

Linalool – a significant contact sensitizer after air exposure

JOHANNA BRÅRED CHRISTENSSON^{1,2}, MIHÁLY MATURA^{2,3}, BIRGITTA GRUVBERGER⁴, MAGNUS BRUZE⁴
AND ANN-THERESE KARLBERG²

¹Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, ²Dermatochemistry and Skin Allergy, Department of Chemistry, University of Gothenburg, Gothenburg, ³Unit of Occupational and Environmental Dermatology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, and ⁴Department of Occupational and Environmental Dermatology, Lund University, Malmö University Hospital, Malmö, Sweden

Background: Linalool is a widely used fragrance terpene. Pure linalool is not allergenic or a very weak allergen, but autoxidizes on air exposure and the oxidation products can cause contact allergy. Oxidized (ox.) linalool has previously been patch tested at a concentration of 2.0% in petrolatum (pet.) in 1511 patients, and 1.3% positive patch test reactions were observed.

Objective: To investigate the optimal patch test concentration for detection of contact allergy to ox. linalool.

Methods: Four concentrations of ox. linalool (2.0%, 4.0%, 6.0%, 11.0% pet.) were tested in 3418 consecutive dermatitis patients.

Results: Ox. linalool 2.0%, 4.0%, 6.0%, and 11.0% pet. detected positive patch test reactions in 0.83%, 3.2%, 5.3%, and 7.2% of the tested patients, respectively. The doubtful reactions increased with rising concentrations but relatively less, giving 5.1%, 6.4%, and 7.3% doubtful reactions, respectively, for ox. linalool 4.0%, 6.0%, and 11.0% pet. Few irritative reactions were seen.

Conclusions: Raising the patch test concentration for ox. linalool gave a better detection of contact allergy, as many as 5–7% positive patch test reactions were detected. We suggest a patch test concentration of ox. linalool 6.0% pet. for future patch testing, giving a dose per unit area of 2.4 mg/cm² when 20 mg test substance is tested in small Finn Chambers®.

Key words: allergic contact dermatitis; autoxidation; dose per unit area; fragrance allergy; hydroperoxides; linalool; oxidation products; patch test concentration; patch testing; terpenes. © John Wiley & Sons A/S, 2010.

Conflicts of interest: Magnus Bruze is a member of the Research Institute for Fragrance Materials (RIFM) Expert Panel (REXPAN).

Accepted for publication 23 September 2009

Linalool is a naturally occurring terpene, present in large amounts in various plants, e.g. in lavender (1, 2). It is also synthetically produced at low costs and widely used as a fragrance chemical in household and hygiene products due to its fresh, flowery odour. According to several studies, linalool is presently among the most common fragrance ingredients in consumer products (3–5). In a recent assessment, an estimated consumer exposure for selected fragrance terpenes was calculated based on

concentrations in 10 types of cosmetic products and on the mode of usage for the product. The maximum daily exposure to linalool was by far the highest compared to the other discussed fragrance terpenes (6).

Like other fragrance terpenes, e.g. limonene, linalyl acetate, and geraniol, linalool has been demonstrated to autoxidize on air exposure at room temperature (7). As a pure compound linalool seldom causes positive patch test reactions (8–10),

while the oxidation products cause contact allergy (9, 11, 12). Several oxidation products such as hydroperoxides, aldehydes, ketones, and alcohols have been identified in the oxidation mixture (11, 12). The primary oxidation products of linalool, linalool hydroperoxides, were found to be the major contact allergens in the oxidation mixture (9, 11, 12). This is in accordance with investigations of autooxidation of other fragrance terpenes, e.g. limonene and linalyl acetate (13, 14).

One earlier multicentre patch test study has been conducted using oxidized (ox.) linalool (9). A patch test preparation of ox. linalool 2.0% in petrolatum (pet.) gave 1.3% positive patch test reactions in 1511 consecutive dermatitis patients (9). We have, in recently performed human irritation studies, shown that ox. linalool exerts very slight irritation at 10% pet., giving no reaction at all in 35% of the persons tested, and a marginal reaction or a slight perceptible erythema in 50% of the persons tested (15). The obtained results gave rise to the question whether the previously used patch test concentration of ox. linalool 2.0% pet. could be raised. The patch test concentration used for screening is important as a higher test concentration normally increases the possibility of diagnosing contact allergy. 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. Guidelines on establishing an appropriate patch test concentration with respect to irritancy and active sensitization have been published in a review on behalf of the European Society of Contact Dermatitis (16).

Fragrances are today common causes of contact allergy, second only to nickel in the baseline patch test series (17, 18). Today's diagnostic tools in the baseline patch test series consist of the Fragrance Mix (FM), *Myroxylon pereirae* (MP) and the more recently introduced Fragrance Mix II (FM II). Concomitant reactions to the different fragrance markers are widely observed in many studies (9, 19, 20).

Concomitant reactions to colophonium are often seen in patients reacting to fragrance markers (9, 19–25). In this study, as in earlier studies on ox. fragrance terpenes, we have chosen to present patch test data on concomitant reactions to colophonium along with the fragrance markers in the baseline series.

