

Contact Allergy to Hydroperoxides of Linalool and D-Limonene in a US Population

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Background: Linalool and D-limonene are common fragrance ingredients that readily oxidize on exposure to air. The resulting hydroperoxides of linalool and D-limonene have been shown to have high frequencies of positive patch test reactions in several European and international studies.

Objective: The aim of the study was to investigate the prevalence of contact allergy to the hydroperoxides of linalool and D-limonene in a US population.

Methods: In this retrospective study, 103 patients with suspected fragrance allergy were patch tested to linalool 10% petrolatum (pet), hydroperoxides of linalool 1% pet, D-limonene 10% pet, and/or the hydroperoxides of D-limonene 0.3% pet between July 9, 2014, and October 25, 2016.

Conclusions: In this study, the frequency of positive patch test reactions to the hydroperoxides of linalool is 20% (19/96), and the frequency of positive reactions to the hydroperoxides of D-limonene is 8% (7/90). These high frequencies suggest that patch testing to the hydroperoxides of linalool and limonene should be performed in all patients with suspected fragrance allergy.

Linalool and D-limonene are fragrance terpenes and are among the most common fragrance ingredients.^{1–3} Linalool is widely used in household and hygiene products for its fresh, flowery odor.¹ Linalool is also found in 88% of essential oils including lavender, ylang-ylang, and rosemary oils.⁴ D-Limonene, a byproduct of the citrus juice industry, is widely used in cosmetics and cleaning products for its citrus odor.² D-Limonene is the main constituent (70%–98%) of peel oil from citrus fruits, including bergamot, grapefruit, lemon, orange, mandarin, and tangerine.⁴ D-Limonene is found in 97% of essential oils and is an important sensitizer in tea tree oil.⁵ In addition to its applications as a fragrance, D-limonene is also used as a solvent and industrial degreasing agent.⁶

Linalool and D-limonene are prehaptenes, which can be transformed by air oxidation into more allergenic compounds.² Thus, although their pure forms are only weakly allergenic, the oxidized forms of D-limonene and linalool have been demonstrated as frequent allergens in several international studies.^{1–3,6–9} Of the various oxidation products of linalool and D-limonene, the most potent allergens are the hydroperoxides, which are the primary oxidation products (Fig. 1).^{10–13} Studies have demonstrated that the frequency of patients with

positive patch test reactions to oxidized linalool and D-limonene is well above that for other single fragrance allergens.^{1,2}

Allergic contact dermatitis to fragrances is well documented. Fragrance mix I, fragrance mix II, and *Myroxylon pereirae* (balsam of Peru) are used to screen for fragrance allergy. Although colophony is not a fragrance allergen, patients with allergy to colophony often also have positive patch tests to fragrances.³ Similar to the fragrance terpenes, colophony can undergo air oxidation to form oxidation products with allergenic potential.³ These screening allergens seem to be less useful in the case of linalool and D-limonene, with more than half of patients going undetected by screening allergens and colophony despite having an allergy to the oxidized fragrance terpenes.^{1,2}

Although studies on the frequency of contact allergy to linalool, D-limonene, and their hydroperoxides have been conducted in Europe and in Canada, this has not yet been investigated in a US population.¹⁴ This study is a retrospective review of data on patients patch tested to these allergens at Duke Dermatology in Durham, NC.

METHODS

This retrospective study was approved by the Duke University Health System Institutional Review Board. Between July 9, 2014, and October 25, 2016, 103 patients were patch tested to linalool, hydroperoxides of linalool, D-limonene, and/or hydroperoxides of D-limonene at Duke Dermatology in Durham, NC. These allergens were tested only when product review suggested high fragrance use, the patient or physician suspected fragrance allergy as the cause of the dermatitis, or linalool or D-limonene was identified in the patient's personal care products. In addition to these fragrance chemicals, all patients were patch tested to the screening fragrance

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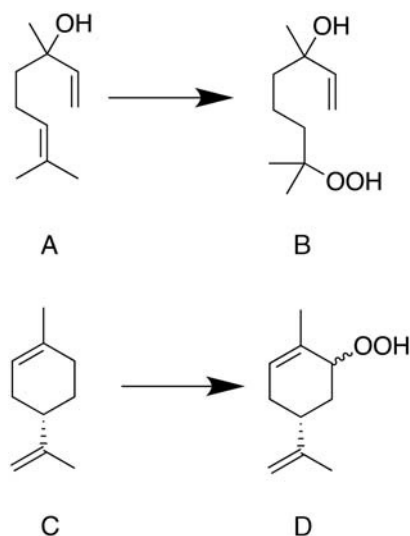


Figure 1. Chemical structures. A, Linalool. B, Linalool 7-hydroperoxide (major hydroperoxide). C, D-Limonene. D, Limonene 2-hydroperoxide (major hydroperoxide).

allergens and colophony, and some were also tested to essential oils that contain linalool or D-limonene.

