

Air-oxidized linalool elicits eczema in allergic patients – a repeated open application test study

Ylva Andersch Björkman^{1,†}, Lina Hagvall^{1,†}, Charlotte Siwmark², Bo Niklasson², Ann-Therese Karlberg³ and Johanna Bråred Christensson^{1,3}

¹Department of Dermatology, Sahlgrenska Academy at University of Gothenburg, 41345 Gothenburg, Sweden, ²Chemotechnique Diagnostics, 23539 Vellinge, Sweden, and ³Dermatochemistry and Skin Allergy, Department of Chemistry and Molecular Biology, University of Gothenburg, 40530 Gothenburg, Sweden

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Summary

Background. Linalool is a commonly used fragrance terpene that forms potent sensitizers upon oxidation. In a recent multicentre study, we found that 7% of 2900 patients showed positive patch test reactions to oxidized linalool at 6.0%. No elicitation studies have been performed.

Objective. To identify threshold concentrations for elicitation of allergic contact dermatitis caused by oxidized linalool in allergic individuals with repeated exposures.

Methods. Repeated open application tests were performed in 6 participants previously diagnosed with contact allergy to oxidized linalool. Creams containing 3.0%, 1.0% and 0.30% oxidized linalool (corresponding to 0.56%, 0.19% and 0.056% linalool hydroperoxides, respectively) and 'fine fragrance' containing 1.0%, 0.30% and 0.10% oxidized linalool (corresponding to 0.19%, 0.056% and 0.019% linalool hydroperoxides, respectively) were used twice daily for up to 3 weeks. Patch testing with a dilution series of oxidized linalool was performed.

Results. Five of 6 participants reacted to the cream containing 3% oxidized linalool. With 1% oxidized linalool, a reaction was seen in 3 (cream) and 4 (fine fragrance) participants, respectively. With 0.3% oxidized linalool, 2 (cream) and 1 (fine fragrance) participants reacted.

Conclusion. Repeated exposure to low concentrations of oxidized linalool can elicit allergic contact dermatitis in previously sensitized individuals.

Key words: allergic eczema; autooxidation; contact allergy; fragrance terpenes; hydroperoxides; linalool; oxidation products; repeated open application test.

Contact allergy to fragrances is very common among dermatitis patients (1, 2). In many studies, 10–15%

of consecutively tested dermatitis patients have shown positive patch test reactions to one or more of the fragrance markers in the baseline series [fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) and *Myroxylon pereirae*] (2–4). However, many common fragrance chemicals are not tested in routine patch testing. A number of fragrance chemicals have been shown to be prehapten or prohaptens, and are thus susceptible to air oxidation or cutaneous metabolism. Strongly sensitizing compounds have been identified among the oxidation products or metabolites (5–9). For these fragrance chemicals, relevant patch test materials have not been available

Correspondence: Johanna Bråred Christensson, Dermatochemistry and skin allergy, Department of Chemistry and Molecular Biology, Box 462, SE 40530 Gothenburg, Sweden. Fax: +46 317723840. E-mail: johanna.brared-christensson@vgregion.se

[†]These authors contributed equally to this work.

Conflicts of interest: Ylva Andersch Björkman, Lina Hagvall, Ann-Therese Karlberg and Johanna Bråred Christensson declare no conflict of interest. Charlotte Siwmark and Bo Niklasson are affiliated to Chemotechnique Diagnostics AB, Vellinge, Sweden.

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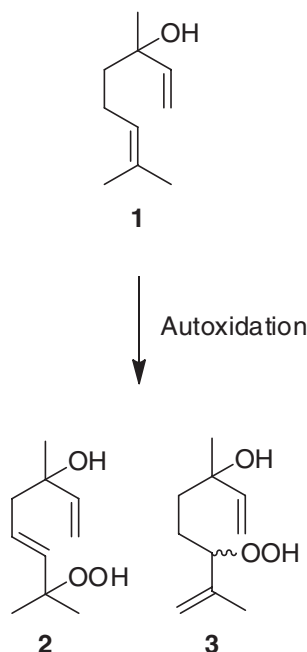


Fig. 1. Chemical structures of **1** linalool (3,7-dimethyl-1,6-octadien-3-ol) and linalool hydroperoxides **2** (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol) and **3** (6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol). These hydroperoxides are the main sensitizers detected in the autoxidation mixture of linalool. Hydroperoxide **2** is the major hydroperoxide detected, as the hydroperoxide fraction of oxidized linalool contains 80% of hydroperoxide **2** (8).

commercially. Thus, fragrance allergy is likely to be more common than previously recognized. Recently, controlled patch test preparations for oxidized limonene and oxidized linalool, with stable concentrations of the main haptens (hydroperoxides), have been made commercially obtainable (Chemotechnique Diagnostics, Vellinge, Sweden).