The aim of the present study was to investigate whether an increased patch test concentration of ox. linalool would detect more cases of contact allergy without rendering more adverse effects, e.g. irritation, and to determine the optimal concentration for detection of contact allergy to ox. linalool in a

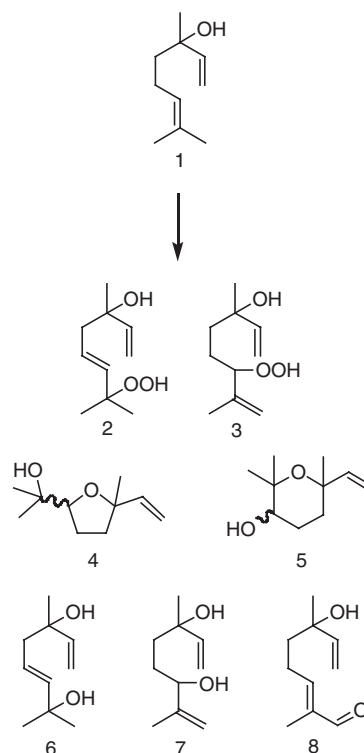


Fig. 1. Identified oxidation products formed by autooxidation of linalool at 45 weeks (11, 12). Linalool (3,7-dimethyl-1,6-octadien-3-ol) **1**; 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol **2**; 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol **3**; 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-ol **4**; 2,2,6-trimethyl-6-vinyltetrahydro-2H-pyran-3-ol **5**; 2,6-dimethylocta-3,7-diene-2,6-diol **6**; 2,6-dimethylocta-1,7-diene-3,6-diol **7**; and 6-hydroxy-2,6-dimethylocta-2,7-dienal **8**.

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 ox. linalool was assessed.

Materials and Methods

Chemicals

Linalool (3,7-dimethyl-1,6-octadien-3-ol) (**1** in Fig. 1) with a stated purity of 97% was obtained from Sigma Aldrich Chemie (Schnelldorf, Germany) and purified by distillation. In order to mimic oxidation that happens during handling and storage conditions, a simplified experimental oxidation model was used according to previous experience (26). The oxidation/degradation process was followed using gas chromatography (GC) and high performance liquid chromatography (HPLC), according to methods previously described (11, 12). After 45 weeks, the oxidation mixture of linalool contained only 30% linalool. At this time, the main oxidation products in the oxidation mixture were highly sensitizing linalool

hydroperoxides and non-sensitizing ethers. Isolated and identified oxidation products after 45 weeks of air oxidation are shown in Fig. 1 (12). At 45 weeks, the oxidation mixture contained 19% linalool hydroperoxides (15% of 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol and 4% of 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol), 20% of 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-ol and less than 4% of 2,2,6-trimethyl-6-vinyltetrahydro-2H-pyran-3-ol. It also contained 2,6-dimethylocta-3,7-diene-2,6-diol, 2,6-dimethylocta-1,7-diene-3,6-diol and 6-hydroxy-2,6-dimethylocta-2,7-dienal (Fig. 1). Details on the chemical analyses of ox. linalool are described elsewhere (11, 12).

Patch test materials

Ox. linalool was stored under argon at -20°C until test preparations were made. Chemical analyses were performed on a regular basis of the stored non-ox. and ox. test materials to ensure that the composition had not changed during storage. New patch test preparations were made every 2–3 months from the same batches. Test preparations of ox. linalool were prepared at 2.0%, 4.0%, 6.0%, and 11.0% (w/w) in non-stabilized white pet. (Kebo Lab A/S, Albertslund, Denmark) and were kept in 5 ml syringes in the refrigerator. Standard allergens used for patch testing were obtained from Chemotechnique Diagnostics AB (Vellinge, Sweden).

Subjects, exposure and evaluation

Consecutive patients undergoing patch testing because of suspected allergic contact dermatitis (ACD) at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, and the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden, were screened with one, two, or three concentrations of ox. linalool simultaneously, in addition to regular patch testing. Patch test preparations of approximately 20 mg (27) were applied in small Finn Chambers[®] (diameter 8 mm, inner area of 0.5 cm^2 , Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape[®] (Norgesplaster A/S, Vennsela, Norway) to the back of the patient and left under occlusion for 2D and then removed by the patient. The dose per unit area was approximately 0.80 mg/cm^2 at ox. linalool 2.0% pet., 1.6 mg/cm^2 at 4.0% pet., 2.4 mg/cm^2 at 6.0% pet., and 4.4 mg/cm^2 at 11.0% pet. Visual readings were scored according to the International Contact Dermatitis Research Group (ICDRG) guidelines (28) on D 3–4 and D 6–7. 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 (28, 29).

Dose–response relationship

Pre-study. To compare the obtained results with those from the earlier multicentre patch test study on ox. linalool 2.0% pet. (9), a pre-study was conducted where the previously used test concentration of ox. linalool, 2.0% pet., was tested in 1343 consecutive patients with suspected ACD. The pre-study was performed in Gothenburg during February 2004–December 2005 and during March–June 2006.

Dose–response studies. The dose–response studies for ox. linalool were performed in three steps in consecutive dermatitis patients with suspected ACD in Gothenburg and Malmö. Between January–March 2005, 350 patients (177 women, 173 men) were tested with ox. linalool at concentrations of 2.0% pet. and 4.0% pet. Between June 2006 and February 2007, 721 patients (463 women, 258 men) were tested with ox. linalool at concentrations of 4.0% pet. and 6.0% pet., and between February–December 2007, 1004 patients (647 women, 357 men) were tested with ox. linalool at 4.0% pet., 6.0% pet., and 11.0% pet. In this last step of the study, concomitant reactions to other fragrance allergens and/or colophonium in the baseline series were compiled. This part of the study was also chosen to investigate on which day of reading the positive patch test reactions were visible for the different concentrations and to study the distribution of positive patch test reactions between men and women.

Statistics

All analyses were carried out using R version 2.6.0 [(2007-10-03), © 2007. The R foundation for Statistical Computing, Vienna, Austria]. Fisher's exact test was used as statistical method.

