

# **Limonene and linalool hydroperoxides review: pros and cons for routine patch testing**

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## **Abstract**

Limonene and linalool are among the most common fragrance terpenes used in products of everyday life. They are pre-haptens forming hydroperoxides (Lim-OOHs, Lin-OOHs) upon oxidation inducing frequent positive patch test reactions in patients with dermatitis. Still, they are not yet routinely tested in Europe. This review evaluates the patch testing experience with Lim-OOHs and Lin-OOHs by answering key questions such as whether hydroperoxide patch testing is warranted, understand difficulties or challenges related to the reading and interpretation of hydroperoxide patch test results with currently available material, assessing their relevance. Studies are increasingly pointing out to high percentages of positive reactions in patients consecutively patch tested with these oxidized products. An association between a positive clinical history and a strong patch test reaction has been described, but problems with doubtful/irritant reactions have also been reported. Considering the high frequencies of relevant positive reactions, the incorporation of Lim-OOHs 0.3% and Lin-OOHs 1% in the baseline series could be discussed and is maybe justified. Since exposure, sensitization and elicitation limits of Lim-OOHs and Lin-OOHs in the products still need to be better determined, an assessment of previous exposure, and possible sensitizations and reactions may help to improve the clinical assessment.

**Key words:** allergic contact dermatitis, hydroperoxides, limonene, linalool, patch test.

## 1 Introduction

Allergic contact dermatitis (ACD) to fragrances is common, affecting 1.1% to 2.6% of the general population in Europe<sup>1-3</sup>. Moreover, some studies report a prevalence that could even reach 15% in patients with a history of dermatitis<sup>4,5</sup>.

Many fragrance materials used today belong to the chemical group of terpenes<sup>6</sup> and, among them, limonene (citrus scent) and linalool (lavender scent) are frequently found in multiple household (hygienic and cosmetic products) and occupational products, essential oils, natural products and in aromatherapy<sup>7-12</sup> which come into contact with the skin daily.

Limonene and linalool are known to be pre-haptens. They oxidize upon contact with air forming hydroperoxides as primary oxidation products and these have been recognized as important contact allergens<sup>13,14</sup>. If the compounds limonene and linalool are patch tested with not deliberately oxidized (“pure”) form, positive patch test reactions are rarely found (Table 1), whereas the hydroperoxides are frequent causes of positive patch test reactions in patients when using standardized patch test materials for oxidized limonene and linalool developed in the last decade (Tables 2 and 3 and references therein).

However, oxidized limonene and linalool are not yet routinely tested in Europe. In recent years, there has been debate about the most useful patch test concentrations to obtain a reliable result when testing oxidized limonene and oxidized linalool. In addition, although limonene and linalool are found in many consumer products, it is often difficult to identify these oxidized terpenes as the culprit ingredients causing ACD. Moreover, the clinical relevance of these hydroperoxides is not easy to specify, these products often induce false-negative patch test reactions when patch tested “as is”, and analyses hardly detect and allow quantification of hydroperoxides in commercial products. Therefore, exposure to oxidation products, such as limonene hydroperoxides (Lim-OOHs) and linalool hydroperoxides (Lin-OOHs), remains largely elusive. Nevertheless, it has also been shown in repeated open application test (ROAT) studies that both oxidized limonene and oxidized linalool can cause ACD in sensitized patients<sup>15,16</sup>. One possible hypothesis of Lim-OOHs and Lin-OOHs sensitization is that there is repeated exposure to the respective allergen from many sources, and even low concentrations of oxidized terpenes in each product can induce cumulative exposure capable of generating ACD in previously sensitized individuals<sup>15</sup>.

The objectives of this review are to identify and try to clarify doubts regarding the need to incorporate oxidized limonene (Lim-OOHs 0.3% pet.) and oxidized linalool (Lin-OOHs 1.0% pet.) in the baseline series for patch testing as it was proposed<sup>17</sup>.

## **2 Methods**

This document is based on a systematic search of all literature published from the first publication on the subject (1985) to 2021. The following bibliographical sources were used for the search: Pubmed, Embase. The literature searches were designed to cover the following items alone and/or combined: allergy, contact dermatitis, eczema, hydroperoxides limonene, hydroperoxides linalool, limonene, linalool, terpenes. The following MeSh terms were used: allergic contact dermatitis and (limonene or limonene-2-hydroperoxide or limonene -1-hydroperoxide) and (linalool or linalool oxide) and patch tests. The manuscript was initially carried out by five recognized experts in chemistry and contact dermatitis due to oxidized terpenes. The primary document was assessed and reviewed by the European Environmental Contact Dermatitis Research Group (EECDRG) members. Then at least two rounds of discussion were performed before reaching consensus on the final document submitted.

## **3 Current knowledge about the sensitizing capacity and the diagnostic system**

### **3.1 Radical mechanisms possibly involved in contact sensitization to Lim-OOHs and Lin-OOHs**

Lin-7-OOH/Lin-6-OOH and Lim-1-OOH/Lim-2-OOH hydroperoxides have been identified as the components in the oxidation mixtures which are responsible for the sensitization potential of autoxidized linalool and *R*-(+)-limonene, respectively (Figure 1). Cutaneous allergens (haptens) are low molecular weight compounds that are unable to stimulate an adaptive immune response, but which become immunogenic after reacting with proteins to form stable hapten-protein conjugates that are subsequently processed and presented to the immune system. The best-known mechanism for hapten-protein interaction is the formation of covalent bonds by two-electrons mechanisms. Indeed, very often the allergen is electrophile and reacts with nucleophilic side chains of amino acids from skin proteins, such

as cysteine and lysine. However, organic hydroperoxides (R-OOHs) do not fit this model and one-electron radical-mediated mechanisms are suspected to be involved<sup>18</sup>. Indeed, sensitizing R-OOHs such as Lin-OOHs and Lim-OOHs can react through radical mechanisms that begin with the cleavage of the O-O bond of weak dissociation energy (*ca.* 175 kJ mol<sup>-1</sup>). Such cleavage is an easy process that allows the initially formed unstable alkoxy radicals (RO<sup>•</sup>) to be efficiently converted into longer half-life carbon-centered radicals through different known rearrangement processes. An example is shown in Figure 2 for Lin-7-OOH<sup>19</sup>. All these radical intermediates could potentially be reactive toward amino acids in the skin.

To understand how R-OOHs can form immunogenic structures once they penetrate into the epidermis, reactivity studies have been performed with amino acids known to be involved in radical processes. These studies were performed in solution (buffer, semi-organic) and were followed by nuclear magnetic resonance (NMR) and liquid chromatography combined to mass spectrometry (LC-MS). It was shown that carbon-radicals emitted by R-OOHs could react with amino acids such as cysteine, histidine, and tryptophan when Fe(II)/Fe(III) redox systems were used to trigger radical initiation<sup>19,20</sup>. Importantly, during these studies it was also noted that oxide-reduction processes are promoted during the reactions inducing chemical modifications of amino acids, thus demonstrating the involvement of oxidative stress.

