

Contact sensitization to limonene and linalool hydroperoxides in Spain: a GEIDAC* prospective study

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Summary

Background. Limonene and linalool are common fragrance terpenes widely used in cosmetic, household and hygiene products. Their primary oxidation products formed after air exposure, the hydroperoxides, have been recognized as important contact haptens.

Objectives. To investigate the prevalence of contact allergy to hydroperoxides of limonene (Lim-OOHs) and hydroperoxides of linalool (Lin-OOHs) in Spain, and to define the optimal concentration for screening in consecutive patients.

Methods. Three different concentrations of Lim-OOHs (0.1%, 0.2% and 0.3% pet.) and Lin-OOHs (0.25%, 0.5% and 1.0% pet.) were simultaneously tested in 3639 consecutive patients at 22 departments of dermatology in Spain.

Results. Lim-OOHs at 0.1%, 0.2% and 0.3% yielded positive patch test reactions in 1.4%, 3.4% and 5.1% of the tested patients, respectively; and Lin-OOHs at 0.25%, 0.5% and 1.0% yielded positive reactions in 1.3%, 2.9% and 4.9% of the tested patients, respectively. Few irritant (1.5–1.9%) and doubtful reactions (0.4–0.5%) to both terpene hydroperoxides were registered at the highest concentrations tested.

Conclusions. Lim-OOHs and Lin-OOHs can be considered as common causes of contact allergy, and their inclusion in an extended baseline patch test series therefore seems to be appropriate. The patch test preparations of Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. are useful tools for screening of contact sensitization.

Key words: allergic contact dermatitis; fragrance allergy; hydroperoxides; limonene; linalool.

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Limonene and linalool are common fragrance terpenes that are present in a wide variety of products that come into close contact with the skin. Owing to the fresh citrus odour of limonene and the fresh flowery aroma of linalool, these compounds are commonly used not only in fine fragrances, but are also very often in many domestic and occupational products (1–4).

Limonene and linalool are known to be prehaptenes (i.e. substances that are prone to be transformed by air oxidation into more allergenic compounds), and their primary oxidation products formed after air exposure, the hydroperoxides, have been recognized as important contact haptens (5–10). Pure (not intentionally oxidized) limonene and linalool have been tested in several studies, with very few positive patch test reactions being recorded in consecutive patients (11–13). However, high prevalences of contact allergy to hydroperoxides of limonene (Lim-OOHs) and hydroperoxides of linalool (Lin-OOHs) have been reported in recent studies (13–15). Owing to their skin-sensitizing capacity, limonene and linalool belong to the group of fragrance chemicals that must be labelled on cosmetic products when used in concentrations >10 ppm in leave-on products and >100 ppm in rinse-off products in the EU (16). These two fragrances, however, are not routinely patch tested in Spain or in other European countries. Moreover, the best patch test preparation and the optimum concentration for testing remain to be established, considering the high number of irritant/doubtful reactions recorded in previous studies (13–15). Currently, testing with Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. is regarded as the best option (13–15, 17).

The aim of the present study was to investigate the prevalence of contact allergy to Lim-OOHs and Lin-OOHs in Spain, and to investigate the optimal concentration for detection of contact allergy to these fragrance terpenes in consecutive patients. Furthermore, the clinical features of patients with positive patch test reactions and, in particular, the relevance for each individual case were assessed.

Materials and Methods

During the period from May 2015 to February 2016, 3639 consecutive patients undergoing patch testing because of suspected allergic contact dermatitis at 22 departments of dermatology belonging to the GEIDAC (Grupo Español de Investigación de Dermatitis de Contacto y Alergia Cutánea – Spanish Contact Dermatitis Research Group network) were screened with Lim-OOHs and Lin-OOHs, in addition to regular patch testing. Three different concentrations of Lim-OOHs (0.1%, 0.2% and 0.3% pet.) and Lin-OOHs (0.25%, 0.5% and 1.0% pet.)

were simultaneously tested. Patients aged <18 years were excluded from the study.

In all participating centres, standard haptens used for patch testing were provided by Chemotechnique Diagnostics (Vellinge, Sweden). The final preparation of the fragrance terpenes was performed in close collaboration with the Department of Dermatochemistry and Skin Allergy, University of Gothenburg, Sweden. Considerable efforts were made to develop the oxidation procedures in such a way that a raw material containing a standardized amount of hydroperoxides with defined stability was achieved. These also included the development of sophisticated high-performance liquid chromatography methods to analyse the content in the raw material and in each released batch of syringes. The test preparations labelled Lim-OOHs contained oxidized *R*-limonene with a validated content of a mix of limonene-1-hydroperoxide and limonene-2-hydroperoxide, and the test preparations labelled Lin-OOHs contained oxidized linalool with a validated content of the major Lin-OOHs and the minor Lin-OOHs.

