Air-oxidized linalool – a frequent cause of fragrance contact allergy

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Summary

Background. Linalool is a common fragrance terpene that, in pure form, is not allergenic or is a very weak allergen. However, linalool autoxidizes on air exposure, and the oxidation products can cause contact allergy. In a Swedish study, oxidized linalool 6.0% in petrolatum (pet.) gave 5% positive patch test reactions in 2,500 dermatitis patients.

Objectives. To investigate whether oxidized linalool 6%, with a stable concentration of the main hapten, the linalool hydroperoxides (Lin-OOHs) in pet., could be a useful tool for the detection of contact allergy in an international setting.

Methods. Oxidized linalool 6.0% (Lin-OOHs 1%) pet. was tested in 2,900 consecutive dermatitis patients in Denmark, the United Kingdom, Singapore, Spain, Sweden, and Australia.

Results. Overall, 6.9% (range 3–13%) of the patients showed positive patch test reactions to oxidized linalool. Doubtful reactions were found in 9.2% of the patients (range 0–36%). Few irritant reactions were seen.

Conclusions. In an international setting, oxidized linalool has been shown to be a common allergen. Oxidized linalool 6.0% (Lin-OOHs 1%) pet. is a useful, standardized and stable tool for the detection of contact allergy in dermatitis patients. Many patients showing positive patch test reactions to oxidized linalool would not have been informed of their fragrance allergy if this specific test had not been performed.

Key words: allergic contact dermatitis; autoxidation; dose per unit area; fragrance; hydroperoxide; linalool; oxidation products; patch test; terpene.

Linalool is a common fragrance terpene that is widely used in household and hygiene products, because of its fresh, flowery odour. In consumer products, linalool is among the most common fragrance ingredients according to several studies (1–3). Estimated consumer exposure to selected fragrance terpenes was recently assessed, based on concentrations in 10 types of cosmetic product and on the mode of usage for the product. The calculations indicated that the maximum daily exposure to linalool was by far the highest among the other discussed fragrance terpenes for high-end users of cosmetic products containing these terpene components (4).

As a pure compound, linalool seldom causes positive patch test reactions (5, 6). However, linalool has been
CONTACT ALLERGY TO OXIDIZED LINALOOL

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possibility of diagnosing contact allergy in dermatitis patients. This is especially true for mixtures in which one or two of the components are the real haptens, which is the case for oxidized linalool. However, with high patch test concentrations, adverse effects such as irritation or sensitization may occur. It is desirable to find a patch test concentration that detects contact allergy with a low incidence of irritancy or doubtful reactions and a safe level with regard to sensitization. A review giving guidelines on establishing an appropriate patch test concentration with respect to irritancy and active sensitization has been published on behalf of the European Society of Contact Dermatitis (15).

The aim of the present study was to investigate whether the suggested patch test concentration of oxidized linalool 6% pet. could be a useful tool for the detection of contact allergy in dermatitis patients in an international setting. We also wanted to assess the adverse effects of the test preparation in a large group of patients. Furthermore, the relationship between contact allergy to other fragrance markers and/or colophonium in the baseline patch test series and positive patch test reactions to the actual test preparation of oxidized linalool was assessed. As the hydroperoxides have been identified as the main haptens, a patch test preparation with a focus on a stable concentration of the hydroperoxides was developed.

Materials and Methods

Chemicals

Linalool (3,7-dimethyl-1,6-octadien-3-ol) (Fig. 1) with a stated purity of 97% was obtained from Sigma Aldrich Chemie (Schnelldorf, Germany), and purified by distillation. In order to mimic the oxidation that occurs during handling and storage, a simplified experimental oxidation model was used, according to previous experience (11, 16). The oxidation/degradation process was followed by the use of gas chromatography and high-performance liquid chromatography, according to methods previously described (7). A maximum concentration of the major hydroperoxide (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol) (Fig. 1) was observed in the oxidation mixture from about 20 weeks until 45 weeks of air exposure. After this time point, the degradation was the dominant process, and the concentration of hydroperoxide decreased. For the present study, a time point of 25 weeks was chosen, at which time the concentration of linalool was 61% and that of the major hydroperoxide was 14.6%. The concentration of remaining linalool in the oxidation mixture in the present study is twice as high as in oxidation mixtures previously used for patch testing (12, 14), as the time for degradation/oxidation was shortened from 45 weeks to 25 weeks. However, the concentration of the major hydroperoxide was in accordance with that of the oxidation mixtures previously used for patch testing (12, 14).

Patch test materials

The standard allergens used for patch testing were obtained from: Chemotechnique Diagnostics, Vellinge, Sweden (Gothenburg, Malmö, Melbourne, and Singapore; also, for some allergens, Odense and Seville); TroLab, Almirall-Hermal, Reinbeck, Germany (Copenhagen, Barcelona, and London); and Allergeaz, Calgary, Canada (Seville). The TRUE Test™ from SmartPractice (Phoenix, AZ, USA) was used in Odense and Seville. Small Finn Chambers® (diameter 8 mm; inner area 0.5 cm²; Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennsela, Norway) patch test material were used in Barcelona, Copenhagen, Göteborg, London, Malmö, Melbourne, Odense, Seville, and Singapore.