The studies mentioning were performed in solution and, of course, a critical step in understanding skin sensitization to these hydroperoxides is to verify if these radicals are really formed in the skin. To achieve this goal, efforts are today focused on the use of electron paramagnetic resonance (EPR) spectroscopy, committed to the study of reactive free radical species, in combination with the spin trapping (ST) technique. EPR-ST is nowadays the best method to identify transient radicals in chemical and biological systems<sup>21</sup>. Hydroperoxide-derived radicals are generally very short-lived or are formed at a too low concentration to be directly detected by EPR. Then, the EPR-ST methodology is employed. EPR-ST is based on the reaction of a spin-trap with a transient radical to form a longer half-life radical adduct detectable by EPR. For many years, EPR-ST experiments were carried out in solution by

activating hydroperoxides with Fe(II) to induce radical initiation. The use of spin-traps such as 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) allowed the identification of different oxygen- and carbon-centered free radicals derived from the target compounds<sup>22</sup>. These studies revealed that several intermediate reactive radicals could be possible depending on the hydroperoxide chemical structure, suggesting that different chemical modifications of immunogenic proteins could lead to sensitization. However, these in-solution studies are far from mimicking real-life scenarios. With the aim to produce the *in vivo* model, a new EPR-ST methodology has been developed to investigate *in situ* the formation and behaviour of hydroperoxide-derived free radicals in a 3D model of reconstructed human epidermis (RHE) (Figure 3)<sup>23,24</sup>. EpiSkin™ RHE (Episkin, Lyon, France) consists of a normal multi-layered culture of keratinocytes, main cell type of the epidermis playing a key role in inflammatory reactions in the skin. EpiSkin™ RHE is a very good replica of human epidermis architecture. It is used for irritation and penetration testing of cosmetic and chemical compounds in replacement of *in vivo* testing<sup>25</sup>. Several oxygen- and carbon-centered radicals were initially identified in this complex environment, using cumene hydroperoxide and ascaridole as proofs of concept. Further, basic triggers for the activation in the skin of hydroperoxides derived from the autoxidation of linalool and limonene were studied using this methodology<sup>22,26</sup>. Experiments were conducted based on a topical application procedure to estimate real-life sensitization. Skin samples were initially loaded with DEPMPO to ensure its presence at the site of radical generation prior to application of the target compounds. The RHE were thus pre-treated with DEPMPO allowing its penetration through the *stratum corneum*. Then, subsequent topical exposure of DEPMPO-pre-treated RHE to different concentrations of hydroperoxides demonstrated that DEPMPO was able to trap carbon-centered radicals formed in this epidermal-like skin environment, together often with the hydroxyl radical and minor alkoxy radicals. Last but not least, no Fe(II) was used for radical initiation when RHE was used to mimic real-life cutaneous allergy situations. Therefore, it could be hypothesized that the skin reaction of hydroperoxides in the presence of one-electron donor agents (e.g., amino and thiol groups present in amino acids, metal complexes, enzymes) may initiate electron transfer processes under radical oxidation conditions and subsequent haptentation of skin proteins.

Major drawback of these studies is the impossibility of accurately assigning the carbon-centered radicals formed in the RHE among the different options resulting from radical rearrangement processes (Figure 2). To overcome this, one possibility is to carry out spin-trapping studies with target compounds site-specific labelled with  $^{13}\text{C}$  at positions precursors of potentially reactive radicals. Recent EPR-ST studies with cumene hydroperoxide  $^{13}\text{C}$ -substituted at methyl positions confirmed the trapping of methyl radicals released by decomposition of the hydroperoxide in the RHE model<sup>27</sup>.

As a conclusion to this section, radicals formed from hydroperoxides are key intermediates that could explain the reactivity with skin proteins and, therefore, their sensitizing potential. Moreover, now we know that these intermediates are formed in a 3D model of epidermis, under conditions closer to what may occur *in vivo*.

### **3.2. Experience from patch testing with Lim-OOHs and Lin-OOHs**

Up to the present, a number of studies have been conducted to evaluate the prevalence of contact allergy to limonene and linalool, both in not deliberately oxidized and oxidized forms (Tables 1, 2 and 3). In some studies, not deliberately oxidized limonene and/or linalool were tested concomitantly with the oxidized forms. Overall, very few patients reacted to not deliberately oxidized limonene or linalool (range 0%-0.88%) (Table 1 and Refs in Table 1).

Oxidized limonene has been tested in different concentrations (Table 2), especially at 3% (containing 0.3% Lim-OOHs), based on experience from a study published in 1997<sup>28</sup>. An early multicenter study with 2273 dermatitis patients using 3% oxidized limonene showed 2.8% positive patch test reactions<sup>13</sup>.

The first multicenter study of patch testing with oxidized linalool 2% pet. (containing 0.33% Lin-OOHs) was published in 2005, showing 1.3% positive patch test reactions in 1511 patients<sup>29</sup>. Subsequently, a dose-response study investigated whether a higher concentration of oxidized linalool could be useful to detect a higher number of allergic reactions, and a patch test concentration of oxidized linalool 6.0% (containing Lin-OOHs at 1.0%) was suggested<sup>30</sup>. Few irritant reactions were identified and there were no cases of active sensitization.

Several multicenter studies have since been conducted using oxidized limonene and oxidized linalool. In 2009, a total of 2900 consecutive dermatitis patients were evaluated in 9 centers dedicated to epicutaneous patch testing (Copenhagen, Malmo, Odense, Barcelona, London, Singapore, Melbourne, Seville and Gothenburg)<sup>31,32</sup>. The overall prevalence of positive reactions to oxidized limonene 3% pet. was 5.2%<sup>32</sup>, ranging from 2.3% to 12.1% in the individual centers. There were notable differences between the northern and southern European centers, with patch testing positive in 3.8% (Gothenburg, Malmo, London, Copenhagen, and Odense) versus 5.5% (Seville and Barcelona). Twenty five percent of patients who had a positive patch test showed a strongly positive (++/+++) reaction and 91% had their maximum reaction at D3-D4 readings. Concomitant reactions between R-limonene and other fragrances and/or colophonium from the baseline series were found in 42% of the oxidized limonene-positive cases and in 19.6% of the patients with doubtful (?+) reactions. Finally, in patients negative to oxidized R-limonene, only 10.6% of patients had positive reactions to fragrances and/or colophonium.

For oxidized linalool 6% pet. (1.0% Lin-OOHs), 6.9% of 2900 tested patients showed positive patch test reactions<sup>31</sup>, with frequencies ranging from 3.3% to 14.3% in the different centers<sup>31</sup>. Of the patients with positive reactions, 28% were strongly positive (++/+++), 86% of them at D3-D4 readings, and 40% were also positive to other baseline series fragrances and/or colophonium, which is statistically significant different from patients with doubtful (?+) or negative reactions (19% and 10%, respectively). No delayed reactions or active sensitization was described.

These studies led to the development of standardized patch test materials of oxidized limonene 3%, with a standardized content of Lim-OOHs at 0.3% (hydroperoxides of Limonene 0.3% pet.®) and oxidized linalool 6%, with a standardized content of Lin-OOHs at 1.0% (hydroperoxides of Linalool 1.0% pet.®) which are manufactured by Chemotechnique Diagnostics AB, Vellinge, Sweden<sup>31,32</sup>. The stability of the patch test material was monitored repeatedly during the study and showed a stable content of hydroperoxides during use.

In subsequent multicenter and single-center studies, patch testing with Lim-OOHs 0.3% pet. has shown positive patch test reactions in 1.2% to 9.4% of cases (Table 2 and references



therein). Likewise, Lin-OOHs 1.0% pet. has been used in many studies, giving 3.9% to 20% positive patch test reactions (Table 3 and references therein). Interestingly, in multicenter studies, wide ranges in rates of positive reactions were found between the test centers<sup>33,34</sup>, as further discussed below.

