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

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Author's contribution

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B.G.B: Registration and management of patients and manuscript revision.

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ABSTRACT

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

Conclusions. Lim-OOHs and Lin-OOHs can actually be considered as common causes of contact allergy, and therefore, their inclusion in an extended baseline patch test series seems 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.

Keywords: allergic contact dermatitis, fragrance allergy, hydroperoxides, limonene, linalool.

INTRODUCTION

Limonene and linalool are common fragrance terpenes present in a wide variety of products that eventually 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 also most often incorporated in many domestic and occupational products (1–4).

Limonene and linalool are known to be prehaptenes (i.e. substances which 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 (non intentionally oxidized) limonene and linalool have been tested in several studies, with very few positive patch test reactions recorded in consecutive patients (11–13). However, significant rates 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 either 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). It should be noted that, nowadays, testing with Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. seems to be the best option (13–15,17).

The aim of the present study was to investigate the prevalence of contact allergy to

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3 limonene and linalool hydroperoxides in Spain, and to investigate the optimal
4 concentration for detection of contact allergy to these fragrance terpenes in consecutive
5 patients. Furthermore, the clinical features of such patients with positive patch test
6 reactions and specially the relevance for each individual case were also assessed.
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11 12 13 14 **MATERIALS AND METHODS**

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16 During the period from May 2015 to February 2016, 3639 consecutive patients
17 undergoing patch testing because of suspected allergic contact dermatitis at 22
18 Departments of Dermatology belonging to the GEIDAC (*Grupo Español de*
19 *Investigación de Dermatitis de Contacto y Alergia Cutánea- Spanish Contact*
20 *Dermatitis Research Group network*) were screened with Lim-OOHs and Lin-OOHs, in
21 addition to regular patch testing. Three different concentrations of Lim-OOHs (0.1, 0.2
22 and 0.3% pet.) and Lin-OOHs (0.25, 0.5 and 1.0% pet.) were simultaneously tested.
23 Patients aged less than 18 years old were excluded from the study.
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36 In all participating centres, standard haptens used for patch testing were provided from
37 Chemotechnique Diagnostics (Vellinge, Sweden). The final preparation of the fragrance
38 terpenes was made in close collaboration with the Department of Dermatochemistry and
39 Skin Allergy, University of Gothenburg, Sweden. Considerable efforts have been made
40 to develop the oxidation procedures to arrive at a raw material containing a controlled
41 amount of hydroperoxides following a specific timetable. This also includes
42 development of sophisticated HPLC methods to analyze the content in the raw material
43 as well as in each released batch of syringes. The test preparations labelled
44 hydroperoxides of limonene contain oxidized R-limonene with a validated content of a
45 mix of Limonene-1-hydroperoxide and Limonene-2-hydroperoxide, while the test
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3 preparations labelled hydroperoxides of linalool contain oxidized linalool with a
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5 validated content of the major Linalool hydroperoxides and the minor Linalool
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7 hydroperoxides.
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11 Patch tests were applied on the patients' upper back using IQ Ultimate chambers
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13 (Chemotechnique) and occluded for 48 hours. Visual readings were made twice, on
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15 days 2 and 4-5, and were scored by using the European Society of Contact Dermatitis
16
17 (ESCD) patch test guidelines (18). The strength of positive (allergic) reactions was
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19 designated as + (weak positive reaction: erythema, infiltration, possibly papules), ++
20
21 (strong positive reaction: erythema, infiltration, papules, vesicles), or +++ (extreme
22
23 positive reaction; intense erythema, infiltrate, coalescing vesicles). Irritant responses
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25 (well-defined inflammation with a lack of infiltrate limited to the exposure area,
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27 composed mostly of papules, whose effect decreases between days 2 and 4), doubtful
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29 (+?) responses, or negative readings were interpreted as non-allergic.
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37 Information regarding clinical features (e.g. age, sex, atopy, site of lesions) was
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39 obtained before testing. In addition, in the case of a positive patch test reaction to Lim-
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41 OOHs and/or Lin-OOHs, the relevance of this positive reaction for the patient's contact
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43 dermatitis was assessed based on the patient's history and the presence of these
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45 compounds on the list of ingredients of one or more products used by the patient, and
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47 accordingly classified as follows: "Present", when the product containing the fragrance
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49 terpene that had elicited the positive patch test was used by the patient at the time of
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51 testing on areas affected by dermatitis; "past", when the suspected product was used at
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53 an earlier time; and "uncertain", when no suspected product could be identified.
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3 All analyses were carried out with the SPSS 15.0 statistical package. The study was
4 approved by the ethic committee (EC-2015/0039/I) of the Hospital del Mar site as
5 promoter and by each involved center and was conducted in accordance with the
6 Declaration of Helsinki and attending local and European regulations. All participants
7 provided written informed consent to participate in the study.
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13 14 15 16 **RESULTS**