The specific test material of oxidized linalool with a controlled content of Lin-OOH was prepared by Chemotechnique Diagnostics (Vellinge, Sweden) in cooperation with Professor Ann-Therese Karlberg, Dermatochemistry and Skin Allergy, Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden. Oxidized linalool was stored under nitrogen at −20°C until test preparations were made, and chemical analyses were performed on a regular basis to ensure that the composition had not changed during storage. Test preparations were made from the same batch in non-stabilized white pet (VWR International AB, Stockholm, Sweden) and were kept in 5-ml syringes in the refrigerator at Chemotechnique Diagnostics, until being distributed to the test centres. The test preparations contained 6.0% oxidized linalool with a validated content of the major hydroperoxide (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol) of 0.8%. The hydroperoxide fraction in autooxidized linalool contains approximately 80% of the major hydroperoxide, and the rest consists of the minor hydroperoxide (6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol) (Fig. 1), according to previous analyses (7). A content of 1% Lin-OOHs in oxidized linalool 6.0% was therefore calculated. This relationship between the two hydroperoxides in linalool oxidized for 25 weeks was further confirmed in more specific analytical studies performed after the actual study.

Prior to the start of the multicentre study, a thorough investigation of the quality and stability of test preparations consisting of oxidized linalool 6.0% (major Lin-OOH 0.8%) in pet. was performed. Series of test preparations
were made and stored under different conditions, that is, in the freezer and in the refrigerator, and handled according to the instructions given for handling of the test preparations at the dermatological test centres during patch testing (see below). Chemical analysis of the content of linalool and the major Lin-OOH was performed after removal of the pet. vehicle. On the basis of this work, the concentrations of linalool and Lin-OOH in the actual test preparations were found to be stable for at least 3 months of recommended usage. Thus, patch test material for an estimated 3 months of usage was sent to the respective test centre at the start of the study. Instructions for storage and handling were distributed with the patch test materials, recommending storage in a freezer at \(-20^\circ C\) until they started to be used in patch testing of patients. When the materials were in use, test centres were asked to minimize the storage time at room temperature, with a maximum of 3–4 hr out of the refrigerator between patient tests. The patch test materials at two test centres (Göteborg and Malmö) were recalled after 4 months of usage and analysed. The concentrations of linalool and Lin-OOH were found to be intact in the test material, and the syringes were sent back to the test centres for continued usage for another 2 months.

### Subjects, exposure, and evaluation

Consecutive patients, undergoing patch testing because of suspected allergic contact dermatitis at the nine test centres, were screened with the patch test preparations of oxidized linalool 6.0% with a validated content of 0.8% of the major Lin-OOH (corresponding to a total content of 1% Lin-OOHs) in pet., in addition to regular patch testing. Patch test preparations of \(\sim 20\) mg (17) were applied in small Finn Chambers® (diameter 8 mm, inner area 0.5 cm²; Epitest Ltd Oy) on Scanpor® tape (Norgesplaster A/S) to the back of the patient, and left under occlusion for 48 hr. The participating test centres and the time periods of patient inclusion in the study are shown in Table 1.

Visual readings were scored according to the International Contact Dermatitis Research Group (ICDRG) guidelines (18) on at least two occasions, D2, D3–4 (mandatory) and/or D6–7. All patch test reactions not fulfilling the criteria to be classified as allergic according to the ICDRG guidelines were differentiated with regard to irritant and doubtful reactions. The day of readings varied between the test centres. Three of nine test centres read the patch tests on three occasions (D2, D3–4, and D7), and two of nine read them on D2 and D3–4 only. Four of nine test centres read the patch tests on D3–4 and D6–7.

A questionnaire about clinical data (i.e. localization of symptoms, history of atopic eczema, and history of adverse reactions to fragrances) was filled out. The patients’ histories of adverse reactions to fragrances in the past were categorized according to the following questions: (i) certain – has reacted with an itching dermatitis to at least one fine perfume or aftershave and also to other scented products; (ii) probable – has reacted to one or more scented products, but a certain perfume has not been identified as the cause of a clinical reaction; (iii) questionable – has reacted to various cosmetics with or without fragrances, and materials other than fragrance constituents may be the cause of reaction; and (iv) none – has never reacted to scented materials [modified after Frosch et al. (19)]. In addition, in the case of a positive patch test reaction to oxidized linalool, an assessment of the relevancy of this positive patch test reaction for the patient’s dermatitis was performed, and a question on whether the patient, to the best of the

### Table 1. Participating test centres, duration of testing, and number of consecutively tested patients

<table>
<thead>
<tr>
<th>Test centre</th>
<th>Duration of testing (day/month/year)</th>
<th>Number of tested patients</th>
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<tbody>
<tr>
<td>Barcelona</td>
<td>12/4/2010 to 10/1/2011</td>
<td>299</td>
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<tr>
<td>Copenhagen</td>
<td>31/5/2010 to 14/12/2010</td>
<td>440</td>
</tr>
<tr>
<td>London</td>
<td>15/5/2010 to 18/8/2010</td>
<td>271</td>
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<tr>
<td>Melbourne</td>
<td>24/5/2010 to 13/12/2010</td>
<td>289</td>
</tr>
<tr>
<td>Odense</td>
<td>8/4/2010 to 14/12/2010</td>
<td>298</td>
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<tr>
<td>Seville</td>
<td>12/4/2010 to 13/6/2011</td>
<td>300</td>
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</table>
patch test-evaluating physician’s knowledge, was using products containing linalool was asked.

Statistics

All analyses were carried out with R version 2.10.1 (14 December 2009; The R foundation for Statistical Computing, Vienna, Austria). Fisher’s exact test was used as the statistical method.