Patch test concentrations used in these studies have been further evaluated. In Spain, the GEIDAC group (*Grupo Español de Investigación de Dermatitis de Contacto y Alergia Cutánea*) conducted a study with 22 Spanish centers and 3639 consecutive patients tested. Overall, 5.1% of the patients showed positive patch test reactions to Lim-OOHs 0.3% pet. with frequencies ranging between 0% and 24.8% at the different test centers<sup>33</sup>. Similarly, Lin-OOHs 1.0% pet. gave 4.9% positive patch test reactions overall, with a frequency ranging between 0% and 13.9%<sup>33</sup>. The GEIDAC study also compared three concentrations of Lim-OOHs at 0.1%, 0.2%, and 0.3% pet. giving 1.4%, 3.4% and 5.1% positive patch test reactions respectively (Table 2). Concomitantly, three concentrations of Lin-OOHs at 0.25%, 0.5%, and 1.0% pet. were tested giving 1.3%, 2.9% and 4.9% positive patch test reactions respectively<sup>33</sup> (Table 3). Overall, 8% of patients responded to one or both hydroperoxides. The prevalence of positive patch tests increased with increasing patch test concentrations for both terpenes. In 30% of the cases of allergy to oxidized terpenes was a concurrent contact allergy to other fragrance markers in the baseline series, suggesting that 70% of the patients reacting to fragrance markers, but in 70% of the allergic patients there was only a contact allergy to oxidized terpenes, indicating that the patients would have gone unnoticed if they had not been tested with the oxidized terpenes patch test. In this study, only 25% of the positive cases were simultaneously positive for both hydroperoxides, supporting the specificity of the reaction.

A British multicenter study was conducted by Wlodek et al. in 2017<sup>34</sup>, in which 4563 consecutive patients were patch tested with Lim-OOHs at 0.1%, 0.2% and 0.3% pet. and with Lin-OOHs at 0.25%, 0.5% and 1.0% pet. Results are given in Tables 2 and 3. For Lim-OOHs 0.3% pet. 5.3% positive patch test reactions were recorded, while for Lin-OOHs 1.0% pet., 7.7% positive patch test reactions were recorded<sup>34</sup>. Positive patch test reactions were considered clinically relevant in approximately 2/3 of the patients, who had a clear current or previous history of fragrance allergy, or dermatitis from a product labelled to contain

limonene or linalool, or dermatitis from a botanical oil with limonene or linalool, or a positive ROAT to such a product. Lim-OOHs at 0.3% pet. and Lin-OOHs at 1.0% pet. were recommended to be included in the British baseline patch test series<sup>34</sup>.

In a recent analysis of 5773 patients tested 2013 to 2020, significantly increasing trends in contact allergy to Lim-OOHs at 0.3% pet. and Lin-OOHs at 1.0% pet. were observed, and the patients with contact allergy to Lim-OOHs and Lin-OOHs were significantly younger than those with contact allergy to other fragrances<sup>35</sup>. There was also a female predominance. Nowadays, cases of sensitization to Lim-OOHs and Lin-OOHs are increasingly described when studying contact allergy to cosmetics, e.g. on the face<sup>36</sup>. Early and continuous exposure to Lim-OOHs and Lin-OOHs has also impact on children as the most frequent contact allergens are nickel sulfate and Lin-OOHs (both 16%), Lim-OOHs (13.5%) and paraphenylenediamine (10.9%)<sup>37</sup>.

### **3.3. Doubtful and irritant patch test reactions. Are doubtful patch test reactions clinically relevant?**

Doubtful and/or irritant reactions in studies on Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. have been a matter of debate. In general, in the multicenter studies, comparatively high rates of doubtful and/or irritant reactions have been recorded at some test sites, while other test sites have had low frequencies of such reactions.

For oxidized limonene and oxidized linalool, irritation studies have been conducted showing very low irritation in the range of tested concentrations of 3% pet. and 6% pet., respectively<sup>38</sup>. However, doubtful reactions to Lin-OOHs 1.0% pet. ranged from 0% to 36% in the international multicenter study<sup>31</sup>, and from 0% to 19% in an UK multicenter study<sup>34</sup>. The corresponding ranges for Lim-OOHs 0.3% pet. were 0% to 24% in the international multicenter study<sup>32</sup> and 0% to 17% in the UK multicenter study<sup>34</sup>. Similar figures were shown in the Spanish multicenter study<sup>33</sup>, where the range of doubtful reactions for Lim-OOHs 0.3% pet. was 0% to 3.6% and for Lin-OOHs 1.0% pet. was 0% to 2.8%, while the range of irritant reactions for Lim-OOHs 0.3% pet. was 0% to 3.6% and for Lin-OOHs 1.0% pet. was 0% to 7.6%<sup>33</sup>. In all of these multicenter studies, when Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. were tested concomitantly, centers that showed high rates doubtful or irritant reactions recorded them for both test preparations, while most centers recorded very low frequencies

of doubtful/irritant reactions. Then, readings may to some extent be the result of differences in the expert interpretation and recording of patch test results or a lack of standardized methodology (e.g., administered dose)<sup>31-34</sup>. Furthermore, it has been shown in dose-response studies when different concentrations of oxidized limonene and/or oxidized linalool were tested, that a proportion of the doubtful reactions at the lower concentration showed a positive reaction at the next higher concentration. In the Swedish dose-response study to oxidized linalool, 25% of the patients who reacted with doubtful reactions to oxidized linalool 4% pet. (0.66% Lin-OOHs) had a positive reaction to 6% pet. (1.0% Lin-OOHs)<sup>30</sup>. Similarly, it was shown in the Spanish multicenter study that 33% and 39% of the doubtful reactions to Lim-OOHs 0.2% pet. and Lin-OOHs 0.5% pet. respectively, were interpreted as positive reactions at the next higher test concentration of the respective test material. A weak allergic reaction that has not yet turned positive, become positive at an increased dose. Therefore it is important to differentiate between irritant and doubtful. The irritant reaction will not change nature but will of course become stronger. Thus, some of the doubtful reactions may, in fact, be weak positive reactions. This is supported by a ROAT study with oxidized limonene, where 2/13 (15%) subjects with doubtful patch test reactions to Lim-OOHs had positive ROAT reactions to Lim-OOHs applied in realistic doses<sup>16</sup>.

There has been discussion regarding the specificity of positive reactions to oxidized patch test materials, due to the overall high rates of reactions to these substances. Many studies have confirmed that positive reactions to the separate oxidation mixtures as well as to their major allergens are specific. In these studies, when oxidized limonene and oxidized linalool are tested concomitantly, in the entire group of positive patients, about 25% will react to both Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet., while the rest (75%) will react to one or to the other<sup>39,40</sup>. These concomitant reactions are in accordance with the frequency of exposure to both fragrances in everyday products, and with the documented common tandem exposure of limonene and linalool in products<sup>7,8,10,11,41</sup>. Considering the total number of tested patients, the majority do not react to either test material, again opposing that the reactions for hydroperoxides are unspecific. Also, for the main oxidized limonene allergens, Lim-1-OOH and Lim-2-OOH (Figure 1), it has been shown that there is specificity for reactions between these two structurally similar hydroperoxides<sup>42</sup>. The statistically significant difference in concomitant contact allergy to other fragrance markers between the groups showing positive,

doubtful, or negative patch test reactions to the oxidized terpenes as described above, also support that the patch test materials for oxidized limonene and linalool are detecting true fragrance allergy.

