Air oxidation of \(d\text{-limonene}\) (the citrus solvent) creates potent allergens

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Products containing as much as 95% of \(d\text{-limonene}\) are used for, e.g., degreasing metal before industrial painting and for cleaning assemblies. Experimental studies on the sensitizing potential of limonene show diverging results. In a previous study, we found that the sensitizing potential of \(d\text{-limonene}\) increased with prolonged air exposure. The aim of this study was to make further chemical analyses, to identify compounds formed by air exposure of \(d\text{-limonene}\) and to study their allergenic potential. \(d\text{-limonene}\) was found to be a sensitizer after prolonged exposure to air according to 2 Freund’s complete adjuvant test (FCAT) experiments and 1 guinea pig maximization test (GPMT) study. No significant response was obtained to \(d\text{-limonene}\) not air exposed, even if the animals were sensitized to oxidized \(d\text{-limonene}\). 5 main oxidation products of \(d\text{-limonene}\) were identified. \((R)(-)-\text{carvone}\) and a mixture of \(c\text{is}\) and \(t\text{rans}\) isomers of \((+)-\text{limonene oxide}\) were found to be potent sensitizers, while no significant reactions were obtained in the animals induced with a mixture of \(c\text{is}\) and \(t\text{rans}\) isomers of \((-)-\text{carveol}\). It can be concluded that air oxidation of \(d\text{-limonene}\) is essential for its sensitizing potential, and that potent allergens are created.

**Key words:** air exposure; carvone; carveol; chemical analysis; contact allergy; FCAT; GPMT; limonene; limonene oxide; sensitizing potential.

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\(d\text{-}(+)-\text{limonene}\) (Fig. 1:1) has long been used for flavouring and as perfume in household products in low concentrations, e.g., in detergents below 0.03% (1). Owing to its solvent capacity, it has also been used as an additive in cleaning products, in a concentration of a few%. However, in recent years \(d\text{-limonene}\) has found new uses, because of the importance of replacing chlorinated hydrocarbons, chlorofluorocarbons (CFC) and other organic solvents with less toxic substances. Products containing as much as 95% of \(d\text{-limonene}\) are used in factories for degreasing metal before industrial painting and for cleaning assemblies. Many histological laboratories use \(d\text{-limonene}\) as a substitute for xylene. Limonene has also been chosen as a solvent for asphalt, heavy oil and rosin for merely technical reasons. Dipentene, the racemic mixture of \(d\text{-limonene}\) and \(l\text{-}(-)-\text{limonene}\) (Fig. 1:2) is offered for sale as a solvent for the paint industry.

\(d\text{-limonene}\) is the main constituent of oil from several fruits of the genus *Citrus*, and also occurs in caraway, dill and celery. It is produced mainly from citrus peel by pressing and distillation, and the distillate (peel oil) usually contains more than 95% of \(d\text{-limonene}\). The contaminants reported are other terpenes such as \(\alpha\text{-and}\beta\text{-pinene, sabinene, myrcene,}\ \delta\text{-carene, camphene, and }\gamma\text{-terpinene. }l\text{-limonene is found for example in turpentine and peppermint oil. Pinus silvestris and Xanthoxylum piperitum contain dipentene. Some products sold as dipentene or limonene contain only*
sensitized the animals. Gas chromatographic (GC) analyses showed that the content of d-limonene had decreased during the exposure, while especially the content of limonene oxide (Fig. 1:3, 4) had increased.

The aim of the present study was to make further chemical analyses and to identify additional compounds formed by air exposure of d-limonene. We also wanted to study the allergenic potential of limonene oxide and other oxidation products.