Results

In total, 200 (6.9%) of the 2900 tested patients showed positive patch test reactions to oxidized linalool 6.0% (Lin-OOHs 1%) pet., 266 of 2900 (9.2%) showed doubtful reactions, and 37 of 2900 (1.3%) showed irritant reactions (Table 2). The individual test results from the participating test centres are also shown in Table 2. The range of percentages of positive reactions between the test centres was 3.3% (Malmö) to 14.3% (Seville). The doubtful reactions ranged between 0% (Barcelona) and 36.2% (Odense), with six of nine centres recording 0–4.3% doubtful reactions among the patients. Two of the test centres had high but more moderate amounts of doubtful reactions (Copenhagen and Singapore), recording 15.4% and 14.4% of doubtful reactions, respectively.

The overall test results for the 2900 patients, also with respect to the strengths of the patch test reactions, are given in Table 3. Overall, 143 of 200 (71.5%) of the patients showing positive patch test reactions to the preparation of oxidized linalool had + as a maximum reaction, and 57 of 200 (28.5%) had ++ or +++ reactions.

Table 2. Total number of patients from each respective test centre and the number and percentage of positive reactions, doubtful reactions and irritant reactions to oxidized linalool 6.0% [linalool hydroperoxides (Lin-OOHs) 1%] pet.

<table>
<thead>
<tr>
<th>Test centre</th>
<th>Total no. tested</th>
<th>No. of positive patch test reactions</th>
<th>%</th>
<th>No. of doubtful patch test reactions</th>
<th>%</th>
<th>No. of irritant patch test reactions</th>
<th>%</th>
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<tbody>
<tr>
<td>Barcelona</td>
<td>299</td>
<td>11 (3.7)</td>
<td></td>
<td>0 (0)</td>
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<td>0 (0)</td>
<td></td>
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<tr>
<td>Copenhagen</td>
<td>440</td>
<td>21 (4.8)</td>
<td>68 (15.4)</td>
<td>24 (5.4)</td>
<td>0 (0)</td>
<td>14 (3.2)</td>
<td>1 (0.25)</td>
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<tr>
<td>Göteborg</td>
<td>397</td>
<td>15 (3.8)</td>
<td>11 (2.8)</td>
<td>1 (0.25)</td>
<td>0 (0)</td>
<td>10 (2.5)</td>
<td>0 (0)</td>
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<tr>
<td>London</td>
<td>271</td>
<td>14 (5.2)</td>
<td>9 (3.3)</td>
<td>0 (0)</td>
<td>13 (4.8)</td>
<td>1 (0.25)</td>
<td>1 (0.25)</td>
</tr>
<tr>
<td>Malmö</td>
<td>300</td>
<td>10 (3.3)</td>
<td>13 (4.3)</td>
<td>0 (0)</td>
<td>10 (3.3)</td>
<td>0 (0)</td>
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<td>Melbourne</td>
<td>289</td>
<td>24 (8.3)</td>
<td>7 (2.4)</td>
<td>0 (0)</td>
<td>9 (3.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td>Odense</td>
<td>298</td>
<td>24 (8.0)</td>
<td>108 (36.2)</td>
<td>7 (2.3)</td>
<td>1 (0.3)</td>
<td>18 (6.0)</td>
<td>0 (0)</td>
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<td>Seville</td>
<td>300</td>
<td>43 (14.3)</td>
<td>6 (2.0)</td>
<td>3 (1.0)</td>
<td>11 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Singapore</td>
<td>306</td>
<td>38 (12.4)</td>
<td>44 (14.4)</td>
<td>2 (0.65)</td>
<td>9 (3.0)</td>
<td>11 (3.6)</td>
<td>0 (0)</td>
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<tr>
<td>Total</td>
<td>2900</td>
<td>200 (6.9)</td>
<td>266 (9.2)</td>
<td>37 (1.3)</td>
<td>143 (49.0)</td>
<td>57 (19.3)</td>
<td>2 (0.7)</td>
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Table 3. Distribution of the strength of positive patch test reactions to oxidized linalool 6.0% [linalool hydroperoxides (Lin-OOHs) 1%] pet. at the different test centres

<table>
<thead>
<tr>
<th>Test centre</th>
<th>Total no. tested</th>
<th>No. positive</th>
<th>+ (%)</th>
<th>++/+ + + (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barcelona</td>
<td>299</td>
<td>11</td>
<td>3/11 (27.2)</td>
<td>8/11 (72.7)</td>
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<tr>
<td>Copenhagen</td>
<td>440</td>
<td>21</td>
<td>17/21 (76.2)</td>
<td>4/21 (19.0)</td>
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<tr>
<td>Göteborg</td>
<td>397</td>
<td>15</td>
<td>8/15 (53.3)</td>
<td>1/15 (6.7)</td>
</tr>
<tr>
<td>London</td>
<td>271</td>
<td>14</td>
<td>1/9/14 (6.4)</td>
<td>5/14 (35.7)</td>
</tr>
<tr>
<td>Malmö</td>
<td>300</td>
<td>10</td>
<td>4/10 (40.0)</td>
<td>6/10 (60.0)</td>
</tr>
<tr>
<td>Melbourne</td>
<td>289</td>
<td>23</td>
<td>9/23/24 (95.8)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>Odense</td>
<td>298</td>
<td>23</td>
<td>4/24 (83.3)</td>
<td>4/24 (16.6)</td>
</tr>
<tr>
<td>Seville</td>
<td>300</td>
<td>22</td>
<td>18/22 (81.8)</td>
<td>0/22 (0.0)</td>
</tr>
<tr>
<td>Singapore</td>
<td>306</td>
<td>15</td>
<td>13/15 (86.7)</td>
<td>2/15 (13.3)</td>
</tr>
<tr>
<td>Total</td>
<td>2900</td>
<td>200</td>
<td>143 (71.5)</td>
<td>57 (28.5)</td>
</tr>
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</table>