Linalool is a fragrance terpene with a fresh flowery aroma. It is found naturally, mainly in lavender (10). Linalool is among the most common fragrance ingredients in everyday products such as cosmetics and household products (11–13). In a recent assessment of estimated consumer exposure to fragrance terpenes, the daily exposure to linalool was estimated to be higher than that to other fragrance terpenes, owing to its frequent occurrence in ordinary products (14).

Pure linalool (Fig. 1) is not allergenic or has a very low sensitization potential (8). It seldom causes positive patch test reactions in dermatitis patients (15, 16). However, linalool has been shown to autoxidize and form oxidation products, some of which have strong or moderate sensitizing potencies when tested in the murine local lymph node assay (8, 17). The hydroperoxides

formed when linalool is oxidized were shown to be the major haptens in the oxidation mixture (8, 9, 17).

Four clinical patch test studies have been performed with oxidized linalool (18–21). When tested at 2.0% in petrolatum (pet.), oxidized linalool gave positive patch test reactions in 1.3% of 1511 tested patients (18). A study in the United Kingdom using oxidized linalool 3% pet. found 2.3% positive patch test reactions in 483 patients (19). Patch test concentrations of oxidized linalool 2.0%, 4.0%, 6.0% and 11.0% pet. were tested in a dose–response study, giving 0.83%, 3.2%, 5.3% and 7.2% positive patch test reactions, respectively. A concentration of 6.0% pet. was suggested as an optimal patch test concentration (20). Recently, an international multicentre study was performed at nine test centres in Europe, Singapore, and Australia, using oxidized linalool 6.0% pet. with a carefully controlled content of linalool hydroperoxides 1% (21). In that study, 6.9% of 2900 tested patients showed positive patch test reactions to oxidized linalool.

The concentrations used in patch testing are often much higher than the concentrations found in commercial products. However, with repeated exposure to a hapten in everyday products, low concentrations may be sufficient to cause or worsen eczema in allergic individuals. To study threshold concentrations for elicitation of contact dermatitis, use tests such as the repeated open application test (ROAT) are of great value (22). The ROAT is a method that has been developed to mimic everyday use of a commercial product, such as a perfume, cream, or deodorant. ROAT studies have been performed for haptens, such as HICC and methyl dibromoglutaronitrile (MDBGN) (23–25). Both HICC and MDBGN were shown to elicit eczema in allergic patients at concentrations used in cosmetic products. Eugenol has also been used in ROAT studies, where patients showed positive reactions to hydroalcoholic model products (26). No similar studies of this type have been performed with oxidized fragrance terpenes.

The aim of the present study was to investigate threshold concentrations for elicitation of allergic contact dermatitis by oxidized linalool in allergic individuals. The study was performed according to an updated ROAT protocol. Two model products containing oxidized linalool were used, one in ethanol to mimic a fine fragrance, and one in a cream base to mimic a cosmetic cream.

Materials and Methods

Chemicals

Glyceryl stearate was obtained from Croda Nordica AB (Malmö, Sweden). Linalool (3,7-dimethyl-1,6-octadien-3-ol) (Fig. 1) with a stated purity of 97% was obtained

		Reading 1												Reading 2						Reading 3								
		/day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21					
Participant 1		%																										
	Cream	3							-	i					16													
		1							-						-						17							
		0.3							-						-						6				i, p		12	
	Fine fragrance	1							-						-						r				13		17	
Participant 2		0.3							-						-										r		13	
		0.1							-						-										2			
	Cream	3		r					11				13												17			
		1					-								-				r				13					
	Fine fragrance	1					-								5				13				13					
Participant 3		0.3					-								-													
		0.1					-								-													
	Cream	3					-				r, p		9		11													
		1					-								-													
	Fine fragrance	1					-								-				1				9					
Participant 4		0.3					-								-													
		0.1					-								-													
	Cream	3					r		12						8													
		1					-								3				8									
	Fine fragrance	1					-								-								9					
Participant 5		0.3					-								-								r				9	
		0.1					-								-													
	Cream	3					-								r				9									
		1					-								-													
	Fine fragrance	1					-								-													
Participant 6		0.3					-								-													
		0.1					-								-													
	Cream	3					-								r				9									
		1					-								-													
	Fine fragrance	1					-								-													

Fig. 2. Reported reactions in the individual participants during the study, and scorings by the physicians at different time-points. The type of reaction as described by the patient is given (i = itching, r = redness, p = papules) at the time noted. At times of reading, the sum of the scoring of reactions according to the criteria in Table 2 is given for each individual. Details of the scores are given in Table S1.

from Sigma Aldrich Chemie (Schnelldorf, Germany), and purified by distillation. In order to mimic oxidation during handling and storage, a simplified experimental oxidation model was used according to previous experience (17). The oxidation/degradation process was followed by the use of high-performance liquid chromatography, according to methods previously described (18). In the present study, the oxidized linalool contained 18.8% linalool hydroperoxides. The hydroperoxide fraction in autoxidized linalool contains ~80% of the major hydroperoxide (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol), and the rest consists of the minor hydroperoxide (6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol) (Fig. 1), according to previous analyses (8) (Fig. 2).

