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Allergic contact dermatitis from (“hypoallergenic”) adhesives containing D-limonene

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Abstract

Background

Besides being a potential component of (some species of) colophonium, D-limonene is also used as a tackifier in the production of adhesives. Hydroperoxides of limonene are well-known skin sensitizers.

Objectives

To show that D-limonene may be present in colophonium-containing but also colophonium-free (“hypoallergenic”) adhesives, and that patients suffering from allergic contact dermatitis (ACD) from both types of adhesives might display positive patch test reactions to limonene hydroperoxides in this regard.

Methods

Five patients with suspected ACD from adhesives were patch tested to the baseline series (containing limonene hydroperoxides 0.3 and 0.2% pet.), additional series and, if available, to the culprit adhesives. The adhesives labelled as containing colophonium (n = 3) or free from it (n = 2) were analysed with gas chromatography - mass spectrometry (GC-MS) for the presence of D-limonene.

Results

All five patients sensitised to adhesives had (strong) positive patch test reactions to limonene hydroperoxides. The presence of D-limonene, and/or related components, could be demonstrated in all three colophonium-containing and, surprisingly, also in two colophonium-free ("hypoallergenic") tapes.

Conclusions

D-limonene may be present in both regular and "hypoallergenic" adhesives, with limonene hydroperoxides potentially contributing to ACD from such medical devices. The use of fragrance chemicals in adhesives deserves further research.

1 INTRODUCTION

Limonene (dipentene) is a racemic mixture of L- and D-limonene and the latter, also known as *p*-mentha-1,8-diene, (+)-limonene or (*R*)-limonene, is commonly used as a fragrance and flavouring chemical, a solvent, and a degreaser. Upon air exposure limonene hydroperoxides are formed, which are strong contact allergens and well-known causes of allergic contact dermatitis (ACD).¹ D-limonene is, however, also a component of (some species of) colophonium and may also be used on its own as a tackifier in resins and in the production of rubbers and polymers (e.g., polyesters and polyurethanes), all used in the manufacture of medical adhesives.²⁻⁵ We here report the clinical and chemical analytical work-up of five patients sensitised to limonene hydroperoxides who developed ACD from "regular" (colophonium-containing) and "hypoallergenic" (colophonium-free) adhesives, all shown to contain D-limonene and/or related components.

2 METHODS

2.1 Patients

We performed a brief retrospective review of the medical files of patients with suspected ACD from medical adhesives and who were patch tested in our clinic in the 2.5-year period between January 2018 and June 2020. We here present five cases of ACD from medical adhesives observed during this period: three young adults and two children (Figures 1 and 2).



FIGURE 1

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(A) Severe allergic contact dermatitis on the back in Case 2, a 3-year-old boy. (B,*) co-attributable to D-limonene in the colophonium-free (“hypoallergenic”) Leukoplast pro LF adhesive. (C,*) Although not confirmed by chemical analysis, D-limonene is possibly also present in the Manuka-honey wound dressing Medihoney Apinate, which also reacted positively; no further information on its composition was available from its label nor from the manufacturer

