

Positive patch test reactions to oxidized limonene: exposure and relevance

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Summary

Background. R-Limonene is a common fragrance terpene found in domestic and industrial products. R-Limonene autoxidizes on air exposure, and the oxidation products can cause contact allergy. In a recent multicentre study, 5.2% (range 2.3–12.1%) of 2900 patients showed a positive patch test reaction to oxidized R-limonene.

Objective. To study the exposure to limonene among consecutive dermatitis patients reacting to oxidized R-limonene in an international setting, and to assess the relevance of the exposure for the patients' dermatitis.

Methods. Oxidized R-limonene 3.0% (containing limonene hydroperoxides at 0.33%) in petrolatum was tested in 2900 consecutive dermatitis patients in Australia, Denmark, the United Kingdom, Singapore, Spain, and Sweden. A questionnaire assessing exposure to limonene-containing products was completed.

Results. Overall, exposure to products containing limonene was found and assessed as being probably relevant for the patients' dermatitis in 36% of the limonene-allergic patients. In Barcelona and Copenhagen, > 70% of the patients were judged to have had an exposure to limonene assessed as relevant.

Conclusions. Oxidized R-limonene is a common fragrance allergen, and limonene was frequently found in the labelling on the patients' products, and assessed as relevant for the patients' dermatitis. A large number of domestic and occupational sources for contact with R-limonene were identified.

Key words: allergic contact dermatitis; autoxidation; fragrance allergy; hydroperoxide; limonene; oxidation products; patch test; relevance; terpenes.

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R-Limonene is the main constituent (98%) of peel oil from citrus fruits, and is widely used in cosmetic, household and hygiene products, owing to its fresh citrus odour. In its pure form, R-limonene is not allergenic or is a very weak allergen. However, R-limonene autoxidizes on air exposure, and the oxidation products can cause contact allergy (1–6).

Along with linalool, R-limonene is among the most common fragrance ingredients according to several

studies, and has been found in up to 80% of examined products (7–9). Concentrations of fragrances in commercial products are often not given, but up to 15–30% of the total volume of a perfume is expected to consist of fragrance material, and up to 0.1–2% of the volume of a liquid detergent or toilet soap (10 and references therein). In addition, *R*-limonene and its enantiomer *S*-limonene are found in several different plant oils, including essential oils from rosemary, eucalyptus, lavender, caraway, lemongrass, and peppermint, which are frequently added to massage oils and aromatherapy products. In such products, the concentrations can be high. Thus, exposure from aromatherapy oils and so-called ‘natural products’, including home-made soaps and fragrances, must also be considered. In addition, the concentration of limonene in hand cleansers and degreasers used both domestically and industrially, can be very high, reaching 10–100%.

Oxidized *R*-limonene has previously been patch tested in two multicentre studies at 3.0% in petrolatum, giving 2–3% positive patch test reactions in consecutive patients (4, 5). In our recent multicentre study (6), a patch test preparation containing oxidized *R*-limonene 3.0% pet., with a specified and controlled content of limonene hydroperoxides (Lim-OOHs) of 0.33%, was tested in 2900 dermatitis patients in Australia, Denmark, the United Kingdom, Singapore, Spain, and Sweden. The results showed that overall, 5.2% (range 2.3–12.1%) of the patients showed a positive patch test reaction to oxidized limonene. Table 1 shows the overall results from the study (6). It was concluded that oxidized limonene 3.0% pet. with a specified content of Lim-OOHs of 0.33%, is a standardized and useful tool with which to detect contact allergy in dermatitis patients.

Since 2005, an important step has been taken to enable consumers to identify potential allergens in consumer products. By EU legislation, limonene is included in a group of 26 fragrance chemicals that must be labelled on cosmetic products in the EU when used in concentrations of > 10 ppm in leave-on products and of > 100 ppm in rinse-off products, according to the Cosmetics Directive (11). In Australia, Singapore, and elsewhere, such labelling is still not mandatory.

In working with contact allergic patients, it is important to determine the exposures of the patient and, if possible, to assess the relevance of a positive patch test reaction to the dermatitis. Few studies have systematically focused on identifying culprit products that are expected to contain the allergen to which the patient has reacted, thereby evaluating the relevance of contact allergy to the dermatitis. Judging relevance is especially challenging with regard to fragrance chemicals, as fragranced products are ubiquitous in our environment, and it is difficult for both the doctor and the patient to suspect that a certain fragrance chemical is causing or worsening the dermatitis. A thorough study was performed regarding the 26 specific fragrance chemicals to be declared on cosmetic products in accordance with the EU cosmetics directive, and high figures for relevance, up to 76%, were found overall for the patients with positive patch test reactions (12). In that study, limonene and linalool in their non-oxidized forms were used, and, consequently, low frequencies of positive patch test reactions were observed. It should also be noted that limonene is used not only as a fragrance ingredient but also in occupational settings, primarily as a solvent. A number of valuable case reports have been written, giving useful indications of sources of limonene exposure in our environment that can cause contact dermatitis (13–17).

Table 1. Total number of patients from each respective test centre and the number and percentage of positive reactions, doubtful reactions and irritant reactions to oxidized *R*-limonene 3.0% [limonene hydroperoxides (Lim-OOHs) 0.33%] pet. Details are published in (6)

Test centre	Oxidized <i>R</i> -limonene 3.0% (Lim-OOHs 0.33%) pet.			
	Total no. tested	No. of positive patch test reactions (%)	No. of doubtful patch test reactions (%)	No. of irritant patch test reactions (%)
Barcelona	299	13 (4.3)	0 (0)	4 (1.4)
Copenhagen	440	16 (3.6)	53 (12.0)	17 (3.9)
Gothenburg	397	10 (2.5)	1 (0.2)	0 (0)
London	271	8 (2.9)	9 (3.3)	0 (0)
Malmö	300	7 (2.3)	11 (3.7)	0 (0)
Melbourne	289	18 (6.2)	11 (3.8)	0 (0)
Odense	298	23 (7.7)	73 (24.5)	3 (1.0)
Seville	300	20 (6.7)	6 (2.0)	0 (0)
Singapore	306	37 (12.1)	40 (13.1)	1 (0.3)
Total	2900	152 (5.2)	204 (7.0)	25 (0.9)

The aim of the present study was to assess exposure to limonene-containing products in patients with positive patch test reactions to oxidized *R*-limonene 3.0% (Lim-OOHs 0.33%) in our multicentre study (6). By characterizing the exposures to limonene, as declared in the products used by the patients on dermatitis areas, an assessment of the relevance of the exposure for the patients' dermatitis was made.

Materials and Methods

Details on chemicals and patch test materials are given in reference (6).