Preparations used in ROATs and for patch testing

The test material for ROATs and patch testing was prepared by Chemotechnique Diagnostics. Oxidized linalool was stored under nitrogen at -20°C until test preparations were made. Chemical analyses were performed before preparation.

Oxidized linalool with a controlled level of hydroperoxides, as described above, was used for the ROAT

preparations. Two types of model product containing oxidized linalool were tested in parallel. Solutions of oxidized linalool (1.0%, 0.30%, and 0.10%, corresponding to 0.19%, 0.056% and 0.019% hydroperoxides, respectively) in ethanol (96%) were produced in order to mimic a fine fragrance. Model creams containing oxidized linalool (3.0%, 1.0%, and 0.30%, corresponding to 0.56%, 0.19% and 0.056% hydroperoxides, respectively) were produced by using cream base (15% glyceryl stearate in water) in order to mimic a scented cosmetic cream.

The same batch of oxidized linalool as used for the ROAT preparations was used for the patch test preparations. A dilution series of oxidized linalool in pet. was prepared in concentrations of 6.0%, 2.0%, 0.70%, 0.20%, and 0.07%. The hydroperoxide concentrations of these preparations were 1.13%, 0.38%, 0.13%, 0.038%, and 0.013%, respectively.

Participants

During 2010 and 2011, 945 patients (282 males; 663 females) were tested with oxidized linalool 6.0% with a controlled concentration of 1% linalool hydroperoxides in pet., in addition to the regular patch testing at

the Department of Dermatology, Sahlgrenska University Hospital. Of these, 34 patients (12 males; 22 females) showed positive patch test reactions to oxidized linalool (22 patients showed + reactions; 12 patients showed ++/+++ reactions). All of these patients were considered as candidates for the study.

Inclusion criteria for participation were a positive patch test reaction to oxidized linalool 6.0% pet. and age between 18 and 75 years. Exclusion criteria included systemic or local immunosuppressant treatment, active eczema, pregnancy, lactation, exposure to the sun or ultraviolet treatment of the test area within 2 weeks of the study.

Of the 34 patients, 27 were eligible according to the exclusion criteria. A letter was sent to the 27 patients. Of these, 6 did not answer, 12 declined to participate, and 9 agreed to further contact. Of these, 1 individual declined to participate for practical reasons, and 1 was excluded at the start of the study because of severe eczema of the body, hands, and arms. Finally, 7 participants were included in the study. One participant discontinued the study after 1 week because of subjective symptoms consisting of discomfort in her left arm. Six individuals completed the study (3 males and 3 females, median age 34 years, range 24–57 years). A questionnaire regarding medical history was filled out before the study. The participants were asked if they had made any changes in their consumer habits and/or experienced any change in their earlier dermatitis after being informed of their allergy to oxidized linalool. For detailed information about the participants, see Table 1. Informed consent from the participants was obtained. The study was performed in 2012.

ROAT procedure

Oxidized linalool in ethanol ('fine fragrance') and oxidized linalool in a cream ('cream') were tested in parallel in each individual. Ethanol (96%) and cream base (15% glyceryl stearate in water) served as negative controls. The fine fragrance was distributed in 1.5-ml Eppendorf Tubes®, and 0.1 ml was applied with a preset automatic pipette. The cream was applied in an amount of 0.1 ml from 1-ml thin syringes with markings at each 0.1 ml. The test preparations were coded in colour, and marked in red, yellow, green, and blue (control). Each participant was supplied with a set of two syringes of cream and two Eppendorf Tubes® of fine fragrance for each concentration of oxidized linalool, and a pipette with 60 disposable tips. New sets of materials were given weekly.

To facilitate application of the cream and fine fragrance, an updated design of the ROAT method was developed. Hypoallergenic 52-mm-wide surgical tape was used, in which circular holes with a diameter of 3.6 cm were cut. The improvement of the method meant that the application of fine fragrance and cream was significantly simplified, the area of application was more precise, and possible loss of skin markings was prevented. Each tape was used for a maximum of 1 week, and changed by the participant if needed. As an alternative, if the tape felt uncomfortable, the participant was instructed to draw a circle of the same size with a skin-friendly marker before removing the dressing. Two of the participants (participants 2 and 6) preferred not to use the surgical tape, and instead, for the greater part of the study, used the skin-friendly marker.