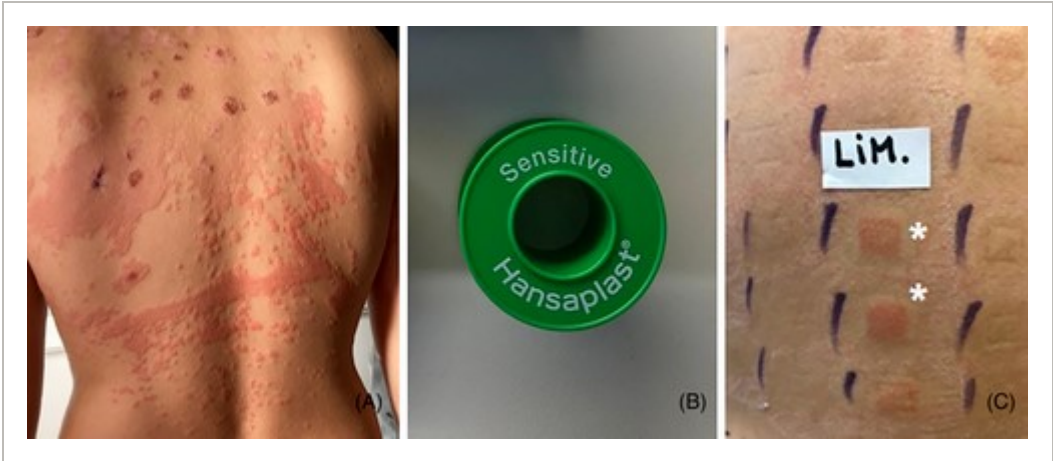


FIGURE 2

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(A) Allergic contact dermatitis on the back in Case 5, a 10-year-old girl (B) caused by several tapes, among which the colophonium-free (“hypoallergenic”), D-limonene-containing Hansaplast sensitive. (C) Strong positive patch test reactions (++) were observed to limonene hydroperoxides 0.3% pet. (upper *) and 0.2% pet. (lower *)

These five patients presented with ACD from six types of adhesives, notably three “regular”, colophonium-containing tapes (Leukoplast Classic, Leukotape Classic, and Tensoplast Classic; BSN Medical, Hamburg, Germany), two “hypoallergenic”, colophonium-free tapes (Leukoplast pro LF; BSN Medical, and Hansaplast Sensitive; Beiersdorf, Hamburg, Germany), and one honey-containing wound dressing (Medihoney Apinate; Spring Medical, Epe, The Netherlands). The relevant clinical data (age, sex, localisation of dermatitis, and type of adhesives used) are summarised in Table 1.

TABLE 1. Overview of the five patients, their culprit adhesives and positive patch tests

Case	Age/sex	Localisation of dermatitis	Culprit adhesive(s) (containing colophonium, D-limonene and/or a	Patch test to the adhesive(s) “as is” (D4)	Patch test to colophonium 20% pet. (D4)	Patch test to limonene hydroperoxides 0.3%/0.2% pet. (D4)	Oth pat (D4)

chemical similar to D-limonene)							
Case	29/F	Feet/legs	Leukoplast	NT	+++	+/+	Myr
1			classic (colophonium ^a , D-limonene ^b , α-terpineol ^b)				per Eve furf Lin hyd Sod met
Case	3/M	Back	Leukoplast pro	+	++	+/+	Frag
2			LF (D- limonene ^b)	++			Pro

Note: Note that two “hypoallergenic” adhesives (Leukoplast pro LF and Hansaplast Sensitive) do not contain colophonium according to their label but do contain D-limonene according to the chemical analyses, and, considering continuous auto-oxidation, thus also limonene hydroperoxides.

Abbreviations: D, day; F, female; IPPD, isopropyl -N-phenyl-4-phenylenediamine; M, male; NT, not tested; PTBP resin, *para*-tertiary butylphenol formaldehyde resin.

- ^a According to the label.
- ^b According to the chemical analyses by means of gas chromatography - mass spectrometry [GC–MS].
- ^c According to the literature.

All five patients were patch-tested to the Belgian baseline series, containing limonene hydroperoxides 0.3% and 0.2% pet. (H-032A and H-032B, respectively, from Chemotechnique Diagnostics, Vellinge, Sweden), but not D-limonene 10% pet. If available, the patients’ own culprit adhesives were also patch-tested “as is” (±2 × 2 cm) as well as any other products concomitantly used (e.g., antiseptics). Additionally, patch tests were also (variably) performed to allergens from a (meth)acrylate, plastics and glues series (containing hydroabietic alcohol or Abitol), isocyanates, and other allergens (e.g., abietic acid), all from Chemotechnique Diagnostics. Patch test preparations were mounted on patch test chambers (AllergEAZE, SmartPractice, Calgary, Canada) and occluded for 2 days on the upper back using Fixomull stretch tape (BSN Medical). The tests were read according to ESCD guidelines on day (D)2, D3/D4, and D7, with readings beyond D4 being performed in person at the clinic, or via the submission of photographs by e-mail.^{6, 7}

2.2 Chemical analyses

All adhesives were chemically analysed, primarily for the presence/absence of D-limonene, using gas chromatography-mass spectrometry (GC-MS). When evaluating the chromatographic peaks, attention was also given to other compounds, and if these stood out, identification was performed using the MS database.