**Material and Methods**

d-limonene (Fig. 1:1) of technical quality was purchased from Frey & Lau, Norderstedt, FRG. The producers quoted a purity of 94.8% according to their GC analysis and optical rotation of +95° to +104°. The main impurities were stated to be α-pinene, sabinene, myrcene and linalool. 2 samples of the product were exposed to air at room temperature for 8 weeks and were stirred 4 x a day for 1 h before the sensitization experiments. Sample B (Table 1) was air-exposed before animal experiment 1 and sample C (Table 1) before experiments 2 and 3. The rest of the limonene was kept in the refrigerator throughout the study.

d-limonene with a purity stated to be 97% and an optical rotation of +106.7° was purchased from Janssen Chimica, Beerse, Belgium. GC analysis showed a content of 98% d-limonene. The product was kept in a refrigerator until the study. (+)-limonene oxide-(1,2) (a mixture of cis and trans isomers), (R)-(−)-carvone and (−)-carveol (a mixture of cis and trans isomers) (Fig. 1:3, 4, 5, 6, 7) were purchased from Aldrich Chemical Company Inc., Milwaukee, Wisconsin, USA. The purity of the limonene oxide was 97% as quoted by the producer. GC analysis showed a content of 56% and 41% of the 2 isomers and 3% identified as d-limonene. The purity of the carvone was 98% as given by the producer and >99% according to our GC analysis. The quoted purity of (−)-carveol was 99%. Analysis by gas chromatography – mass spectrometry (GC-MS) revealed a con-

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**Fig. 1.** d-limonene [(+)-limonene] (1); l-limonene [(−)-limonene] (2); cis-(+)-limonene oxide (3); trans-(+)-limonene oxide (4); (R)-(−)-carvone (5); cis-(−)-carveol (6); trans-(−)-carveol (7).
Table 1. GC analyses of 3 samples of d-limonene from Frey & Lau; the analyses were performed before and after 8 weeks air exposure

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Relative retention time</th>
<th>% of combined peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sample A(^a)</td>
</tr>
<tr>
<td>1.</td>
<td>0.38 (carvone and</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>carveol)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>0.63 (limonene oxide)</td>
<td>n.d.</td>
</tr>
<tr>
<td>3.</td>
<td>0.64 (limonene oxide)</td>
<td>n.d.</td>
</tr>
<tr>
<td>4.</td>
<td>1.00 (limonene)</td>
<td>97%</td>
</tr>
</tbody>
</table>

n.d. = not detected.

\(^a\) Used in a previous study (20).

\(^b\) Used in FCAT experiment 1 (Table 2).

\(^c\) Used in FCAT experiment 2 (Table 3) and in the GPMT experiment (Table 4).

The content of 0.9% carvone and 0.01% butylated hydroxytoluene (BHT) in the carvone.

Freund's Complete Adjuvant (FCA) was purchased from Difco, Detroit, Mich., USA. Olive oil was purchased from Kebo Lab AB, Stockholm, Sweden.

**Purification of (−)-carveol**
The carveol was purified twice by preparative thin-layer chromatography (TLC) (DC Fertigplatten Kieselgel 60 ohne Fluoreszenzindikator 200 × 200 mm from Merck, Darmstadt, FRG) to remove the content of carvone. The eluent consisted of hexane: diethylether 65:35. Iodine was used for TLC detection. After purification, GC-MS analysis showed a content of >99% carveol and 0.07% carvone.

**Analysis by gas chromatography (GC)**
The analyses were performed on an HP 5890 gas chromatograph equipped with a splitless injector and an FID detector, using a fused silica column (SE-30; 30 m, 0.25 mm i.d.) and nitrogen as a carrier gas (0.67 ml min\(^{-1}\)). The injector temperature was 190°C, the column temperature 100°C and the detector temperature 190°C. The 2 samples of d-limonene (Samples B and C) were analysed at the start and also at the end of the air exposure just before the respective animal tests. Co-chromatography with (+)-limonene oxide-(1,2), R(--)-carvone and (−)-carveol, respectively, was performed.