Results

The results from the pre-study and the three steps in the dose–response study are given in Table 1. The total number of patients tested with each patch test concentration of ox. linalool in the study and the overall number and (%) of positive reactions, doubtful reactions, and irritant reactions to each respective concentration are given in Table 2. The overall (%) of positive, doubtful, and irritant patch test reactions to the four concentrations of ox. linalool are shown in Fig. 2. The distribution of strength of the positive reactions is shown in Table 3.

Statistically, a significant increase in the number of patients showing positive patch test reactions

Table 1. Number (no.) of patients, positive patch test reactions, doubtful reactions, and irritant reactions for each tested concentration of ox. linalool in the different study periods of the dose–response study for ox. linalool

Ox. linalool (w/w% pet.)	Total no tested	No. of positive patch test reactions (%)		No. of doubtful patch test reactions (%)		No. of irritant patch test reactions (%)	
Pre-study	2.0	1343	13 (0.97)	25 (1.9)	0		
First study period	2.0	350	1 (0.29)	8 (2.3)	0		
	4.0	350	10 (2.8)	23 (6.6)	4 (1.1)		
Second study period	4.0	721	27 (3.7)	35 (4.8)	0		
	6.0	721	36 (5.0)	44 (6.1)	1 (0.14)		
Third study period	4.0	1004	30 (3.0)	48 (4.8)	3 (0.30)		
	6.0	1004	55 (5.5)	67 (6.7)	3 (0.30)		
	11.0	1004	72 (7.2)	73 (7.3)	7 (0.70)		

Table 2. Total number of patients tested with each patch test concentration of ox. linalool in the study and the number and (%) of positive reactions, doubtful reactions, and irritant reactions to each respective concentration in the dose–response study for ox. linalool^a

Ox. linalool (w/w% pet.)	Total no. tested	Positive (%)		Doubtful (%)		Irritant (%)	
2.0	1693	14/1693	(0.83)	33/1693	(1.9)	0	
4.0	2075	67/2075	(3.2) ^b	106/2075	(5.1) ^c	7/2075	(0.34%)
6.0	1725	91/1725	(5.3) ^d	111/1725	(6.4)	4/1725	(0.23%)
11.0	1004	72/1004	(7.2) ^e	73/1004	(7.3) ^f	7/1004	(0.7%)

^aAll statistics calculated with Fisher's exact test.

^bIncrease in positive patch test reactions compared with positive reactions to ox. linalool 2.0% pet. $P = 1.81 \times 10^{-7}$.

^cIncrease in doubtful patch test reactions compared with doubtful reactions to ox. linalool 2.0% pet. $P = 9.19 \times 10^{-8}$.

^dIncrease in positive patch test reactions compared with positive reactions to ox. linalool 4.0% pet. $P = 0.0019$.

^eIncrease in positive patch test reactions compared with positive reactions to ox. linalool 6.0% pet. $P = 0.054$.

^fIncrease in doubtful patch test reactions compared with doubtful reactions to ox. linalool 4.0% pet. $P = 0.018$.

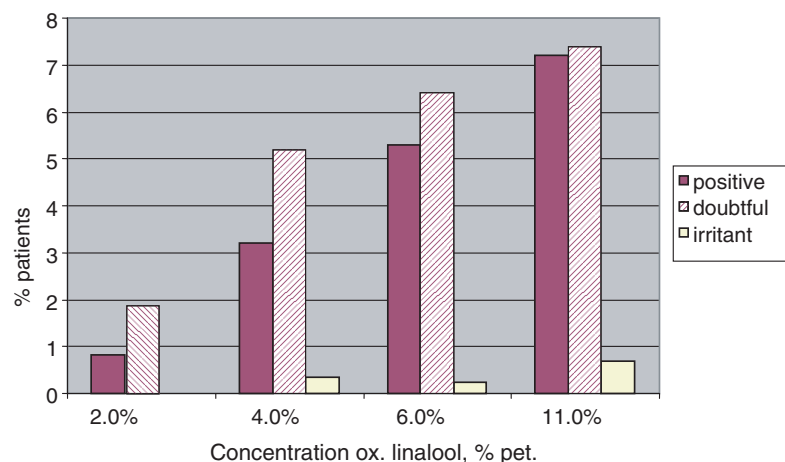


Fig. 2. Percentage (%) of positive, doubtful, and irritant patch test reactions to four concentrations of ox. linalool in pet. in 3418 consecutively patch-tested dermatitis patients. Numerical values are given in Table 2.

could be demonstrated as the concentrations were increased, Table 2. Although an increase in doubtful reactions was recorded as the concentration of ox. linalool was increased, the ratios of positive reactions to doubtful reactions increased with rising concentrations giving 0.44 (pos reactions/doubtful reactions = 0.83/1.9) for ox. linalool 2.0% pet., 0.63 (3.2/5.1) for 4.0% pet., 0.83 (5.3/6.4) for 6.0% pet., and 0.99 (7.2/7.3) for ox. linalool 11.0% pet.

Tables 4a–d show how the reactions appeared in the individual patients at the different concentrations of ox. linalool. Table 4a compares ox. linalool 2.0% and 4.0% pet. in 350 consecutive dermatitis patients. It was found that 5/8 (62.5%) patients showing doubtful reactions at 2.0% pet. showed positive

Table 3. Distribution of strength of positive patch test reactions for the four tested concentrations of ox. linalool in the dose–response study for ox. linalool

Ox linalool (w/w% pet.)	Total no. tested	No positive	+	(%)	++/+	+++ (%)
2.0	1693	14	8/14	(57)	6/14	(43)
4.0	2075	67	46/67	(69)	21/67	(31)
6.0	1725	91	66/91	(72)	25/91	(27)
11.0	1004	72	53/72	(74)	19/72	(26)

reactions at 4.0% pet. Also, four patients negative at 2.0% pet. became positive at 4.0% pet. Of the 10 positive patch test reactions at 4.0% pet., only 1 (10%) would be detected at 2.0% pet.