Eight circular areas of 10.2 cm² each were identified, four on the volar side of each forearm. Each test area on the forearms was assigned to one of the test preparations,

Table 1. Description of the participants in the study, including age, sex, earlier patch test reactions to oxidized linalool, and concomitant reactions to other fragrance markers in previous patch testing

Participant	Sex	Age (years)	Earlier patch test		Concomitant reactions to fragrance markers	Documented exposure to linalool-containing products	Effect of avoidance of linalool
			Year tested	Result			
1	F	41	2011	+	–	Many products containing essential oils	Clearance of dermatitis
2	M	26	2010	+++	<i>Myroxylon pereirae</i> , fragrance mix I, fragrance mix II	Hygiene products, lotion, sunscreen	Improvement of dermatitis
3	M	57	2010	+++	<i>Myroxylon pereirae</i> , fragrance mix I, fragrance mix II	Hygiene products	No improvement
4	F	24	2010	+	–	Occupational exposure	Avoidance not possible, recurring dermatitis
5	F	27	2010	+	–	Cosmetics, hygiene products	Improvement of dermatitis
6	M	43	2011	+	–	Hygiene products	Improvement of dermatitis

F, female; M, male.



Fig. 3. Photograph of the reactions in participant 1 at D21. On the left, reactions to creams in decreasing concentrations are shown from top to bottom. The 'fine fragrance' solutions are shown on the right, also in decreasing concentrations from top to bottom. The corresponding controls are located over the wrists of the patient, on the areas marked by the hypoallergenic surgical tape. Note that the cream at the highest concentration (3% oxidized linalool) has also caused allergic eczema on the upper arm, corresponding to the skin fold at the elbow.

which, in turn, were colour coded. The cream was applied on the right forearm and the fine fragrance on the left. The study was single-blinded, as the participants were not informed what the colour codes represented. A colour-coded diagram of application areas on the lower arms was provided.

At the start of the study, the participants were instructed how to use the pipette and syringes and allowed to practise together with the project leader until they could perform the application correctly. The participants were

instructed to apply 0.1 ml of the cream, as marked on the syringe, and 0.1 ml of the perfume, as measured by the pipette, to the allocated skin areas, and to gently spread the test material using one specific finger, each finger being used only on one area. This procedure was performed twice daily (morning and evening). If there was too much cream, the participants were instructed to remove the excess with a paper towel. They were advised to avoid contact with water directly after applying the material. Nothing else was applied to the test area during the study.

At each reading, the remaining test materials from the previous week were brought in by the participant, and the remaining volume of test material in syringes and tubes was measured and recorded.

The application of the preparations continued for a maximum of 3 weeks or until a reaction occurred. The participants were instructed to contact the clinic if symptoms such as redness, papules, vesicles or itching in the test area appeared. They were instructed to continue application until the same day on which the reaction was evaluated and documented. When a reaction was evaluated as positive, they were instructed to stop further application to that specific test area.

Evaluation

During the study period, the skin was examined once weekly, or earlier at the request of the participant. Evaluation was performed with a scale (Table 2) developed by Duus Johansen et al. (22, 27, 28). Scoring points were given for the following morphological features: erythema, papules/infiltration, vesicles, and extent of area involved. The ROAT was considered to be positive at a score of 5 points. During the weekly examination, additive scoring to some concentrations was performed even if the application in the ROAT for that concentration was finished. This was done in order to observe the development of the reactions (increase or decrease) during the days nearest to those when the exposure to the hapten had ceased.

Photographs were taken of all reactions for documentation. The total amount of each preparation that had been used was determined by measuring the volumes of remaining fine fragrance and cream in the syringes and tubes (Table 3).

Two volunteers without skin problems and with negative reactions to oxidized linalool (6.0% pet.) performed a ROAT with the highest concentrations of oxidized linalool (3.0% in cream and 1.0% in ethanol) and the vehicles according to the same ROAT procedure as described above.

Table 2. Reading scale of the repeated open application test proposed by Johansen et al. (22, 27, 28); a positive reaction is defined as a score of a minimum of 5 points

Area involvement		Erythema				Papules/infiltration		Vesicles	
%	Score	Involvement	Score	Strength	Score	Score	Score	Score	Score
90–100	4	Homogeneous	2	Strong	3	Homogeneous	4	Confluent	4
50–89	3	Spotty	1	Medium	2	> 10	3	> 10	3
25–49	2	None	0	Weak	1	5–10	2	5–10	2
1–24	1	–	–	–	–	< 5	1	< 5	1
None	0	–	–	–	–	None	0	None	0

Table 3. The used test materials in their containers were brought in by the participants at readings, and the volume that had been used was measured