**Analysis by gas chromatography – mass spectrometry (GC – MS)**
For analysis, the GC-MS system used consisted of a Varian 3400 gas chromatograph interfaced to a Finnigan MAT INCOS 50 mass spectrometer. The gas chromatograph was equipped with a J&W DB-5 capillary column (30 m, 0.25 mm i.d., 0.10 mm thickness of stationary phase) and a split/splitless injector. The temperature of the injector was 250°C. The GC programming was as follows: 35°C and splitless with a delay of 0.5 min, followed by a linear temperature gradient of 5°C/minute up to 185°C. The temperature of the GC-MS interface was 250°C. The ion-source temperature was kept at 150°C. Prior to analysis, all samples were dissolved in distilled dichloromethane.

**Sensitization experiments in guinea pigs**
2 animal experiments were carried out using Freund’s complete adjuvant test (FCAT) method (21) in the modified version with closed challenge testing (22) and 1 experiment using the guinea pig maximization test (GPMT) method (13). Challenge testing was performed using Finn Chambers® (Epitest, Helsinki, Finland). The patches were removed
after 24 h and the reactions were assessed at 48 and 72 h after the start of exposure. The experiments were performed on female Dunkin-Hartley albino guinea pigs from AB Sahlns Försöksdjursfarm, Malmö, Sweden. The animals weighed 300–350 g.

In the FCAT experiments, 3 intradermal injections were used for induction. The substance to be investigated was dissolved in FCA for the exposed animals, while the controls received FCA only.

Experiment 1 (FCAT). One group of animals (I) was induced with (+)-limonene oxide-(1,2) (mixture of cis and trans isomers) and another group (II) with oxidized d-limonene (Sample B with air exposure for 8 weeks, Table 1). The concentration used for induction was 5% in FCA in both groups. Before challenge testing, the maximum non-irritating concentrations were determined by giving each of 6 animals one intradermal injection of FCA. 1 week later, 3 of the animals were tested with limonene oxide and 3 with d-limonene in the concentrations 10, 5, 2, 0.1, 0.05, 0.01% in olive oil (w/w). Skin irritation was seen at 10% for both preparations and the test concentrations were thus set at 5.0, 1.0 and 0.1% in olive oil for limonene oxide (corresponding to $33 \times 10^{-2}$, $6.6 \times 10^{-2}$, $6.6 \times 10^{-3}$ mmol/g) and 5% (37 $\times 10^{-2}$ mmol/g) for d-limonene.

Experiment 2 (FCAT). One group of animals (III) was induced with (R)-(−)-carvone, one group (IV) with (−)-carveol (mixture of cis and trans isomers) and a 3rd group (V) with oxidized d-limonene (sample C with air exposure for 8 weeks, Table 1). The concentrations used for induction were 5% in FCA. The maximum non-irritating challenge concentrations for carvone and carveol were determined by testing 3 FCA-treated animals with each compound in the concentrations 10, 5, 1, 0.1% in olive oil (w/w). Irritation was seen at 10% and the test concentrations were thus set at 5.0, 1.0 and 0.2% in olive oil (corresponding to $33 \times 10^{-2}$, $6.6 \times 10^{-2}$, $1.3 \times 10^{-2}$ mmol/g) for carvone and (−)-carveol respectively. The challenge concentration for d-limonene was 5% according to the experience from experiment I.

Experiment 3 (GPMT). The animals were induced with oxidized d-limonene (Sample C with air exposure for 8 weeks, Table 1), intradermally 5% in FCA, water and arachid oil, and epidermally 20% in pet. The concentration for epidermal induction was determined by testing 5 FCA-treated animals with d-limonene.

Table 2. Experiment 1 (FCAT) on (+)-limonene oxide-(1,2) and oxidized d-limonene

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>oxidized d-limonene (5%)</th>
<th>(+)-limonene oxide (5% 1% 0.1%)</th>
<th>Vehicle control olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group I (n = 15)</td>
<td>13</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>$p$ (exp/co)</td>
<td>$&lt;$0.001</td>
<td>$&lt;$0.001</td>
<td>$&lt;$0.001</td>
</tr>
<tr>
<td>group II (n = 15)</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$p$ (exp/co)</td>
<td>$&lt;$0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 15)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The number of animals with positive test reactions at 72 h after application of the challenge test is given.