Table 4. Tables showing how individual positive, doubtful, and irritant reactions in patients appeared in other simultaneously tested patch test concentrations of ox. linalool

(a) Ox. linalool 2.0% versus 4.0% pet.^a

		Ox linalool 2.0%			
Ox linalool 4.0%		Positive	Doubtful	Irritant	Negative
	Pos	1	5	0	4
	Doubtful	0	3	0	20
	Irritant	0	0	0	4
	Negative	0	0	0	313

(b) Ox. linalool 4.0% versus 6.0% pet.^b

		Ox linalool 4.0% pet.			
Ox linalool 6.0% pet.		Positive	Doubtful	Irritant	Negative
	Positive	28	12	0	15
	Doubtful	2	35	0	30
	Irritant	0	0	3	0
	Negative	0	1	0	878

(c) Ox linalool 6.0% versus 11.0% pet.^b

		Ox linalool 6.0% pet.			
Ox linalool 11.0% pet.		Positive	Doubtful	Irritant	Negative
	Positive	53	13	0	6
	Doubtful	2	44	0	27
	Irritant	0	2	3	2
	Negative	0	8	0	844

(d) Ox linalool 4.0% versus 11.0% pet.^b

		Ox linalool 4.0% pet.			
Ox. linalool 11.0% pet.		Positive	Doubtful	Irritant	Negative
	Positive	28	19	0	25
	Doubtful	2	24	0	47
	Irritant	0	1	3	3
	Negative	0	4	0	848

(a) Shows pattern of reactions to ox. linalool 2.0% and 4.0% pet. (b–d) show pattern of reactions to ox. linalool 4.0%, 6.0%, and 11.0% pet.

^aFirst part of the dose–response study, $n = 350$.

^bThird part of the dose–response study, $n = 1004$.

Tables 4b–d refer to the third part of the study when ox. linalool 4.0%, 6.0% and 11.0% pet. were tested simultaneously in 1004 patients. Table 4b compares ox. linalool 4.0% pet. and 6.0% pet. Of the 48 patients with doubtful reactions at 4.0%, 12 (25.0%) showed positive reactions at 6.0% pet. Also, 15 patients who were negative at 4.0% pet. showed positive patch test reactions at 6.0% pet. Of the 55 positive patch test reactions at 6.0% pet., 28 (50.9%) would be detected at 4.0% pet.

Table 4c compares ox. linalool 6.0% pet. and 11.0% pet. Of the 67 patients showing doubtful reactions to ox. linalool 6.0% pet., 13 (19.4%) showed positive reactions to 11% pet. Here, six of the negative reactions at 6.0% pet. became positive at 11.0% pet. Of the 72 positive patch test reactions at 11.0% pet., 53 (73.6%) would be detected at 6.0% pet.

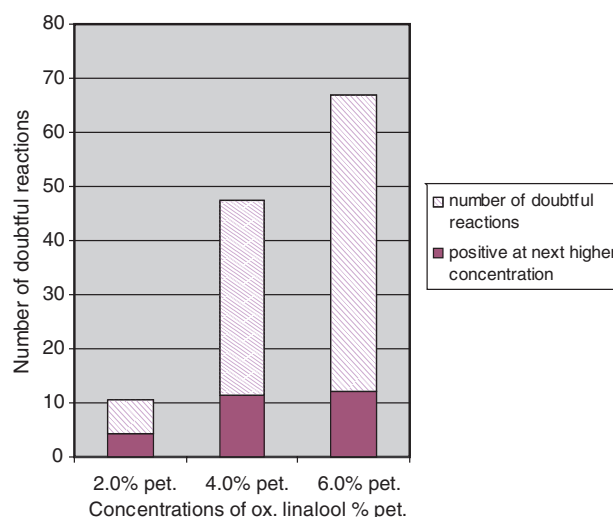


Fig. 3. Number of patients with doubtful reactions at ox. linalool 2.0% ($n = 350$), and 4.0%, and 6.0% pet. ($n = 1004$), and the subgroups showing a simultaneous positive patch test reaction to the next higher concentration.

Table 4d compares the reactions to ox. linalool 4.0% pet. and 11.0% pet. Of the 48 patients showing doubtful reactions to 4.0% pet., 19 (39.6%) showed positive reactions to 11.0% pet. Also, 25 patients who were negative at 4.0% pet. showed positive patch test reactions at 11.0% pet. Of the 72 positive patch test reactions at 11.0% pet., 28 (38.9%) would be detected at 4.0% pet.

Figure 3 shows the number of doubtful reactions at 2.0% pet. (in 350 patients), 4.0% pet., and 6.0% pet. (in 1004 patients) and marks the subgroup which showed positive reactions to the next higher concentration in the dose–response study. Figure 4 shows the positive reactions at each tested concentration of ox. linalool and how the reactions read at the next lower concentration tested in the dose–response study.

The positive patch test reactions in the third step of the study ($n = 1004$) were differentiated as to which day of reading they appeared (Table 5). The majority of the positive patch test readings were visible on D 3–4 or at both readings. Only 6–10% appeared exclusively at reading D 6–7.