Vehicle	Concentration (%)	Participant 1		Participant 2		Participant 3		Participant 4		Participant 5		Participant 6	
		Total expected use (ml)	Used (% of expected use)	Total expected use (ml)	Used (% of expected use)	Total expected use (ml)	Used (% of expected use)	Total expected use (ml)	Used (% of expected use)	Total expected use (ml)	Used (% of expected use)	Total expected use (ml)	Used (% of expected use)
Cream	3	2.6	100	1.1	173	1.8	100	1.0	120	2.4	96	3.8	68
	1	3.4	100	3.1	NR	3.8	100	3.0	107	3.8	97	3.8	66
	0.3	4.0	103	3.9	NR	3.8	100	3.8	105	3.8	97	3.8	68
	Control	4.0	100	3.9	NR	3.8	103	3.8	105	3.8	97	3.8	66
Fine fragrance	1	3.2	103	3.1	NR	3.8	105	3.8	103	3.8	87	3.8	63
	0.3	4.0	103	3.9	NR	3.8	97	3.8	97	3.8	82	3.8	66
	0.1	4.0	105	3.9	NR	3.8	111	3.8	97	3.8	71	3.8	71
	Control	4.0	105	3.9	NR	3.8	103	3.8	97	3.8	76	3.8	71

NR, not recorded.

Participant 2 did not bring the used ROAT preparations at reading 3, and a total use volume could not be calculated. However, the participant used 120–124% of the expected doses of the creams and 76–80% of the expected doses of the fine fragrances during weeks 1–2.

At the end of the study, the total volume used was calculated. This table shows the expected use volumes of test preparations, and the actual volumes used expressed as percentages of the expected use volumes at the end of the study. The total expected use volumes differ between the participants, as they stopped using the test preparations as reactions occurred. Also, the starting times differed slightly between participants, giving a different total dose.

Patch testing

All of the participants except participant 1 were patch tested after 2 weeks of ROAT application. Participant 1 was tested after 3 weeks of ROAT application, for practical reasons. Patch test preparations of ~20 mg (29) were applied in IQ Ultra Chambers™ (8 × 8 mm, inner area of 0.64 cm²; Chemotechnique Diagnostics) to the back of the participant, left under occlusion for 48 hr, and then removed by the participant. Readings were performed on D3 and D7 according to the International Contact Dermatitis Research Group recommendations (30).

The study was approved by the local Ethics Committee.

Results

In the 3 weeks of ROAT application, 5 of the 6 participants reacted to the cream and 4 also reacted to the fine fragrance at any concentration. One participant

(participant 6) reacted neither to the cream nor to the fine fragrance, and did not show any positive patch test reaction at retesting.

The questionnaire prior to the study showed that 5 of 6 patients avoided contact with linalool-containing products after their diagnosis of contact allergy to oxidized linalool. Four of these 5 participants answered that they had experienced improvement of their earlier dermatitis after having stopped using products labelled as containing linalool (Table 1).

ROAT study

The results from the ROAT study are summarized in Table 4, and details are given in Table S1. Five of the participants reacted to the cream containing 3% oxidized linalool (0.56% linalool hydroperoxides) (Table S1). A reaction was seen to 1% oxidized linalool (0.19% linalool

Table 4. Expected dose per unit area of oxidized linalool per application and vehicle

	Cream			Fine fragrance			Vehicle controls
Concentration of oxidized linalool (%)	3.0	1.0	0.30	1.0	0.30	0.1	–
Content of linalool hydroperoxides (%)	0.56	0.19	0.056	0.19	0.056	0.019	–
Dose of oxidized linalool per unit area (µg/cm ²)	273	91	27	77	23	7.7	–
Responding individuals (n)	5	3	2	4	1	0 ^a	0

The table also shows the number of individuals responding to each dose. All application areas were 10.2 cm². The density of the cream was found to be 0.93 g/ml.

^aParticipant 1 showed some erythema and papules at reading at day 21, although not enough for a positive repeated open application test reading; see Table S1.

hydroperoxides) in 3 of the participants when tested with the cream and in 4 of the participants when tested with the fine fragrance. For the preparations containing 0.3% oxidized linalool (0.056% linalool hydroperoxides), 2 participants reacted to the cream and 1 reacted to the fine fragrance. A tendency to react to the fine fragrance containing 0.1% oxidized linalool (0.019% linalool hydroperoxides) was seen in 1 participant. None of the participants reacted to the control ROAT materials, that is, the cream and fine fragrance without oxidized linalool.

The time required for elicitation of reactions is shown in Table S1 and Fig. 2. The first symptoms reported by the participants were erythema (redness) and itching, followed by infiltration of the area (Fig. 2). Almost all of the reactions that were read and determined to be positive ROAT reactions in between the regular reading times continued to develop into strong reactions at later readings after application of the model product had stopped (Table S1; Fig. 2).