The following allergens were obtained from Chemotechnique Diagnostics (Vellinge, Sweden): linalool 10% petrolatum (pet), hydroperoxides of linalool 1% pet, D-limonene 10% pet, hydroperoxides of D-limonene 0.3% pet, fragrance mix I 8% pet, fragrance mix II 14% pet, *M. pereirae* 25% pet, colophony 20% pet, propolis 10% pet, tea tree oil 5% pet, ylang-ylang 2% pet, and lavender absolute 2% pet. The remaining allergens were obtained from Allergeaze (Smartpractice, Calgary, Alberta, Canada): oil of bergamot 2% pet, oil of lemon 2% pet, orange oil 2% pet, and oil of rosemary 0.5% pet. Allergens were applied with Finn Chambers (SmartPractice, Phoenix, AZ) on Scanpor tape (Norgesplaster Alpha AS, Vennesla, Norway). Readings were carried out on D2 and D4/D5 and designated negative (–), doubtful (+/– or ?), weak positive (+), strong positive (++), extreme positive (+++), or irritant (IR) in accordance with International Contact Dermatitis Research Group criteria.¹⁵ Relevance was designated definite, probable, possible, or past in accordance with previously defined criteria.¹⁶

A retrospective review of these patients' charts was completed. Patch test results to the allergens of interest were recorded and used to calculate frequencies of positive reactions.

All statistical analyses were carried out using SAS software package version 9.4 (SAS Institute, Cary, NC) by the Duke Department of Biostatistics and Bioinformatics.

RESULTS

Of 556 patients patch tested at Duke Dermatology between July 9, 2014, and October 25, 2016, 103 (19%) were tested to linalool, hydroperoxides of linalool, D-limonene, and/or hydroperoxides of D-limonene. Of these 103 patients, 96 (93%) were tested to linalool and hydroperoxides of linalool, 93 (90%) were tested to D-limonene,

and 90 (87%) were tested to hydroperoxides of D-limonene. All patients who were tested to linalool were also tested to hydroperoxides of linalool, and all but 3 patients who were tested to D-limonene were also tested to hydroperoxides of D-limonene.

The male, occupational, atopic, hand, leg, face, age > 40 (MOAHLFA) index for the tested patients is shown in Table 1. Mean age was 48.2 years, range 19 to >89. For 7 patient records in which no history of atopic dermatitis was mentioned, omission was interpreted as a negative history.

Patch test results can be reviewed in Table 2. Of the 96 patients patch tested to linalool, 1 (1%) had a + patch test. Of the 96 patients tested to hydroperoxides of linalool, 19 (20%) had a positive patch test. Of these, 15 (79%) had a + reaction, and 4 (21%) had a ++ reaction.

Of the 93 patients patch tested to D-limonene, there were no positive patch tests. Of the 90 patients patch tested to hydroperoxides of D-limonene, 7 (8%) had a positive patch test. All of these patients had a + reaction.

The rates of concomitant positive reactions between the hydroperoxides of linalool and D-limonene and the screening fragrance allergens (fragrance mix I, fragrance mix II, and *M. pereirae*) and colophony are reported in Table 3.

Concomitant positive reactions between the hydroperoxides of linalool and D-limonene occurred in 5 (24%) of the 21 patients who had a positive patch test to either allergen. This represents 26% of the patients with positive reactions to hydroperoxides of linalool and 71% of the patients with positive reactions to hydroperoxides of D-limonene.

Several other linalool-containing allergens were tested. Ylang-ylang had a positive patch test in 2 (2%) of 103 patients, including 1 (5%) of 19 patients with a positive patch test to hydroperoxides of linalool. Lavender was positive in 1 (2%) of 45 patients tested, and this patient was 1 (5%) of 19 patients with a positive patch test to hydroperoxides of linalool. Oil of rosemary was negative in all 27 patients who were tested. Propolis had a positive patch test in 3 (3%) of 103 patients but was negative in all patients with a positive patch test to hydroperoxides of linalool.