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18 Of the 3639 patients tested, 292 (8.0%) showed positive patch test reactions to one or
19 both hydroperoxides (at any concentration): 187 (5.1%) patients to Lim-OOHs, 179
20 (4.9%) to Lin-OOHs and 74 (2.0%) to both terpene hydroperoxides.
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27 The number of patients with positive, doubtful and irritant patch test reactions to Lim-
28 OOHs and Lin-OOHs at each tested concentration, along with the distribution of the
29 strength of the positive reactions, is shown in Table 1. Overall, when the patch test
30 concentration for both terpene hydroperoxides was higher, an increasing number of
31 patients showing positive and irritant reactions and a decreasing frequency of doubtful
32 reactions were registered. For the higher concentration tested, 187 (5.1%) patients had
33 positive patch test reactions to Lim-OOHs 0.3% pet. and 179 (4.9%) to Lin-OOHs 1.0%
34 pet. Sixty-three of the 187 (33.7%) patients with positive reactions to Lim-OOHs 0.3%
35 pet. and 73 of the 179 (40.8%) patients with positive reactions to Lin-OOHs 1.0% pet.
36 would not have been diagnosed using only the immediate lower patch test
37 concentration. Likewise, 33.3% and 39.3% of the doubtful reactions to Lim-OOHs
38 0.2% pet. and to Lin-OOHs 0.5% pet., respectively, were interpreted as positive
39 reactions at the highest concentration. ~~Regarding the strength of positive reactions,~~
40 ~~approximately 60-70% of the patients reacting to Lim-OOHs or to Lin-OOHs had + as a~~
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3 ~~maximum reaction, and 30-40% had ++ or +++ reactions, with similar results for both~~
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5 ~~terpenes and for the three tested concentrations.~~
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10 Table 2 shows the number of patients with positive, doubtful and irritant reactions to
11 Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. from each participating centre. Lim-
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13 OOHs 0.3% pet. (range: 0- 24.8%) and Lin-OOHs 1% pet. (range: 0-13.3%) showed a
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15 wide range in the frequency of positive reactions between the 22 centres. Conversely
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17 Lim-OOHs 0.3% pet. (range: 0-3.6%) and Lin-OOHs 1% pet. (range: 0-2.8%) showed a
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19 narrow range in the frequency of doubtful reactions.
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25 Table 3 shows the MOAHLFA index (Male, Occupational, Atopic dermatitis, Hand
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27 eczema, Leg dermatitis, Facial dermatitis, and Age \geq 40 years) and the physician's
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29 assessment of the relevance of the contact allergy for the patient's dermatitis registered
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31 from patients with positive reactions to Lim-OOHs and Lin-OOHs at the highest tested
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33 concentration. The mean age of the patients with positive reactions to Lim-OOHs 0.3%
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35 pet. (n=187) was 46 years (range 18-89). One hundred and thirty-one (70.1%) patients
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37 were female and 34 (18.2%) showed occupational contact allergy ~~and 40 (21.4%)~~
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39 ~~presented atopic features. The most common sites affected for these patients were the~~
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41 ~~hands (40.6%) and the face (27.3%).~~ Patients showing allergic contact dermatitis to Lin-
42
43 OOHs 1.0% pet. (n=179), showed a mean age of 50 years (range 18-90). One hundred
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45 and two (57.0%) patients were female and 25 (14.0%) showed occupational contact
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47 dermatitis ~~and 33 (18.4%) were atopic.~~ **The most common sites affected for both**
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49 **terpene hydroperoxides** were the hands (41%) and the face (27-32%).
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3 Regarding relevance, a present exposure to one or several products containing
4 limonene, used on the dermatitis area, was registered in 86 of the 187 (46.0%) patients
5 with positive reactions to Lim-OOHs 0.3% pet., while a past exposure was registered in
6
7 5 (2.7%) patients. In the same way, 84 (46.9%) and 3 (1.7%) of the 179 patients
8 showing positive reactions to Lin-OOHs 1% pet. were judged to have a present and past
9 relevance, respectively, for their contact dermatitis. The most common products
10 containing limonene and/or linalool of clinical relevance to the patients' allergic
11 reactions were cosmetics and fine fragrances (judged to be relevant in 57 patients),
12 soaps (n=21), hair products (n=18), moisturizers (n=17), and detergents (n=12). Less
13 common products include deodorants (n=8), essential oils (n=5), toothpaste (n=4), and
14 wet wipes (n=2).
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29 Table 4 gives the number and percentage of patients showing positive reactions to Lim-
30 OOHs and to Lin-OOHs, at each tested concentration, who also reacted to other
31 fragrance markers (fragrance mix I [FM I], fragrance mix II [FM II], hydroxyisohexyl
32 3-cyclohexene carboxaldehyde [HICC], and *Myroxylon pereirae*) and/or colophonium
33 in the baseline patch test series, which were tested concomitantly. The overall frequency
34 of concomitant reactions to other fragrance markers and/or colophonium in patients
35 with positive reactions to Lim-OOHs 0.3% pet. was 31.0% (58/187), while in patients
36 showing positive reactions to Lin-OOHs 1.0% pet. the frequency was 33.0% (59/179).
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38 ~~Twenty two of the 74 (29.7%) patients with positive reactions to both Lim-OOHs and~~
39 ~~Lin-OOHs showed a concomitant reaction to other fragrances and/or colophonium.~~
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DISCUSSION