Patch tests were applied on the patient's upper back with IQ Ultimate™ chambers (Chemotechnique), and occluded for 48 h. Visual readings were performed twice, on day (D) 2 and D4/D5, and were scored according to the ESCD patch test guideline (18). The strength of positive (allergic) reactions was designated as + (weak positive reaction: erythema, infiltration, and possibly papules), ++ (strong positive reaction: erythema, infiltration, papules, and vesicles), or +++ (extreme positive reaction: intense erythema, infiltrate, and coalescing vesicles). Irritant responses (well-defined inflammation limited to the exposure area, without infiltrate, composed mostly of papules, which decreased between D2 and D4), doubtful (?+) responses or negative readings were interpreted as non-allergic.

Information regarding clinical features (e.g. age, sex, atopy, and site of lesions) was obtained before testing. In addition, in the case of a positive patch test reaction to Lim-OOHs and/or Lin-OOHs, the relevance of this positive reaction for the patient's contact dermatitis was assessed on the basis of the patient's history and the presence of these compounds in the list of ingredients of one or more products used by the patient, and accordingly classified as follows: 'present', when the product containing the fragrance terpene that had elicited the positive patch test reaction was used by the patient at the time of testing on areas affected by dermatitis; 'past', when the suspected product was used at an earlier time; and 'uncertain', when no suspected product could be identified.

All analyses were carried out with the SPSS 15.0 statistical package. The study was approved by the ethics

committee (EC-2015/0039/I) of the Hospital del Mar site as promoter and by each involved centre, and was conducted in accordance with the Declaration of Helsinki and related local and European regulations. All participants provided written informed consent to participate in the study.

Results

Of the 3639 patients tested, 292 (8.0%) had positive patch test reactions to one or both hydroperoxides (at any concentration): 187 (5.1%) patients to Lim-OOHs, 179 (4.9%) to Lin-OOHs, and 74 (2.0%) to both terpene hydroperoxides.

The numbers of patients with positive, doubtful and irritant patch test reactions to Lim-OOHs and Lin-OOHs at each tested concentration, along with the distribution of the strength of the positive reactions, are shown in Table 1. Overall, when the patch test concentrations of both terpene hydroperoxides were higher, an increasing number of patients with positive and irritant reactions and a decreasing frequency of doubtful reactions were noted. For the highest concentrations tested, 187 (5.1%) patients had positive patch test reactions to Lim-OOHs 0.3% pet. and 179 (4.9%) to Lin-OOHs 1.0% pet. Sixty-three of the 187 (33.7%) patients with positive reactions to Lim-OOHs 0.3% pet. and 73 of the 179 (40.8%) patients with positive reactions to Lin-OOHs 1.0% pet. would not have been diagnosed if only the next lower patch test concentrations had been used. Likewise, 33.3% and 39.3% of the doubtful reactions to Lim-OOHs 0.2% pet. and to Lin-OOHs 0.5% pet., respectively, were interpreted as positive reactions at the highest concentrations.

The numbers of patients with positive, doubtful and irritant reactions to Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. from each participating centre are shown in Table 2. Lim-OOHs 0.3% pet. (range: 0–24.8%) and Lin-OOHs 1% pet. (range: 0–13.3%) showed a wide range in the frequency of positive reactions between the 22 centres. Conversely, Lim-OOHs 0.3% pet. (range: 0–3.6%) and Lin-OOHs 1% pet. (range: 0–2.8%) showed a narrow range in the frequency of doubtful reactions.

The MOAHLFA index (Male, Occupational, Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age ≥ 40 years) and the physician's assessment of the relevance of the contact allergy for the patient's dermatitis registered from patients with positive reactions to Lim-OOHs and Lin-OOHs at the highest tested concentrations are shown in Table 3. The mean age of the patients with positive reactions to Lim-OOHs 0.3% pet.

($n = 187$) was 46 years (range: 18–89 years). One hundred and thirty-one (70.1%) patients were female, and 34 (18.2%) had occupational contact allergy. Patients with contact allergy to Lin-OOHs 1.0% pet. ($n = 179$) had a mean age of 50 years (range: 18–90 years). One hundred and two (57.0%) patients were female, and 25 (14.0%) had occupational contact dermatitis. The most common sites affected for both terpene hydroperoxides were the hands (41%) and the face (27–32%).