Beside the culprit adhesives, we also analysed Fixomull stretch tape, which is used in our clinic as a fixation for the patch tests as well as the following patch test materials from Chemotechnique Diagnostics: D-limonene (10% pet.), limonene hydroperoxides (0.3% pet.), linalool hydroperoxides (1.0% pet.), colophonium (20% pet.), *Myroxylon pereirae* resin (25% pet.), fragrance mix I (FM I; 8% pet.), and fragrance mix II (FM II; 14% pet.).

One gram of each adhesive sample was cut into pieces and placed in test tubes filled with 7 mL heptane and then heated at 50°C for 24 hours, after which they were centrifuged (20 minutes at 14 000 rpm) at room temperature; 3 µL of the supernatants was injected manually in the GC-MS. One gram of the patch test materials was diluted in 10 mL heptane and sonicated for 1 hour, after which they were centrifuged (20 minutes at 14 000 rpm) at 0°C; 1 µL of the supernatants was injected automatically into the GC-MS. An extra evaporation step was applied for two products (Hansaplast Sensitive adhesive and Medihoney Apinate wound dressing) to concentrate the extracts and to allow us to visualise chromatographic peaks, that is, to detect low amounts of all potential ingredients. This was done using a rotavapor VARIO PC 2001 (Vacuubrand GmbH & Co. KG, Wertheim, Germany), with automatic pressure and 95 rpm at 25 °C. After adding 200 µL heptane, 3 µL was manually injected into the GC-MS.

The GC-system consisted of a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific Inc., Waltham, Massachusetts, US), fitted up with a 30m-long Restek Rxi-5HT capillary column (Chrom Tech Inc., Minnesota, US), with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The carrier gas was helium (Liquid Helium, Praxair Technology Inc., Danbury, Connecticut, US) with a constant flow rate of 0.7 mL/min. The inlet was heated to 200°C, the injection volume was 3 µL, the injection was with a split ratio of 20 and a 14 mL/min split flow. The temperature program continued as follows: 2 minutes isothermal at 40°C, raising the temperature by 1.5°C/min till 120°C, finishing with 10 minutes isothermal at 120°C.

Mass spectrometry was performed with a DSQ mass spectrometer (Thermo Fisher). The MS operated in electron impact mode with an ionisation energy of 70 eV. Full scan mode was used, measuring ions with m/z values within a range of 50–650 u, scanning starting after 5 minutes. The transfer line temperature was 250°C, the temperature of the ion source was 220°C. As a reference standard, 0.01% (m/V) of D-limonene (purity of 97%) (Sigma Aldrich, Schnelldorf, Germany) was used, showing a mass spectrum peak at 19.77 minutes.