The clinical relevance of a positive contact allergen can be assessed in many ways, and a common method is to interview the patient and search for exposure that can be expected to contribute to the patient's dermatitis. The relevance assessment can be difficult when interpreting positive patch test to Lim-OOH and Lin-OOH because the suspected responsible agents, the terpene hydroperoxides are not labelled or identified as it is in the products being this fact a limitation. Nevertheless, it is very difficult not to find a product that does not contain terpenes that potentially could be oxidized. For patients with positive reactions to Lim-OOHs 0.3% pet., in about 35-70% of the positive patients, an exposure to products containing listed limonene has been assessed to be relevant for the dermatitis<sup>12,40</sup>. For Lin-OOHs 1.0% pet., similar assessments have been made and similar estimates of relevance have been reported<sup>31,40,43</sup>. The GEIDAC study<sup>33</sup> reported that at least 50% of the reactions were relevant. The difficulty of assessing the relevance would benefit from an *in vitro* or *in vivo* test that would be able to detect the responsible hydroperoxide in the specific product.

### **3.4. Sources of exposure and thresholds of elicitation**

Commercial notification of the presence of fragrances is optional in some countries (such as Australia and Singapore), so their presence, both qualitative and quantitative, is unknown. In Europe, since 2005, the Cosmetics Directive determines that 26 fragrances must be declared in cosmetic products when their concentration exceeds 10 ppm in leave-on and 100 ppm in rinse-off cosmetics, respectively<sup>44</sup>. This mandatory declaration of content is not applicable in other parts of the world.

Limonene and linalool have been identified in up to 80% of common products (mainly personal hygiene products, cosmetics, and cleaning household products) either by their labelling or by chemical analyses<sup>7,8,10</sup>, with limonene also being present e.g., in industrial soaps and citrus-based solvents<sup>45</sup> and adhesives<sup>46-48</sup>. Linalool has been found to be the fragrance with the highest exposure, based on daily cumulative exposure from many

sources<sup>4</sup>. Limonene and linalool were also found to be the most common tandem exposure and are frequently used together in fragranced/scented products<sup>11,41,49</sup>. Both are also common components of essential oils, and they are found in aromatherapy and natural products. Limonene is a component of citrus peel oil (genus *Citrus*, family *Rutaceae*) as well as eucalyptus essential oils (leaf of *Eucalyptus*, family *Myrtaceae*), whereas linalool is a major component of lavender oil (distillate of *LavandulaAngustifoli*), which consists of approximately 50% linalool<sup>50</sup>. It has been shown that lavender oil of natural origin will autoxidize similarly to synthetic lavender oil (made by blending the three main components linalool, linalyl acetate, and caryophyllene), and in turn, in a similar way to its separate components<sup>14,50,51</sup>.

With this scenario, to establish the relevance of Lim-OOHs and Lin-OOHs positive patch tests, it would be ideal to assess the minimal levels of the oxidized forms capable of sensitizing individuals as well as eliciting contact dermatitis in sensitized individuals. To study elicitation levels, ROATs have been performed for oxidized linalool and limonene. In these studies, the daily use of a product (“cream” or “perfume”) is simulated by repeated skin applications of the products containing the allergen(s) in low concentrations. In the case of oxidized linalool, individuals who earlier had been shown to be allergic in patch testing, reacted in the ROAT to a concentration as low as 0.3% oxidized linalool, which contained 560 µg/g Lin-OOHs both in perfume and cream base<sup>15</sup>. In the case of oxidized limonene, allergic individuals reacted to as low as 140 µg/g in the ROAT, while three allergic patients reacted to concentrations as low as 24 µg/g Lim-OOHs in a series of patch test dilutions<sup>16</sup>. These studies demonstrate that allergic individuals react to very low concentrations of hydroperoxides.

Isolated cases of contact dermatitis to Lim-OOHs have been reported. Some were due to occupational exposure, with a histology technician that developed a recalcitrant hand eczema after being in contact with a limonene-based solvent agent<sup>52</sup>. The patient turned out negative to patch test with limonene and the solvent agent at the appropriate concentrations, while positive to patch test with Lim-OOHs. Chemical analysis failed to detect Lim-OOHs in the solvent agent used by the patient, so the authors suggested that oxidation of limonene may

occur during the handling of the product, especially in presence of oxidant stains (frequently used in histological laboratories).

Another case showed a lymphomatoid contact dermatitis due to hygiene products demonstrated by patch and provocation tests<sup>53</sup>. Two cases of dermatitis in patients allergic to oxidized linalool (Lin-OOHs 1.0% pet.) in which the culprit products were analyzed have been reported<sup>54,55</sup>.

### **3.5. Quantification of hydroperoxides in products**

Hydroperoxides are very difficult to quantify and at present, detection limit values in products have not been decided. In a study 2019<sup>56</sup> evaluated 104 samples of consumer products that patients suspected could trigger their symptoms, as well as products containing essential oils, including old and new samples of the same brand. Interestingly, limonene and linalool were not declared in 10 of the products in which they were confirmed by analysis. In all, four samples (3.8%) contained >50 µg/g of at least one of the hydroperoxides by the screening method. Samples recovered from patch test positive patients were below the detection limit for all target analytes. However, analysis by the screening method, specifically analysis by liquid chromatography-mass spectrometry (LC-MS) methods, showed levels <50 µg/g of the different hydroperoxides. In a previous study, a geometric mean of 30 µg/g was determined in 22/39 (56%) samples investigated by LC-MS analysis<sup>57</sup>. New methods could help to assess even lower amounts of such hydroperoxides and elucidate the clinical relevance of positive patch tests<sup>58</sup>.

Using a two-dimensional LC-MS method, a content of 8.4 µg/g Lin-6-OOH and 5.6 µg/g Lin-7-OOH was demonstrated in the deodorant that caused eczema in the axilla of a female patient with a solitary reaction to oxidized linalool (Lin-OOHs 1.0% pet.). The use test showed clear clinical relevance<sup>55,58</sup>. In another case, a content of 0.2 µg/g Lin-OOHs was detected in a shampoo that caused eyelid dermatitis in a child<sup>54</sup>. A cumulative exposure could be the reason for sensitization to Lim-OOHs and Lin-OOHs.

Taken together, the results obtained so far from chemical analyses of limonene and linalool hydroperoxides in scented products indicate that the content of these hydroperoxides may

cause elicitation in sensitized individuals. About 4% of the products analyzed by Natsch et al.<sup>56</sup> contained >50 µg/g of either hydroperoxide. Considering the widespread use of products containing limonene and linalool, there are many opportunities to reach amounts of hydroperoxides capable of eliciting ACD in daily exposures. The ROAT, as well as previous case reports, support that very low amounts of hydroperoxides can elicit ACD.

One question raised concerns the timing of terpene oxidation. Between ordering and receiving essential oils, oxidation had already occurred at the time of delivery<sup>59</sup>. Moreover, essential oils oxidize in any environment (stored in the dark, in the refrigerator, etc.)<sup>59</sup>. Thus, oxidation of a product can occur both before formulation of the product (during production or transport from the fragrance producer), during formulation, during transport of the consumer product (e.g., shampoo or cream), during storage, and during use. The presence of antioxidants is considered a protective factor for chemical degradation, but their usefulness has been shown to last for a limited time period. In the case of limonene, it has been demonstrated that the antioxidant butylated hydroxytoluene (BHT) will prevent degradation during a certain period, but will be consumed, after which the oxidation of the terpene will occur<sup>60</sup>.

