral or its components, 25% to 34% reacted to FM II and 61% reacted to oxidized geraniol.

Conclusions: Patch testing with citral, its components, or oxidized geraniol detects contact allergic reactions not detected using the baseline series. Patch testing with pure geraniol was shown to be of little value. Geranial and neral, although closely chemically related, are concluded to be separate haptens.

KEYWORDS

allergic contact dermatitis, autoxidation, citral, fragrance mix I, fragrance mix II, geranial, geraniol, neral, oxidized geraniol, patch testing, RRID:SCR_001905

1 | INTRODUCTION

Geraniol and citral are commonly used fragrance materials in cosmetics as well as in household and hygiene products. Both compounds are found in natural plant oils and are produced industrially. In patch testing, geraniol is included in fragrance mix I (FM I) and citral in fragrance mix II (FM II); both are thus part of the European baseline

series for standardized patch testing of dermatitis patients with suspected contact allergy. Both are also included among the 26 fragrances that need to be identified on the label according to the Cosmetic Directive 76/768/EEC amended by 2003/15/EC.¹

Geraniol has been found on labels or detected by chemical analyses in about 40% to 60% of cosmetics and household and hygiene products.²⁻⁴ Geraniol is found in several natural oils, including

citronella oil, rose oil, and palmarosa oil.^{5,6} Citral is frequently used both as a fragrance chemical and as a flavor substance, and consists of a mixture of two aldehydes, geranial and neral in a ratio of 2:1. Geranial and neral are often produced by oxidation of geraniol⁷ (Figure 1). Citral has been detected in 25% of domestic and occupational products.^{3,4} Citral is the major component of lemongrass (*Cymbopogon citratus*) and *Litsea cubeba* oil, and is present in lemon and lime leaf oils.^{5,6}

Contact allergy to geraniol and citral has been frequently reported through the years. For citral, frequencies of 0.39% to 1.2% positive reactions have been reported from Sweden, Denmark, and England when tested at concentrations of 1.5% or 2.0% pet.⁸⁻¹⁰ Purity and test dose were not always reported, which is a possible source for variation. Citral 3.5% pet. has been patch tested in a study at the University of Gothenburg, giving 0.92% positive reactions in 655 patients. In the same study, geranial and neral were tested 3.5% pet., yielding 2.0% and 0.92% positive reactions, respectively.¹¹ Irritation was investigated before the study and found to be low; patch test concentrations were subsequently chosen according to guidelines.¹²

Concomitant reactions to fragrance haptens are often recorded and can be explained by coupled exposure from scented products; however, elicitation due to cross-reactivity has also been discussed.^{13,14} It is therefore difficult to measure true cross-reactivity between fragrance haptens in dermatitis patients. Today, sensitization studies in the guinea pig are the only way to explore cross-reactivity. Recent research has revealed a complicated pattern of activation and formation of skin sensitizers involving geraniol and citral. Geraniol is prone to autooxidation, forming geraniol hydroperoxides and the isomeric aldehydes geranial and neral (components of citral) which are all skin sensitizers.¹⁵ Geraniol is also metabolized by skin enzymes to geranial and neral¹⁶ (Figure 1). It is important to investigate these findings clinically, as this can have an impact on diagnosis and prevention of contact allergy to citral and geraniol.

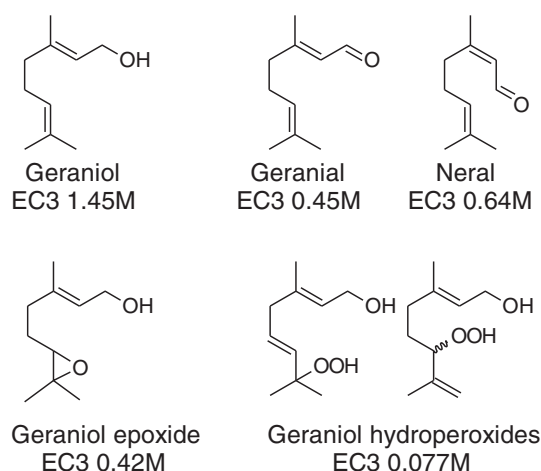


FIGURE 1 Molecular structures of geraniol and skin-sensitizing oxidation products identified after autooxidation or bioactivation of geraniol. Geranial and neral constitute the fragrance citral. The sensitizing potencies of each compound in the local lymph node assay are indicated below each structure as EC3 value