Subjects, exposure, and evaluation

Consecutive patients undergoing patch testing because of suspected allergic contact dermatitis at the nine test centres (Barcelona, Copenhagen, Gothenburg, London, Malmö, Melbourne, Odense, Seville, and Singapore) were screened with patch test preparations of oxidized *R*-limonene 3.0% pet. with a validated content of 0.33% Lim-OOHs, in addition to undergoing regular patch testing. Visual readings were scored according to International Contact Dermatitis Research Group (ICDRG) guidelines (18) on at least two occasions: D2, D3–4 (mandatory), and/or D6–7. All patch test reactions not fulfilling the criteria for being classified as allergic according to ICDRG guidelines were differentiated with regard to irritant and doubtful reactions. The day of readings varied between the test centres. Three of nine test centres read the patch tests on three occasions (D2, D3–4, and D7), two of nine read them on D2 and D3–4, and four of nine read them on D3–4 and D6–7.

A questionnaire about clinical data concerning localization of eczema, history of atopic dermatitis and history of adverse reactions to fragrances was completed. The patients' histories of adverse reactions to fragrances in the past were categorized according to the following questions: (A) certain – has reacted with an itching dermatitis to at least one fine perfume or aftershave and also to other scented products; (B) probable – has reacted to one or more scented products, but a certain perfume has not been identified as the cause of a clinical reaction; (C) questionable – has reacted to various cosmetics with or without fragrances, and materials other than fragrance constituents may be the cause of the reaction; and (D) none – has never reacted to scented materials [modified after Frosch et al. (19)]. For comparison of the patients' histories of adverse reactions to fragranced products, as a measurement of clinical relevance, calculations were performed for the group of patients positive to oxidized

limonene in total ($n = 152$), patients positive to oxidized limonene but not to any of the fragrance markers and/or colophonium, and those with no reaction to any fragrance material [negative to oxidized limonene and all fragrance markers in the baseline series, and/or colophonium, also excluding test protocols showing doubtful and irritant reactions to oxidized limonene and reactions to oxidized linalool, tested in parallel for another study (20)] ($n = 1737$), at eight test centres (Barcelona, Copenhagen, Gothenburg, London, Melbourne, Odense, Seville, and Singapore). The negative protocols from Malmö were not submitted, and were thus not included in the calculations.

In the case of a positive patch test reaction to oxidized *R*-limonene 3.0% (Lim-OOHs 0.33%), a question was asked regarding whether the patient was using products labelled as containing limonene, to the best of the patch test evaluating physicians' knowledge. In many cases, patients brought in products to the test centre for inspection. Alternatively, patients searched for the word limonene on the labelling of their products after being given information about the contact allergy, and subsequently reported their findings to the test centre.

The questionnaire also included a question regarding whether the contact allergy to oxidized limonene was, in the physician's view, relevant for the patient's dermatitis. The relevance of the positive patch test reaction to the individual patient's dermatitis was classified by answer alternatives 'present', 'past', and 'uncertain'. 'Present' meant that a product used by the patient at the time of the patch test on areas affected by dermatitis was identified as containing limonene. 'Past' meant that such a product had been in use at an earlier time. 'Unknown' meant that no such product could be identified. In many cases, the suspected product was given by brand name or product type on the questionnaire.

Results

Table 1 shows the overall results from the study; details are given in reference (6). Table 2 gives a description of the 152 patients with positive patch test reactions to oxidized *R*-limonene 3.0% (Lim-OOHs 0.33%) in the multicentre study with regard to age, atopic dermatitis, and a history of adverse reactions to fragranced products. Fifty-two males and 97 females (sex not given in three patch test record sheets), with a mean age of 47.4 years, were diagnosed with positive patch test reactions to oxidized *R*-limonene. Test results from the individual patients are shown in Table S1, along with the location of dermatitis, the physician's assessment of relevance, and a listing of products in which limonene was found, mostly by the physician reading the labelling, or by the patient

Table 2. Description of patients showing positive patch test reactions to oxidized R-limonene 3.0% (limonene hydroperoxides 0.33%) pet. in the multicentre study, regarding history of problems with fragranced products, atopic dermatitis, use of fragranced products, assessed relevance of contact allergy to R-limonene for dermatitis, and exposure to limonene in products

Patients reacting to oxidized limonene, number		Gender ^a		History of problems with fragranced products?						Atopic dermatitis				Using fragranced products ^a		Relevance ^f / exposure				
				Male	Female	Certain ^b	Probable ^c	Questionable ^d	None ^e	Not marked	Yes	No	Uncertain	Not given	Yes	No	Present relevance	Past relevance	Uncertain relevance	Not given
Test centre		Mean age																		
Barcelona	13	50.5	3	9	4	3	1	4	1	1	12	0	0	10	2	8	1	3	1	10
Copenhagen	16	53.1	12	3	1	4	0	10	1	2	14	0	0	15	1	10	0	4	2	13
Göteborg	10	50.9	2	7	1	0	1	6	2	3	5	1	1	6	0	3	0	4	3	4
London	8	50.7	1	7	0	0	0	1	7	3	5	0	0	7	1	0	1	5	2	0
Malmö	7	42.6	5	2	0	1	0	6	0	2	4	0	1	7	0	0	0	7	0	0
Melbourne	18	40.6	1	17	3	0	3	5	7	5	10	2	1	13	3	5	1	9	3	5
Odense	23	46.3	6	17	2	4	1	12	4	8	13	2	0	19	3	3	0	12	8	11
Seville	20	50.5	9	11	0	1	1	18	0	1	19	0	0	20	0	8	0	12	0	8
Singapore	37	41.6	13	24	1	4	3	24	5	13	22	1	1	13	22	2	1	21	13	5
Total	152	47.4	52	97	12	17	10	86	27	38	104	6	4	110	32	39	4	77	32	56

^aIn three cases, gender was not recorded in forms.^bA. Certain: has reacted with an itching dermatitis to at least one fine perfume or aftershave, and has reacted to other scented products.^cB. Probable: has reacted to one or more scented products, but a certain perfume has not been identified by the patient as the cause of a clinical reaction.^dC. Questionable: has reacted to various cosmetics with and without fragrances; other materials than fragrance constituents may be the cause of the reaction.^eD. None: has never reacted to scented materials.^fIf positive reaction to oxidized limonene: is contact allergy relevant for the patient's dermatitis (physician's assessment)?^gExposure: is the patient using products that contain limonene (citrus fragrance)?

Responses A–D (history of problems with fragranced products) are modified after Frosh et al. (19).

searching for the word limonene on the labelling of the products after being given information about the contact allergy.