#### **4. Challenges in Lim-OOHs and Lin-OOHs path testing**

Patch testing with standardized patch test material of oxidized limonene 3% (standardized content of Lim-OOHs at 0.3%; hydroperoxides of limonene 0.3% pet.®) and oxidized linalool 6% (standardized content of Lin-OOHs at 1.0%; hydroperoxides of linalool 1.0% pet.®) (Chemotechnique Diagnostics AB, Vellinge, Sweden<sup>31,32</sup>) has now been performed in most large studies, showing consistent and stable results. *Both selected Lim-OOH and Lin-OOH concentrations allow to identify better doubtful and irritant reactions than lower concentrations.*<sup>33</sup> A large part of the positive reactions has been judged relevant by the clinicians.

A great number of patients with positive patch test reactions to Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. show no concomitant reactions to other fragrances or related substances. If Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. are not used for routine patch test,

especially in patients without other fragrance allergies, a large part of the fragrance-allergic patients will not be informed of fragrance allergy, exposing them to the risk of a relapsing episodes of clinical ACD, without the possibility of secondary prevention strategies. Therefore, Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. should routinely be tested in patients suspected of having fragrance allergy.

At present it seems that positive patch test reactions to Lim -OOHs and Lin-OOHs still do not fulfill the necessary criteria to justify their inclusion in the European baseline series, although some countries already use them in their national baseline series<sup>17</sup>. One challenge when performing Lim-OOHs and Lin-OOHs patch tests is the misinterpretation of positive reactions due to the considerable percentage of doubtful and irritant reactions. In cases of weak patch test reactions, it is important to rule out a false positive, irritant reaction. A ROAT may then help to distinguish true contact allergies from irritant reactions<sup>12,16,61</sup>.

How to reliably assess Lim-OOHs and Lin-OOHs exposure is still difficult, and sensitization and elicitation limits of Lim-OOHs and Lin-OOHs in products remain unknown.

Finally, further patch test materials for assessing fragrance allergy need to be developed to be up to date with current exposures to fragrances. Many other fragrance terpenes are known to be pre- or prohaptens (e.g., geraniol, citronellol, linalyl acetate, eugenol) and are known to be altered by air oxidation and/or skin metabolism and patch test materials need to be adjusted for this<sup>62</sup>.

## **5. Conclusions**

We recommend that Lim-OOHs 0.3% pet. and Lin-OOHs 1.0% pet. should be patch tested in all patients with suspected fragrance allergy. Additionally, it should be considered to include them in the baseline series<sup>33,34</sup>, as positive reactions are frequently relevant detecting additional cases of fragrance allergy. However, the routine conditions for patch testing of these materials, and their sources of exposure should be better clarified before "allowing" them in a "reference series" that should be practical/usable by "all" dermatologists, also the less experienced ones. Testing simultaneously lower concentrations, such as Lim-OOHs 0.2% and Lin-OOHs 0.5%, may help in interpretation of patch test results, discovering



contact allergies. Moreover, weak (+) reactions should be still interpreted with caution as it might not be excluded that they, in fact, represent irritant ones. Relevance assessment should include an in-depth evaluation of exposure to limonene and linalool in different types of products and thus a comprehensive exposure history. Therefore, the development of patient questionnaires in which exposure and skin problems are assessed prior to testing may allow for better evaluation of clinical relevance<sup>12</sup>. Moreover, more work should be done to develop stable and reliable methods for detection of hydroperoxides in complex consumer products to identify and confirm the actual exposure sources causing sensitization and elicitation of ACD.

It should be kept in mind that the discussion on the general interest about when and how patch testing for some common skin contact allergens should be performed transcends the boundaries of our daily practice and has a direct impact on the health of our patients and on all preventive measures designed to avoid a public and multidisciplinary problem.

#### **Credit (Contribution Roles of authors)**

Isabel A Ogueta, Johanna Brared Christensson, Elena Giménez-Arnau: conceptualization, data curation, formal analysis, methodology, Writing – Original Draft Preparation and review  
Richard Brans, Mark Wilkinson, Luca Stingeni, Caterina Foti, Olivier Aerts, Cecilia Svedman, Margarida Gonçalo: EECDRG member involved in the supervision, validation, visualization, Writing – Review & Editing

Ana Giménez-Arnau: EECDRG member conceptualization, data curation, formal Analysis, methodology, project Administration, validation, Writing – Original Draft Preparation, Writing – Review & Editing

## References

1. Thyssen JP, Linneberg A, Menné T, Nielsen NH, Johansen JD. The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol*. 2009;161(1):95-101. doi:10.1111/j.1365-2133.2009.09157.x
2. Uter W, Johansen JD, Börje A, et al. Categorization of fragrance contact allergens for prioritization of preventive measures: clinical and experimental data and consideration of structure-activity relationships. *Contact Dermatitis*. 2013;69(4):196-230. doi:10.1111/cod.12117
3. Diepgen TL, Ofenloch R, Bruze M, et al. Prevalence of fragrance contact allergy in the general population of five European countries: a cross-sectional study. *Br J Dermatol*. 2015;173(6):1411-1419. doi:10.1111/bjd.14151
4. Marks JG, Belsito DV, DeLeo VA, et al. North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens. *Journal of the American Academy of Dermatology*. 1998;38(6):911-918. doi:10.1016/S0190-9622(98)70587-0
5. Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Dermatitis*. 2008;58(3):134-141. doi:https://doi.org/10.1111/j.1600-0536.2007.01287.x
6. de Groot AC, Schmidt E. Essential Oils, Part III: Chemical Composition. *Dermatitis*. 2016;27(4):161-169. doi:10.1097/DER.000000000000193
7. Buckley DA. Fragrance ingredient labelling in products on sale in the U.K. *Br J Dermatol*. 2007;157(2):295-300. doi:10.1111/j.1365-2133.2007.08018.x
8. Rastogi SC, Heydorn S, Johansen JD, Basketter DA. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis*. 2001;45(4):221-225. doi:10.1034/j.1600-0536.2001.450406.x
9. Rastogi SC, Menné T, Johansen JD. The composition of fine fragrances is changing. *Contact Dermatitis*. 2003;48(3):130-132. doi:https://doi.org/10.1034/j.1600-0536.2003.00035.x
10. Bennike NH, Oturai NB, Müller S, et al. Fragrance contact allergens in 5588 cosmetic products identified through a novel smartphone application. *J Eur Acad Dermatol Venereol*. 2018;32(1):79-85. doi:10.1111/jdv.14513
11. Yazar K, Johnsson S, Lind ML, Boman A, Lidén C. Preservatives and fragrances in selected consumer-available cosmetics and detergents. *Contact dermatitis*. 2010;64:265-272. doi:10.1111/j.1600-0536.2010.01828.x