3 RESULTS

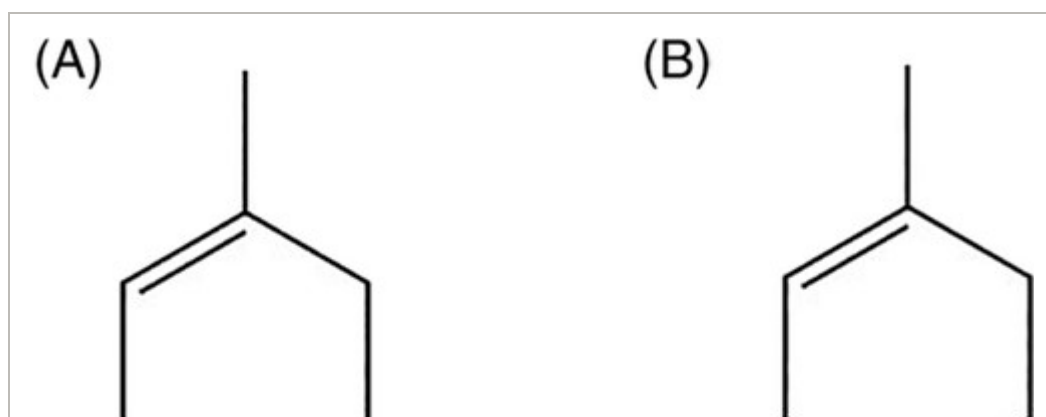
3.1 Patients

According to our patch test database 57 out of 1264 patients (4.5%) had been patch-tested in the 2.5-year study period because of a suspicion of ACD from medical adhesives: (43 females, 14 males; age range 4–75 years; mean and median ages of 39 and 41 years, respectively). A majority (40/57 patients, 70%), was shown to be sensitised to limonene hydroperoxides: seven doubtful (?+), 25 weak (+) and eight strong (++) reactions, respectively; 10 patch-tested negatively, while seven had not been patch-tested to the allergens. Many patients co-reacted to other fragrance allergens, that is, to linalool hydroperoxides (31/57 patients; 54%), *Myroxylon pereirae* resin (20/57; 35%), FM I (14/57; 25%) and FM II (4/57; 7%). Colophonium showed positive patch test reactions in 20 out of 57 patients (35%) with 17 of 20 patients equally reacting to limonene hydroperoxides.

The results of the patch tests of the five patients detailed in the current report are summarised in Table 1. All but one patient (Case 5) showed strong (++) or extreme (+++) reactions to colophonium 20% pet., and some to related components such as abietic acid (major allergen of colophonium) (Case 3) and/or Abitol (modified colophonium) (Cases 3 and 4). All five patients had weak (+) or strong (++) reactions to limonene hydroperoxides 0.3% and 0.2% pet, and all were also co-sensitised to linalool hydroperoxides. Many other fragrance chemicals gave positive reactions, including *Myroxylon pereirae* resin, propolis, evernia furfuracea, and fragrance mix I and II. However, none of the five patients reported here had ever noticed any skin problems when using scented products.

3.2 Chemical analyses

GC-MS confirmed the presence of D-limonene in the colophonium 20% pet. patch test preparation as well as in the colophonium-containing tapes Leukoplast Classic and Tensoplast Classic (both 10–50 ppm), but, surprisingly, not in the (equally colophonium-containing) Leukotape Classic. The latter contained a component very similar to D-limonene (Figure 3A), that is, *p*-menth-1-en-8-ol, known as α -terpineol (Figure 3B), which was also found in the colophonium patch test material and in the two other colophonium-containing adhesives (Leukoplast Classic and Tensoplast Classic).