D-Limonene-containing allergens were also tested. Tea tree oil had a positive patch test in 2 (2%) of 103 patients, including 2 (11%) of the 19 patients with a positive patch test to hydroperoxides of linalool. However, none of the 7 patients with a positive patch test to the hydroperoxides of D-limonene had a positive patch test to tea tree oil. Oils of bergamot, orange, and lemon were also tested, and no patients had a positive patch test reaction.

TABLE 1. The MOAHLFA for Tested Patients

	No. Patients (n = 103)	Percent
Male	12	12
Occupational	6	6
Atopic	47	46
Hand	13	13
Leg	11	11
Face	43	42
Age >40 y	68	66

TABLE 2. Number and Frequency of Positive Patch Test Reactions

Allergen	No. Patients Tested	No. Positive Reactions	Frequency of Positive Reactions, %
Linalool 10% pet	96	1	1
Hydroperoxides of linalool 1% pet	96	19	20
D-Limonene 10% pet	93	0	0
Hydroperoxides of D-limonene 0.3% pet	90	7	8
Fragrance mix I 8% pet	103	17	17
Fragrance mix II 14% pet	103	12	12
<i>M. pereirae</i> 25% pet	103	20	19
Colophony 20% pet	103	6	6
Propolis 10% pet	103	3	3
Tea tree oil 5% pet	103	2	2
Ylang-ylang oil 2% pet	103	2	2
Lavender absolute 2% pet	45	1	2
Oil of bergamot 2% pet	10	0	0
Orange oil 2% pet	24	0	0
Oil of lemon 2% pet	24	0	0
Oil of rosemary 0.5% pet	27	0	0

Four patients (21%) with a + reaction to hydroperoxides of linalool would have been undetected by the screening fragrance allergens (fragrance mix I, fragrance mix II, *M. pereirae*) and colophony. None of these patients were positive for any of the tested linalool-containing essential oils or lavender absolute.

No patients with a + reaction to hydroperoxides of D-limonene would have been undetected by the screening fragrance allergens or colophony.

Clinical relevance for the one patient with a positive reaction to linalool was described as “possible.” For the 19 patients with positive reactions to hydroperoxides of linalool, relevance was “definite” in 1 (5%), “probable” in 6 (31%), and “possible” in 12 (63%). Relevance for the 7 patients with positive reactions to hydroperoxides of D-limonene was “probable” in 3 (42%) and “possible” in 4 (57%).

DISCUSSION

In this study, there were high frequencies of positive patch test reactions to hydroperoxides of linalool (19%) and hydroperoxides of D-limonene (7%). The frequencies in this nonconsecutive patient

group are higher than those reported in several large multicenter studies involving consecutive patients, in which positive reactions to oxidation products of linalool occurred in 1.7% to 6.9% of patients and to oxidation products of D-limonene in 1.1% to 5.2% of patients.^{1-3,6-9}

One explanation for the high frequencies of positive reactions is that in this study linalool, D-limonene, and their hydroperoxides, as well as other extended fragrance allergens, were tested only when product review suggested high fragrance use, the patient or physician suspected fragrance allergy as a cause of the dermatitis, or the patient's personal care products contained these fragrance chemicals. Thus, patients were at a higher risk of fragrance allergy and thus more likely to have a positive patch test to a fragrance chemical.

Concomitant reactions between the hydroperoxides of linalool (79%) and D-limonene (100%) with the screening fragrance allergens (fragrance mix I, fragrance mix II, and *M. pereirae*) and colophony occurred frequently. These frequencies of concomitant reactions are higher than those found in large multicenter studies, which found the frequency of concomitant reactions to be 40% for oxidized linalool and 42% for oxidized D-limonene.^{1,2} This may be related to the characteristics of the group of patients being patch tested in this study, given that there was a high suspicion of fragrance allergy. However, even with the high frequencies of concomitant positive reactions demonstrated in this study, there were 4 patients (21%) with positive patch tests to the hydroperoxides of linalool that were undetected by the screening fragrance allergens and colophony.