The reactivity at 48 h after application was in accordance with that of 72 h.

Statistical analysis of exposed/control animals.

NS: not significant.

Induction: group I: (+)-limonene oxide-(1,2) (a mixture of cis and trans isomers) 5% (w/w), group II: d-limonene (sample B) 5% (w/w) exposed to air for 8 weeks (see Material and Methods).
10, 20, 30, 40, 60 and 80% in arachidic oil (w/w). 10% gave weak erythema in 2/5 animals while 20% gave weak to moderate erythema in 4/5 animals. The animals were challenge tested with oxidized d-limonene (sample C with air exposure for 8 weeks, Table 1), d-limonene with minimal air exposure from Janssen Chemica and Frey & Lau, (+)-limonene oxide-(1,2) and (R)-(−)-carvone in olive oil. The test concentrations were chosen according to the experience from earlier experiments.

**Statistical methods**
The results from the animal experiments were analysed using the Fisher exact test.

**Results**

**Chemical analyses**
The content of d-limonene decreased during air exposure for 8 weeks (Table 1). The results obtained in the analyses (samples B and C) accord with the results obtained in our previous study (sample A) (20). A small decrease in the content of d-limonene was also seen during the storage with minimal air exposure for each experiment. The experiments were performed one after the other and samples A, B and C were all taken from the same bottle of limonene kept in the refrigerator throughout the study. Complicated chromatograms were obtained for the air-exposed samples of d-limonene, but 5 main compounds were identified by GC and GC-MS. The compounds identified were carvone, cis- and trans-limonene oxide (1,2), cis- and trans-carveol (Fig. 1:2, 3, 4, 5, 6, 7). The optical rotation was not determined for the identified compounds but following identifications in the literature (Schenk 23) (R)-(−)-carvone, a mixture of cis- and trans- (+)-limonene oxide (1,2) and a mixture of cis- and trans-(−)-carveol were chosen for the sensitization experiments.

**Sensitization experiments**

**Experiment 1 (FCAT).** (+)-limonene oxide-(1,2) sensitized the animals (Table 2). A significant response was found to the 5% and 1% concentrations, but not to 0.1%. A significant response was obtained to oxidized d-limonene (sample B) in both animal groups.

**Experiment 2 (FCAT).** (R)-(−)-carvone was a sensitizer (Table 3). A significant response was obtained to all 3 challenge concen-

**Table 3.** Experiment 2 (FCAT) on R (−)-carvone, (−)-carveol and oxidized d-limonene

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>oxidized d-limonene</th>
<th>(R)-(−)-carvone</th>
<th>(−)-carveol</th>
<th>Vehicle control olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed⁶⁶</td>
<td>5%</td>
<td>5%</td>
<td>1%</td>
<td>0.2%</td>
</tr>
<tr>
<td>group III (n=14)</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>p (exp/co)¹¹</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>group IV (n=14)</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>p (exp/co)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>group V (n=14)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>p (exp/co)</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Controls⁶⁶</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ The number of animals with positive test reactions at 72 h after application of the challenge test is given.

² The reactivity at 48 h after application was in accordance with that of 72 h.

³ Statistical analysis of exposed/control animals.

⁴ NS: not significant.

Induction: group III: (R)-(−)-carvone 5% (w/w), group IV: (−)-carveol (a mixture of cis and trans isomers) 5% (w/w), group V: d-limonene (sample C) 5% (w/w) exposed to air for 8 weeks (see Material and Methods)
trations. A significant response was obtained to oxidized \(d\)-limonene (sample C) in animal groups III and V. The animals induced with \((-\)\)-carveol (group IV) gave no significant response, but animals sensitized to carvone reacted when challenged tested with carveol.

Experiment 3 (GPMT). Oxidized \(d\)-limonene (sample C) was shown to be a sensitizer. No significant reactions were obtained to the \(d\)-limonene not exposed to air for a prolonged time (Table 4).