For the question regarding a history of adverse reaction to fragranced products, patch test record sheets were completed for 125 of the 152 (82.2%) positive patients. The results are shown in Tables 2 and 3. When calculating together the 'certain' and 'probable' patient histories (responses A and B), the test centres found values ranging between 0% (London) and 58% (Barcelona). Thirty-three per cent of patients in Copenhagen, 32% of patients in Odense and 27% of patients in Melbourne with positive reactions to oxidized limonene were recorded as alternatives A or B. In Gothenburg, Malmö, Seville, and Singapore, between 5% and 20% of the allergic patients were recorded as alternatives A or B. Overall, 23.2% had responses A or B. Also, the results of responses A–D for the subgroup of patients reacting to oxidized limonene but with no reactions to any fragrance markers [Fragrance Mix I (FM I), Fragrance Mix II (FM II), *Myroxylon pereirae*, colophonium in the baseline series, or oxidized

linalool], as well as for the patients with negative test results for all fragrance materials tested, are given in Table 3. The number of patients answering with alternative A or B among those not reacting to oxidized limonene or any fragrance marker was statistically significantly smaller than the number of patients answering with alternative A or B among those with positive reactions to oxidized limonene but with no other reactions to fragrance markers ($p < 0.01$).

A question regarding the use of fragranced products was asked, and the responses are given in Table 2. Here, 110 patients of 142 (10 of 152 not given) (77.5%) were using fragranced products, and 32 of 142 (22.5%) were not using fragranced products.

An assessment of the probable relevance of the diagnosed contact allergy to oxidized limonene in relation to the patient's dermatitis (as history of exposure), including the identification of actual products containing limonene among those used by the patient, is also given in Table 2; details are given in Table S1. In 39 of the 120 (32.5%) cases for which the patch test record sheet was completed

Table 3. Comparison between the frequency of responses (A–D) (19) between the patients with positive patch test reactions to oxidized R-limonene and those with no reactions to any fragrance markers in the baseline series [fragrance mix I (FM I), fragrance mix II (FM II), *Myroxylon pereirae*, and colophonium] or to oxidized linalool

	No. of patients (not given)	Used for calculation	Response							
			A ^a		B ^b		C ^c		D ^d	
			No.	%	No.	%	No.	%	No.	%
Positive for oxidized R-limonene (all)	152 (27)	125	12	9.6	17	13.6	10	8.0	86	68.8
Positive for oxidized R-limonene, positive for any fragrance marker	66 (15)	51	7	13.7	9	17.6	5	9.8	30	58.8
Positive for oxidized R-limonene but no reactions to fragrance markers	86 (12)	74	5 ^e	6.7	8 ^e	10.8	5 ^e	6.7	56 ^e	75.7
Negative for oxidized R-limonene, fragrance markers, and oxidized linalool	1737 (150)	1587	51 ^e	3.2	79 ^e	5.0	148 ^e	9.3	1309 ^e	82.5

^aCertain: has reacted with an itching dermatitis to at least one fine perfume or aftershave, and has reacted to other scented products.

^bProbable: has reacted to one or more scented products, but a certain perfume has not been identified by the patient as the cause of a clinical reaction.

^cQuestionable: has reacted to various cosmetics with and without fragrances; materials other than fragrance constituents may be the cause of the reaction.

^dNone: has never reacted to scented materials.

^eThe number of patients answering A or B was statistically significantly smaller in the group not reacting to any fragrance marker, oxidized R-limonene or oxidized linalool than in those reacting to oxidized R-limonene but no other fragrance marker; $p < 0.01$, Fisher's exact test, two-tailed.

for this question, a present exposure to one or several products containing limonene, used on the dermatitis area, was documented, supporting relevance of the contact allergy to oxidized limonene to the patient's dermatitis. In an additional 4 cases, a past relevance was found. Thus, overall, the evaluating physicians found a present or past relevance for the contact allergy to oxidized limonene with regard to the patient's dermatitis in 35.8% (43 of the 120 patients showing positive patch test reactions to oxidized R-limonene and for whom patch test record sheets were completed) in this multicentre study. In some test centres (Barcelona and Copenhagen), > 70% of the patients were judged to have a relevant exposure to limonene [75% (9 of 12 patients) and 71% (10 of 14 patients), respectively] (Table 2). Of the non-EU test centres, Melbourne reported 40% present or past relevance (6 of 15 marked protocols) and Singapore 12.5% (3 of 24 marked protocols). Thirty-two (23.0%) of the 152 patch test record sheets were not completed for this question.

Among the 152 patients with positive patch test reactions to oxidized limonene, 56 (36.8%) were found to be using products containing limonene or in other ways coming into contact with limonene (Tables 2 and S1). Ten of 13 patients (76.9%) in Barcelona with positive patch test reactions to oxidized R-limonene were found to be using limonene-containing products, 13 of 16 (81.2%) in Copenhagen, 11 of 23 (47.8%) in Odense, and 4 of 10 (40%) in Gothenburg. In the non-EU test centres, Melbourne found limonene in products in 5 of 18 cases

(27.8%), and Singapore found limonene in products in 5 of 37 cases (13.5%).

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 multicentre study, some test centres found up to 70–80% of the patients with positive patch test reactions to oxidized R-limonene to be, or to have been, exposed to limonene-containing products judged to be probably relevant for their dermatitis. At these test centres, limonene was found to be present in the patients' products in 75–80% of the cases, thus showing the ubiquity of this fragrance terpene in common products. Overall, the contact allergy to oxidized limonene was assessed as being likely to be relevant for the patients' dermatitis in 36% of the limonene-allergic patients. On a similar note, in our parallel study on oxidized linalool, the individual test centres found relevance for the contact allergy to oxidized linalool in 20–80% of the cases, with four of nine centres recording relevant exposures in $\geq 50\%$ of the cases (20).

Limonene, like linalool, is a ubiquitous fragrance chemical that is expected to be found in 60–80% of domestic, cleansing, cosmetic and occupational products (7–9). In the patch test record forms of the patients reacting to oxidized limonene in this study, products

such as perfumes, shampoos, soaps, body creams and domestic cleaners were frequently listed as sources of limonene judged to be relevant for the patients' dermatitis. Similar products were implicated in an earlier study on relevant cosmetic exposures to fragrance allergens (12). However, creams for massage, sunscreens and anti-septic tea tree oil-based products were also implicated. In occupational settings, detergents and domestic cleaners were implicated (Table S1). Among the patients showing positive patch test reactions, a laboratory technician using limonene occupationally, and several masseurs, were identified. These listings show the breadth of use of limonene in the patients' environments, and the challenge in giving information to the patient on where to expect to be exposed to limonene. The frequency of limonene in everyday products adds to the risk of being sensitized, as well as having problems with elicitation at low concentrations, as a person will be potentially in contact with oxidized limonene from many sources in a day.