It is known that testing with the oxidation mixture of geraniol will detect more cases than testing with pure geraniol.¹⁷⁻¹⁹ We also know that patch testing with citral at a higher test concentration in parallel to FM II will identify more cases of contact allergy.⁹ Moreover, our previous investigations have suggested that geranial, the major isomer of citral, might be a better screening substance for contact allergy compared with citral.^{11,17} The pattern of concomitant reactions to these compounds has been investigated in a single-center study on consecutive dermatitis patients.¹³

The aim of this study was to investigate frequencies of positive reactions to citral, geranial and neral 3.5% pet. The pattern of concomitant reactions to these fragrance sensitizers as well as geraniol and oxidized geraniol, all patch tested in parallel, was also studied. The results were evaluated together with results from oxidized geraniol, tested concomitantly but published separately.¹⁹

2 | MATERIALS AND METHODS

Geranial (98%), neral (>99%), and citral (neral 34% and geranial 66%; Figure 1) were obtained from Bedoukian Research (Danbury, Connecticut). High-performance liquid chromatography (HPLC) analyses were conducted on an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, California) with a 1260 diode array detector VL+ detector. An Eclipse Plus C₈ column (3.5 μ m, 4.6 $\times$ 150 mm; Agilent Technologies) was used. Analysis of geranial, neral, and citral was conducted with external standards as previously described.¹¹ All chemicals were distilled prior to use. No contaminants could be detected in the analysis of the distilled chemicals.

2.1 | Patch test preparations

Based on previous experience,²⁰ non-stabilized pet. (Prolabo, Chester, Pennsylvania) was used as vehicle in the patch test preparations. The patch test preparations consisted of neral 3.5% wt/wt, citral 3.5% wt/wt, and geranial 3.5% wt/wt, all in pet. The preparation of citral 3.5% thus contained geranial 2.3% and neral 1.2%. Test preparations of the fragrance markers of the baseline series were obtained from Chemotechnique Diagnostics (Vellinge, Sweden). The substances were stored in a freezer at -20°C at the Department of Dermatology, University of Gothenburg (Gothenburg, Sweden) and patch test preparations were prepared shortly before the start of the study and sent out to the other participating clinics. The patch test preparations were stored in a refrigerator when in use. New preparations were prepared after 6 months and distributed to the participating clinics.

2.2 | Test centers and patients

This study included five dermatology clinics in Sweden: Sahlgrenska University Hospital (Gothenburg), Skåne University Hospital (Malmö), Northern Sweden University Hospital (Umeå), Uddevalla Hospital (Uddevalla), and University Hospital of Örebro (Örebro), all members of the Swedish Contact Dermatitis Research Group. The participants

TABLE 1 Positive (+, ++, +++) and doubtful (?) patch test reactions in 1476 patients patch tested with geranial, neral, and citral in five dermatology clinics

Site	Total tested	Citral 3.5%					Geranial 3.5%					Neral 3.5%							
		Positive n	Positive %	CI	Doubtful n	Doubtful %	CI	Positive n	Positive %	CI	Doubtful n	Doubtful %	CI	Positive n	Positive %	CI	Doubtful n	Doubtful %	CI
Gothenburg	388	14	3.6	2.0-5.9	6	1.5	0.6-3.2	13	3.4	1.8-5.5	2	0.5	0.09-1.7	11	2.8	1.5-4.9	2	0.5	0.09-1.7
Malmö	672	16	2.4	1.4-3.8	32	4.8	3.3-6.6	18	2.7	1.6-4.2	46	6.8	5.1-9.1	7	1.0	0.46-2.1	30	4.4	3.1-6.3
Uddevalla	182	8	4.4	2.0-8.3	3	1.6	0.42-4.5	11	6.0	3.1-11	6	3.3	1.3-6.9	7	3.8	1.7-7.6	4	2.2	0.7-5.3
Umeå	134	2	1.5	0.25-4.9	3	2.2	0.57-6.1	3	2.2	0.57-6.1	3	2.2	0.57-6.1	1	0.7	0.04-3.7	3	2.2	0.57-6.1
Örebro	100	3	3	0.76-8.2	2	2	0.34-6.6	5	5	1.8-11	5	5	1.8-11	2	2	0.34-6.6	0	0	-
All	1476	43	2.9	2.1-3.9	46	3.1	2.3-4.1	50	3.4	2.5-4.4	62	4.2	3.2-5.3	28	1.9	1.3-2.7	39	2.6	1.9-3.6

Note: The bold indicates highlights the most important parts of the table.
Abbreviation: CI, confidence interval.

in the study were patients who were referred for patch testing because of suspected allergic contact dermatitis during 2012. A total of 1476 patients, 984 (67%) women and 492 (33%) men, were patch tested.