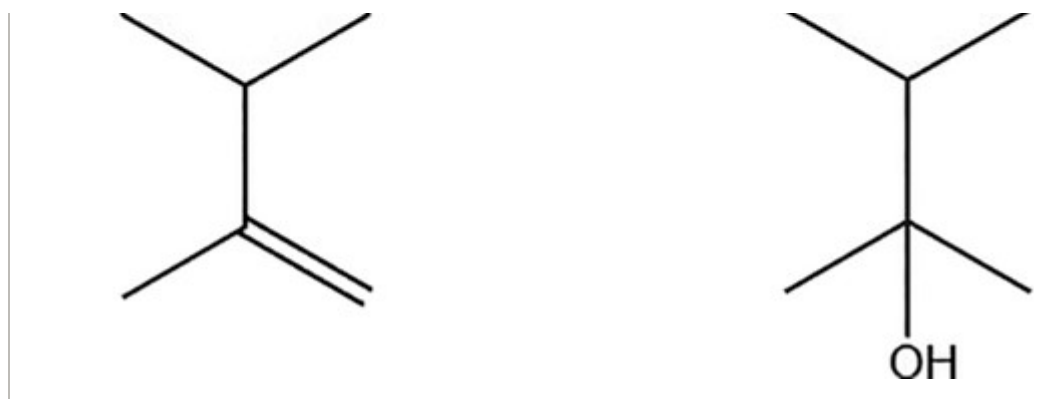


FIGURE 3

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The chemical structures of **(A)** limonene (syn. *P*-mentha-1,8-diene) and **(B)** the related component α-terpineol (syn. *P*-menth-1-en-8-ol)

Neither D-limonene nor α-terpineol could be demonstrated in our patch test fixation material (Fixomull stretch tape), nor in the Medihoney Apinate wound dressing.⁸

Interestingly, D-limonene was also found in the colophonium-free (“hypo-allergenic”) tapes Leukoplast pro LF (50-100 ppm) and Hansaplast Sensitive (1-5 ppm).

In all adhesives in which D-limonene was detected, its concentrations ranged from 1–100 ppm.

Contrary to the patch test preparation of colophonium (20% pet.) and the colophonium-containing adhesives, the patch test preparations of D-limonene (10% pet.) and limonene hydroperoxides (0.3% pet.) did not contain the component α-terpineol.

Linalool hydroperoxides (1% pet.), FM I (8% pet.), FM II (14% pet.), and *Myroxylon perei* resin (25 % pet.) did not contain D-Limonene nor α-terpineol.

Based on identification via the MS database, β-farnesene was also present in Tensoplast Classic.

4 DISCUSSION

Although only few data exist on the epidemiology of contact dermatitis from medical devices and adhesives, in particular, a recent study showed that approximately 5% of patients had developed contact dermatitis, including ACD, from adhesive substances in the glucose sensor FreeStyle Libre.⁹ This figure concurs well with the share of patients that presented at our patch test clinic during the period between January 2018 and the first half of 2020 with suspected ACD from adhesives in general (4.5%).

Interestingly, previous studies have indicated that patients with ACD from medical adhesives are sometimes surprisingly co-sensitised to fragrance chemicals, an observation for which

an explanation is currently lacking. For example, out of 26 patients with ACD from the above-mentioned glucose sensor and related diabetic devices, 61% were shown to be sensitised to fragrances, particularly terpenes.^{10, 11}

In the current report, colophonium, the most well-known contact allergen in adhesives, with hydroperoxides of abietic acid as the most sensitising compounds of the resin, showed positive patch test reactions in 20 out of 57 patients (35%), a large majority of them (17/20) also being sensitised to limonene hydroperoxides. Our patch test database showed a strikingly high number of patients with skin problems from adhesives who appeared to be sensitised to limonene hydroperoxides (ie, 40/57, 70%). Positive co-reactions were also often observed, albeit it to a lesser extent, to other fragrance materials, that is, linalool hydroperoxides (31/57 patients, 54%), *Myroxylon pereirae* resin (20/57, 35%), FM I (14/57, 25%), and FM II (4/57, 7%), as well as to other contact allergens potentially present in adhesives (e.g. acrylates, rubber allergens, and isocyanates).

For all five cases reported herein, all displaying suspected ACD from adhesives, there was no clear alternative explanation for the positive patch test reactions to limonene as scented products were apparently well tolerated. It might be hypothesised that the prolonged and/or repeated application of D-limonene-containing (and by continuous auto-oxidation, limonene hydroperoxide-containing) adhesives, under occlusion, on compromised skin (ie, wounds) might have facilitated the development of ACD. Similar to cosmetics, even low amounts of limonene hydroperoxides might then even be sufficient to provoke contact allergy, especially in highly sensitised individuals (e.g., the Hansaplast Sensitive adhesive, containing only 1-5 ppm limonene, used by Case 5 who was shown to be strongly [++] positive to limonene hydroperoxides).¹²

As demonstrated, D-limonene is also used on its own as an independent tackifier in the production of so-called "hypoallergenic" (colophonium-free) tapes (ie, Hansaplast Sensitive and Leukoplast pro LF). Both, according to their label, claimed to be free from "resin" (ie, free from colophonium).^{4, 13} However, as shown in Cases 2 and 5, D-limonene can then still be a relevant culprit contact allergen contributing to ACD from such "hypoallergenic" tapes, even in those patients who are not contact allergic to colophonium (Case 5).