Table 4. Distribution of positive patch test reactions by day of reading

<table>
<thead>
<tr>
<th>Oxidized linalool 6.0% (Lin-OOHs 1%) pet.</th>
<th>No. positive</th>
<th>Positive only on D2</th>
<th>Positive on both D2 and D3–4</th>
<th>Positive only on D3–4</th>
<th>Positive on both D3–4 and D6–7</th>
<th>Positive only on D6–7</th>
<th>Positive on both D3–4, and D6–7</th>
<th>Only read once</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barcelona</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>21</td>
<td>2</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Göteborg</td>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>London</td>
<td>14</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2</td>
</tr>
<tr>
<td>Malmö</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>Melbourne</td>
<td>24</td>
<td>1</td>
<td>5</td>
<td>18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>Odense</td>
<td>24</td>
<td>NA</td>
<td>NA</td>
<td>14</td>
<td>7</td>
<td>3</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>Seville</td>
<td>43</td>
<td>3</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Singapore</td>
<td>38</td>
<td>NA</td>
<td>NA</td>
<td>27</td>
<td>2</td>
<td>9</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>Overall</td>
<td>200</td>
<td>13</td>
<td>28</td>
<td>96</td>
<td>26</td>
<td>14</td>
<td>21</td>
<td>2</td>
</tr>
</tbody>
</table>

Lin-OOH, linalool hydroperoxides; NA, not applicable.
Table 5. Number of patients and percentage showing positive patch test reactions to oxidized linalool 6.0% [linalool hydroperoxides (Lino- OOHHs) 1%] pet. who also reacted to other fragrance markers [fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), and Myroxylon pereirae] and/or colophonium in the baseline patch test series tested concomitantly in the multicentre study

<table>
<thead>
<tr>
<th>Test centre</th>
<th>Patients reacting to oxidized linalool (no.)</th>
<th>Concomitant positive patch test reactions</th>
<th>Fragrance mix I</th>
<th>Fragrance mix II</th>
<th>HICC</th>
<th>Myroxylon pereirae</th>
<th>Colophonium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Barcelona</td>
<td>11</td>
<td>3/11 (27.3)</td>
<td>3/11 (27.3)</td>
<td>0 (0)</td>
<td>2/11 (18.2)</td>
<td>0 (0)</td>
<td>4/11 (36.4)</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>21</td>
<td>4/21 (19.0)</td>
<td>2/21 (9.5)</td>
<td>1/21 (4.8)</td>
<td>2/21 (9.5)</td>
<td>2/21 (9.5)</td>
<td>7/21 (33.3)</td>
</tr>
<tr>
<td>Göteborg</td>
<td>15</td>
<td>2/15 (13.3)</td>
<td>2/15 (13.3)</td>
<td>0 (0)</td>
<td>2/15 (13.3)</td>
<td>0 (0)</td>
<td>3/15 (20)</td>
</tr>
<tr>
<td>London</td>
<td>14</td>
<td>4/14 (28.6)</td>
<td>4/14 (28.6)</td>
<td>1/14 (7.1)</td>
<td>0 (0)</td>
<td>3/14 (21.4)</td>
<td>5/14 (35.7)</td>
</tr>
<tr>
<td>Malmö</td>
<td>10</td>
<td>3/10 (30)</td>
<td>4/10 (40)</td>
<td>0 (0)</td>
<td>2/10 (20)</td>
<td>1/10 (10)</td>
<td>5/10 (50)</td>
</tr>
<tr>
<td>Melbourne</td>
<td>24</td>
<td>7/24 (29.2)</td>
<td>3/24 (12.5)</td>
<td>0 (0)</td>
<td>7/24 (29.2)</td>
<td>4/24 (16.7)</td>
<td>12/24 (50)</td>
</tr>
<tr>
<td>Odense</td>
<td>24</td>
<td>8/24 (33.3)</td>
<td>8/24 (33.3)</td>
<td>7/24 (29.2)</td>
<td>2/24 (8.3)</td>
<td>2/24 (8.3)</td>
<td>9/24 (37.5)</td>
</tr>
<tr>
<td>Seville</td>
<td>43</td>
<td>9/43 (20.9)</td>
<td>6/43 (13.9)</td>
<td>3/43 (7)</td>
<td>2/43 (4.6)</td>
<td>2/43 (4.6)</td>
<td>16/43 (37.2)</td>
</tr>
<tr>
<td>Singapore</td>
<td>38</td>
<td>12/38 (31.5)</td>
<td>8/38 (21.0)</td>
<td>3/38 (7.9)</td>
<td>11/38 (28.9)</td>
<td>2/38 (5.3)</td>
<td>18/38 (47.4)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>52/200 (26.0)</td>
<td>40/200 (20.0)</td>
<td>15/200 (7.5)</td>
<td>30/200 (15.0)</td>
<td>16/200 (8.0)</td>
<td>79/200 (39.5)</td>
</tr>
</tbody>
</table>

The distribution of positive patch test reactions by day of reading is shown in Table 4. Reading on D 3–4 detected 171 of 198 (86.3%) positive patch test reactions, whereas 13 of the 111 read at D 2 (11.7%) were visible only on D 2, and 14 of 162 (8.6%) only on D 6–7. Two of the patients with positive readings were read only once, and were not used to compare the days of reading.