Regarding relevance, present exposure to one or several products containing limonene, used on the dermatitis area, was documented for 86 of the 187 (46.0%) patients with positive reactions to Lim-OOHs 0.3% pet., and past exposure for 5 (2.7%) patients. Similarly, for 84 (46.9%) and 3 (1.7%) of the 179 patients with positive reactions to Lin-OOHs 1% pet., the reactions were judged to have present and past relevance, respectively. The most common products containing limonene and/or linalool of clinical relevance to the patients' allergic reactions were cosmetics and fine fragrances (judged to be relevant in 57 patients), soaps ($n = 21$), hair products ($n = 18$), moisturizers ($n = 17$), and detergents ($n = 12$). Less common products included deodorants ($n = 8$), essential oils ($n = 5$), toothpaste ($n = 4$), and wet wipes ($n = 2$).

Table 4 shows the number and percentage of patients with positive reactions to Lim-OOHs and to Lin-OOHs, at each tested concentration, who also reacted to other fragrance markers [fragrance mix I (FM I), fragrance mix II (FM II), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), and *Myroxylon pereirae*] and/or colophonium in the baseline patch test series, which were tested concomitantly. The overall frequency of concomitant reactions to other fragrance markers and/or colophonium in patients with positive reactions to Lim-OOHs 0.3% pet. was 31.0% (58/187), and in patients with positive reactions to Lin-OOHs 1.0% pet. the frequency was 33.0% (59/179).

Discussion

Limonene and linalool are ubiquitous allergens, and are among the most common fragrance ingredients according to several studies (2–4). Although these fragrance terpenes are present in a significant number of personal care products, contact dermatitis will only occur in the vast majority of the patients if these compounds have been previously oxidized after air exposure. Importantly, quantifiable levels of Lim-OOHs and Lin-OOHs could be present in commercially available fine fragrances and cosmetics, owing to autoxidation (19, 20).

Table 1. Numbers and percentages of patients with positive, doubtful and irritant patch test reactions to hydroperoxides of limonene (Lim-OOHs) and hydroperoxides of linalool (Lin-OOHs) at each respective concentration. The distribution of the strength of positive reactions is also shown

Fragrance terpene	Patch test concentration	No. of positive patch test reactions (%)			No. of doubtful patch test reactions (%) ^a	No. of irritant patch test reactions (%) ^a
		Total (%) ^a	+	++/+++		
Lim-OOHs	0.1% pet.	51 (1.4)	34/51 (66.7)	17/51 (33.3)	25 (0.7)	11 (0.3)
	0.2% pet.	124 (3.4)	90/124 (72.6)	34/124 (27.4)	21 (0.6)	30 (0.8)
	0.3% pet.	187 (5.1)	112/187 (59.9)	75/187 (40.1)	14 (0.4)	55 (1.5)
Lin-OOHs	0.25% pet.	46 (1.3)	33/46 (71.7)	13/46 (28.3)	31 (0.9)	9 (0.2)
	0.5% pet.	106 (2.9)	64/106 (60.4)	42/106 (39.6)	28 (0.8)	42 (1.2)
	1.0% pet.	179 (4.9)	109/179 (60.9)	70/179 (39.1)	17 (0.5)	70 (1.9)

^aRegarding the total of patients tested (n = 3639).**Table 2.** Total numbers of patients from each respective test centre and the numbers and percentages of positive, doubtful and irritant patch test reactions to hydroperoxides of limonene (Lim-OOHs) 0.3% pet. and hydroperoxides of linalool (Lin-OOHs) 1.0% pet.