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5 Limonene and linalool are ubiquitous allergens in our environment, and are among the
6
7 most common fragrance ingredients according to several studies (2–4). Although these
8
9 fragrance terpenes are present in a significant number of personal care products, contact
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11 dermatitis will only occur in the vast majority of the patients if these compounds have
12
13 been previously oxidized after air exposure. Importantly, quantifiable levels of Lim-
14
15 OOHs and Lin-OOHs could be present in commercially available fine fragrances and
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17 cosmetics owing to auto-oxidation (19,20).
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23 The present study shows a significant rate of contact allergy to limonene and linalool
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25 hydroperoxides in the Spanish population: 5.1% of the tested patients had a positive
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27 patch test reaction to Lim-OOHs 0.3% pet. and 4.9% to Lin-OOHs 1.0% pet. Similar
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29 rates of contact allergy have been found in a study from the United Kingdom (5.0% of
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31 patients showed positive reactions to Lim-OOHs 0.3% pet. and 5.9% to Lin-OOHs
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33 1.0% pet.) (13) and **in a recent international multicentre study including 9 clinics in**
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35 **Australia, Denmark, Singapore, Spain, Sweden and the United Kingdom** (5.2% of the
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37 patients showed positive reactions to oxidized limonene 3.0% pet.- containing 0.33%
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39 limonene hydroperoxides- and 6.9% to oxidized linalool 6.0% pet.-containing 1.0%
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41 linalool hydroperoxides) (14,15). These rates place limonene and linalool
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43 hydroperoxides among the most common contact haptens throughout Europe. As with
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45 the aforementioned studies, a large difference in the prevalence of contact allergy to
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47 limonene and linalool hydroperoxides between the participating test centres was
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49 observed. These regional differences in the prevalence of sensitivity have also been
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51 observed for other fragrance haptens of the European baseline series (21), and are most
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53 likely attributable to regional variations in exposure (i.e. different preferences regarding
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3 fragrance notes), differences of tested populations (e.g. centres that deals only with
4 occupational cases), and/or differences in the reading/interpretation of patch test
5 reactions between centres.
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11 Patch testing to non-oxidized terpenes has proved to be a less useful screening method
12 to detect contact allergy to limonene and linalool. In a previous study from the United
13 Kingdom, only 0.2% of the patients had positive reactions to stabilized (non-oxidized)
14 limonene, and 0.3% to stabilized linalool (13). Similarly, a IVDK study (Information
15 Network of Departments of Dermatology: Germany, Switzerland and Austria) found
16 that only 0.1% of the patients reacted to stabilized limonene and 0.3% to stabilized
17 linalool (12). For this reason, testing to the terpene hydroperoxides, which have been
18 demonstrated to be the main haptens in the oxidation mixture (5–9), is a good tool to
19 diagnose contact allergy to these compounds. The patch test with the best raw material
20 and concentration used for screening should also be established, as a higher test
21 concentration usually increases the possibility of diagnosing contact allergy; however,
22 adverse effects such as irritation or active sensitization may occur and must be
23 controlled. In the present study, the higher patch test concentration used for Lim-OOHs
24 (0.3% pet.) and Lin-OOHs (1.0% pet.) allowed diagnosing the largest number of cases
25 of contact allergy. A fairly high percentage of these cases, approximately 30-40%,
26 would not have been diagnosed using the immediate lower patch test concentration for
27 both terpenes. In addition, with higher patch test concentrations, only a slight increase
28 of irritant reactions were observed, with an overall rate of 1.5% (when testing to Lim-
29 OOHS 0.3% pet.) and 1.9% (when testing to Lin-OOHs 1.0% pet.). Moreover, a large
30 number of doubtful reactions were interpreted as positive reactions at higher
31 concentrations, thus indicating that a certain number of the doubtful reactions are very
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3 weak positive patch test responses, similarly to previous studies in this field (17).