Table 5 gives the number of patients and the percentage showing positive patch test reactions to oxidized linalool who also reacted to other fragrance markers (fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde, and Myroxylon pereirae) and/or colophonium in the baseline patch test series, tested concomitantly in the multicentre study. The overall frequency of positive reactions to other fragrance markers and/or colophonium in patients showing positive patch test reactions to oxidized linalool was 79 of 200 (39.5%). The patients showing positive patch test reactions to oxidized linalool had statistically significantly higher frequencies of positive reactions to one or more other fragrance markers and/or colophonium (Table 7). The patients showing doubtful patch test reactions to oxidized linalool had statistically significantly higher frequencies of positive reactions to one or more other fragrance markers and/or colophonium in the baseline series than the patients with no reactions to oxidized linalool ($p < 0.0001$).

A description of patients showing positive patch test reactions to oxidized linalool in the multicentre study, with regard to age, atopic dermatitis, and history of adverse reactions to fragranced products, is given in Table 8. A history of atopic dermatitis was found for 47 of 193 patients (24.3%) (seven patch test record sheets were not filled in for this question), and 140 of 193 (72.5%) denied having had atopic dermatitis. Regarding history of adverse reaction to fragranced products, 26 of 200 (13.0%) patch test record sheets were not completed for this question. In 14 of the 174 (8.0%) completed patch test record sheets, a certain history of itching dermatitis when in contact with a perfume or aftershave was confirmed (alternative A). Twenty-three of 174 patients (13.2%) had reacted to at least one scented product or perfume, but no certain link had been made (alternative B), and 14 patients (8.0%) had experienced reactions to scented products that were more questionably related to the fragrance components (alternative C). One hundred and twenty-three of 174 patients (70.7%) had not noted any problems when using scented products.

Questions regarding the use of fragranced products, an assessment of the relevance of contact allergy to oxidized linalool in relation to the patients’ dermatitis and the identification of actual products containing linalool...
Table 6. Patients showing doubtful or irritant reactions to oxidized linalool 6.0% [linalool hydroperoxides (Lin-OOHs) 1%] pet., at the different test centres, and the concomitant positive patch test reactions to the fragrance markers [fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), and Myroxylon pereirae] and colophonium tested in the baseline patch test series.

<table>
<thead>
<tr>
<th>Patients with doubtful or irritant reactions to oxidized linalool</th>
<th>Concomitant positive patch test reactions</th>
<th>One or more fragrance markers and/or colophonium</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Doubtful</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barcelona</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>68</td>
<td>6 (8.8)</td>
</tr>
<tr>
<td>Göteborg</td>
<td>11</td>
<td>0 (0)</td>
</tr>
<tr>
<td>London</td>
<td>9</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Malmö</td>
<td>13</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Melbourne</td>
<td>11</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Odense</td>
<td>109</td>
<td>10 (9.2)</td>
</tr>
<tr>
<td>Seville</td>
<td>6</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Singapore</td>
<td>44</td>
<td>5 (11.4)</td>
</tr>
<tr>
<td>Total</td>
<td>271</td>
<td>23 (8.5)</td>
</tr>
<tr>
<td>Irritant*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copenhagen</td>
<td>24</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Göteborg</td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Odense</td>
<td>7</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Seville</td>
<td>3</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Singapore</td>
<td>2</td>
<td>2 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>3 (8.1)</td>
</tr>
</tbody>
</table>

*Barcelona, London, Malmö and Melbourne recorded no irritant reactions.

Table 7. Patients showing negative reactions to oxidized linalool 6.0% [linalool hydroperoxides (Lin-OOHs) 1%] pet. at the different test centres, and the concomitant positive patch test reactions to the fragrance markers [fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), and Myroxylon pereirae] and colophonium tested in the baseline patch test series.

<table>
<thead>
<tr>
<th>Patients negative to oxidized linalool</th>
<th>Concomitant positive patch test reactions</th>
<th>One or more fragrance markers and/or colophonium</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Barcelona</td>
<td>288</td>
<td>19 (6.6)</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>327</td>
<td>25 (7.6)</td>
</tr>
<tr>
<td>Göteborg</td>
<td>365</td>
<td>23 (6.3)</td>
</tr>
<tr>
<td>London</td>
<td>248</td>
<td>10 (4.0)</td>
</tr>
<tr>
<td>Malmö</td>
<td>277</td>
<td>9 (3.2)</td>
</tr>
<tr>
<td>Melbourne</td>
<td>254</td>
<td>19 (7.5)</td>
</tr>
<tr>
<td>Odense</td>
<td>160</td>
<td>6 (3.7)</td>
</tr>
<tr>
<td>Singapore</td>
<td>221</td>
<td>11 (5.0)</td>
</tr>
<tr>
<td>Seville</td>
<td>248</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>Total</td>
<td>2388</td>
<td>125 (5.2)</td>
</tr>
</tbody>
</table>

among those used by the patient are also shown in Table 8; details are given in Table S1. One hundred and fifty-two of 189 patients for whom an answer was given (80.4%) were using fragranced products, and 37 of 189 (19.6%) were not using fragranced products. Eleven of the 200 patch test record sheets were not completed for this question. The relevance of the positive patch test reaction to the individual patient’s dermatitis was assessed by the physician, and, in 60 of the 169 (35.5%) cases, present relevance of the contact allergy was found. In an additional 11 cases, past relevance was found. Thirty-one of the 200 (15.5%) patch test record sheets were not completed for this question. Among the 200 patients testing positive for oxidized linalool, 76 (38.0%) were found to be using products containing linalool or coming into contact with linalool in other ways.
Table 8. Description of patients showing positive patch test reactions to oxidized linalool in the multicentre study