The test substances were applied to the upper part of the back in addition to the ordinary patch test preparations. Approximately 20 mg of test materials were applied in small Finn Chambers (Epitest, Tuusula, Finland; 8 mm diameter, 0.5 cm²) on Scanpor tape (Norgesplaster, Vennesla, Norway). Test preparations were applied to the chambers directly before application on the back of the patients. After 48 hours of occlusion, the patches were removed by the patients. Readings were performed on day (D) 3 or D4 and on D7, according to the recommendations of the ESCD.^{21,22}

Recently, 16 factors of possible significance for the outcome of multicenter patch test studies were published together with a ranking of the study based on scoring of the 16 factors.²³ The present study is ranked as a study with excellent quality with regard to patch testing with neral, citral, geranial, geraniol, and oxidized geraniol. The highest scores were obtained for all factors except for variation in adhesive tapes, no control of the adhesiveness after 48 hours, as well as lack of calibration of reading and monitoring.

2.3 | Statistical analysis

Analyses were performed using R version 3.0.3 (The R foundation for Statistical Computing, Vienna, Austria; RRID:SCR_001905). All tests were two-sided and $P < .05$ was considered statistically significant. The exact binomial test was used to test for differences in positive reactions between the test materials. Confidence intervals of frequencies of contact allergy were calculated using the Mid-P exact test and OpenEpi (<http://openepi.com>). Venn diagrams were drawn using eulerAPE.²⁴

3 | RESULTS

A summary of patch test results is presented in Table 1. Geranial 3.5% pet. yielded the highest frequency of positive reactions (3.4%). The frequencies of contact allergy to geranial and citral were found to be significantly higher than that of neral ($P < .001$ and $P = .004$, respectively). No significant difference could be seen in frequencies of contact allergy to geranial and citral ($P = .28$). Positive reactions were detected early; only 2%, 6%, and 4% of positive reactions appeared on day 7 for citral, geranial, and neral, respectively. Doubtful reactions are also presented in Table 1. Irritant reactions were reported in a total of four patients at three clinics (0.3%). One patient showed an irritant reaction to neral only, two patients showed irritant reactions to all three patch test preparations, and one showed irritant reactions to neral and citral.

The distribution of strength of positive reactions to geranial 3.5%, neral 3.5% and citral 3.5% is presented in Table 2. Patch test results stratified according to gender are presented in Table 3. Contact allergy to either of geranial, neral, or citral was more frequent in

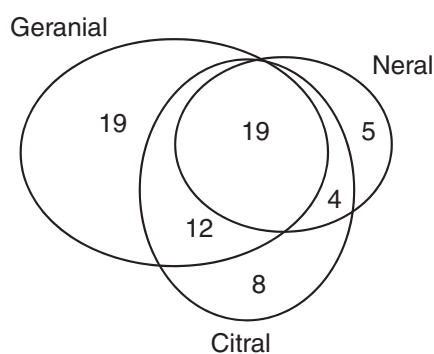
TABLE 2 Positive reactions to geranial, neral, and citral differentiated stratified for strength of the reaction

	Total positive	+	% +	++/+++	% ++/+++
Citral 3.5%	43	29	67	14	33
Geranial 3.5%	50	40	80	10	20
Neral 3.5%	28	19	68	9	32

TABLE 3 Positive reactions to geranial, neral, and citral stratified for gender

	Tested men	Positive men n (%)	CI	Tested women	Positive women n (%)	CI	Gender differences (P-value)
Citral 3.5%	492	8 (1.6)	0.76-3.1	984	35 (3.6)	2.5-4.9	.047
Geranial 3.5%	492	11 (2.2)	1.2-3.9	984	39 (4.0)	2.9-5.4	.094
Neral 3.5%	492	3 (0.61)	0.15-1.7	984	25 (2.5)	1.7-3.7	.008

Abbreviation: CI, confidence interval.