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5 Regarding sensitization, no patients were, to our knowledge, actively sensitized during
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7 the present study, since no cases of late-appearing reactions were reported. Based on
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9 these results, and according to previous recommendations (13–15,17), it seems that
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11 patch test preparations of Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. are useful
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13 tools for screening of contact sensitization.
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18 The relevance of the positive patch test reactions to limonene and linalool in relation to
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20 the patients' dermatitis was assessed by the physician. It should be noted that, especially
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22 for fragrance chemicals, which are ubiquitous in our environment, clinical relevance is
23
24 always difficult to evaluate and allergen avoidance is difficult to achieve (21). In this
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26 study, contact allergy to limonene and linalool hydroperoxides was assessed as being
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28 likely to be relevant in almost 50% of the patients. Specific products associated with
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30 allergy to limonene and linalool hydroperoxides were also recorded in all patients. Fine
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32 fragrances and cosmetics were the most frequently products containing limonene and/or
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34 linalool judged to be relevant for the patients' dermatitis. Other products, such as soaps,
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36 shampoos, deodorants and moisturizers were also frequently listed as sources of these
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38 terpenes. In occupational settings, products like detergents and domestic cleaners, which
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40 are sources that patients might not suspect to contain fragrances, were also implicated.
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42 Thus, the frequency of limonene and linalool in everyday products adds to the risk of
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44 being sensitized, as a person will be potentially in contact with these compounds from
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46 many sources in a day. Although the actual concentration of limonene and linalool and
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48 its oxidized products used in different consumer products are commonly unknown
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50 (14,22), it has been demonstrated that with a repeated exposure to these fragrance
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52 terpenes, low concentrations may be sufficient to elicit or worsen eczema in previously
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3 sensitized individuals (22). Therefore, allergic patients may be at continuous risk to
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5 develop contact dermatitis due to the ubiquity of these compounds in common products.
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10 Contact allergy to fragrances is quite frequent, affecting between 1.1% and 2.3% of the
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12 general population in Europe (23). Quality of life is considerably impaired in young
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14 women, and especially if sensitizations are multiple and of high degree (24). The sites
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16 affected, the female predominance and the age group in patients with positive reactions
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18 to limonene and linalool hydroperoxides reflect those affected by fragrance allergy in
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20 general. Regarding atopy, its role in the induction of allergic contact dermatitis,
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22 especially for fragrance haptens, has been widely debated. However, as demonstrated
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24 for FM I (25), atopy does not appear to confer an overall increased risk of sensitization
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26 to the terpene hydroperoxides, since only 20% of patients with allergic reactions to
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28 limonene and/or linalool presented atopic features.
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34 Today's fragrance markers in the baseline series, mainly the two mixes FM I and FM II,
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36 HICC and *Myroxylon pereirae*, are expected to detect a large proportion of patients with
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38 contact allergy to fragrance chemicals (26–28). Other materials, such as colophonium,
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40 have been also shown to be associated with higher reactivity in fragrance-sensitive
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42 individuals (29). Concomitant reactions between these fragrance markers have been
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44 described in many studies. Thus, patients showing positive patch test reactions to a
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46 certain fragrance marker will, in many cases, also have reactions to other markers
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48 (28,30). These concomitant reactions indicate multiple sensitizations, since most of
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50 these fragrance chemicals are simultaneously present in many types of consumer
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52 products. In the present study, approximately 30-33% of the patients with allergic
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54 reactions to Lim-OOHs 0.3% pet. and/or to Lin-OOHs 1.0% pet. showed concomitant
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2
3 reactions to the fragrance markers in the baseline patch test series. Similar figures were
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5 obtained in previous works (14,15,31), and this means that almost 70% of the patients
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7 showing allergic reactions to limonene and/or linalool would not have been informed of
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9 any fragrance allergy if these specific patch tests had not been performed. This would
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11 lead to the patients continuing to use risky products, and thus still being exposed to
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13 fragrance materials eliciting or worsening their dermatitis. Considering the wide array
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15 of materials used for perfuming, it is not surprising that many cases of contact allergy to
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17 fragrances are not diagnosed by using the current markers of the baseline series. Since
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19 the use of fragrances varies over time, new relevant markers for fragrance contact
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21 allergy need to be developed. Another interesting finding from our study is that 25%
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23 (74/292) of the patients with positive patch test reactions presented simultaneous
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25 reactions to both terpene hydroperoxides, with similar results for all the test
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27 concentrations. Similar values were also found in two recent multicentre studies
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29 (13,32). In other words, the majority of patients (approximately 75%) reacted only to
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31 one of the hydroperoxides, thus supporting the specificity of the reactions.
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39 In summary, Lim-OOHs and Lin-OOHs can nowadays be considered as common
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41 causes of contact allergy, and therefore, their inclusion in an extended baseline patch
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43 test series seems appropriate. The patch test preparations of Lim-OOHs 0.3% pet. and
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45 Lin-OOHs 1.0% pet. are useful methods for screening of contact sensitization. Since
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47 individuals could be potentially in contact with these compounds from many sources in
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49 a day, the identification of allergic patients by using specific patch tests would be
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51 necessary. Further studies focusing in the thresholds definition for eliciting allergic
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53 contact dermatitis, as well as the minimum concentration of terpene hydroperoxides
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55 able to induce sensitization, should be conducted. The knowledge of these features
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3 would help to find the best and more pure preparation to be used to patch test in order to
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5 avoid adverse effects.
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TABLE 1. Number and percentage of patients with positive, doubtful and irritant patch test reactions to hydroperoxides of limonene (Lim-OOHs) and 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 (% *)	No. of irritant patch test reactions (% *)
		Total (% *)	+	++/+++		
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)