Figure 2 shows the times of the first reactions to respective concentrations of cream and fine fragrance in each participant. The morphological score at readings for each concentration of the cream and perfume is also shown in Fig. 2. It has been suggested that a reaction is required to affect > 25% of the test area for a ROAT test to be positive. This criterion was met in all reactions considered to be positive.

One participant (participant 1) reacted to all concentrations of oxidized linalool for both the cream and the fine fragrance, although the reaction for the 0.1% perfume was weak, and was thus not registered as a positive reaction. Figure 3 shows participant 1 on D21. No reactions were observed in the controls.

Compliance data

The compliance of the participants was assessed by measurement of the volume of test material used. The results are shown in Table 3. In the monitoring of usage of

Table 5. Result of the confirmatory patch test, performed at the end of the repeated open application test, with a dilution series of oxidized linalool (6.0%, 2.0%, 0.70%, 0.20% and 0.07% pet.)

Concentration of oxidized linalool (% in pet.)	Participant number					
	1	2	3	4	5	6
6.0	+++ ^a	+++	+++	++	++	–
2.0	+++	+++	++	++	?	–
0.70	+++	++	++	+	–	–
0.20	++	–	–	+	–	–
0.07	+	–	–	–	–	–

The hydroperoxide concentrations of these preparations were 1.13%, 0.38%, 0.13%, 0.038%, and 0.013%, respectively. The table shows results of the D3 reading. Reading at D7 gave no additional information

^aReadings according to the International Contact Dermatitis Research Group guidelines (29).

model products, it was shown that most of the participants were compliant with the instructions given, and were able to use the model products accurately, defined here as the intended dose $\pm$ 20% (Table 3). Participant 6 used lower amounts than intended ($\sim$ 70% of the intended dose) of all model products. Participant 2 did not bring the used ROAT preparations at reading 3, and a total use volume could not be calculated.

Patch testing

Five of 6 participants showed positive patch test reactions at retesting. All reactions to the highest concentration (6.0% pet.) of oxidized linalool were strong (++/+++), whereas reactions to the lower concentrations of oxidized linalool were weaker (Table 5). One doubtful reaction was registered for participant 5 with 2.0% pet. oxidized linalool.

Discussion

This ROAT study shows that oxidized linalool can elicit contact allergic reactions when applied repeatedly in low

concentrations in allergic individuals, as in everyday use of cosmetic products. As low a concentration as 0.3% oxidized linalool (0.056% linalool hydroperoxides) in both cream base and fine fragrance gave reactions in participants within the test period of 3 weeks. As expected, the highest concentrations of oxidized linalool, in this case a cream at 3.0%, gave most responders and the quickest response (Table 4). For the lower concentrations, not all participants responded during the time allocated, and longer exposure times were required to elicit a reaction than with the higher test concentrations used. The results showed no overall difference in time of reaction or reaction pattern when the same concentrations of oxidized linalool in different vehicles were compared (Table S1).

On the basis of the above results, it can be expected that common consumer products containing oxidized linalool will elicit allergic eczema in sensitized individuals. This is supported by the fact that the participants in this study had experienced subjective problems with fragranced products and improvement in their dermatitis when they avoided products declared to contain linalool (Table 1). The actual concentrations of linalool used in different consumer products are not well described. In earlier studies, the concentrations of individual fragrance compounds in cosmetics and cleansing products have been shown to be in the range 0.1–2% (12, 13). In fine fragrances, the composition is often confidential. For the cream with oxidized linalool at 3% (linalool hydroperoxides 0.56%) and 1% (linalool hydroperoxides 0.19%), pronounced dermatitis was recorded in 5 and 3 cases, respectively. Likewise, 4 of 6 participants showed strong reactions to the fine fragrance at 1% oxidized linalool. One participant reacted to all tested concentrations, although weakly to the lowest concentration (0.3% oxidized linalool) in the study period.

Overall, most reactions appeared in the third week (Fig. 2). Especially at the lower concentrations of the test material, slight reactions sometimes began to appear at the D13–D14 reading, whereas a positive reading was not visible until D20–D21. Thus, a longer study period would be preferable to obtain an adequate assessment of eczematous reactions among allergic individuals, especially when reactions to low concentrations of oxidized linalool are evaluated. In a recent ROAT study on eugenol, a study length of 4 weeks was found to be useful, as dermatitis was not elicited during the first 3 weeks (26).

The multitude of common products containing linalool causes exposure from many sources every day for high-end users of scented products (14). As a formulated product ages, oxidation products are expected to be formed from fragrance ingredients with a chemical structure prone to autoxidation, for example linalool, limonene, geraniol, and linalyl acetate (5, 7–9). The

primary oxidation products, the hydroperoxides, formed by autoxidation possess strong sensitizing properties (31). They form specific antigens, and cross-reactivity between hydroperoxides is seen only for those compounds that are closely related with regard to their overall chemical structure (32, 33). This is analogous to the cross-reactivity seen for other haptens. Also, secondary oxidation products formed from hydroperoxides can be allergenic, thus further increasing the sensitization potential of the autoxidation mixture (31). To what extent the formation of radicals by autoxidation can activate other compounds in the chemical mixture of a fragranced product has never been investigated. Addition of an antioxidant will prevent the degradation of components, but it has been shown that this effect will last for only a certain time, as the antioxidant is consumed. The rate of antioxidant depletion depends on the type of fragrance compound and its initial purity (34).

