

Non-mix fragrances are top sensitizers in consecutive dermatitis patients – a cross-sectional study of the 26 EU-labelled fragrance allergens

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Summary

Background. For cosmetics, it is mandatory to label 26 fragrance substances, including all constituents of fragrance mix I (FM I) and fragrance mix II (FM II). Earlier reports have not included oxidized *R*-limonene [hydroperoxides of *R*-limonene (Lim-OOH)] and oxidized linalool [hydroperoxides of linalool (Lin-OOH)], and breakdown testing of FM I and FM II has mainly been performed in selected, mix-positive patients.

Objectives. To report the prevalence of sensitization to the 26 fragrances, and to assess concomitant reactivity to FM I and/or FM II.

Methods. A cross-sectional study on consecutive dermatitis patients patch tested with the 26 fragrances and the European baseline series from 2010 to 2015 at a single university clinic was performed.

Results. Of 6004 patients, 940 (15.7%, 95%CI: 14.7–16.6%) were fragrance-sensitized. Regarding the single fragrances, most patients were sensitized to Lin-OOH (3.9%), *Evernia furfuracea* (3.0%), Lim-OOH (2.5%), and hydroxyisohexyl 3-cyclohexene carboxaldehyde (2.1%). Significantly fewer patients were 'FM I-positive and constituent-positive' than 'FM II-positive and constituent-positive' (32.7% versus 57.0%, $p < 0.0001$). Additionally, significantly more patients were 'FM II-negative but constituent-positive' than 'FM I-negative but constituent-positive' (12.4% versus 3.2%, $p = 0.0008$).

Conclusions. Non-mix fragrances are the most important single fragrance allergens among consecutive patients. The test concentration of the single FM I constituents should be increased when possible.

Key words: 26 fragrances; clinical relevance; concomitant reactivity; contact allergy; fragrance mix; non-mix fragrances; oxidized limonene; oxidized linalool.

Fragrance substances are recognized as some of the most common causes of contact allergy, both in the general population and among dermatitis patients (1–3). It has

previously been shown that fragrance allergy causes a decreased quality of life, especially among younger female dermatitis patients and in patients sensitized to multiple fragrance allergens (4). We have recently shown that contact allergy to fragrance mix I (FM I), the most important screening marker for fragrance allergy, has been increasing among dermatitis patients within recent years, irrespective of sex and age (5).

Since 2005, it has been mandatory to label 26 well-established fragrance allergens within the EU if they are present at ≥ 10 ppm in leave-on cosmetic products,

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and at ≥ 100 ppm in rinse-off cosmetic products or household detergents (6). Fourteen of these 26 fragrance allergens constitute the single fragrance ingredients present in FM I and fragrance mix II (FM II), both of which are present in the current European baseline series of contact allergens (7). FM I 8% pet. contains seven fragrance chemicals (amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, and isoeugenol), and the natural extract *Evernia prunastri*. In addition, FM I 8% pet. contains the emulsifier sorbitan sesquileate (SSO), which can also cause contact allergy, at 5% (8). FM II 14% pet. was introduced in 2005, and consists of six fragrance chemicals: citronellol 0.5%, citral 1.0%, coumarin 2.5%, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (Lyrall[®]) 2.5%, farnesol 2.5%, and hexyl cinnamal 5.0% (9, 10). In contrast to FM I, for which the concentrations of single constituents for breakdown testing vary between manufacturers, breakdown patch testing with the single constituents of FM II uses double the concentrations of those found in the mix. Previous investigations into breakdown testing with the single constituents of FM I and FM II have mainly been performed in patients with either a positive patch test reaction to the respective mix or in patients with fragrance allergy (11–16).