<table>
<thead>
<tr>
<th>Test centre</th>
<th>Mean age (years)</th>
<th>Sex</th>
<th>History of problems with fragranced products?</th>
<th>Atopic dermatitis</th>
<th>Using fragranced products&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relevance&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (no.)</td>
<td>Female (no.)</td>
<td>Certain&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Probable&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Questionable&lt;sup&gt;e&lt;/sup&gt;</td>
<td>None&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Barcelona</td>
<td>11</td>
<td>52.6</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>21</td>
<td>53.1</td>
<td>10</td>
<td>11</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Göteborg</td>
<td>15</td>
<td>43.7</td>
<td>4</td>
<td>11</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>London</td>
<td>14</td>
<td>48.9</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Malmö</td>
<td>10</td>
<td>49.2</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Melbourne</td>
<td>24</td>
<td>38.9</td>
<td>6</td>
<td>18</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Odense</td>
<td>24</td>
<td>45.9</td>
<td>5</td>
<td>19</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Seville</td>
<td>43</td>
<td>48.7</td>
<td>16</td>
<td>27</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Singapore</td>
<td>38</td>
<td>43.2</td>
<td>11</td>
<td>27</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

History of problems with fragranced products is modified after Frosch et al. (19).

<sup>a</sup>Does not always add up to full number; 'not given' have not been recorded in forms.

<sup>b</sup>Relevance: Is the patient using products that contain linalool (lavender fragrance)?

History of positive reaction to oxidized linalool: is contact allergy relevant for the patient’s dermatitis (physician’s assessment)?

<sup>c</sup>A. Certain: Has reacted with an itching dermatitis to at least one fine perfume or after-shave, and has reacted to other scented products.

<sup>d</sup>B. Probable: Has reacted to one or more scented products, but a certain perfume has not been identified by the patient as the cause of a clinical reaction.

<sup>e</sup>C. Questionable: Has reacted to various cosmetics with and without fragrances; materials other than fragrance constituents may be the cause of the reaction.

<sup>f</sup>None: Has never reacted to scented materials.
Table S1 shows the individual test results for the patients with positive patch test reactions to oxidized linalool at the different test centres.

No patients were, to the best of our knowledge, sensitized during this study, as no reports of late-appearing reactions (>D7), indicating active sensitization, were reported to the test centres.

Discussion

In this study, we confirmed the earlier results of patch testing with oxidized linalool 6.0% pet., with an overall frequency of reactions of 6.9% in the tested patients, as compared with 5.3% in the earlier study (14). The range between the test centres was wide, between 3% (Malmö) and 14% (Seville). However, the overall figure places oxidized linalool 6.0% (Lin-OOHs 1%) among the most common causes of contact allergy, and, at least, still well above other single fragrance hapten described to this date.

The differences in frequency of positive reactions to fragrance allergens between test centres have been described earlier in multicentre studies. Similar scenarios have been found in several multicentre studies regarding fragrance materials as well as other patch test materials (19–23). In a multicentre study on oxidized limonene, the range of positive patients was between 0.3% and 6.5% (21, 22). A difference in exposure was suggested, perhaps resulting from different preferences regarding fragrance notes. Another possibility is, of course, the effect of a cut-off at 300–400 patients, which may give large variations in frequency for a low number of patients. Longer study periods and larger numbers of patients are always to be preferred, but, for practical reasons, a limit has to be set. It should be noted that the distribution of positive patch test reactions to the different fragrance markers in the baseline series also showed great variation among the patients reacting to oxidized linalool at the different test centres, as well as among the patients negative to oxidized linalool (Table 5–7).

The distribution of strength of the positive patch test reactions in Table 3 is, interestingly, very similar to that in the earlier study on oxidized linalool 6.0% pet. (14), where 72% had a + reaction (71.5% in the present study), and 27% had a ++ or +++ reaction (28.5% in the present study). The distribution of the reactions according to strength shows a large variation between the different test centres, and, at three of nine test centres, the stronger reactions dominated among the positive patients.

The days of reading varied between the test centres. Three of nine test centres read the patch tests on three occasions (D2, D3–4, and D7), whereas two of nine read on D2 and D3–4 only. Overall, reading on D3–4 detected 85% of the positive patients (Table 4). Few had reactions only visible on D2 or D6–7. This is in accordance with the earlier patch test study on oxidized linalool (14).

The relevance of the findings in the present study was supported by a high frequency (39.5%) of positive reactions to other fragrance markers and/or colophonium in patients showing positive patch test reactions to oxidized linalool (Table 5). The frequency of concomitant reactions to other fragrance markers is similar to that found in other studies of fragrance materials, and to the previous study on oxidized linalool, where 41.8% of the positive patients had concomitant reactions to a fragrance marker and/or colophonium (14). This, in our view, supports the view that we are detecting patients who have a true contact allergy to fragrances and not recording unspecific (false-positive) patch test reactions. Conversely, 60.5% of the patients showing positive patch test reactions to oxidized linalool would not have been identified if this specific patch test had not been performed, and thus would not have been informed of their fragrance allergy. In Table S1, the reactions to other fragrance markers among the individual patients showing positive patch test reactions to oxidized linalool are shown. Among the positive patients, many had several concomitant reactions to fragrance markers.