The animals sensitized to \((+\)\)-limonene oxide (group I, Table 2) and \((R\)-(\(-\)\))-carvone (group III, Table 3) reacted when tested with the oxidized limonene, but the animals sensitized to oxidized limonene did not react when tested with these substances.

Discussion

Oxidation products of \(d\)-limonene, identified as potent allergens, were found after prolonged air exposure of \(d\)-limonene. In a recent study (20), the sensitizing potential of \(d\)-limonene itself was shown to be very low. However, the sensitizing capacity was highly increased after exposure to air for 8 weeks at room temperature. In this study, the experiment was repeated 2 \(\times\) and the results were confirmed. \(d\)-limonene was found to be a sensitizer after prolonged exposure to air according to 2 FCAT experiments and 1 GPMT study (Tables 2-4). No significant response was obtained to oxidized \(d\)-limonene not air exposed, even if the animals were sensitized to oxidized \(d\)-limonene (Table 4). Hellerström et al. (24) observed that limonene needed much oxygen to give reactions in humans sensitive to turpentine. However, Pirilä et al. (25) considered that the allergenicity of autooxidized turpentine was due only to oxidized \(d\)-carene.

The \(d\)-limonene tested contained small amounts of other terpenes, since we wanted to investigate \(d\)-limonene used in industry. No significant response was found in animals induced with limonene which had not been purposely exposed to air (20), and the animals sensitized to oxidized limonene did not react when challenged with 2 types of non-air-exposed limonene (Table 4).

\(d\)-limonene is considered to be a contact allergen (4, 5), but the experimental results are not very conclusive. In a human maximization test, \(d\)-limonene gave no sensitization in 25 volunteers (17). In a comprehensive study of animal methods, sensitization was obtained to limonene \((d\) or \(l\) not given) in FCAT, GPMT and open epicutaneous test (OET) but not in the Draize test (18). No sensitization was obtained when \(d\)-limonene was tested in vitamin-A-fed mice (19). The difference between the results may be due to an interspecies variation and/or to sensitization to impurities such as oxidation products and not to \(d\)-limonene itself.

Autooxidation of monocyclic terpenes readily occurs to give a variety of oxygenated compounds (3, 26). In this study, 5 main oxidation products, carvone, the \(cis\) and \(trans\) isomers of limonene oxide-(1,2) and of carveol (Fig. 1: 3, 4, 5, 6, 7), were identified. These compounds have earlier been identified in autooxidized \(d\)-limonene (2, 23, 27, 28). In the present study, \((R\)-(\(-\)\))-carvone and a mixture of \(cis\) and \(trans\) isomers of \((+\)\)-limonene oxide-(1,2) were found to be potent sensitizers, while no significant reactions were obtained in the animals induced with \((-\)\)-carveol (a mixture of \(cis\) and \(trans\) isomers) (Tables 2, 3). No sensitization studies or reports on cases of contact allergy to \((+\)\)-limonene oxide-(1,2) or \((-\)\)-carveol have been published, to the best of our knowledge. Carvone is the main constituent in spearmint which is used in most toothpastes. It has been described as causing positive patch test reactions in a selected group of patients with sore mouth, stomatitis and/or dermatitis around the mouth and in dental personnel (29). No sensitization was found when a human maximization test was performed with carvone (30). According to the literature, \((R\)-(\(-\)\))-carvone is most likely formed when \(d\)-limonene is oxidized (23), which is why this substance was used for our sensitization experiments. However, it
Table 4. Experiment 3 (GPMT) on oxidized \( d \)-limonene

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>oxidized ( d )-limonene</th>
<th>( d )-limonene(^a) Frey &amp; Lau</th>
<th>( d )-limonene(^b) Janssen</th>
<th>(( + ))-limonene oxide</th>
<th>(R)-((-)) carvone</th>
<th>Vehicle control olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed(^a) (( n = 20 ))</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>( p ) (exp/contro)(^a)</td>
<td>&lt;0.001</td>
<td>NS(^a)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Controls(^b) (( n = 20 ))</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Limonene with min. air exposure.