12. Bråred Christensson J, Andersen KE, Bruze M, et al. Positive patch test reactions to oxidized limonene: exposure and relevance. *Contact Dermatitis*. 2014;71(5):264-272. doi:10.1111/cod.12285
13. Matura M, Goossens A, Bordalo O, et al. Oxidized citrus oil (R-limonene): A frequent skin sensitizer in Europe. *J Am Acad Dermatol*. 2002;47(5):709-714. doi:10.1067/mjd.2002.124817
14. Sköld M, Börje A, Matura M, Karlberg AT. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis*. 2002;46(5):267-272. doi:https://doi.org/10.1034/j.1600-0536.2002.460504.x
15. Björkman YA, Hagvall L, Siwmark C, Niklasson B, Karlberg AT, Christensson JB. Air-oxidized linalool elicits eczema in allergic patients – a repeated open application test study. *Contact Dermatitis*. 2014;70(3):129-138. doi:https://doi.org/10.1111/cod.12163
16. Bennike NH, Palangi L, Christensson JB, et al. Allergic contact dermatitis caused by hydroperoxides of limonene and dose-response relationship—A repeated open application test (ROAT) study. *Contact Dermatitis*. 2019;80(4):208-216. doi:https://doi.org/10.1111/cod.13168
17. Wilkinson SM, Badulici S, Giménez-Arnau A, et al. The European baseline series: Criteria for allergen inclusion with reference to formaldehyde releasers. *Contact Dermatitis*. 2021;85(2):125-128. doi:10.1111/cod.13836
18. Lepoittevin JP. Molecular aspects in allergic and irritant contact dermatitis. In: *Contact Dermatitis*. 5th edition. Springer-Verlag; 2011:91-110.
19. Kao D, Chaintreau A, Lepoittevin JP, Giménez-Arnau E. Synthesis of allylic hydroperoxides and EPR Spin-Trapping studies on the formation of radicals in iron systems as potential initiators of the sensitizing pathway. *J Org Chem*. 2011;76(15):6188-6200. doi:10.1021/jo200948x
20. Kao D, Chaintreau A, Lepoittevin JP, Giménez-Arnau E. Mechanistic studies on the reactivity of sensitizing allylic hydroperoxides: investigation of the covalent modification of amino acids by carbon-radical intermediates. *Toxicol Res*. 2014;3(4):278-289. doi:10.1039/C3TX50109D
21. Lauricella R, Tuccio B. Detection and characterization of free radicals after spin trapping. In: *Electron Paramagnetic Resonance Spectroscopy*. Springer, Cham; 2020:51-82.
22. Kuresepi S, Vileno B, Lepoittevin JP, Giménez-Arnau E. Mechanistic insights on skin sensitization to linalool hydroperoxides: EPR evidence on radical intermediates formation in reconstructed human epidermis and <sup>13</sup>C NMR reactivity studies with

- thiol residues. *Chem Res Toxicol*. 2020;33(7):1922-1932. doi:10.1021/acs.chemrestox.0c00125
23. Kuresepi S, Vileno B, Turek P, Lepoittevin JP, Giménez-Arnau E. Potential of EPR spin-trapping to investigate in situ free radicals generation from skin allergens in reconstructed human epidermis: cumene hydroperoxide as proof of concept. *Free Radical Research*. 2018;52(2):171-179. doi:10.1080/10715762.2017.1420906
  24. Sahli F, Sousa MSE, Vileno B, et al. Understanding the skin sensitization capacity of ascaridole: a combined study of chemical reactivity and activation of the innate immune system (dendritic cells) in the epidermal environment. *Arch Toxicol*. 2019;93(5):1337-1347. doi:10.1007/s00204-019-02444-3
  25. Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method. Accessed February 25, 2021. [https://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation-reconstructed-human-epidermis-test-method\\_9789264242845-en](https://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation-reconstructed-human-epidermis-test-method_9789264242845-en)
  26. Lichter J, Silva E Sousa M, Peter N, et al. Skin sensitization to fragrance hydroperoxides: interplay between dendritic cells, keratinocytes and free radicals. *Br J Dermatol*. Published online November 18, 2020. doi:10.1111/bjd.19685
  27. Sahli F, Godard A, Vileno B, Lepoittevin JP, Giménez-Arnau E. Formation of methyl radicals derived from cumene hydroperoxide in reconstructed human epidermis: an EPR spin trapping confirmation by using <sup>13</sup>C-substitution. *Free Radic Res*. 2019;53(7):737-747. doi:10.1080/10715762.2019.1624741
  28. Karlberg AT, Dooms-Goossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis*. 1997;36(4):201-206. doi:10.1111/j.1600-0536.1997.tb00270.x
  29. Matura M, Sköld M, Börje A, et al. Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis*. 2005;52(6):320-328. doi:<https://doi.org/10.1111/j.0105-1873.2005.00605.x>
  30. Bråred Christensson J, Matura M, Gruvberger B, Bruze M, Karlberg AT. Linalool – a significant contact sensitizer after air exposure. *Contact Dermatitis*. 2010;62(1):32-41. doi:<https://doi.org/10.1111/j.1600-0536.2009.01657.x>
  31. Bråred Christensson J, Andersen KE, Bruze M, et al. Air-oxidized linalool: a frequent cause of fragrance contact allergy. *Contact Dermatitis*. 2012;67(5):247-259. doi:10.1111/j.1600-0536.2012.02134.x
  32. Bråred Christensson J, Andersen KE, Bruze M, et al. An international multicentre study on the allergenic activity of air-oxidized R-limonene. *Contact Dermatitis*. 2013;68(4):214-223. doi:<https://doi.org/10.1111/cod.12036>

33. Deza G, García-Bravo B, Silvestre JF, et al. Contact sensitization to limonene and linalool hydroperoxides in Spain: a GEIDAC\* prospective study. *Contact Dermatitis*. 2017;76(2):74-80. doi:<https://doi.org/10.1111/cod.12714>
34. Wlodek C, Penfold CM, Bourke JF, et al. Recommendation to test limonene hydroperoxides 0.3% and linalool hydroperoxides 1.0% in the British baseline patch test series. *British Journal of Dermatology*. 2017;177(6):1708-1715. doi:<https://doi.org/10.1111/bjd.15648>
35. Sukakul T, Bruze M, Mowitz M, et al. Contact allergy to oxidized linalool and oxidized limonene: Patch testing in consecutive patients with dermatitis. *Contact Dermatitis*. 2021, online ahead of print. doi:10.1111/cod.13980
36. Bruusgaard-Mouritsen MA, Garvey LH, Johansen JD. Facial contact dermatitis caused by cosmetic-relevant allergens. *Contact Dermatitis*. 2021, online ahead of print. doi:10.1111/cod.13966
37. Noë E, Huygens S, Morren MA, Garmyn M, Goossens A, Gilissen L. Contact allergy in a paediatric population observed in a tertiary referral centre in Belgium. *Contact Dermatitis*. doi:10.1111/cod.13975. doi:10.1111/cod.13975
38. Bråred Christensson J, Forsström P, Wennberg AM, Karlberg AT, Matura M. Air oxidation increases skin irritation from fragrance terpenes. *Contact Dermatitis*. 2009;60(1):32-40. doi:10.1111/j.1600-0536.2008.01471.x
39. Bråred Christensson J, Karlberg AT, Andersen KE, et al. Oxidized limonene and oxidized linalool - concomitant contact allergy to common fragrance terpenes. *Contact Dermatitis*. 2016;74(5):273-280. doi:10.1111/cod.12545
40. Audrain H, Kenward C, Lovell CR, et al. Allergy to oxidized limonene and linalool is frequent in the U.K. *British Journal of Dermatology*. 2014;171(2):292-297. doi:<https://doi.org/10.1111/bjd.13037>
41. Uter W, Yazar K, Kratz EM, Mildau G, Lidén C. Coupled exposure to ingredients of cosmetic products: I. Fragrances. *Contact Dermatitis*. 2013;69(6):335-341. doi:<https://doi.org/10.1111/cod.12125>
42. Bråred Christensson J, Johansson S, Hagvall L, Jonsson C, Börje A, Karlberg AT. Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis*. 2008;59(6):344-352. doi:<https://doi.org/10.1111/j.1600-0536.2008.01442.x>
43. Dittmar D, Schuttelaar MLA. Contact sensitization to hydroperoxides of limonene and linalool: Results of consecutive patch testing and clinical relevance. *Contact Dermatitis*. 2019;80(2):101-109. doi:10.1111/cod.13137
44. LexUriServ.pdf. Accessed February 25, 2021. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:066:0026:0035:EN:PDF>