Work has been performed to confirm the presence of hydroperoxides from fragrance terpenes in complex products. However, the analysis of hydroperoxides in commercial products has proved to be difficult, as hydroperoxides are labile compounds that are not easily detected in the complicated matrices of consumer products with existing separation techniques. Recently, a breakthrough was achieved in the development of analytical methods for the detection and quantification of specific fragrance terpene hydroperoxides, as a new method using liquid chromatography coupled with tandem mass spectrometry was developed (35). With this method, linalyl acetate hydroperoxides and linalool hydroperoxides were identified in petigrain oil; limonene hydroperoxides were likewise identified in sweet orange oil, both after normal storage and after air oxidation (35). Earlier studies have shown that sensitizing hydroperoxides are formed in air-oxidized lavender oil, where linalool hydroperoxides have been identified after air exposure. No difference was found between the oxidation patterns in lavender oil of natural origin and those in a synthetic model, where the three main components of lavender oil, (linalool, linalyl acetate, and caryophyllene) had been purified and mixed together prior to oxidation (36).

In ROAT studies, it is important to provide detailed compliance data. Most participants used the test materials in a compliant way. However, participant 4 initially used a higher dose than intended of the 3.0% cream, which might have shortened the reaction time for this specific model product. One participant (participant 6) consistently used 30% less than the intended doses of all model products. As this participant did not react at patch testing to any concentrations, it is possible that he would not have

reacted in the ROAT even if the correct doses of test materials had been used. The present study is the first in which surgical tape was used to confine the test areas. We consider that monitoring of compliance data was important for the interpretation of the obtained results, and should be emphasized in investigations with similar study designs.

In ROATs, as well as in patch testing, the question of irritancy is of great interest. In this study, no irritant reactions to the vehicles were seen in any of the participants or controls. The two control persons were also tested with the highest concentrations of oxidized linalool (3.0% cream, 1.0% fine fragrance) in both vehicles, and no irritation was seen. Oxidized linalool has previously been studied in concentrations of 2.5–20% in an irritation study, and very little irritation was seen, except for a slight increase in irritation recorded at 20% pet. (37).

There was no clear correlation between the strength of reaction at the earlier patch test and the time of reaction in the ROAT. Towards the end of the study, we found that 3 of the participants had a stronger patch test reaction to 6.0% oxidized linalool than in earlier patch tests. It has been shown that there can be contact allergic priming of the test areas, leading to stronger reactions at later testing times (38). However, the universally increased tendency to react to an allergenic compound after local stimulation on another part of the body, for example by repeated applications locally on the arms, as in our study, has, to our knowledge, not been extensively studied.

In accordance with earlier ROAT studies (22–25), this study shows that, in allergic patients reacting to oxidized linalool 6.0% pet., reactions to much lower concentrations occurs through repeated applications. As the recommended patch test concentration of 6.0% pet. oxidized linalool (controlled content of 0.8–1.1% hydroperoxides) (20) is high as compared with the concentrations to which individuals are expected to be exposed, the clinical relevance of the positive patch test reactions has been discussed. The results from the present study support the relevance of oxidized linalool as a cause of allergic dermatitis, and indicate that many allergic consumers may be at risk for allergic contact dermatitis if sensitized to oxidized linalool. They also

suggest that additional cases of sensitization in users of scented consumer products can be expected. The frequent use of linalool and chemically related fragrance terpenes in scented consumer products is an important health issue that should be further investigated and assessed.

Conclusion

In this study, we have shown that low concentrations of oxidized linalool can elicit eczematous reactions in dermatitis patients with contact allergy to oxidized linalool. Thus, the results of the present study support the relevance of oxidized linalool in causing allergic contact dermatitis, as well as being an important cause of sensitization in dermatitis patients. New methods for more precise monitoring of exposure doses and areas exposed were found to be useful. To further investigate the threshold for elicitation of contact dermatitis, a study in a larger group of participants should be performed with a prolonged study time of 4 weeks. Studies of the effect of repeated application of oxidized fragrance terpenes in everyday, consumer-like settings are important, and need to be developed for better assessment of safe levels of fragrance compounds in common consumer products.

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Supporting Information

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

Table S1. Readings of skin reactions to oxidized linalool in cream base (3%, 1%, and 0.3%) and fine fragrance (ethanol) (1%, 0.3%, and 0.1%).