**FIGURE 2** Venn diagram representing the pattern of concomitant reactions to geranial 3.5%, neral 3.5%, and citral 3.5% among the 67 patients with positive reactions to any of these test materials

women. The difference was significant for neral ($P = .008$) and citral ($P = .047$). No significant difference in frequencies of contact allergy between genders could be seen for geranial ($P = .051$).

The patterns of concomitant reactions to geranial, neral, and citral are presented in Figure 2. In total, 67 patients reacted to any of the patch test materials, with the highest proportion of contact allergy detected for geranial. Patch testing with only geranial would detect 74% of cases, whereas patch testing with neral or citral would detect 41% and 64% of cases, respectively. Many patients reacted to all three test preparations. Between the isomers geranial and neral, 19 of 51 (35%) patients reacting to geranial also reacted to neral, and 19 of 29 (67%) reacting to neral also reacted to geranial (Figure 2). Concomitant reactions to FM II, containing citral at 1%, were observed in 25% to 34% of the cases (Table 4). The association was strongest for patients with contact allergy to neral, where 34% reacted to FM II. Concomitant reactions to FM I were more common, with 33% to 45% concomitant reactions. Concomitant reactions to any of the fragrance markers of the baseline series were observed in 45% to 62% of the cases.

Patch testing with distilled geraniol 6.0% and oxidized geraniol 11.0% pet. (containing geranial at 0.5% and neral at 0.1%) was performed in the same cohort, and has been previously reported.¹⁹

Concomitant reactions to oxidized geraniol 11.0%, among patients with positive patch test reactions to geranial, neral, or citral are presented in Figure 3. The majority of patients, in all 61% of those reacting to geranial, neral, or citral ($n = 67$), also reacted to oxidized geraniol. Furthermore, concomitant reactions to geranial, neral, or citral were found in 53% of patients with positive reactions to pure geraniol ($n = 15$). Conversely, concomitant reactions to pure geraniol were found in 12% of patients with positive reactions to geranial, neral, or citral.

4 | DISCUSSION

Frequencies of positive patch test reactions to citral, geranial, and neral are all higher than in previous studies. Looking specifically at citral, the frequency of 2.9% is higher than that found in the earlier study (0.92%) in Gothenburg, using the same patch test dose and test method.¹¹

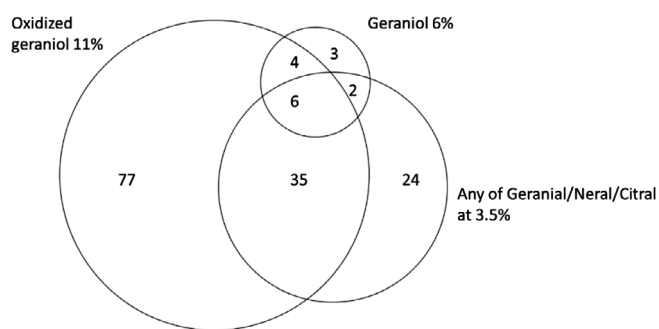
Using Finn chambers and a dose of 1.4 mg/cm², geranial and citral gave positive reactions in 4.2% of the tested patients. Of these patients, 3.4% would have been detected if testing with geranial only and 2.9% if only citral had been used for testing. Neral gave significantly fewer positive reactions compared with geranial and citral, which makes neral a less important alternative for screening. This may be a result of a lower exposure to neral when exposed to citral as a fragrance, as the ratio of neral to geranial is 1:2. The sensitizing potencies of geranial and neral are similar (0.45M and 0.64M, respectively) according to the local lymph node assay.⁸ As in earlier studies, it was found that citral can be patch tested at 3.5% pet. (1.4 mg citral/cm²) without causing irritation, and additional cases of contact allergy are detected using this dose. In both the present study and in earlier studies, very few cases of irritation are recorded and larger studies using this patch test concentration are desirable to validate this test concentration.

Geranial and neral are structural isomers and thus patterns in patch test reactions are interesting to study. First, concomitant reactions to geranial and neral should be expected due to simultaneous

TABLE 4 Concomitant reactions to the fragrance markers of the Swedish baseline series of 2012 in the 67 patients reacting to geranial, neral, or citral

	Positive reactions	Fragrance mix I		Fragrance mix II		HICC		<i>Myroxylon perei</i>		Colophonium		≥1 Fragrance marker	
		n	%	n	%	n	%	n	%	n	%	n	%
Citral 3.5%	43	15	35	12	28	4	9	6	14	3	7	21	49
Geranial 3.5%	51	17	33	13	25	4	8	6	12	3	6	23	45
Neral 3.5%	29	13	45	10	34	4	14	4	14	2	7	18	62

Note: The bold indicates highlights the most important parts of the table.
Abbreviations: HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.