Arriving at a diagnosis of allergic contact dermatitis is a three-step procedure (21). The first step is establishing contact allergy; the second is demonstration of present exposure to the sensitizer or possibly cross-reacting substances; and the final step is assessment of clinical relevance, which means that the demonstrated exposure should explain and/or contribute to the dermatitis under investigation with regard to the exposure and localization, type and course of the dermatitis. Contact allergy to oxidation products of limonene is common, but, at present, as discussed below, there is insufficient knowledge on the presence, qualitatively and quantitatively, of these oxidation products in consumer products. In this study, exposure to limonene has been considered as an approximation of exposure to oxidation products of limonene.

Although the information on present exposure to the actual oxidation products of limonene is insufficient, the study design enables other ways to assess clinical relevance. The use of a questionnaire with questions on exposure and skin problems answered by all patients before either the patients or the test-reading dermatologists know whether there is any contact allergy will provide unbiased answers on factors of significance for the assessment of clinical relevance (22). In this study, a questionnaire with questions on fragrance exposure and itching dermatitis was responded to by all tested patients before the testing. Expectedly, and in agreement with previous studies on fragrance allergy (23–25), a high number of those with contact allergy to oxidation products of limonene [29/125 (27 not given); 23%] reported a certain or probable history of adverse reactions to fragranced products (responses A or B). Among these patients, there is no statistically significant

difference between the number of individuals with allergy to oxidized *R*-limonene with a certain or probable history (responses A or B) among those without simultaneous contact allergy to other fragrance markers (13/74) and the number of individuals with simultaneous allergy to the other fragrance markers (16/51). However, on comparison of those 13 of 74 (responding with alternatives A or B with contact allergy to oxidation products of limonene but without simultaneous allergy to the other fragrance markers) with those 130 of 1587 (responding with alternatives A or B, all with negative test results to both oxidized *R*-limonene and the other fragrance markers, including oxidized linalool), there are significantly more individuals with contact allergy to oxidized *R*-limonene who have a certain or probable history of adverse reactions to scented products than individuals without allergy who have this history (13/74 versus 130/1587; $p < 0.01$, Fisher's exact test, two-sided). This strongly indicates the clinical relevance of contact allergy to oxidation products of limonene.

The reaction profiles, expressed as the strength of reaction to oxidized *R*-limonene, differed between the test centres. Associations were found between stronger (++)/+++ reactions and 'certain' relevance (i. e. Barcelona and Copenhagen) and weaker (+) reactions and uncertain relevance. This association between positive history and greater reaction strength has been found in earlier studies (26). In the search for relevance, a strong reaction may increase the motivation of both the patient and the testing physician or nurse to look for exposure. Another aspect is that a strong reaction may be the result of a strong and recently triggered allergy, and may thus be a sign of more current exposure. In our experience, many patients with + reactions tell us that their dermatitis has cleared or improved after avoidance of products containing the implicated fragrance.

In earlier studies, stronger reactions have been more often confirmed by a positive repeated open application test (ROAT) result, whereas weaker, doubtful reactions have been less often confirmed or supported by the ROAT (27). This may imply that weaker reactions are less reliable, and could be false positive. In this study, no doubtful reactions were included in the analysis for relevance. In our recent ROAT for oxidized linalool, patients with earlier reactions (at patch testing 2–3 years earlier) of strengths + and +++ were included. In 3 weeks of repeated open application testing, no clear difference in reaction in the ROAT or at the confirmatory patch test was found between those with + or +++ reactions in the original patch test (28).

In clinical studies on oxidized fragrance terpenes, there are often the questions of how the patients have

been exposed to the oxidation products causing sensitization and whether the oxidation products can be expected to be formed in consumer products. Showing the presence of hydroperoxides and quantifying them in formulated products has been difficult for technical reasons, as hydroperoxides are labile compounds, and will disintegrate if heated, for example, for gas chromatographic analyses. Recently, with the use of new methodology which has proven to be effective, Lim-OOHs have been shown to be present in natural sweet orange oil (29). Lim-OOHs were shown to be already present in the sweet orange oil on delivery from the producer, and further oxidation, with increasing amounts of hydroperoxides, was observed after storage and handling (29). Linalyl acetate hydroperoxides and linalool hydroperoxides were likewise identified in petigrain oil (29). We have previously shown that both linalool and linalyl acetate will oxidize and form hydroperoxides in lavender oil of both natural and synthetic origin (30). We have also recently published the results of a ROAT in which low concentrations of oxidized linalool caused eczema in allergic patients, providing more evidence for the clinical relevance of the positive patch test reactions (28). Thus, although they are not intended to be present by the producer, oxidation products can most likely be found in formulated products. The oxidation could have taken place before the fragrance components were added to the product [as in the sweet orange oil (29)], or at a later time, during storage and handling. Addition of an antioxidant will prevent the degradation of components, but it has been shown that this may be efficient only for a certain time, after which the antioxidant will have been consumed, as has been shown for limonene and butylated hydroxytoluene (31).

As in the earlier publication on this multicentre study on oxidized limonene, concomitant reactions to other fragrance markers (FM I, FM II, and *M. pereirae*) and/or colophonium in the baseline patch test series were commonly (in 42%) found in the positive patients (6). However, almost 60% of the patients showing positive patch test reactions to oxidized *R*-limonene would not have been informed of any fragrance allergy if this specific test had not been performed. This would lead to the patients continuing to use fragranced products, and thus still being potentially exposed to fragrance materials that could cause or worsen their dermatitis. For oxidized *R*-limonene and oxidized linalool, controlled patch test material has recently been made available for patch testing. The development of new and relevant patch test materials for common allergens is important work that needs to be continued.

As single fragrance ingredients, oxidized limonene and oxidized linalool gave, respectively, 5.2% and almost 7%

(6.9% of 2900 patients) positive patch test reactions in the tested patients (6, 20). A recent study showed that oxidized geraniol 6.0% pet. gave positive patch test reactions in 2.3% of the tested patients (32). A thorough analysis of 14 patients reacting to oxidized and/or pure geraniol supported the relevance of the finding of the fragrance allergy, as 11 of 14 cases were judged to have relevant exposure to geraniol-containing products in a domestic or occupational setting (33). A number of other common fragrance chemicals, namely linalyl acetate, α -terpineol, and cinnamyl alcohol, have been shown to be prehapten; that is, they are prone to be transformed by air oxidation into more allergenic compounds (34–37). Considering the number of fragrance additives on the market that may be prehapten and that are not covered in their oxidized form by traditional test materials, fragrance allergy may be a widely underestimated problem among dermatitis patients, owing to a lack of diagnostic tools.