\(^b\) The number of animals with positive test reactions at 72 h after application of the challenge test is given. The reactivity at 48 h after application was in accordance with that of 72 h.

\(^c\) Statistical analysis of exposed/control animals.

\(^d\) NS: not significant.

*Induction: 5% (w/w) intradermally and 20% epidermally of \( d \)-limonene (sample C) exposed to air for 8 weeks (see Material and Methods).*

cannot be excluded that \( S (+) \)-carvone is also formed. The study of carvone in \( d \)-limonene, and also in other products, continues. \((-)\)-carvone was not found to be allergenic, but the animals induced with carvone reacted when tested with carvone. This is probably due to the oxidation of small amounts of carvone to carvone.

The studies showed that guinea pigs sensitized to limonene oxide or to carvone reacted when challenge tested with oxidized limonene. However, the animals sensitized to oxidized limonene showed no significant response when challenge tested with limonene oxide and carvone. There might be different explanations of this. Other allergens with higher reactivity or concentrations may be formed by air exposure of \( d \)-limonene. The content of limonene oxide and carvone was determined to 1–4% each by GC. At an induction concentration of 5% oxidized \( d \)-limonene, the concentration of these allergens might be too low to cause sensitization. However, when the animals were sensitized to the single allergens, the content was high enough to give an elicitation reaction. Limonene oxide and carvone are both considered secondary oxidation products of \( d \)-limonene, while the primary ones may be the hydroperoxides. Different hydroperoxides of \( d \)-limonene have been identified as their corresponding alcohols (23, 31). Hydroperoxides of terpenes, identified in colophony and turpentine, are known to be potent sensitizers (25, 32, 33). GC analyses will not detect hydroperoxides, since they are too unstable. Studies of the hydroperoxides in oxidized \( d \)-limonene are in progress.

Quenching is a process used in the perfume industry to suppress contact sensitization from cinnamic aldehyde and citral, usually by adding eugenol or \( d \)-limonene (34). Histological studies and radiolabel experiments demonstrated a quenching effect of \( d \)-limonene to some extent in animals sensitized to citral, although no difference in the reactivity was seen at challenge testing (35, 36). Different hypotheses have been presented concerning the mechanism of quenching (37). In one report (38), \( d \)-limonene is stated to give immunosuppression in mice fed with this substance. Basketter & Allenby (39) found little evidence of the quenching of delayed contact hypersensitivity reactions using \( d \)-limonene and eugenol in a comprehensive series of studies. No evidence was found in the present study for the quenching effect of \( d \)-limonene on the elicitation phase of carvone and limonene oxide, since the animals sensitized to these compounds reacted to oxidized limonene. However, a specific quenching at the induction
phase of these secondary oxidation products cannot be excluded.

The studies describing skin problems from the citrus fruit industry are old and not very conclusive (7–9). The exposure was complex with many factors contributing to the dermatitis. Thus, the cutaneous hazards also included extraneous allergens, mechanical trauma, irritation and secondary infections from wet work. Most cases reported were irritant contact dermatitis. There are very few reports on skin problems due to d-limonene or dipentene traditionally used for flavouring, as perfume and as an additive in cleaning products in low concentrations (15, 16, 40, 41). The use of d-limonene in high concentrations in industry is likely to cause more problems, especially since there is no attempt to avoid air oxidation. The method for air exposure used in this study was a simple model of the way the product might be handled in daily routine in the industry. To avoid air oxidation, for technical reasons, the producers often add an antioxidant, usually butylated hydroxytoluene (BHT) to the product. BHT is described as a contact allergen (42). The effect of this antioxidant on the oxidation and the allergenicity of d-limonene is at present being studied in our laboratory.

From this study, it can be concluded that air oxidation of d-limonene is essential for its sensitizing potential, and that potent allergens are created. However, questions still remain to be resolved, especially that of the primary sensitizers.

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We wish to thank Mrs. Gunnel Hagelthorn and Mr. Peter Fernström for skilful technical assistance.

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