**FIGURE 3** Venn diagram representing the pattern of concomitant reactions to geranial/neral/citral, oxidized geranial 11%, and geranial 6.0% among the 151 patients with positive reactions to any of these test materials

exposure when citral is added to products. Second, cross-reactivity is a possibility due to the similarity in their chemical structures. Third, geranial and neral are structural isomers, differing only in the *cis* or *trans* orientation of the double bond and an oxidative or metabolic conversion between the two has been shown experimentally,²⁵ but previously not studied clinically.

In experimental studies of autooxidation of geranial (*trans*-oriented double bond; Figure 1) not only geranial (*trans*-oriented double bond) but also neral (*cis*-oriented double bond) was detected, showing that neral was formed through isomerization during the oxidative spontaneous reaction.⁸ The same was observed during *in vitro* experiments of cutaneous metabolism of geranial.⁹ However, isomerization of geranial to neral or vice versa was not confirmed in the present study as contact allergy to only one of the two isomers was common (Figure 2). Only 35% of patients reacting to geranial also reacted to neral and 67% reacting to neral also reacted to geranial (Figure 2). This is a large overlap but still indicates that patients are reacting to the respective isomer.

Concomitant reactions to geranial and neral do not show a symmetrical pattern. There are several examples in the literature of pairs of diastereomers and enantiomers where sensitization to one isomer in either patients or guinea pigs does not lead to elicitation when exposed to the other isomer of the pair.^{26–28} Thus, very small differences in structure are sufficient for the immune system to perceive compounds as different. In the Gothenburg study, the overlap of reactions to geranial and neral was less, with 27% of those positive to geranial and 50% of those positive to neral also positive to the other

isomer.¹¹ The frequencies of concomitant reactions in the clinical studies are similar to those between different fragrance compounds or mixes.^{12,18} Thus, geranial and neral are not likely to cause extensive cross-reactivity and could be considered as separate haptens, although this has not been studied experimentally. It should be noted that eight of 43 patients reacted only to citral and not to either geranial or neral. This could be due to the enhanced effect of mixtures, requiring lower test concentrations to elicit a contact allergic reaction.²⁹

In the mixture of oxidation products from autooxidized geranial, strongly sensitizing geranial hydroperoxides were detected in addition to geranial and neral. The oxidation mixture of geranial at 10 weeks contained geranial 59% (wt/wt), geranial 4.6%, neral 1.2%, and geranial hydroperoxide 3.7%.¹⁹ Only 34% of patients with positive reactions to oxidized geranial also reacted to one or more of the aldehydes (Figure 3). It is thus likely that the geranial hydroperoxides rather than the aldehydes are the major culprit of the reactions to oxidized geranial. Conversely, among patients with positive reactions to the aldehydes, 61% of these patients also showed reactions to oxidized geranial. This can be explained by the lower test concentration of aldehydes in the preparations of oxidized geranial. The majority of the patients reacting to geranial showed positive reactions to the aldehydes or to oxidized geranial 11% (Figure 3), thus indicating that geranial is mainly a prehapten and prohapten and not a sensitizer on its own. This is in line with findings from the studies of differences in activation of dendritic cells during exposure to the pure terpenes linalool and limonene and the hydroperoxides, respectively.³⁰ It was shown that the pure terpenes induced a limited activation, but that mainly linalool hydroperoxides and limonene hydroperoxide induced a significant activation.

In FM II, citral is included at a concentration of 1%. The pattern of concomitant reactivity to FM II was investigated in patients reacting to citral 3.5% pet. We found that only 28% of the citral-positive patients also reacted to FM II. Thus, 72% of the patients allergic to citral 3.5% would not have been detected using only FM II. Similar discrepancies have been recorded when patch testing with citral 2.0%, where 60% to 70% of citral-positive patients showed concomitant reactions to FM II.^{8–10} Thus, the larger the difference in patch test dose, the larger the difference in the result of concomitant patch testing is to be expected.