Of the 26 fragrances not present in either FM I or FM II, that is, the 'non-mix' fragrances, the fragrance terpenes linalool and R-limonene are among the most extensively used fragrance substances in scented consumer products such as personal care products, household detergents, and hand cleansing agents (17). It has been established that both fragrance terpenes are prehaptenes, with oxidation of these resulting in the formation of specific allergenic hydroperoxides (18). Recent multicentre trials have shown that contact allergy to oxidized R-limonene and oxidized linalool, with stable and standardized concentrations of the main allergenic hydroperoxides, is common (19–22). Previous reports on patch testing of consecutive dermatitis patients with the 26 EU-labelled fragrances have not included results on the oxidized forms of R-limonene and linalool (23–27).

The aim of this study was to assess the prevalence of contact allergy to the 26 fragrance allergens from 2010 to 2015, including results for oxidized R-limonene and oxidized linalool from 2012 to 2015. In addition, results concerning concomitant reactions with FM I and/or FM II were analysed.

Materials and Methods

Data for this cross-sectional registry study were obtained from the clinical database on contact allergy at the

Department of Dermatology and Allergy, Copenhagen University Hospital Herlev-Gentofte, Denmark (28). We included available patch test results for FM I, FM II and 25 of the 26 EU-labelled fragrances from consecutive dermatitis patients who were patch tested with our baseline series between January 2010 and December 2015, irrespective of age. Patients had not been tested with methyl 2-octynoate, which may cause active sensitization (29). For R-limonene and linalool, we report patch test results from January 2012, when consecutive patch testing with the oxidized forms of these fragrance allergens was implemented, replacing patch testing with the unoxidized fragrance terpenes. For the current investigation, we did not include patch test results for balsam of Peru (*Myroxylon pereirae*) and colophonium from the European baseline series, owing to the wide variation in the importance of these allergens as screening markers for fragrance allergy (30).

In addition to the European baseline series, baseline patch testing at our department was performed with our fragrance series from Trolab[®], provided during the study period by Almirall Hermal (Reinbek, Germany), consisting of the 25 fragrance ingredients and SSO 20% pet. The oxidized forms of linalool and R-limonene, with standardized and stable concentrations of hydroperoxides of limonene (Lim-OOH) 0.3% pet. and hydroperoxides of linalool (Lin-OOH) 1% pet., respectively, were supplied by Chemotechnique Diagnostics (Vellinge, Sweden). Between 45.4–53.1% of patients aged <18 years (n = 262) were not tested with the single constituents of our fragrance series, but only with FM I, FM II, and HICC, owing to the limited space on their back. Patch testing was performed with Finn Chambers[®] (8 mm; SmartPractice, Phoenix, AZ, USA) applied on the upper back for 48 h with Scanpor[®] tape (Norgesplaster, Vennesla, Norway). Patch test readings were performed on day (D) 2, D3 or D4, and D7, and the maximum reactions are presented here. Grading of positive allergic reactions as weak (+), strong (++), and extreme (+++), and the scoring of doubtful (?+) and irritant reactions (IRs), were performed according to international guidelines, which, retrospectively, are compliant with the current criteria implemented by the ESCD in 2015 (7, 31). In the assessment of concomitant reactivity to FM I and FM II and their single constituents, patch test reactions to the respective mix were grouped as either positive, doubtful (?+), or negative (including IRs).

The clinical characteristics of patch tested patients were available according to the MOAHLEA index, describing the proportion of patients with regard to sex, occupational relevance of dermatitis, a lifetime prevalence of atopic dermatitis assessed by the treating dermatologist,

Table 1. Demographic and clinical characteristics according to MOAHLFA for all patients tested, fragrance-sensitized patients, and patients with no positive patch test reactions to the investigated fragrance allergens