* Regarding the total of patients tested (n=3639)

TABLE 2. Total number of patients from each respective test centre and the number and percentage 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 (H. Mar)	296	11 (3.7)	0 (0)	0 (0)	18 (6.1)	0 (0)	0 (0)
Barcelona (H. Sant Pau)	166	5 (3.0)	0 (0)	0 (0)	7 (4.2)	0 (0)	0 (0)
Cadiz	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 (F. Jiménez Díaz)	227	18 (7.9)	2 (0.9)	1 (0.4)	13 (5.7)	0 (0)	1 (0.4)
Madrid (H. 12 Octubre)	107	2 (1.9)	1 (0.9)	0 (0)	2 (1.9)	0 (0)	0 (0)
Madrid (H. La Princesa)	130	5 (3.8)	0 (0)	3 (2.3)	7 (5.4)	0 (0)	2 (1.5)
Murcia (H. Massager)	83	11 (13.3)	3 (3.6)	1 (1.2)	11 (13.3)	2 (2.4)	1 (1.2)
Murcia (H. V. 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 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)

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

Fragrance terpene	Patch test concentration	No. patients with positive reactions	M No. (%)	O No. (%)	A No. (%)	H No. (%)	L No. (%)	F No. (%)	A No. (%)	Relevance		
										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. Number of patients showing positive patch test reactions to hydroperoxides of limonene (Lim-OOHs) and 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. patients with positive reactions	Concomitant positive patch test reactions					≥1 fragrance marker 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)

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