Legislation regarding contact allergy from medical adhesives and tapes mainly focuses on the presence of colophonium, and colophonium-free ("hypoallergenic") adhesives are often considered as safer alternatives (ie, free of allergenic components). However, here we showed that adhesives in general, and "hypoallergenic" ones in particular, may also (or still) contain D-limonene as a tackifier. Contrary to cosmetic products, in which the presence of D-limonene is strictly regulated by the Cosmetics Regulation (ie, leave-on and rinse-off products must be labelled if their limonene concentrations exceed 10 ppm [0.001%] and 100 ppm [0.01%], respectively), no such legislation exists regarding adhesives in medical products or devices. However, similar concentrations of D-limonene (1-100 ppm) have now also been demonstrated in (hypoallergenic) adhesives, which, additionally, are often used

for a prolonged application time on compromised skin.

Although we were unable to confirm the presence of D-limonene in the wound dressing containing Manuka honey, it has been shown that this type of honey may contain D-limonene.^{8, 14} Factors related to the chemical analytical set-up might perhaps explain this: as the wound dressing is a different type of matrix than the five adhesives, a different extraction protocol might have been required to reliably demonstrate the presence of D-limonene (e.g., longer extraction times, higher temperatures, etc.).

In the colophonium-containing Leukotape Classic (Case 4) no D-limonene could be found either, possibly because no D-limonene and/or another type of colophonium (ie, free from limonene) was used in its production. However, the related substance α -terpineol (syn. *p*-menth-1-en-8-ol), also present in colophonium, could be demonstrated in it, as well as in the other two colophonium- and D-limonene-containing tapes (Leukoplast Classic, Tensoplast Classic). How then can this be linked to Case 4 who demonstrated a strong (++) positive patch test to limonene hydroperoxides? It could be possible that the production process of the Leukotape Classic might have involved D-limonene (syn. *p*-mentha-1,8-diene) that converted into α -terpineol (syn. *p*-menth-1-en-8-ol),³ leaving only low, analytically undetected amounts of D-limonene still capable of eliciting ACD; this, however, was not investigated in our study. Alternatively, if D-limonene is effectively absent, and only α -terpineol is present, it might be hypothesised that cross-reactivity occurred between the oxidation products of limonene and α -terpineol (Figure 3A,B), although, according to general radical mechanism rules, no sensitizing autoxidation products in common can be formed. A third option would be that the observed contact allergy to oxidised limonene (ie, limonene hydroperoxides 0.3% in the oxidised limonene preparation from Chemotechnique Diagnostics) might not be relevant to the observed dermatitis in this particular patient (Case 4).

Concomitant positive patch test reactions to limonene hydroperoxides and colophonium have often been considered as "cross-reactions". Christensson et al. investigated the hypothesis of a specific cross-reactivity between hydroperoxides of colophonium, especially abietic acid, and the hydroperoxides of limonene involving a radical mechanism forming non-specific antigens as a possible explanation. However, they concluded that there was no evidence for this theory.¹⁵ In addition, according to the literature, and as also shown in this study, D-limonene is, on its own, a component of some species of colophonium, thus automatically present in some colophonium-containing tapes and adhesives (e.g., Leukoplast Classic and Tensoplast Classic); hence, its concomitant presence may lead to concomitant sensitization; co-reactions to colophonium and limonene hydroperoxides are not an illustration of cross-reactivity.