45. Bennike NH, Zachariae C, Johansen JD. Non-mix fragrances are top sensitizers in consecutive dermatitis patients - a cross-sectional study of the 26 EU-labelled fragrance allergens. *Contact Dermatitis*. 2017;77(5):270-279. doi:10.1111/cod.12822
46. Dendooven E, Foubert K, Goossens A, et al. Concomitant positive patch test reactions in FreeStyle-allergic patients sensitized to isobornyl acrylate. *Contact Dermatitis*. 2021;84(3):166-174. doi:10.1111/cod.13706
47. Mowitz M, Fornander L, Hosseiny S, Ryberg K, Bruze M. Patch Testing with Isobornyl Acrylate in 16 Swedish Patients with Contact Dermatitis from Glucose Sensors and/or Insulin Pumps. *Acta Derm Venereol*. 2019;99(13):1286-1287. doi:10.2340/00015555-3256
48. Thomas AF, Bessière Y. Limonene. *Nat Prod Rep*. 1989;6(3):291-309. doi:10.1039/NP9890600291
49. Nanyan P. Fragrance Allergens in Hair Removal Cosmetic Products. *Dermatitis*. 2019;30(4):268-271. doi:10.1097/DER.0000000000000491
50. Hagvall L, Sköld M, Bråred hristensson J, Börje A, Karlberg AT. Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis*. 2008;59(3):143-150. doi:https://doi.org/10.1111/j.1600-0536.2008.01402.x
51. Sköld M, Hagvall L, Karlberg AT. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis*. 2008;58(1):9-14. doi:10.1111/j.1600-0536.2007.01262.x
52. Foti C, Zambonin CG, Conserva A, Casulli C, D'Accolti L, Angelini G. Occupational contact dermatitis to a limonene-based solvent in a histopathology technician. *Contact Dermatitis*. 2007;56(2):109-112. doi:10.1111/j.1600-0536.2007.00995.x
53. Gatica-Ortega ME, Pastor-Nieto MA, Schoendorff-Ortega C, Mollejo-Villanueva M, Giménez-Arnau A. Lymphomatoid contact dermatitis caused by limonene hydroperoxides confirmed by an exposure provocation test with the involved personal hygiene products. *Contact Dermatitis*. 2018;78(3):230-233. doi:10.1111/cod.12908
54. Elliott JF, Ramzy A, Nilsson U, Moffat W, Suzuki K. Severe intractable eyelid dermatitis probably caused by exposure to hydroperoxides of linalool in a heavily fragranced shampoo. *Contact Dermatitis*. 2017;76(2):114-115. doi:https://doi.org/10.1111/cod.12738
55. Isaksson M, Karlberg AT, Nilsson U. Allergic contact dermatitis caused by oxidized linalool in a deodorant. *Contact Dermatitis*. 2019;81(3):213-214. doi:10.1111/cod.13276

56. Natsch A, Nägelin M, Leijds H, et al. Exposure source for skin sensitizing hydroperoxides of limonene and linalool remains elusive: An analytical market surveillance. *Food Chem Toxicol.* 2019;127:156-162. doi:10.1016/j.fct.2019.03.028
57. Kern S, Dkhil H, Hendarsa P, Ellis G, Natsch A. Detection of potentially skin sensitizing hydroperoxides of linalool in fragranced products. *Anal Bioanal Chem.* 2014;406(25):6165-6178. doi:10.1007/s00216-014-8066-3
58. Ramzi A, Ahmadi H, Sadiktsis I, Nilsson U. A two-dimensional non-comprehensive reversed/normal phase high-performance liquid chromatography/tandem mass spectrometry system for determination of limonene and linalool hydroperoxides. *J Chromatogr A.* 2018;1566:102-110. doi:10.1016/j.chroma.2018.06.056
59. Rudbäck J, Islam MN, Börje A, Nilsson U, Karlberg AT. Essential oils can contain allergenic hydroperoxides at eliciting levels, regardless of handling and storage. *Contact Dermatitis.* 2015;73(4):253-254. doi:https://doi.org/10.1111/cod.12427
60. Karlberg AT, Magnusson K, Nilsson U. Influence of an anti-oxidant on the formation of allergenic compounds during auto-oxidation of d-limonene. *Ann Occup Hyg.* 1994;38(2):199-207. doi:10.1093/annhyg/38.2.199
61. Johansen JD, Andersen KE, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis.* 1996;34(6):414-418. doi:10.1111/j.1600-0536.1996.tb02244.x
62. Karlberg AT, Börje A, Duus Johansen J, et al. Activation of non-sensitizing or low-sensitizing fragrance substances into potent sensitizers – prehapten and prohaptens. *Contact Dermatitis.* 2013;69(6):323-334. doi:10.1111/cod.12127
63. Schnuch A, Uter W, Geier J, Lessmann H, Frosch PJ. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis.* 2007;57(1):1-10. doi:10.1111/j.1600-0536.2007.01088.x
64. van Oosten EJ, Schuttelaar MLA, Coenraads PJ. Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis.* 2009;61(4):217-223. doi:10.1111/j.1600-0536.2009.01605.x
65. Uter W, Geier J, Frosch P, Schnuch A. Contact allergy to fragrances: Current patch test results (2005-2008) from the Information Network of Departments of Dermatology. *Contact Dermatitis.* 2010;63:254-261. doi:10.1111/j.1600-0536.2010.01759.x
66. Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis.* 1987;16(2):93-95. doi:10.1111/j.1600-0536.1987.tb01386.x

67. de Groot AC, Liem DH, Nater JP, van Ketel WG. Patch tests with fragrance materials and preservatives. *Contact Dermatitis*. 1985;12(2):87-92. doi:10.1111/j.1600-0536.1985.tb01059.x
68. de Groot AC, Coenraads PJ, Bruynzeel DP, et al. Routine patch testing with fragrance chemicals in The Netherlands. *Contact Dermatitis*. 2000;42(3):184-185.
69. Ung CY, White JML, White IR, Banerjee P, McFadden JP. Patch testing with the European baseline series fragrance markers: a 2016 update. *Br J Dermatol*. 2018;178(3):776-780. doi:https://doi.org/10.1111/bjd.15949
70. Nath NS, Liu B, Green C, Atwater AR. Contact Allergy to Hydroperoxides of Linalool and d-Limonene in a US Population. *Dermatitis*. 2017;28(5):313-316. doi:10.1097/DER.0000000000000318
71. Bennike NH, Lepoittevin JP, Johansen JD. Can contact allergy to p-phenylenediamine explain the high rates of terpene hydroperoxide allergy? - An epidemiological study based on consecutive patch test results. *Contact Dermatitis*. 2017;76(2):67-73. doi:10.1111/cod.12618
72. Sabroe RA, Holden CR, Gawkrödger DJ. Contact allergy to essential oils cannot always be predicted from allergy to fragrance markers in the baseline series. *Contact Dermatitis*. 2016;74(4):236-241. doi:https://doi.org/10.1111/cod.12528
73. Christensson JB, Hellsén S, Börje A, Karlberg AT. Limonene hydroperoxide analogues show specific patch test reactions. *Contact Dermatitis*. 2014;70(5):291-299. doi:https://doi.org/10.1111/cod.12195



## Figure legends

Figure 1. Chemical structures of linalool, R-(+)-limonene and corresponding sensitizing hydroperoxides.