This study supports the importance of considering air oxidation when discussing contact allergy to fragrance compounds. The pure compound might not be the actual sensitizer. This is the case for limonene, as patch testing with pure limonene has shown no (4, 5) or very few (38, 39) positive patch test reactions. Within the EU regulatory work on the classification and labelling of dangerous substances (led by the European Chemicals Bureau), *R*-limonene and *S*-limonene, and the racemic form dipentene, are classified as skin sensitizers, effectively on the basis of the formation of skin sensitizers resulting from air exposure of limonene.

Overall, finding limonene or linalool in patients' products, and assessing the relevance to the allergic individual, are demanding tasks. In an ideal situation, products should be brought for inspection, or the name of the culprit fragrance should be given to the patient, who should search for it, using information given by the clinic. The overall figures for exposure judged as being potentially relevant for the patients' dermatitis for both limonene and linalool (36% and 38%, respectively) (20) are most likely low estimates. In the non-EU test centres in Australia and Singapore, relevance is even more difficult to assess, as labelling of individual fragrances is not mandatory. The importance of assessing relevance in clinical practice is thus supported, not least to aid the patients to identify exposures that may worsen their dermatitis.

Conclusions

In this multicentre study, limonene was frequently found in the labelling on the patients' products and the contact allergy to oxidized *R*-limonene was frequently judged to be relevant for the patients' dermatitis. A large number

of sources of contact with limonene, such as cosmetic, cleansing and occupational exposures, were identified. At some test centres, up to 80% of the patients with contact allergy to oxidized limonene were using products containing limonene on areas of skin affected by dermatitis. The continued widespread use of the easily oxidized fragrance terpene limonene in domestic and occupational products suggests that further sensitizations and widespread elicitation of allergic contact dermatitis in allergic individuals can be expected. With ubiquitous allergens, assessing exposure is an important step. As a result, better information can be given on avoiding the allergen, which will help to prevent allergic dermatitis in contact allergic patients. Further studies, such as ROATs aimed at investigating thresholds for the elicitation of allergic contact dermatitis, need to be performed.

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Supporting Information

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

Table S1. Description of individual patients showing positive patch test reactions to oxidized R-limonene 3.0% (limonene hydroperoxides 0.33%) pet.

References

- Karlberg A-T, Boman A, Melin B. Animal experiments on the allergenicity of d-limonene – the citrus solvent. *Ann Occup Hyg* 1992; **35**: 419–426.
- Karlberg A-T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992; **26**: 332–340.
- Nilsson U, Bergh M, Shao L P, Karlberg A-T. Analysis of contact allergenic compounds in oxidized d-limonene. *Chromatographia* 1996; **42**: 199–205.
- Karlberg A-T, Dooms-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.
- Bråred Christensson J, Andersen K E, Bruze M et al. An international multicentre study on the allergenic activity of air-oxidized R-limonene. *Contact Dermatitis* 2013; **68**: 214–223.
- Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001; **45**: 221–225.
- Rastogi S C, Menné T, Johansen J D. The composition of fine fragrances is changing. *Contact Dermatitis* 2003; **48**: 130–132.
- Buckley D A. Fragrance ingredient labelling in products on sale in the UK. *Br J Dermatol* 2007; **157**: 295–300.
- Johansen J D. Fragrance contact allergy: clinical and experimental investigations of the fragrance mix and its ingredients. *Contact Dermatitis* 2002; **46** (Suppl. 3): 1–31.
- 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.
- Heisterberg M V, Menné T, Johansen J D. Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. *Contact Dermatitis* 2011; **65**: 266–275.
- Foti C, Zamboni C G, Conserva A, Casulli C, D'Accolti L, Angelini G. Occupational contact dermatitis to a limonene-based solvent in a histopathology technician. *Contact Dermatitis* 2007; **56**: 109–112.
- Kerre S, Matura M, Goossens A. Allergic contact dermatitis from a degreaser. *Contact Dermatitis* 2006; **55**: 117–118.
- Topham E J, Wakelin S H. D-Limonene contact dermatitis from hand cleansers. *Contact Dermatitis* 2003; **49**: 108–109.
- Rycroft R J. Allergic contact dermatitis from dipentene in honing oil. *Contact Dermatitis* 1980; **6**: 325–329.
- Meding B, Barregård L, Marcus K. Hand eczema in car mechanics. *Contact Dermatitis* 1994; **30**: 129–134.
- Wahlberg J. Patch testing. In: *Textbook of Contact Dermatitis*, 2nd edition, Rycroft R, Menné T, Frosch P (eds): Berlin, Springer-Verlag, 1995: pp. 241–261.
- Frosch P J, Johansen J D, Menné T et al. Lyril is an important sensitizer in patients sensitive to fragrances. *Br J Dermatol* 1999; **141**: 1076–1083.
- Bråred Christensson J, Andersen K E, Bruze M et al. Air oxidized linalool – a frequent cause of fragrance contact allergy. *Contact Dermatitis* 2012; **67**: 247–259.
- Bruze M. What is a relevant contact allergy? *Contact Dermatitis* 1990; **23**: 224–225.
- Bruze M, Edman B, Björkner B, Möller H. Clinical relevance of contact allergy to gold sodium thiosulfate. *J Am Acad Dermatol* 1994; **31**: 579–583.
- Thyssen J P, Carlsen B C, Menné T, Johansen J D. Trends of contact allergy to

- fragrance mix I and Myroxylon pereirae among Danish eczema patients tested between 1985 and 2007. *Contact Dermatitis* 2008; **59**: 238–244.
- 24 Schnuch A, Lessmann H, Geier J, Frosch P J, Uter W, IVDK. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK. *Contact Dermatitis* 2004; **50**: 65–76.
 - 25 Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Dermatitis* 2008; **58**: 134–141.
 - 26 Frosch P J, Pilz B, Andersen K E et al. Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995; **33**: 333–342.
 - 27 Johansen J D, Andersen K E, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis* 1996; **34**: 414–418.
 - 28 Andersch Björkman Y, Hagvall L, Siwmark C, Niklasson B, Karlberg A-T, Bråred Christensson J. Air oxidized linalool elicits eczema in allergic patients – a repeated open application test study. *Contact Dermatitis* 2014; **70**: 129–138.
 - 29 Rudbäck J, Islam M N, Nilsson U, Karlberg A T. A sensitive method for determination of allergenic fragrance terpene hydroperoxides using liquid chromatography coupled with tandem mass spectrometry. *J Sep Sci* 2013; **36**: 1370–1378.
 - 30 Hagvall L, Sköld M, Bråred Christensson J, Börje A, Karlberg A-T. Lavender oil lacks natural protection against autooxidation, forming strong contact allergens on air exposure. *Contact Dermatitis* 2008; **59**: 143–150.
 - 31 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.
 - 32 Hagvall L, Karlberg A-T, Bråred Christensson J. Finding the optimal patch test material and test concentration to detect contact allergy to geraniol. *Contact Dermatitis* 2013; **68**: 224–231.
 - 33 Hagvall L, Karlberg A-T, Bråred Christensson J. Contact allergy to air-exposed geraniol: clinical observations and report of 14 cases. *Contact Dermatitis* 2012; **67**: 20–27.
 - 34 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.
 - 35 Rudbäck J, Bergström M A, Börje A, Nilsson U, Karlberg A T. alpha-Terpinene, an antioxidant in tea tree oil, autoxidizes rapidly to skin allergens on air exposure. *Chem Res Toxicol* 2012; **25**: 713–721.
 - 36 Sköld M, Hagvall L, Karlberg A-T. Autooxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 2008; **58**: 9–14.
 - 37 Niklasson I B, Delaine T, Islam M N, Karlsson R, Luthman K, Karlberg A T. Cinnamyl alcohol oxidizes rapidly upon air exposure. *Contact Dermatitis* 2013; **68**: 129–138.
 - 38 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.
 - 39 Bruze M, Svedman C, Andersen K E et al. Patch test concentrations (doses in mg/cm²) for the 12 non-mix fragrance substances regulated by European legislation. *Contact Dermatitis* 2012; **66**: 131–136.