Apart from limonene hydroperoxides, patch tests with other fragrance chemicals might be of interest in the work-up of patients with suspected ACD from (medical) adhesives. Examples include carvone, menthol, and other mint-derivatives (all closely related to

limonene),¹⁶ but also β -farnesene (related to farnesol, a component of FM II) which was shown to be present in the Tensoplast adhesive and which, according to the literature, is also used as a tackifier in adhesives.^{17, 18}

Apart from colophonium, D-limonene and related components, also acrylates and many other potential sensitisers can still be present in medical adhesives, some of which may also have contributed to the overall picture of ACD in the cases presented here. Sulfites, for example, might be contained in some rubber-containing consumer items, including natural rubber latex-containing tapes (as used by Case 1), although the presence of these antioxidants in rubber items still needs to be formally confirmed.¹⁹ Equally so, *para*-tertiary butylphenol formaldehyde resin and isothiazolinone derivatives might also play a role in cases of adhesive contact allergy (e.g., Cases 3 and 5) as these chemicals can also be present in some glues and adhesives.^{10, 20}

4.1 Limitations

A first limitation of this study relates to the patch materials. The patients described herein all reacted to the commercialised patch test materials H-032A ("limonene hydroperoxides 0.3% pet.") and H-032B ("limonene hydroperoxides 0.2% pet.") from Chemotechnique Diagnostics. These patch test preparations contain, besides limonene itself, the primary oxidation products (ie, the hydroperoxides at 0.3% or 0.2% pet.), but also secondary oxidation products (e.g., carvone). Although the hydroperoxides are considered the most prominent sensitisers, we did not patch test them separately, for example, in another vehicle. D-limonene, which acts as a penetration enhancer, cannot be left out as this might reduce the elicitation potential of the hydroperoxides. Moreover, the hydroperoxides are unstable and by using the oxidation mixture we use a material similar to nature for patch testing. Additionally, the secondary oxidation products can also be involved in some cases of ACD from limonene-containing products, and these have not been specifically addressed in the present study.

A second limitation relates to the chemical analyses. Four out of five culprit adhesives were shown to contain D-limonene, used by the industry as a tackifier. However, D-limonene evidently differs from its primary and secondary oxidation products contained in the patch test preparations to which the patients reacted. This difference, in general, affects the way "relevance" of positive patch test reactions to the above-mentioned commercialised patch test materials is currently discussed. The reasoning is that products that contain D-limonene will, to a certain extent (e.g., through auto-oxidation), also contain its oxidation products, including the hydroperoxides, in concentrations sufficient to induce or elicit ACD. As regards cosmetics, some authors^(e.g.,²¹) have recently demonstrated that D-limonene-containing cosmetics apparently contain only little amounts of hydroperoxides. In the present study, however, we have not undertaken additional chemical analyses to identify or quantify limonene oxidation products (incl. hydroperoxides) in the adhesives.

5 CONCLUSION

Patients experiencing ACD from regular (colophonium-containing) and "hypoallergenic" (colophonium-free) adhesives may show positive patch test reactions to the commercialised test preparations containing limonene hydroperoxides (0.3% and 0.2% pet.), because D-limonene and/or related components may not only be present in certain types of colophonium but may also themselves be used as tackifiers. The apparent overrepresentation of contact allergy to fragrance chemicals in some patients experiencing ACD from adhesives suggests that these chemicals might also play a role in adhesive allergy and underlines the need for further research concerning the potential link between them.

CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

Ella Dendooven: Conceptualization (equal); data curation (lead); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal). **Kenn Foubert:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal). **Tania Naessens:** Investigation (equal); methodology (equal); project administration (equal). **Luc Pieters:** Supervision (equal); validation (equal). **Julien Lambert:** Supervision (equal); validation (equal). **An Emilie Goossens:** Formal analysis (equal); validation (equal). **Olivier Aerts:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); supervision (lead); validation (lead).

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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