As Table 2 shows, the number of reactions classified as doubtful (?) varied considerably among the centres. A high range of doubtful reactions, where no further development of positive or irritant reactions occurred, was registered at three of the nine centres (Odense, Copenhagen, and Singapore). However, in earlier studies, such as a multicentre study on fragrance mix II, the readings at the two Danish departments have likewise shown a high proportion of doubtful reactions, which has been attributed to differences in the reading of patch test reactions (23). In these departments, all differences from normal skin at the patch test site are recorded, and a slight erythema with or without papules is graded as doubtful. In readings in other departments, these might be scored as negative or possibly read as doubtful. Historical perspectives on the use of visual grading scales in evaluating skin irritation and sensitization, as well as available bioengineering measures, have recently been reviewed (24).

The comparatively high patch test concentration of oxidized linalool 6.0% (Lin-OOHs 1%) pet. must, however, be discussed, as a patch test concentration giving rise to problematic readings, and possibly irritation, should be avoided. In the earlier irritation study for oxidized linalool (13), as well as the earlier dose–response study (14), irritation caused by oxidized linalool 6% pet.
CONTACT ALLERGY TO OXIDIZED LINALOOL

was low or, at most, moderate. Moreover, the higher concentration of non-oxidized linalool in the present test preparation than in the preparation used in the earlier dose–response study (14) should not affect the irritancy, as pure linalool was shown to be non-irritating up to a concentration of 40% pet. (13). The earlier dose–response study for oxidized linalool 6.0% pet. showed 6.4% doubtless reactions among the tested patients to the current patch test concentration (14). When patients were tested concomitantly with higher patch test concentrations, the doubtful reactions at 2.0% pet. became positive at oxidized linalool 4.0% pet. in 62% of the patients, thus indicating that the doubtful reaction marked a very weak positive response. In the same study, 19.4% of the doubtful reactions at oxidized linalool 6.0% pet. became positive at 11.0% pet. (14). Thus, a certain number of the doubtful reactions are probably very weak positive patch test responses.

Irritant reactions were overall less frequent than doubtful reactions, but above the earlier frequency of 0.23% (14). Irritant reactions may appear in many different forms, and may thus be interpreted in different ways. Again, a difference in the criteria for reading is possible, and may explain the range of frequencies (0–5.4%).

The patients were asked about earlier problems with fragranced products. This is a difficult question to answer, and the answers should be regarded as an indication. However, 21% of the patients showing positive patch test reactions to oxidized linalool had experienced a certain or probable reaction in relation to scented products. Interestingly, almost 20% of the patients showing positive patch test reactions to oxidized linalool for whom the patch test record sheets were completed responded that they were not using fragranced products, possibly because they had suspected a connection with their skin problems. The multitude of scented products commercially available and the lower number of fragrance-free alternatives suggest that these patients had made an active choice when purchasing their consumer goods. However, for 7 of 38 (18%) patients who answered that they did not use fragranced products, linalool was nonetheless found in their products (Table S1).

In the present study, the importance of the positive patch test reactions to oxidized linalool in relation to the patients’ dermatitis was assessed in some questions regarding relevance. This is also a difficult question to answer, and the answers should again be regarded as an indication. However, in 35% of the patients for whom an answer was given, present relevance was indicated, and in an additional 6% past relevance was found. This indicates that a distinctive overall relevance for oxidized linalool was found in 41% of the patients, and in the separate Table S1, the products in which linalool was found are, in many cases, indicated. The listing gives an interesting overview of the types of product that are important in this respect. It can be noted that shampoos, soaps and facial and body creams are frequently listed in Table S1 as sources of linalool judged to be relevant for patients’ dermatitis.

For 38% of the patients, linalool was found in their products, showing how common the fragrance chemical is (Tables 8 and S1). Finding linalool in patients’ products is a tedious task, and requires that the patient bring in all relevant products for inspection, or a search for it using information given by the clinic. Noteworthy is that Barcelona found present or past relevance in 73% of the positive patients, and Copenhagen found relevance for oxidized linalool in 66% of the positive patients. It can be assumed that the overall figure for relevance (35%) is a low estimate; however, the true extent is difficult to know, as linalool is ubiquitously found in many types of product. In the EU, linalool belongs 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, owing to its skin-sensitizing capacity according to the Cosmetics Directive (25). However, in Australia and Singapore, such labelling of the name of the fragrance is optional, and relevance is thus more difficult to assess in these countries.

There has been some discussion of the discrepancy between the suggested patch test concentration of oxidized linalool (6%) and the presumably lower concentrations to which patients are exposed in everyday life. However, it is difficult to know the actual range of concentrations that are frequent in consumer products. Depending on the product, many different fragrances, at different concentrations, may be added. In perfumes, up to 15–30% of the total volume may consist of fragrance material (26 and references therein). Lavender oil is common in massage oils and aromatherapy products, and the concentrations of lavender oil, in which linalool and linalyl acetate are the main components (27), may also be high. In a study on autoxidation of lavender oil, no difference was found between the autoxidation in natural lavender oil and in synthetically prepared linalyl acetate and linalool, separately or in a mixture (27), so the presence of Lin-OOHs is to be expected in air-oxidized lavender oil. Thus, exposure from aromatherapy oils and so-called ‘natural products’ must also be considered. Furthermore, fragrance compounds may make up 0.1–2% of the volume of liquid detergents and toilet soaps (26). It can hence be stipulated that a consumer may come into contact with 6% linalool and...
higher in products, and may also be exposed repeatedly to concentrations in the range of 2% and below (1–3). It must be considered how ubiquitous a fragrance chemical is, as the added exposures from many sources need to be calculated. This was performed in a recent exposure assessment, where linalool showed the highest maximum daily exposure of all fragrance terpenes for high-end users of cosmetic products containing these terpene components (4). The present study supports the need for adequate risk assessment with regard to what people actually come into contact with.