Table S1. Description of individual patients showing positive patch test reactions to oxidized *R*-limonene 3.0% (limonene hydroperoxides 0.33%) pet.

Positive patch test reactions															Exposure/relevance limonene	
Patient data					Fragrance markers in baseline series											
					Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.					Colophonium						
Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4	D7	FM I ¹	FM II ²	HCC ³	MP ⁴	Using limonene	Notes	Relevance for dermatitis		
Barcelona (a)	50 (F)	A	No	Hands, wrist	Yes	++	+++	+++	++	++	-	-	Positive	Yes	Massage creams, masseuse	Present, occupational
	48 (F)	B	No	Trunk	Yes	+	+++	+	-	-	-	-	Yes	Yes	-	Present
	20 (M)	B	No	Legs, arms	Yes	-	++	+	+++	-	+++	-	Yes	Yes	-	Present
	41 (F)	-	Yes	Trunk	Yes	-	++	+	-	-	-	-	Uncertain	-	-	Uncertain
	74 (F)	A	No	Lower leg	Yes	+	++	++	++	-	++	-	Yes	Yes	Leg cream	Present
	45 (F)	D	No	Back, legs, trunk	No	+	++	++	-	-	-	-	Yes	Yes	-	Past
	Age not given (F)	D	No	Trunk, legs, face	Yes	++	++	-	-	-	-	-	Yes	Yes	Shampoo	Present
	35 (F)	D	No	Fingertips	Yes	+	++	++	-	-	-	-	Yes	Yes	Domestic cleaner	Present
	79 (M)	A	No	Dorsum both fingers	Yes	+	+	+	-	-	-	-	Yes	Yes	Domestic cleaner	Present
	75 (M)	A	No	Trunk, arms	No	+	+	+	-	-	-	-	No	-	-	Uncertain
32 (F)	B	No	Trunk, legs	Yes	-	+	-	-	-	-	-	Yes	Yes	-	-	-
	37 (gender not given)	D	No	Fingertips, three fingers both hands	Yes	-	+	-	-	-	-	-	Yes	Yes	Laboratory technician	Present, occupational
		70 (F)	C	No	Arms, legs, fingers, hands, perineal	Yes	-	+	+	-	-	+	-	Uncertain	-	-

Table S1. Continued

Positive patch test reactions																			
Patient data					Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.					Fragrance markers in baseline series					Exposure/relevance limonene				
Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4	D7	FM I ¹	FM II ²	HCC ³	MP ⁴	Colophonium	Using limonene	Notes	Relevance for dermatitis				
Copenhagen (b)	51 (M)	D	No	Face	Yes	-	++	+	-	-	?	++	No	-	Uncertain				
	B	Yes	Hands, body	Yes	++	++	?	?	-	-	Yes	-	Yes	Hand cream	Present				
	57 (F)	D	No	Hands, arms, buttocks	Yes	?	++	?	++	?	?	-	No	Not found in products	Uncertain				
	18 (F)	D	No	Hands	Yes	?	++	?	-	-	-	-	Yes	Shampoo hairdresser apprentice	Present				
	56 (F)	D	No	Face	Yes	++	+	?	Yes	?	Yes	-	Yes	Shampoo	-				
	48 (F)	D	No	Face	Yes	-	+	?	?	-	?	-	Yes	Shampoo, lotion	Present				
	34 (F)	B	No	Hands	Yes	+	?	?	-	-	-	-	Yes	Hand cream	Present				
	55 (F)	A	No	Hands	No	-	+	?	-	-	?	?	No	-	-				
	68 (M)	?	No	Face	Yes	-	+	+	-	-	-	-	Yes	Shaving gel, deodorant	Present				
	69 (F)	D	No	Body	Yes	?	+	-	+	-	-	-	Yes	Shampoo	Present				
	56 (M)	D	No	Feet	Yes	-	+	+	-	-	-	-	Yes	Deodorant	Uncertain				
	81 (F)	D	No	Around stoma	Yes	?	+	-	++	-	++	?	Yes	Shampoo	Uncertain				
	34 (F)	D	Yes	Eyes, neck	Yes	?	+	-	-	-	-	-	Yes	Eye makeup remover	Present				
	92 (F)	B	No	Trunk	Yes	+	+	?	-	-	?	-	Yes	Body cream	Present				
	52 (F)	D	No	Trunk, scalp	Yes	?	+	-	?	-	-	-	Yes	Shampoo	Present				
	25 (F)	B	No	Hands	Yes	?	+	?	-	-	-	Yes	Yes	Hairspray, deodorant	Present				

Table S1. Continued

Positive patch test reactions																	
Patient data					Positive patch test reactions												
					Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.				Fragrance markers in baseline series				Exposure/relevance limonene				