Figure 2. Radical decomposition of Lin-7-OOH.

Figure 3. EPR-ST methodology developed in RHE EpiSkin<sup>TM</sup> and applied here to Lin-OOHs using DEPMPO as spin-trap. Experimental EPR spectra are analyzed by means of computer simulation using labmade scripts based on Easyspin toolbox under Matlab (Mathworks) environment. It is shown the EPR experimental spectrum (Exp) of Lin-OOHs (10mM)/DEPMPO (250 mM) in RHE, together with computer simulation (Sim) and deconvolution affording spectra of adducts representing trapping of hydroxy, carbon and alkoxy radicals formed in the epidermis<sup>22</sup>.

Table 1. Prevalence of positive reactions to linalool and limonene (“pure” or not intentionally oxidized / not deliberately oxidized) when patch tested at different

	<b>Test Concentration (%)</b>	<b>Number tested</b>	<b>Positive cases (%)</b>	<b>Study</b>
<b>Limonene</b>	10	4731	<b>0.2</b>	Audrain et al. (2014) <sup>40</sup>
	2	2396	<b>0.1</b>	Schnuch et al. (2007) <sup>63</sup>
	2	320	<b>0</b>	van Oosten et al. (2009) <sup>64</sup>
	2	1241	<b>0.88</b>	Uter et al. (2010) <sup>65</sup>
	2	1200	<b>0</b>	Santucci et al. (1987) <sup>66</sup>
<b>Linalool</b>	30	179	<b>0</b>	de Groot et al. (1985) <sup>67</sup>
	20	1825	<b>0.2</b>	de Groot et al. (2000) <sup>68</sup>
	10	320	<b>0.6</b>	van Oosten et al. (2009) <sup>64</sup>
	10	985	<b>0.16</b>	Uter et al. (2010) <sup>65</sup>
	10	2401	<b>0.2</b>	Schnuch et al. (2007) <sup>63</sup>
	10	4731	<b>0.3</b>	Audrain et al. (2014) <sup>40</sup>

concentrations in various studies

Table 2. Prevalence of positive reactions to oxidized R-limonene/Lim-OOHs when patch tested at different concentrations in various studies.

Test Concentration of Lim-OOHs (%)	Number tested	Positive cases %	Doubtful cases %	Irritative cases %	Study
0.1*	3639	1.4	0.7	0.3	Deza et al. (2017) <sup>33</sup>
	4563	1.3	1.2	0.6	Wlodek et al. (2017) <sup>34</sup>
0.2**	3639	3.4	0.6	0.8	Deza et al. (2017) <sup>33</sup>
	4563	3.2	2.1	0.9	Wlodek et al. (2017) <sup>34</sup>
0.3***	5773	5.1	7.9	0.1	Sukakul et al. (2021) <sup>35</sup>
	821	9.4	17.2	0.9	Dittmar & Schuttelaar (2019) <sup>43</sup>
	2084	4.3	1.4	1.4	Ung et al. (2018) <sup>69</sup>
	103	8	--	--	Nath et al. (2017) <sup>70</sup>
	3639	5.1	0.4	1.5	Deza et al. (2017) <sup>33</sup>
	4563	5.3	2.4	2	Wlodek et al. (2017) <sup>34</sup>
	6004 <sup>63</sup> 3843 <sup>64</sup>	2.5	13.7	5.8	Bennike et al. (2016, 2017) <sup>45,71</sup>
	1292	5	4.3	9.8	Sabroe et al. (2016) <sup>72</sup>
	4731	5	--	3.9	Audrain et al. (2014) <sup>40</sup>
	763	1.2	--	--	Christensson et al. (2014) <sup>73</sup>
	2900	5.2	7	0.9	Christensson et al. (2010) <sup>32</sup>
	2273	2.8	--	--	Matura et al. (2002) <sup>13</sup>

\* Patch test material of oxidized limonene containing Lim-OOHs at 0.1%

\*\* Patch test material of oxidized limonene containing Lim-OOHs at 0.2%

\*\*\* Patch test material of oxidized limonene containing Lim-OOHs at 0.3%

Table 3. Prevalence of positive reactions to oxidized linalool/Lin-OOHs when patch tested at different concentrations in various studies.

Test Concentration of Lin-OOHs (%)	Number tested	Positive cases (%)	Doubtful cases (%)	Irritative cases (%)	Study
0.25*	3639	1.3	0.9	0.2	Deza et al. (2017) <sup>33</sup>
	4563	2.5	2.9	1.1	Wlodek et al. (2017) <sup>34</sup>
0.33% **	1693	0.83	1.9	0	Christensson et al. (2010) <sup>30</sup>
0.33%**	1511	1.3	--	--	Matura et al. (2005) <sup>29</sup>
0.5***	3639	2.9	0.8	1.2	Deza et al. (2017) <sup>33</sup>
	4563	5.1	3.4	2	Wlodek et al. (2017) <sup>34</sup>
0.66%****	2075	3.2	5.1	0.34	Christensson et al. (2010) <sup>30</sup>
1.0% *****	5773	7.0	10.1	0.2	Sukakul et al. (2021) <sup>35</sup>
	821	11.7	21.9	1.9	Dittmar & Schuttelaar (2019) <sup>43</sup>
	2084	7.4	1.5	1.5	Ung et al. (2018) <sup>69</sup>
	3639	4.9	0.5	1.9	Deza et al. (2017) <sup>33</sup>
	4563	7.7	2.9	3.9	Wlodek et al. (2017) <sup>34</sup>
	103	20	--	--	Nath et al. (2017) <sup>70</sup>
	6004	3.9	20.9	7.2	Bennike et al. (2016, 2017) <sup>45,71</sup>
	4731	5.9	--	5.9	Audrain et al. (2014) <sup>40</sup>
	2900	6.9	9.2	1.3	Christensson et al. (2012) <sup>31</sup>
	1725	5.3	3.4	0.23	Christensson et al. (2010) <sup>30</sup>
	1292	9.8	6.6	13.6	Sabroe et al. (2016) <sup>72</sup>
1.8%***** *	1004	7.2	7.3	0.7	Christensson et al. (2010) <sup>30</sup>

\* Patch test material of oxidized linalool containing Lin-OOHs at 0.25%

\*\* Patch test material of oxidized linalool 2.0% containing Lin-OOHs at 0.33%

\*\*\* Patch test material of oxidized linalool containing Lin-OOHs at 0.5%

\*\*\*\* Patch test material of oxidized linalool 4.0% containing Lin-OOHs at 0.66%

\*\*\*\*\* Patch test material of oxidized linalool containing Lin-OOHs at 1.0%

\*\*\*\*\* Patch test material of oxidized linalool 11% containing Lin-OOHs at 1.8%