Concomitant reactions to other fragrance markers and/or to colophonium in the baseline series were common in patients reacting to ox. linalool and are given in Table 6. For all concentrations, the patients (men and women calculated together) showing positive patch test reactions to ox. linalool had statistically significantly higher frequencies of positive reactions to one or more other fragrance marker and/or colophonium in the baseline series, compared with the patients not showing positive patch test reactions to ox. linalool ($P < 0.001$), Table 6.

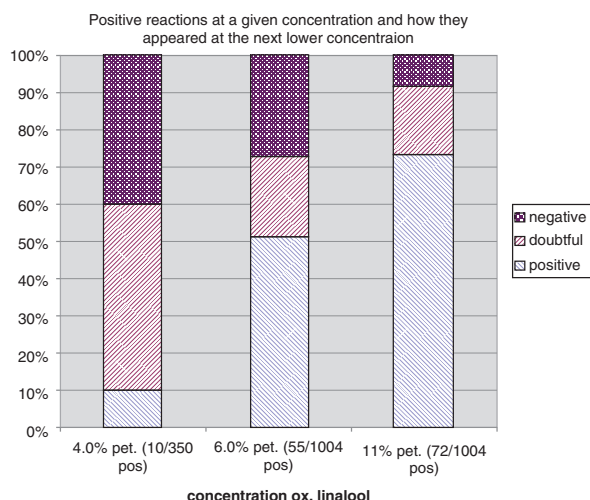


Fig. 4. Positive reactions at each tested concentration of ox. linalool and how the reactions read at the next lower concentration tested in the dose–response study. When 4.0% pet. is compared to 2.0% pet., 90% of the patients would not have been diagnosed using the lower test concentration. Likewise, for 6.0% pet. compared to 4.0% pet., 49% of the patients would not have been diagnosed using the lower test concentration. In a similar way, for 11.0% pet. compared to 6.0% pet., 26% of the patients would not have been diagnosed using the lower test concentration only.

Table 5. Distribution of positive patch test reactions by day of reading in third part of the study ($n = 1004$)

Ox. linalool (w/w%)	Pos only D3–4	Pos both D3–4 and D6–7	Pos only D6–7
4.0	18 ^a /30	10/30	2/30
6.0	35 ^a /55	17/55	3/55
11.0	45 ^a /72	20/72	7/72

^aOne patient was only read D3.

Concomitant reactions to fragrance markers and/or colophonium in the baseline series for the subgroups of patients showing + or stronger (++/+ + +) positive patch test reactions and for patients showing doubtful or negative reactions to ox. linalool in the third step of the dose–response study are shown in Fig. 5. The subgroup of patients showing positive patch test reactions to one or more other fragrance marker and/or colophonium in the baseline series is very high in the +++/+ + + group (75–80%), and still high in the + group (28% at 11.0% pet., 36% at 6.0% pet., and 43% at 4.0% pet.), compared with 11.9% in the patients negative to ox. linalool at all tested concentrations.

The frequency of positive patch test reactions to ox. linalool, as well as to fragrance markers and colophonium, separated for men and women in the third step of the dose–response study, is shown in Table 7. There was no statistically significant difference between the frequencies of positive patch test reactions to ox. linalool between men and women at any given concentration. No statistical

difference was found between men and women in FM, FM II, or colophonium, while the different frequencies for men and women in positive patch test reactions to MP gave $P = 0.012$.

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 observed positive patch test reactions to ox. linalool in a greater frequency than earlier recognized. As many as 5–7% of the patients tested showed positive patch test reactions to ox. linalool at 6.0% pet. or 11.0% pet. patch test concentration. This places ox. linalool among our most common contact allergens. In relation to an earlier survey over Swedish patch test data, positive patch test reactions to ox. linalool are as prevalent as positive patch test reactions to the FM or MP (30). The relevance of the findings in the present study was supported by a high frequency of positive reactions to other fragrance markers and/or colophonium in patients showing positive patch test reactions to ox. linalool (Table 6, Fig. 5). This, in our view, supports that we are detecting patients who have a true contact allergy to fragrances and not recording unspecific patch test reactions, even at the relatively high patch test concentration of 11.0% pet.

In raising the patch test concentration, the gain in positive reactions was great between 2.0% and 4.0%, and still large between 4.0% and 6.0% pet. For the tested lower concentrations (2.0% pet., 4.0% pet., 6.0% pet.), 62%, 25%, and 19%, respectively, of the doubtful reactions became positive at the next higher concentration (Tables 4a–c). Likewise, for the raised test concentrations (4.0% pet., 6.0% pet., 11.0% pet.), 90%, 49%, and 26%, respectively, of the patients would not have been diagnosed using the next lower test concentration only (Fig. 4).

Based on the results of this study, we suggest a patch test concentration of ox. linalool of 6.0% pet. for future screening of dermatitis patients for contact allergy. This gives a dose per unit area of 2.4 mg/cm^2 when 20 mg of patch test preparation is applied in small Finn Chambers® (27). The 11.0% pet. (4.4 mg/cm^2) patch test concentration could probably also be used in screening or as a supplementary test if a doubtful reaction is seen to the 6.0% pet. patch test concentration and it is important to verify or exclude contact allergy to ox. linalool. It is however of great importance to consider the risk of active sensitization, a risk which is always present in patch testing but which should be minimized (16).