Recently, the American Contact Dermatitis Society added lavender absolute, which contains linalool, to its Core Allergen Series.¹⁷ However, even with lavender included, none of the 4 patients who went undetected by the screening fragrance allergens and colophony in this study would have been detected by the linalool-containing essential oils on the Core Allergen Series. Although no patients with a positive patch test to hydroperoxides of D-limonene went undetected by screening fragrance allergens and colophony in this study, more than half of such patients went undetected in another large study.² These findings, in conjunction with the high rates of positive patch test reactions to the hydroperoxides of linalool and D-limonene, support the addition of these allergens to a baseline series.

Concomitant positive reactions between the hydroperoxides of linalool and the hydroperoxides of D-limonene also occurred frequently (24%). A similar value is reported in recent large multicenter studies.^{7,18} This is likely related to patients' frequent simultaneous

TABLE 3. Concomitant Positive Patch Test Reactions Between the Oxidized Fragrance Terpenes and the Fragrance Screening Allergens and/or Colophony

	Fragrance Mix I, n(%)	Fragrance Mix II, n(%)	<i>M. pereirae</i> , n(%)	Colophony, n(%)	≥1 of the Screening Fragrance Allergens and/or Colophony, n(%)
Hydroperoxides of linalool	6 (32)	6 (32)	6 (32)	3 (16)	15 (79)
Hydroperoxides of D-limonene	3 (43)	3 (43)	3 (43)	2 (29)	7 (100)

There were a total of 19 positive patch test reactions to hydroperoxides of linalool and 7 positive patch test reactions to hydroperoxides of D-limonene.

exposure to these fragrance chemicals in consumer products, resulting in sensitization to both allergens.¹⁸ Although they frequently coreact, the hydroperoxides of linalool and D-limonene are not thought to have cross-reactivity given their lack of close similarity in overall structure.¹⁸

The finding that nonoxidized linalool (1%) and D-limonene (0%) had only rare positive patch test reactions is consistent with previous studies.⁷ Not only is contact allergy to these chemicals uncommon, but also almost all patients who have contact allergy to the nonoxidized chemicals will also have allergy to their oxidation products. In a multicenter study, testing to the terpene hydroperoxides alone captured 97% of positive reactions.⁷ In addition, linalool and D-limonene oxidize readily on exposure to air, resulting in quantifiable levels of hydroperoxides of linalool and D-limonene in commercially available fragrances.^{7,19,20} Given this information, patch testing exclusively to the hydroperoxides of linalool and D-limonene without testing the nonoxidized chemicals should not dramatically impact the ability to detect contact allergy to linalool and D-limonene.

This study demonstrates high frequencies of atopic history in patients with positive reactions to hydroperoxides of linalool (63%) and hydroperoxides of D-limonene (71%). However, this is likely attributable to the high frequency of atopy in the 103 patients patch tested to these fragrances (77%). One reason for this high frequency may be that this is a referral-only patient population, which includes many patients referred by allergists as well as dermatologists. In addition, atopic history is self-reported by patients and may not be accurate. Multicenter studies have found atopic history to be present in 18.4% to 45.0% of patients with positive patch test reactions to hydroperoxides of linalool and 21.4% to 43.0% of patients with positive reactions to hydroperoxides of D-limonene.^{7,9} In general, atopy is not thought to confer increased risk of contact allergy to the hydroperoxides of linalool and D-limonene or to fragrance mix I.^{7,9}

One limitation of this study is that patients were nonconsecutive, which limits conclusions that can be drawn regarding the addition of hydroperoxides of linalool and D-limonene to a baseline screening panel as has been suggested by other studies.^{1,2,7,9} Another limitation is that in this single-center study, there were a limited number of patients who were tested to the hydroperoxides of linalool and D-limonene during the study period. Both of these limitations could be addressed by a future study in which consecutive patients are patch tested to these allergens.

In summary, this retrospective study of 103 patients who were patch tested to linalool, D-limonene, and their hydroperoxides demonstrates high frequencies of positive patch tests to hydroperoxides of linalool (20%) and hydroperoxides of D-limonene (8%). These frequencies are comparable with those of the screening fragrance allergens and colophony. Given the ubiquity of linalool and D-limonene as fragrance ingredients, these findings suggest that patch testing to the hydroperoxides of linalool and limonene should be performed in all patients with suspected fragrance allergy.

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