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Table S1. Readings of skin reactions to oxidized linalool in cream base (3%, 1%, and 0.3%) and fine fragrance (ethanol) (1%, 0.3%, and 0.1%)

		Participant number									
		1		2		3		4		5	6
a. Cream 3.0% oxidized linalool (0.56% hydroperoxides)											
First reaction (day)		11		2		8		5		12	–
End of application (day)		14		6		10		6		13	20
Day of reading		14	6	13	9	13	6	13	13	20	
Scoring ^a	Area (0–4)	4	2	4	2	2	4	4	4	–	–
	Erythema/quality (0–2)	2	1	2	1	1	2	2	1	–	–
	Erythema/strength (1–3)	3	2	3	2	2	2	2	1	–	–
	Papules/infiltration (0–4)	4	3	4	3	3	4	–	3	–	–
	Vesicles (0–4)	3	3	0	1	3	0	0	0	–	–
	Total score	16	11	13	9	11	12	8	9	–	–
b. Cream 1% oxidized linalool (0.19% hydroperoxides)											
First reaction (day)		17		15		–		13		–	–
End of application (day)		18		16		20		16		20	20
Day of reading		17	21	16	20	20	13	20	20	20	
Scoring ^a	Area (0–4)	2	4	3	4	–	2	3	–	–	–
	Erythema/quality (0–2)	1	2	2	2	–	1	1	–	–	–
	Erythema/strength (1–3)	1	3	2	3	–	1	1	–	–	–
	Papules/infiltration (0–4)	2	4	3	4	–	0	3	–	–	–
	Vesicles (0–4)	0	4	3	4	–	0	0	–	–	–
	Total score	6	17	13	17	–	4	8	–	–	–
c. Cream 0.3% oxidized linalool (0.056% hydroperoxides)											
First reaction (day)		18		–		–		20		–	–
End of application (day)		21		20		20		20		20	20
Day of reading		21		20		20		20		20	20
Scoring ^a	Area (0–4)	4		–		–		4		–	–
	Erythema/quality (0–2)	2		–		–		1		–	–
	Erythema/strength (1–3)	2		–		–		1		–	–
	Papules/infiltration (0–4)	4		–		–		3		–	–
	Vesicles (0–4)	–		–		–		–		–	–
	Total score	12		–		–		9		–	–
d. Fine fragrance 1.0% oxidized linalool (0.19% hydroperoxides)											
First reaction (day)		16		13		16		18		–	–
End of application (day)		17		16		20		20		20	20
Day of reading		17	21	13	16	20	16	20	20	20	20
Scoring ^a	Area (0–4) ^a	3	4	1	3	4	0	2	4	–	–
	Erythema/quality (0–2) ^a	2	2	1	2	2	0	2	2	–	–
	Erythema/strength (1–3) ^a	2	3	1	2	3	0	2	2	–	–
	Papules/infiltration (0–4) ^a	4	4	2	3	4	1	2	4	–	–
	Vesicles (0–4) ^a	2	4	0	3	0	0	1	0	–	–
	Total score	13	17	5	13	13	1	9	12	–	–
e. Fine fragrance 0.3% oxidized linalool (0.056% hydroperoxides)											
First reaction (day)		18		–		–		–		–	–
End of application (day)		21		20		20		20		20	20
Day of reading		21		20		20		20		20	20
Scoring ^a	Area (0–4) ^a	4		–		–		–		–	–
	Erythema/quality (0–2) ^a	2		–		–		–		–	–
	Erythema/strength (1–3) ^a	2		–		–		–		–	–
	Papules/infiltration (0–4) ^a	4		–		–		–		–	–
	Vesicles (0–4) ^a	1		–		–		–		–	–
	Total score	13		–		–		–		–	–

Table S1. Continued

		Participant number					
		1	2	3	4	5	6
f. Fine fragrance 0.1% oxidized linalool (0.019% hydroperoxides)							
First reaction (day)		21	–	–	–	–	–
End of application (day)		21	20	20	20	20	20
Day of reading		21	20	20	20	20	20
Scoring ^a	Area (0–4) ^a	0	–	–	–	–	–
	Erythema/quality (0–2) ^a	0	–	–	–	–	–
	Erythema/strength (1–3) ^a	1	–	–	–	–	–
	Papules/infiltration (0–4) ^a	1	–	–	–	–	–
	Vesicles (0–4) ^a	0	–	–	–	–	–
	Total score	2	–	–	–	–	–

Weekly readings were performed on days 6–7, 13–14, and 20–21. In the case of a reaction between these reading times, an additional reading was performed. Scoring criteria were according to Johansen et al. (22, 27, 28) (Table 2). A positive reaction is defined as a minimum of 5 points.

^aCriteria given in Table 2.