Table 6. Number of patients and (%) of 1004 consecutively tested dermatitis patients in the third part of the dose–response study, showing positive or negative patch test reactions to ox. linalool, also reacting to other fragrance markers (FM, FM II, MP) and/or colophonium in the baseline patch test series^a

Concomitant reactions to:	Number of patients and (%) with positive reactions to ox. linalool (total <i>n</i> = 75) showing concomitant reactions to fragrance markers and/or colophonium in the baseline patch test series						Number of patients (%) not positive to ox. linalool at any concentration, <i>n</i> = 929, showing concomitant reactions to fragrance markers and/or colophonium	
	4.0% pet. <i>n</i> = 30		6.0% pet. <i>n</i> = 55		11.0% pet. <i>n</i> = 72			
Fragrance mix (FM)	8/30	(26.7)	12/55	(21.8)	14/72	(19.4)	56/929	(6.0)
Fragrance mix II (FM II)	5/30	(16.7)	8/55	(14.5)	9/72	(12.5)	29/929	(3.1)
<i>Myroxylon pereirae</i> (MP)	10/30	(33.3)	11/55	(20)	14/72	(19.4)	45/929	(4.8)
Colophonium	5/30	(16.7)	8/55	(14.5)	9/72	(12.5)	24/929	(2.6)
≥1 Fragrance markers and/or colophonium	14/30	(46.7) ^b	23/55	(41.8) ^c	28/72	(38.9) ^d	111/929	(11.9) ^{b,c,d}

^aAll statistics calculated with Fisher's exact test.

^bStatistically significant concomitant reactions to other fragrance markers and/or colophonium in subjects showing positive patch test reactions ox. linalool 4.0% compared to those with negative reactions to ox. linalool ($P = 4.76 \times 10^{-6}$).

^cStatistically significant concomitant reactions to other fragrance markers and/or colophonium in subjects showing positive patch test reactions ox. linalool 6.0% compared to those with negative reactions to ox. linalool ($P = 8.86 \times 10^{-8}$).

^dStatistically significant concomitant reactions to other fragrance markers and/or colophonium in subjects showing positive patch test reactions ox. linalool 11.0% compared to those with negative reactions to ox. linalool ($P = 3.13 \times 10^{-8}$).

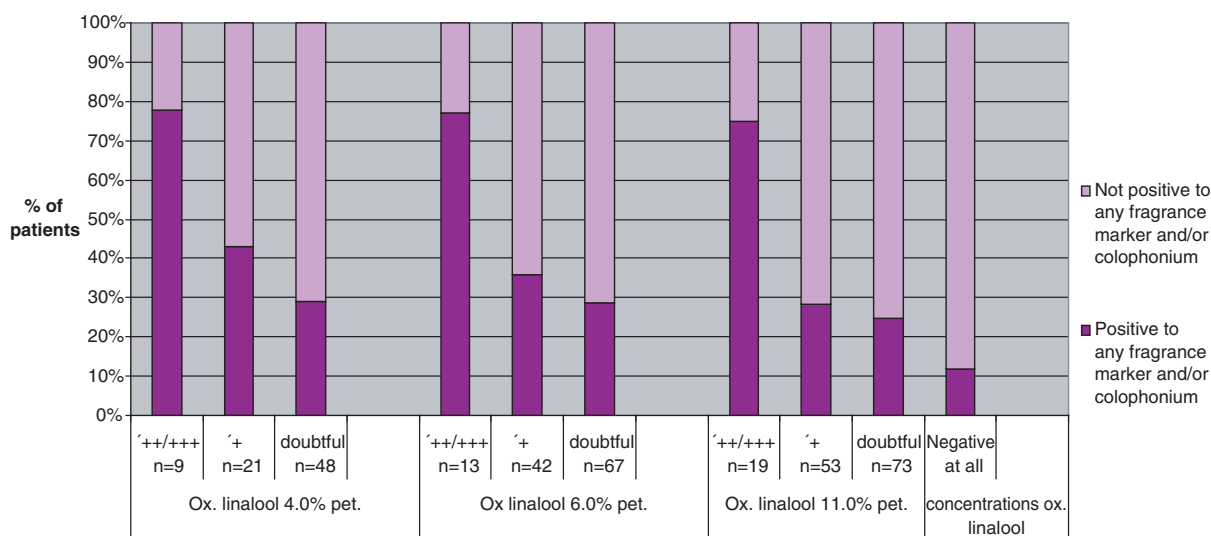


Fig. 5. Percent (%) of patients showing concomitant reactions to other fragrance markers and/or colophonium in the baseline series in patients with positive, doubtful or negative patch test reactions to ox. linalool. The patients reacting to ox. linalool are differentiated as to strength of reaction ++/+++ or +.

Table 7. Distribution of women and men [number of patients, (%)] showing positive patch test reactions to ox. linalool, fragrance markers and/or colophonium in the baseline patch test series in the third part of the dose–response study (*n* = 1004)

	Ox. linalool			FM	FMII	MP	Colophonium
	4% pet.	6% pet.	11% pet.				
Women %	19/647 2.9	32/647 4.9	43/647 6.6	48/628 7.6	30/625 4.8	34/627 5.4 ^a	35/639 5.5
Men %	11/357 3.1	23/357 6.4	29/357 8.1	20/353 5.7	8/345 2.3	7/352 2.0 ^a	16/354 4.5
Total positive %	30/1004 3	55/1004 5.5	72/1004 7.2	68/981 6.9	38/970 3.9	41/979 4.2	51/993 5.1

^aStatistically significant difference between men and women ($P = 0.012$).

It is generally desirable to patch test with defined compounds rather than natural products or mixtures. However, in clinical practice, patch testing with carefully controlled oxidation mixtures may be the

most efficient way to detect contact allergy to fragrance terpenes that autoxidize (9, 21, 24). As the composition of the oxidation mixtures varies with time, and both oxidation method and batch

size influence the oxidation process, standardization of appropriate test material with respect to the main allergens is necessary to ensure the reliability and reproducibility of the testing. Also, storage conditions as well as use and handling may affect the test material (31). Further studies to ensure the stability of patch test material of ox. linalool to be utilized under patch testing conditions are planned.