Test centre	Total no. tested	Lim-OOHs 0.3% pet.			Lin-OOHs 1.0% pet.		
		No. of positive patch test reactions (%)	No. of doubtful patch test reactions (%)	No. of irritant patch test reactions (%)	No. of positive patch test reactions (%)	No. of doubtful patch test reactions (%)	No. of irritant patch test reactions (%)
Alcorcon	228	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alicante	177	7 (4.0)	0 (0)	17 (9.6)	5 (2.8)	0 (0)	11 (6.2)
Badalona	168	1 (0.6)	0 (0)	0 (0)	3 (1.8)	0 (0)	0 (0)
Barcelona (Hospital del Mar)	296	11 (3.7)	0 (0)	0 (0)	18 (6.1)	0 (0)	0 (0)
Barcelona (Hospital Sant Pau)	166	5 (3.0)	0 (0)	0 (0)	7 (4.2)	0 (0)	0 (0)
Cádiz	77	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Canarias	107	3 (2.8)	0 (0)	0 (0)	5 (4.7)	0 (0)	1 (0.9)
Fuenlabrada	162	6 (3.7)	0 (0)	3 (1.9)	2 (1.2)	0 (0)	6 (3.7)
Guadalajara	211	10 (4.7)	1 (0.5)	3 (1.4)	27 (12.8)	6 (2.8)	16 (7.6)
León	191	9 (4.7)	3 (1.6)	0 (0)	9 (4.7)	2 (1.0)	0 (0)
Madrid (Fundación Jiménez Díaz)	227	18 (7.9)	2 (0.9)	1 (0.4)	13 (5.7)	0 (0)	1 (0.4)
Madrid (Hospital 12 Octubre)	107	2 (1.9)	1 (0.9)	0 (0)	2 (1.9)	0 (0)	0 (0)
Madrid (Hospital La Princesa)	130	5 (3.8)	0 (0)	3 (2.3)	7 (5.4)	0 (0)	2 (1.5)
Murcia (Hospital Massager)	83	11 (13.3)	3 (3.6)	1 (1.2)	11 (13.3)	2 (2.4)	1 (1.2)
Murcia (Hospital Virgen Arrixaca)	82	0 (0)	1 (1.2)	2 (2.4)	8 (9.8)	0 (0)	0 (0)
Navarra	128	3 (2.3)	0 (0)	1 (0.8)	9 (7.0)	0 (0)	1 (0.8)
Santiago de Compostela	269	10 (3.7)	0 (0)	5 (1.9)	4 (1.5)	0 (0)	9 (3.3)
Sevilla	214	53 (24.8)	0 (0)	3 (1.4)	26 (12.1)	2 (0.9)	2 (0.9)
Toledo	183	21 (11.5)	3 (1.6)	0 (0)	10 (5.5)	5 (2.7)	0 (0)
Valencia	113	1 (0.9)	0 (0)	3 (2.7)	1 (0.9)	0 (0)	2 (1.8)
Vigo	97	2 (2.1)	0 (0)	5 (5.2)	1 (1.0)	0 (0)	3 (3.1)
Vitoria	223	8 (3.6)	0 (0)	8 (3.6)	11 (4.9)	0 (0)	15 (6.7)
Total	3639	187 (5.1)	14 (0.4)	55 (1.5)	179 (4.9)	17 (0.5)	70 (1.9)

The present study shows a high prevalence of contact allergy to Lim-OOHs and Lin-OOHs in the Spanish population: 5.1% of the tested patients had positive patch test reactions to Lim-OOHs 0.3% pet. and 4.9% to Lin-OOHs 1.0% pet. Similar prevalences of contact allergy were found in a study from the United Kingdom, in which 5.0% of patients had positive reactions to Lim-OOHs 0.3% pet. and 5.9% to Lin-OOHs 1.0% pet. (13), and in a recent international multicentre study including nine clinics in Australia, Denmark, Singapore, Spain, Sweden, and the

United Kingdom, with 5.2% of the patients having positive reactions to oxidized limonene 3.0% pet. (containing 0.33% Lim-OOHs) and 6.9% to oxidized linalool 6.0% pet. (containing 1.0% Lin-OOHs) (14, 15). These findings place Lim-OOHs and Lin-OOHs among the most common contact allergens throughout Europe. As in the aforementioned studies, a large difference in the prevalence of contact allergy to Lim-OOHs and the prevalence of contact allergy to Lin-OOHs between the participating test centres was observed. These regional differences in the

Table 3. The Male, Occupational, Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, Age ≥ 40 years (MOAHLFA) index of patients with positive patch test reactions to hydroperoxides of limonene (Lim-OOHs) and hydroperoxides of linalool (Lin-OOHs) at the highest tested concentrations, along with the physician's assessment of the relevance of the contact allergy for the patient's dermatitis