Table S1. Continued

Positive patch test reactions																
Patient data			Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.					Fragrance markers in baseline series				Exposure/relevance limonene				
			Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4	D7	FM I ^a				FM II ^a	HC ^a
Malmö (e)	49 (M)	D	No	Knee, thigh, lower arms, hands	Yes	NR	+++	+++	Yes	–	–	Yes	–	Uncertain	–	Uncertain
	58 (M)	D	No	Dorsum of hands	Yes	NR	–	++	Yes	Yes	–	–	–	Uncertain	–	Uncertain
	49 (M)	D	–	–	Yes	NR	++	+	–	–	–	–	–	Uncertain	–	Uncertain
	43 (M)	D	Yes	Generalized	Yes	NR	++	++	–	?	–	–	–	Uncertain	–	Uncertain
	24 (F)	D	No	Feet, hands, lower arms	Yes	NR	+	–	–	–	–	–	–	Uncertain	–	Uncertain
Melbourn (e and f)	51 (M)	B	No	Palms, fingertips, feet	Yes	NR	+	?	?	?	–	–	–	Uncertain	–	Uncertain
	24 (F)	D	Yes	Antecubital fossae, face	Yes	NR	+	?	–	–	–	–	–	Uncertain	Tea tree oil	Uncertain
	35 (F)	D	Yes	Neck, elbow crease	Yes	–	++	–	–	–	–	–	–	Uncertain	–	Uncertain
	42 (F)	D	No	Right forearm	Yes	+	+	–	Yes	–	–	Yes	Yes	Uncertain	–	Past
	32 (F)	C	No	Face	Yes	+	+	–	–	–	–	–	–	Yes	Lemongrass cream	Present
	39 (F)	–	Yes	–	–	+	+	–	–	–	–	+	–	Yes	Tea tree oil	Present
	31 (F)	C	No	–	Yes	–	+	–	–	–	–	–	–	Uncertain	–	Uncertain
	46 (F)	–	?	Oral	Yes	–	+	–	Yes	–	–	Yes	Yes	Uncertain	–	Uncertain
	45 (F)	–	–	–	–	–	+	–	–	–	–	–	Yes	Yes	Lotion, shower gel, body oil, hair products	Present
	15 (F)	–	Yes	Hands	No	–	+	–	–	–	–	–	Yes	Uncertain	–	Uncertain
	60 (F)	–	No	Hand, lower leg	Yes	–	+	–	–	–	–	–	–	–	–	–
	56 (F)	A	?	Palms	No	+	+	–	Yes	Yes	Yes	Yes	–	No	Makes candles	Uncertain

Table S1. Continued

Odense (g)	Positive patch test reactions										Exposure/relevance limonene						
	Patient data				Fragrance markers in baseline series												
	Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4	D7	FM I ^a	FM II ^b		HCC ^c	MP ^d	Colophonium	Using limonene	Notes	Relevance for dermatitis
	57 (M)	C	No	Arms, hands	Yes	-	+	-	-	-	-	-	-	Uncertain	-	Uncertain	
	65 (F)	-	No	Lips	Yes	-	+	-	-	-	-	Yes	-	Uncertain	-	Uncertain	
	46 (F)	D	No	Neck, feet, limbs	Yes	-	+	-	-	-	-	+	-	Yes	Hairspray, hairdye	-	
	26 (F)	A	Yes	Lateral upper arm, cheeks, axillae	Yes	-	+	-	-	-	-	+	-	Uncertain	-	Present	
	20 (F)	-	No	Perioral	Yes	+	+	-	-	-	-	Yes	-	Uncertain	-	Uncertain	
	50 (F)	A	Yes	Face, neck, chest, antecubital fossa	Yes	-	+	-	-	-	-	-	-	Uncertain	-	-	
	33 (F)	D	No	Hands	No	-	+	-	Yes	Yes	Yes	Yes	Yes	Yes	Face wash, mouth wash	Present	
	32 (F)	D	No	Eyelids	Yes	+	?	-	-	-	-	-	-	Uncertain	-	Uncertain	
	48 (F)	B	No	Hands, feet	Yes	-	+	+++	?	-	-	-	-	Yes	Uncertain	-	-
	42 (F)	B	Yes	Face, body, arms, thighs	Yes	-	++	+	+	+	-	+	+	++	Uncertain	-	Uncertain
	57 (F)	D	No	Hands	Yes	-	++	+	-	-	-	-	-	-	Yes	-	-
	51 (M)	D	No	Hands, legs	No	-	++	+	-	-	-	-	-	-	No	-	Uncertain
	21 (F)	A	Yes	Head, neck, hands, arms	No	-	+	-	-	-	-	-	-	-	Yes	-	Present
	28 (F)	-	Yes	Periorbital	Yes	-	+	+	-	-	-	-	-	-	Uncertain	-	-
	75 (M)	D	No	Face, arms, trunk	Yes	-	+	-	-	-	-	-	-	-	Yes	Deodorant	Uncertain
	62 (M)	D	?	Hands, feet	Yes	-	+	-	-	Yes	Yes	-	-	-	Uncertain	-	-
	27 (M)	D	Yes	Hands, elbows	Yes	-	+	+	-	-	-	-	-	-	Yes	Body lotion	Uncertain
	54 (M)	-	No	Legs, trunk	Uncertain	-	+	+	+	-	-	-	-	-	Yes	-	-
59 (M)	D	No	Axillae, legs	Yes	-	+	?	+	+	-	-	-	-	Uncertain	-	Uncertain	

Table S1. Continued

Positive patch test reactions																	
Patient data			Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.					Fragrance markers in baseline series									
			Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4	D7	FM I ¹	FM II ²	HCC ³	MP ⁴	Colophonium	Using limonene	Notes
Seville (h)	62 (F)	–	?	Hands	Yes	–	+	?	+	+	+	–	–	–	Yes	Hair products	Uncertain
	22 (F)	D	No	Scalp, neck	Yes	–	+	–	–	–	–	–	–	–	Uncertain	–	Uncertain
	45 (F)	D	No	Perianal	Yes	–	+	?	–	–	–	–	–	–	Yes	–	–
	28 (F)	B	Yes	Hands	Yes	–	?	+	–	–	–	–	–	–	Yes	Shower gel	Present
	40 (F)	–	Yes	Face, eyelids, hands	Yes	–	+	–	Yes	–	–	–	–	–	Uncertain	–	Uncertain
	68 (F)	D	Yes	Hands	Yes	–	+	Irritant	–	–	–	–	–	–	Yes	Sunscreen	Present
	23 (F)	D	No	Hands	Yes	–	+	?	–	–	–	–	–	–	Yes	15 products: lotions, creams, sun lotion, shampoo, gel wash, deodorant	Uncertain
	58 (F)	A	No	Arms, neck, axillae, lower legs	Yes	–	+	+	Yes	–	–	–	–	–	No	–	Uncertain
	28 (F)	D	No	Hands	Yes	–	+	–	–	–	–	–	–	–	Yes	Deodorant	–
	51 (F)	B	Yes	Arms, photo-exposed	Yes	–	+	–	–	–	–	–	Yes	Yes	Uncertain	–	Uncertain
	54 (F)	D	No	Hands, feet	Yes	–	+	?	–	Yes	Yes	–	–	–	Uncertain	–	–
	63 (F)	C	No	Legs, arms, trunk	no	–	+	+	–	–	–	–	–	–	No	–	Uncertain
	65 (F)	D	No	Palms, eyelids	Yes	+++	+++	+++	+	–	–	–	–	–	Yes	Hand cream	Present
	57 (M)	D	No	Genital	Yes	+	++	+	+	–	–	–	+	–	Uncertain	–	Uncertain
	43 (F)	D	Yes	Feet	Yes	–	++	+	+	++	–	–	–	–	Uncertain	–	Uncertain
36 (M)	D	No	Hands, palms	Yes	–	++	–	–	–	–	–	–	–	Uncertain	Scalp gel	Uncertain	
27 (F)	D	No	Generalized	Yes	–	++	+	+	–	–	–	–	–	Yes	Beautician	Present, occupational	
65 (M)	D	No	Leg, generalized	Yes	++	++	+	+	+	++	++	+++	–	Uncertain	Cosmetic products	Uncertain	