This study supports the importance of considering the problem of air oxidation when patch testing with fragrance terpenes, as the pure compound added to the perfumed product in many cases is not the actual sensitizer. Linalool and limonene belong to the group of fragrance chemicals that must be labelled on cosmetic products when used in concentrations >10 p.p.m. in leave-on products and >100 p.p.m. in rinse-off products, due to their skin sensitizing capacity according to the Cosmetics Directive within the EU (32). However, for linalool, patch testing with the pure compound has shown no (9) or very few (8, 10) positive patch test reactions, and as a result, the selection of linalool as a common fragrance allergen to be labelled is questioned (10). The very low frequency of positive patch test reactions [7/2396, 0.3% (10)], when not testing with a controlled oxidation mixture, is, in our view, in accordance with experimental studies (11, 12) and with earlier clinical studies (8, 9). Furthermore, the possibility of reaction to oxidation products formed in the test material during long test periods, giving the few positive patch test reactions, was not discussed (10). Within the EU regulatory work on the classification and labelling of dangerous substances (led by the European Chemicals Bureau), *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 (33). Such a statement needs to be included in the Cosmetics Directive regarding linalool, limonene and other chemicals that form allergenic compounds on air exposure.

Based on the autoxidation potential of linalool, the International Fragrance Research Association (IFRA) Standard states that linalool should only be used when the peroxide level is as low as possible, and, at maximum, 20 mmol/l, e.g. by use of an antioxidant at production of the raw material (34). It has been shown that an antioxidant, such as butylated hydroxytoluene (BHT), can prevent the formation of oxidation products from limonene for several months, but that the oxidation process starts immediately at the consumption of the antioxidant (35). The purity of the raw material as well as storage temperatures had impact on the time until onset of autoxidation. No correlation was seen between the amount of BHT added and the length of time before

autoxidation began (35). It is thus important that the stability of a fragrance terpene known to autoxidize is controlled not only at production but also in the finalized product as well as after a certain time of use and handling. New routines for usage and handling, involving, e.g. low storage temperatures or smaller and more air-tight containers, may be in order.

Linalool is a ubiquitous fragrance chemical, and contact allergy to linalool may have wide implications for the patient. Both men and women are affected alike, interestingly with slightly higher figures for men, unlike the other fragrance markers presented in Table 7. With the widespread use of linalool in domestic and occupational products, both further sensitizations as well as widespread elicitation of ACD in allergic individuals may be expected. It was shown in a recent study (2) that also natural lavender oil, which consists of linalyl acetate and linalool as main components, autoxidizes in the same way as the synthetically prepared components, separately or in mixture. Thus, exposure from aromatherapy oils and so-called 'natural products' must also be considered. The present study supports the need for adequate risk assessment with regard to what people actually come in contact with. As the use of fragrances varies over time (36), new relevant markers for fragrance contact allergy have to be developed. The actual chemicals to which the population is exposed need to be continuously reassessed, revalidated, and studied with regard to allergenicity. The baseline patch test series used for screening for contact allergy in dermatitis patients should be continuously revised and updated when needed. The results from this study indicate that ox. linalool is an important allergen, which, in our opinion, should be included in routine testing.

Most toxicological and dermatological data on fragrance terpenes published so far concern the non-ox. terpenes as shown in a recent review on linalool and other terpene alcohols (6). As both the irritant potential (15) and the sensitizing potential (11, 12, 26) have been shown to differ between ox. and non-ox. forms of linalool and limonene as well as for geraniol (37), further studies of the effect of autoxidation on toxicological and dermatological aspects of other common fragrance terpenes are important.

Conclusions

In conclusion, this study shows that contact allergy to ox. linalool is more common than earlier recognized. As many as 5–7% of the consecutive dermatitis patients tested in our populations showed positive patch test reactions to ox. linalool at 6.0% or 11.0% pet. This frequency of positive patch test reactions

places ox. linalool among the most common contact allergens, in our test population as common as reactions to the FM or the MP. For future patch testing, a test concentration of 6.0% pet. is suggested. Studies to further investigate the clinical relevancy of the positive reactions as well as of the doubtful reactions should be conducted, possibly using repeated open application test (ROAT) or other use-tests.

Acknowledgments

The skilful technical assistance of Petri Karhunen and statistical analyses by Martin Gillstedt are gratefully acknowledged.

Funding

The work was financially supported by the Swedish Asthma and Allergy Association, Stockholm, Sweden, the Welander-Finsen Foundation, Stockholm, Sweden, and the Research Institute for Fragrance Materials Inc. (RIFM), New York, USA. RIFM is a research institute that is funded by the manufacturers of fragrance and consumer products containing fragrances.