Fragrance terpene	Patch test concentration	No. of patients with positive reactions								Relevance		
			M No. (%)	O No. (%)	A No. (%)	H No. (%)	L No. (%)	F No. (%)	A No. (%)	Present No. (%)	Past No. (%)	Uncertain No. (%)
Lim-OOHs	0.3% pet.	187	56 (29.9)	34 (18.2)	40 (21.4)	76 (40.6)	33 (17.6)	51 (27.3)	116 (62.0)	86 (46.0)	5 (2.7)	96 (51.3)
Lin-OOHs	1.0% pet.	179	77 (43.0)	25 (14.0)	33 (18.4)	74 (41.3)	33 (18.4)	57 (31.8)	127 (70.9)	84 (46.9)	3 (1.7)	92 (51.4)
Both hydroperoxides		74	28 (37.8)	16 (21.6)	12 (16.2)	30 (40.5)	15 (20.3)	22 (29.7)	52 (70.3)	38 (51.4)	0 (0)	36 (48.6)

Table 4. Numbers of patients with positive patch test reactions to hydroperoxides of limonene (Lim-OOHs) and hydroperoxides of linalool (Lin-OOHs) at each respective concentration who also reacted to other fragrance markers [fragrance mix I (FM I), fragrance mix II (FM II), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), and *Myroxylon pereirae*] and/or colophonium in the baseline patch test series tested concomitantly

Fragrance terpene	Patch test concentration	No. of patients with positive reactions	Concomitant positive patch test reactions					One or more fragrance markers and/or colophonium No. (%)
			FM I No. (%)	FM II No. (%)	HICC No. (%)	<i>Myroxylon pereirae</i> No. (%)	Colophonium No. (%)	
Lim-OOHs	0.1% pet.	51	6 (11.8)	4 (7.8)	2 (3.9)	2 (3.9)	3 (5.9)	13 (25.5)
	0.2% pet.	124	14 (11.3)	12 (9.7)	6 (4.8)	14 (11.3)	4 (3.2)	30 (24.2)
	0.3% pet.	187	23 (12.3)	23 (12.3)	10 (5.3)	18 (9.6)	9 (4.8)	58 (31.0)
Lin-OOHs	0.25% pet.	46	8 (17.4)	6 (13.0)	1 (2.2)	4 (8.7)	4 (8.7)	13 (28.3)
	0.5% pet.	106	16 (15.1)	14 (13.2)	4 (3.8)	13 (12.3)	8 (7.5)	32 (30.2)
	1.0% pet.	179	27 (15.1)	22 (12.3)	5 (2.8)	20 (11.2)	13 (7.3)	59 (33.0)
Both hydroperoxides		74	11 (14.9)	11 (14.9)	4 (5.4)	10 (13.5)	7 (9.5)	22 (29.7)

prevalence of sensitivity have also been observed for other fragrance haptens of the European baseline series (21), and are most likely attributable to regional variations in exposure (i.e. different preferences regarding fragrance notes), differences in tested populations (e.g. centres treating only occupational cases), and/or differences in the reading/interpretation of patch test reactions between centres.

Patch testing with non-oxidized terpenes has proven to be a less useful screening method for detecting contact allergy to limonene and linalool. In a previous study from the United Kingdom, only 0.2% of the patients had positive reactions to stabilized (non-oxidized) limonene, and 0.3% to stabilized linalool (13). Similarly, an Information Network of Departments of Dermatology study found that only 0.1% of the patients reacted to limonene and 0.3% to linalool, both of which were not deliberately oxidized (12). For this reason, testing with the terpene hydroperoxides, which have been shown to be the main haptens in the oxidation mixture (5–9), is a good method for diagnosing contact allergy to these compounds.

The suitability of raw material and concentration used for patch test screening had also been addressed; although

a higher test concentration usually increases the possibility of diagnosing contact allergy, adverse effects such as irritation or active sensitization may occur, and must be considered. In the present study, the highest patch test concentrations used for Lim-OOHs (0.3% pet.) and Lin-OOHs (1.0% pet.) allowed diagnosis of the highest number of cases of contact allergy. A fairly high percentage of these cases, approximately 30–40%, would not have been diagnosed by use of the next lower patch test concentrations for both terpenes. In addition, with higher patch test concentrations, only slight increases in the numbers of irritant reactions were observed, with an overall proportion of 1.5% (when testing was performed with Lim-OOHs 0.3% pet.) and 1.9% (when testing was performed with Lin-OOHs 1.0% pet.). Moreover, a large number of doubtful reactions were interpreted as positive reactions at higher concentrations, indicating that a certain number of these doubtful reactions were very weak positive patch test responses, as in previous studies in this field (17). Regarding sensitization, no patients were, to our knowledge, actively sensitized during the present study, as no cases of late-appearing reactions were reported. On the basis of these results, and according to previous recommendations (13–15, 17), it seems

that patch test preparations of Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. are useful tools for screening of contact sensitization.