Table S1. Continued

Positive patch test reactions																	
Patient data			Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.					Fragrance markers in baseline series				Exposure/relevance limonene					
			Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4	D7	FM I ¹	FM II ²	HCC ³	MP ⁴	Colophonium	Using limonene	Notes
Singapore (i)	47 (M)	D	No	Hands	Yes	+	++	+	+	-	-	++	-	-	Uncertain	-	Uncertain
	60 (M)	B	No	Face, neck, genitals	Yes	++	+	-	-	-	+++	++	-	-	Yes	Cleaner	Present
	63 (F)	D	No	Right leg	Yes	+	++	+	+	-	-	-	-	-	Uncertain	Perfume	Uncertain
	48 (M)	D	No	Abdomen	Yes	+	-	-	-	-	-	-	-	-	Uncertain	-	Uncertain
	56 (F)	D	No	Hands	Yes	-	+	+	-	-	-	-	-	-	Yes	Body cream	Present
	57 (M)	D	No	Right hand	Yes	-	+	+	+	-	+	-	-	-	Yes	Cleaner's machine	Present, occupational
	47 (F)	D	No	Scalp	Yes	+	+	+	-	-	-	-	-	-	Yes	Hair dye	Present
	66 (M)	D	No	Hands	Yes	-	+	+	-	-	-	-	-	-	Yes	Hand cream	Present
	23 (F)	D	No	Hands	Yes	-	+	+	-	-	-	-	-	-	Uncertain	-	Uncertain
	55 (F)	D	No	Generalized	Yes	-	+	+	-	-	-	-	-	-	Uncertain	-	Uncertain
	34 (F)	D	No	Hands	Yes	-	+	+	-	-	-	-	-	-	Yes	Hand cream	Present
	62 (F)	D	No	Arms, hands	Yes	-	+	+	+	+	-	-	-	-	Uncertain	-	Uncertain
	37 (F)	C	No	Dorsum of left hand	Yes	+	+	+	-	-	-	-	-	++	Uncertain	-	Uncertain
	62 (M)	D	No	General	Yes	+	-	-	-	-	-	-	-	-	Uncertain	-	Uncertain
	23 (F)	C	Yes	Right fingers, palms	Yes	NR	+++	-	-	-	+	+	-	-	Uncertain	Perfume, lotion	Uncertain
	51 (F)	-	No	Axillae	Yes	NR	++	++	-	++	++	++	++	-	Uncertain	-	Present
	17 (F)	D	Yes	Elbow	Yes	NR	++	++	-	-	-	-	-	-	No	-	Uncertain
	32 (F)	D	?	Face	Yes	NR	+	+	-	-	-	-	-	-	No	-	-
	50 (F)	-	Yes	Limbs, buttocks	Yes	NR	+	+	-	Yes	NT ⁶	NT	Yes	-	Yes	Dish washing liquid	Present
	21 (M)	-	Yes	Hands, feet	No	NR	+	+	-	-	-	-	Yes	-	Yes	-	Uncertain
67 (M)	D	No	Scalp, jaw, thighs, feet	No	NR	+	+	-	-	-	-	-	-	-	-	-	-
21 (F)	-	-	-	-	NR	+	+	-	+	+	-	+	++	Uncertain	-	-	Uncertain

Table S1. Continued

Patient data			Positive patch test reactions							Exposure/relevance limonene																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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			Age (gender)	History	Atopic eczema	Dermatitis location	Use of fragranced products	D2	D3 or D4			D7	FM I ¹	FM II ²	HCC ³	MP ⁴	Colophonium	Using limonene	Notes	Relevance for dermatitis																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
39 (M)	C	Yes	Neck	No	NR	+	NR	+	NR	+	Yes	+	+	+	No	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+</

Table S1. Continued

Age (gender)	Patient data				Positive patch test reactions					Exposure/relevance limonene			
	History	Atopic eczema	Dermatitis location	Use of fragranced products	Oxidized limonene 3.0% (limonene hydroperoxides 0.33%) pet.			Fragrance markers in baseline series					
					D2	D3 or D4	D7	FM I ¹	FM II ²		HCC ³	MP ⁴	
60 (F)	D	No	Hands	No	NR	+	–	–	–	–	No	–	–
20 (F)	A	No	Upper limbs	Yes	NR	+	–	–	Yes	–	Yes	–	–
20 (M)	D	No	None	Yes	NR	+	–	–	–	–	–	No	–
28 (F)	D	No	Hands, waist	Yes	NR	–	–	–	–	–	–	Uncertain	–
59 (F)	B	Yes	Face, palms	No	NR	+	–	Yes	Yes	–	–	Uncertain	–
60 (M)	D	No	Scalp, ears, face	No	NR	+	–	–	–	+	–	No	–
39 (F)	D	No	Arms, legs	Yes	NR	+	+	–	–	–	–	Yes	Face cream
30 (F)	D	No	Face	No	NR	+	–	–	–	–	–	Uncertain	–
23 (F)	D	No	Fingers	No	NR	+	–	–	–	–	–	No	–
63 (F)	B	No	Lower limbs	No	NR	+	–	–	–	–	–	Yes	Tea tree oil with limonene
51 (F)	B	Yes	Face	No	NR	+	–	?	–	–	–	No	–
45 (F)	C	Yes	Hands	No	NR	+	?	Yes	–	–	–	Uncertain	–
													Uncertain

FM I, Fragrance Mix I; FM II, Fragrance Mix II; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; MP, *Myroxylon pereirae*; NR, not read; NT, not tested.

In patient data below, 'history' (with responses A–D) signifies the patient's response to questions regarding previous problems with scented products, which are categorized as: (A) certain – has reacted with an itching dermatitis to at least one fine perfume or aftershave, and also to other scented products; (B) probable – has reacted to one or more scented products, but no certain product has been identified as the cause of a clinical reaction; (C) questionable – has reacted to various cosmetics with or without fragrances, that is, materials other than fragrance constituents may be the cause of the reaction; and (D) none – has never reacted to scented materials [modified after Frosch et al. (19)].