References

- Casabianca H, Graff J B, Faugier V, Fleig F, Grenier C. Enantiomeric distribution studies of linalool and linalyl acetate. A powerful tool for authenticity control of essential oils. *J High Resolution Chromatogr* 1998; 21: 107–112.
- Hagvall L, Sköld M, Bråred Christensson J, Börje A, Karlberg A-T. Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis* 2008; 59: 143–150.
- Rastogi S C, Johansen J D, Frosch P et al. Deodorants on the European market: quantitative chemical analysis of 21 fragrances. *Contact Dermatitis* 1998; 38: 29–35.
- Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001; 45: 221–225.
- Buckley D A. Fragrance ingredient labelling in products on sale in the U.K. *Br J Dermatol* 2007; 157: 295–300.
- RIFM EXPERT Panel: Belsito D, Bickers D, Bruze M, Calow P, Greim H, Hanifin J M et al. A toxicologic and dermatologic assessment of cyclic and non-cyclic terpene alcohols when used as fragrance ingredients. *Food Chem Toxicol* 2008; 46(Suppl. 11): S1–S71.
- Karlberg A-T, Bergström M A, Börje A, Luthman K, Nilsson J L. Allergic contact dermatitis—formation, structural requirements, and reactivity of skin sensitizers. *Chem Res Toxicol* 2008; 21: 53–69.
- Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis* 1987; 16: 93–95.
- Matura M, Sköld M, Börje A et al. Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis* 2005; 52: 320–328.
- Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis* 2007; 57: 1–10.
- Sköld M, Börje A, Matura M, Karlberg A-T. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis* 2002; 46: 267–272.
- Sköld M, Börje A, Harambasic E, Karlberg A-T. Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem Res Toxicol* 2004; 17: 1697–1705.
- Sköld M, Hagvall L, Karlberg A-T. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 2008; 58: 9–14.
- Nilsson U, Bergh M, Shao L P, Karlberg A-T. Analysis of contact allergenic compounds in oxidized d-limonene. *Chromatographia* 1996; 42: 199–205.
- Bråred Christensson J, Forsström P, Wennberg A-M, Karlberg A-T, Matura M. Air oxidation increases skin irritation from fragrance terpenes. *Contact Dermatitis* 2009; 60: 32–40.
- Bruze M, Condé-Salazar L, Goossens A, Kanerva L, White I R. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis* 1999; 41: 241–250.
- ESSCA Writing Group. The European Surveillance System of Contact Allergies (ESSCA): results of patch testing the standard series, 2004. *J Eur Acad Dermatol Venereol* 2008; 22: 174–181.
- Thyssen J P, Linneberg A, Menné T, Johansen J D. The epidemiology of contact allergy in the general population—prevalence and main findings. *Contact Dermatitis* 2007; 57: 287–299.
- Nardelli A, Carbonez A, Ottroy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Dermatitis* 2008; 58: 134–141.
- Brasch J, Uter W, Geier J, Schnuch A. Associated positive patch test reactions to standard contact allergens. *Am J Contact Dermat* 2001; 12: 197–202.
- Karlberg A-T, Doms-Goossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 1997; 36: 201–206.
- Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjö K, Karlberg A-T. Oxidized citrus oil (R-limonene): a frequent sensitizer in Europe. *J Am Acad Dermatol* 2002; 47: 709–714.
- Matura M, Sköld M, Börje A, Andersen K E, Bruze M, Frosch P, et al. Not only oxidized R-(+)- but also S-(–)-limonene is a common cause of contact allergy in dermatitis patients in Europe. *Contact Dermatitis* 2006; 55: 274–279.
- Matura M, Goossens A, Bordalo O, Garcia-Brava B, Magnusson K, Wrangsjö K, Karlberg A-T. Patch testing with oxidized R-(+)-limonene and its hydroperoxide fraction. *Contact Dermatitis* 2003; 49: 15–21.
- Lu X, Li L F, Wang W, Wang J. A clinical and patch test study of patients with positive patch test reactions to fragrance mix in China. *Contact Dermatitis* 2005; 52: 188–191.
- Karlberg A-T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992; 26: 332–340.
- Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007; 56: 281–285.
- Wahlberg J. Patch testing. In: *Textbook of Contact Dermatitis*, 2nd edition, Rycroft R, Menné T, Frosch P (eds): Berlin, Germany, Springer-Verlag, 1995: 241–261.
- Fregert S. *Manual of Contact Dermatitis*, 2nd edition, Copenhagen, Denmark, Munksgaard, 1981: 71–80.
- Lindberg M, Edman B, Fischer T, Stenberg B. Time trends in Swedish patch test data from 1992 to 2000. A multi-centre study based on age- and sex-adjusted results of the Swedish standard series. *Contact Dermatitis* 2007; 56: 205–210.
- Nilsson U, Magnusson K, Karlberg O, Karlberg A-T. Are contact allergens stable in patch test preparations? Investigation of the degradation of d-limonene hydroperoxides in petrolatum. *Contact Dermatitis* 1999; 40: 127–132.
- Directive 2003/15/EC of the European Parliament and of the council of 27 February 2003 amending Council Directive

- 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Off J Eur Union* 2003; L66: 26–35.
33. Basketter D, Dooms-Goossens A, Karlberg A-T, Lepoittevin J P. The chemistry of contact allergy: why is a molecule allergenic? *Contact Dermatitis* 1995; 32: 65–73.
34. IFRA standards, found at <http://www.ifraorg.org>. (Accessed 23 November 2009).
35. Karlberg A T, Magnusson K, Nilsson U. Influence of an anti-oxidant on the formation of allergenic compounds during auto-oxidation of d-limonene. *Ann Occup Hyg* 1994; 38: 199–207.
36. Rastogi S C, Menné T, Johansen J D. The composition of fine fragrances is changing. *Contact Dermatitis* 2003; 48: 130–132.
37. Hagvall L, Bäcktorp C, Svensson S, Nyman G, Börje A, Karlberg A-T. Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization. *Chem Res Toxicol* 2007; 20: 807–814.

Address:

Johanna Bråred Christensson
Department of Dermatology
Sahlgrenska University Hospital
413 45 Gothenburg
Sweden
e-mail: johanna.brared-christensson@vgregion.se