The relevance of the positive patch test reactions to limonene and linalool in relation to the patients' dermatitis was assessed by the physician. It should be noted that, especially for fragrance chemicals, which are ubiquitous in our environment, clinical relevance is always difficult to evaluate, and allergen avoidance is difficult to achieve (21). In this study, contact allergy to Lim-OOHs and Lin-OOHs was assessed as probably relevant in almost 50% of the patients. Specific products associated with allergy to Lim-OOHs and Lin-OOHs were also recorded for all patients. Fine fragrances and cosmetics were the most frequent products containing limonene and/or linalool judged to be relevant for the patients' dermatitis. Other products, such as soaps, shampoos, deodorants, and moisturizers, were also frequently listed as sources of these terpenes. In occupational settings, products such as detergents and domestic cleaners, which are sources that patients might not suspect of containing fragrances, were also implicated. Thus, the frequency of the presence of limonene and linalool in everyday products adds to the risk of being sensitized, as a person will be potentially in contact with these compounds from many sources in the course of one day. Although the actual concentrations of limonene and linalool and their oxidized products used in different consumer products are commonly unknown (14, 22), it has been shown that, with repeated exposure to these fragrance terpenes, low concentrations may be sufficient to elicit or worsen eczema in previously sensitized individuals (22). Therefore, allergic patients may be at continuous risk of developing contact dermatitis, owing to the ubiquity of these compounds in common products.

Contact allergy to fragrances is quite frequent, affecting between 1.1% and 2.3% of the general population in Europe (23). Quality of life is considerably impaired in young women, especially if sensitization is multiple and intense (24). The sites affected, the female predominance and the age group of patients with positive reactions to Lim-OOHs and Lin-OOHs reflect those affected by fragrance allergy in general. Regarding atopy, its role in the induction of allergic contact dermatitis, especially for fragrance haptens, has been widely debated. However, as shown for FM I (25), atopy does not appear to confer an overall increased risk of sensitization to the terpene hydroperoxides, as only 20% of patients with allergic reactions to limonene and/or linalool had atopic features.

Today's fragrance markers in the baseline series, mainly FM I, FM II, HICC, and *M. pereirae*, are supposed

to detect a large proportion of patients with contact allergy to fragrance chemicals (26–28). Other materials, such as colophonium, have also been shown to be associated with higher reactivity in fragrance-sensitive individuals (29). Concomitant reactions between these fragrance markers have been described in many studies. Thus, patients showing positive patch test reactions to a certain fragrance marker will, in many cases, also have reactions to other markers (28, 30). These concomitant reactions indicate multiple sensitizations, as most of these fragrance chemicals are simultaneously present in many types of consumer products. In the present study, approximately 30–33% of the patients with allergic reactions to Lim-OOHs 0.3% pet. and/or to Lin-OOHs 1.0% pet. had concomitant reactions to the fragrance markers in the baseline patch test series. Similar figures were obtained in previous studies (14, 15, 31), and this means that almost 70% of the patients with allergic reactions to limonene and/or linalool would not have been informed of any fragrance allergy if these specific patch tests had not been performed. Hence, patients would continue to use products containing these allergens, so elicitation or worsening of their dermatitis would be possible. Considering the wide array of materials used for perfuming, it is not surprising that many cases of contact allergy to fragrances are not diagnosed with use of the current markers of the baseline series. As the use of fragrances varies over time, new relevant markers for fragrance contact allergy need to be developed. Another interesting finding from our study is that 25% (74/292) of the patients with positive patch test reactions had simultaneous reactions to both terpene hydroperoxides, with similar results being obtained for all test concentrations. Similar values were also found in two recent multicentre studies (13, 32). In other words, the majority of patients (~75%) reacted only to one of the hydroperoxides, supporting the specificity of the reactions.

In summary, Lim-OOHs and Lin-OOHs can be considered as common causes of contact allergy, and their inclusion in an extended baseline patch test series therefore seems appropriate. The patch test preparations of Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. are useful for screening of contact sensitization. As individuals could potentially come into contact with these compounds from many sources, the identification of allergic patients by using these haptens seems necessary. Further studies focusing on the thresholds for eliciting allergic contact dermatitis, and the minimum concentrations of terpene hydroperoxides that are able to induce sensitization, should be conducted. Knowledge about these features would also help in optimizing the patch test preparation.

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