It is generally desirable to patch test with defined compounds and controlled levels of allergens rather than natural products or mixtures, to optimize the repeatability of patch test results. However, in clinical practice, patch testing with carefully controlled oxidation mixtures may be the most efficient way to detect contact allergy to oxidized fragrance terpenes (12, 14, 21, 22). As the composition of the oxidation mixture varies with time, it is important to standardize the composition of the test material with respect to the main hapten. Thus, the hydroperoxides, which have been demonstrated to be the main hapten in oxidized linalool, were kept at a defined concentration in the patch test preparation in the present study. However, it is important not only to test with the main hapten but to keep the composition of the total oxidation mixture stable. Other components may increase the bioavailability, as terpenes are known skin penetration enhancers. Furthermore, minor hapten can also cause sensitization in some individuals. In a previous study, it was shown that some individuals reacted to oxidized linalool but not to the hydroperoxide fraction (12). It is also very important to ensure the stability of the patch test material during storage and usage, especially when dealing with unstable hapten such as hydroperoxides. Prior to the present study, the stability of the test preparations under different handling and storage conditions was thoroughly checked by repeated analyses. Good stability was shown for 3 months. Further analysis of test material that had been in use at two of the test centres showed that the test material was intact after 4 months. Thus, the main hapten (the hydroperoxides) should be present at a specific concentration under clinical patch testing conditions and storage when handled as in the test centres. Handling according to the instructions given in this study must, however, be ensured when oxidized linalool is used in future routine testing, to give reliable results.

This study further supports the importance of considering the problem of air oxidation when discussing contact allergy to fragrance terpenes. The pure compound is not the actual sensitizer. Evidence for this has been provided by experimental studies, where pure linalool gave an EC3 (eliciting concentration needed to give a stimulating index of 3) of 46.2 in the LLNA (7), thus placing it as a very weak sensitizer. The air-oxidized linalool at 45 weeks gave an EC3 value of 4.8, thus placing oxidized linalool as a strong sensitizer (7). In accordance with this, patch testing with pure linalool has shown no (12) or very few (5, 6) positive patch test reactions, and, as a result, the labelling of linalool on cosmetics and hygiene products according to the Cosmetics Directive within the EU (25) has been questioned (5). Within the EU regulatory work on the classification and labelling of dangerous substances (led by the European Chemicals Agency), R-limonene and S-limonene, as well as the racemic form dipentene, are classified as skin sensitizers, effectively based on the formation of skin sensitizers resulting from air exposure of limonene. This also needs to be the case for linalool and other chemicals that form allergic compounds on air exposure. Furthermore, in cosmetic products, a limitation on the permitted concentration of skin-sensitizing oxidation products need to be considered.

Most toxicological and dermatological data on fragrance terpenes published so far concern the non-oxidized terpenes, as shown in a recent review on linalool and other terpene alcohols (5). As both the irritant potential (13) and the sensitizing potential (7, 11, 12, 28) have been shown to differ between oxidized and non-oxidized forms of linalool, limonene, linalyl acetate, and geraniol (29), further studies of the effect of autooxidation on toxicological and dermatological aspects of other common fragrance terpenes are important.

Conclusions

Oxidized linalool 6.0% (Lin-OOHs 1%) has, in an international setting, been shown to be a valuable mix of hapten, frequently eliciting contact allergy in patch testing. The test preparation of oxidized linalool 6.0% pet. with a standardized content of Lin-OOHs of 1% is a useful tool with which to detect contact allergy in dermatitis patients. The patients reacting to oxidized linalool have, to a great extent, documented exposure to linalool-containing cosmetics and hygiene products, which have been judged to be relevant for their dermatitis. A large number of concomitant reactions to fragrance markers and/or colophonium in the baseline patch test series supports the relevance of the positive patch test reactions to oxidized linalool. However, it should be observed that, for about half of the patients showing positive patch test reactions to oxidized linalool, fragrance allergy would
not have been identified if this specific test had not been performed, and thus they would not have been informed of their fragrance allergy.

Linalool is a ubiquitous fragrance chemical, and contact allergy to linalool may have wide implications for the patient. The widespread use of linalool in domestic and occupational products suggests that both further sensitization and widespread elicitation of allergic contact dermatitis in allergic individuals may be expected. Chemical analyses regarding the presence of Lin-OOHI s in consumer products should be performed in order to investigate and control the exposure of the population.

The results obtained from this multicentre patch test study, together with the investigations of the patch test materials prior to and during the study, show that there now is a possibility of performing routine testing with a standardized material of oxidized linalool. Further studies with scientifically conducted repeated open application tests should be conducted as a basis for the decision on whether oxidized linalool meets the requirements to be included in international baseline patch test series (15).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Description of individual patients showing positive patch test reactions to oxidized linalool. History (A–D) signifies the patient’s response to questions regarding previous problems with scented products, and are categorized as: (A) certain – has reacted with an itching dermatitis to at least one fine perfume or aftershave, and also to other scented products; (B) probable – has reacted to one or more scented products, but no certain product has been identified as the cause of a clinical reaction; (C) questionable – has reacted to various cosmetics with or without fragrances, that is, materials other than fragrance constituents may be the cause of reaction; and (D) none – has never reacted to scented materials (modified after Frosch et al. (19)).

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