TABLE 5 Seven cases with positive reactions to geranial, neral, citral, oxidized geranial, or pure geraniol with exposure assessment judged as clinically relevant

Site	Sex	Age	Occupation	Positive reactions to study materials	Geraniol exposure	Citral exposure	Reaction to fragrance markers, baseline series	Localization of dermatitis
Gothenburg	F	47	Masseur, teacher	Geranial, neral, citral, oxidized geraniol	Rose-scented hand soap, essential oils	Dishwashing product, rose-scented hand soap, essential oils	Myroxylon pereirae, FM I	Hands, feet
Gothenburg	F	54	Masseur	Citral, oxidized geraniol	Massage oils	Massage oils	-	Hands, arms
Malmö	F	23	?	Citral, oxidized geraniol	Silicon drops for hair care	-	FM I	Scalp, face, hands
Malmö	F	40	Office	Oxidized geraniol	Body lotion	-	-	Hands
Uddevalla	F	39	?	Geranial, neral, citral, oxidized geraniol, geraniol	Essential oils, massage balm tonic	Massage balm tonic	FM I, FM II	Face, eyelids, lips, arms, hands, legs
Uddevalla	F	25	?	Oxidized geraniol	Hand soap	-	-	Hands
Umeå	F	24	Spa therapist	Geranial, neral, citral, oxidized geraniol	Massage oils	Massage oils	FM I, FM II, HICC	Hands

Abbreviations: ?, not known; FM, fragrance mix; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Interestingly, of the patients positive to citral or its components, more patients reacted to FM I than to FM II (Table 4). Besides, in the previous clinical study of citral and its components, concomitant reactivity to FM I was more common than that to FM II; however, the frequencies of concomitant reactions were lower.¹¹ This is probably due to concomitant sensitization to the fragrance compounds of FM I, as the present study suggests that patch testing with purified geraniol in itself does not detect contact allergy to citral (Figure 3). Further, frequencies of concomitant reactions to FM I and FM II are similar to those of other studied fragrance compounds, namely, oxidized linalool, oxidized limonene, and oxidized linalyl acetate,³¹⁻³³ probably reflecting a broad exposure to many fragrance compounds in fragranced products. In addition, in these instances, a higher frequency of concomitant reactions to FM I was seen.

Investigations of clinical relevance of positive reactions among the patients identified rinse-off products and essential oils as the most common sources of exposure to geraniol and citral (Table 5). Although a small sample, this is in accordance with other studies on oxidized fragrance terpenes.^{31,34,35} In the European Union regulation, geraniol and citral are included in a group of 26 fragrance chemicals that must be labeled on cosmetic products in the European Union when used in concentrations greater than 10 ppm in leave-on products and greater than 100 ppm in rinse-off products, according to the Cosmetics Regulation.¹ This information should make it possible for patients with allergies to these fragrance materials to avoid products where the respective fragrance is listed among the labeled contents. In other parts of the world, such labeling is still not mandatory.

Patch testing with citral 3.5% pet. detects more positive patch test reactions than today's patch test material included in FM II. If we are to detect allergic patients and to realistically map the frequencies of contact allergy to fragrance materials, it is important to use reliable and appropriate tools. Otherwise, the scope of contact allergy will not be correctly represented and problems will not be identified. Using geranial along with citral will identify even more patients, and using this diagnostic tool could be of importance when investigating fragrance allergy in a patient. The many cases of contact allergy in this study not detected by FM I and FM II show the importance of patch testing for fragrance allergy with a broad set of fragrance haptens, especially as fragrance allergy can be difficult to suspect based on the patients' history.^{10,36,37}

5 | CONCLUSION

Patch testing with oxidized geraniol, citral, or the aldehydes geranial and neral all show contact allergic reactions not detected with the baseline fragrance materials used today. Using both citral and geranial in parallel detects 4.2% positive patch test reactions in consecutive dermatitis patients. Patch testing with pure geraniol is of little value compared to patch testing with the materials listed earlier. Geranial and neral, although closely chemically related, are concluded to be separate haptens. It is likely that the geraniol hydroperoxides rather than the aldehydes are the major culprits concerning reactions to oxidized geraniol. Further work should be performed with regard to

evaluation of patch test materials of oxidized geraniol. Geraniol and citral at higher concentrations as used in this study should be made available for routine patch testing of dermatitis patients.

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CONFLICTS OF INTEREST

Magnus Bruze is a member of an expert panel for fragrance safety ([www.http://fragrancesafetypanel.org/](http://fragrancesafetypanel.org/)).

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