	All patients N = 6004	Fragrance-positive* n = 940	Fragrance-negative n = 5064	Fragrance-positive versus fragrance-negative, <i>p</i> -value [†]
Age (years), mean (SD)	47.1 (18.1)	50.5 (16.3)	46.5 (18.3)	<0.0001
Age ≥40 years, n (%)	3900 (65.0)	698 (74.3)	3202 (63.2)	<0.0001
Male, n (%)	1865 (31.1)	245 (26.1)	1620 (32.0)	0.0003
Occupational dermatitis, n (%)	1205 (20.1)	174 (18.5)	1031 (20.4)	0.19
Atopic dermatitis, n (%)	1250 (20.8)	184 (19.6)	1066 (21.1)	0.31
Hand dermatitis, n (%)	2301 (38.3)	376 (40.0)	1925 (38.0)	0.25
Leg dermatitis, n (%)	102 (1.70)	28 (2.98)	74 (1.46)	0.0009
Face dermatitis, n (%)	1653 (27.5)	321 (34.2)	1332 (26.3)	<0.0001

SD, standard deviation.

*Contact allergy to at least one of the investigated fragrance allergens (excluding the emulsifier sorbitan sesquileate).

[†]Chi² test for categorical variables and Mann–Whitney *U*-test for continuous variables.

a point prevalence of hand, leg and facial dermatitis, and the proportion of patients aged >40 years (32). In patients with a positive patch test reaction to any of the investigated fragrance allergens, the clinical relevance of sensitization (i.e. the presence of dermatitis related temporally and anatomically to an established exposure to the relevant allergen) was assessed according to current guidelines (7). The current clinical relevance of a positive patch test reaction was mainly established through the patient's medical history or examination of declarations on relevant consumer products, and more rarely through patch or use tests with relevant products or chemical analyses of these.

Data management and statistical analyses were performed with SAS® Enterprise Guide®, version 7.1 (SAS Institute, Cary, NC, USA), following guidelines for the presentation of contact allergy data (33). Comparisons of patient subgroups were performed with the chi² test for categorical variables, and with the Mann–Whitney *U*-test for continuous variables. Figures were prepared with GRAPHPAD PRISM version 7.02 (GraphPad Software, La Jolla, CA, USA). For temporal trends, sensitization prevalences were standardized according to sex and age over/under 40 years (34). Patch test results for individuals not tested because of known sensitivity or not tested for other unknown reasons were treated as missing, and excluded from analyses. Two-sided *p*-values of <0.05 were considered to be statistically significant. All data were extracted from the database in June 2016 and handled anonymously.

Results

During the study period, 6058 dermatitis patients were patch tested with the investigated fragrance allergens. Among these, 6004 patch test results (99.1%) were

obtained for consecutive unselected patients, constituting the study population. A total of 940 (15.7%, 95%CI: 14.7–16.6%) patients were sensitized to at least one of the investigated fragrance allergens (i.e. not including the emulsifier SSO). Demographic and clinical characteristics according to MOAHLFA for the total study population, fragrance-sensitized patients and patients with no positive patch test reactions to any of the investigated fragrance allergens are shown in Table 1. Fragrance-sensitized patients were significantly older, more likely to be female, and significantly more often suffered from leg and face dermatitis. The age range for the total study population was 3–96 years, and that for fragrance-sensitized patients was 5–91 years.

Patch test reactions to the investigated allergens, including the patch test concentrations used, are shown in Table 2 in descending order by prevalence of sensitization. For the individual fragrances, the highest prevalences of contact allergy were observed for Lin-OOH (3.9%, 95%CI: 3.2–4.5%), *Evernia furfuracea* (3.0%, 95%CI: 2.6–3.5%), Lim-OOH (2.5%, 95%CI: 2.0–3.0%), and HICC (2.1%, 95%CI: 1.7–2.5%). High proportions of doubtful patch test reactions were seen especially for Lin-OOH (20.9%), FM I (15.4%), and Lim-OOH (13.7%), and to a lesser extent for FM II (8.9%), as compared with the remaining allergens. Benzyl benzoate 1% pet. was the only fragrance allergen with no positive patch test reactions during the study period. The established clinical relevance in patients with positive patch test reactions to the investigated fragrance allergens is shown in Table 3. For the fragrance mixtures, current and/or past relevance was established in 79.5% of FM I-sensitized and 82.4% of FM II-sensitized patients, respectively, with the majority of these being of current clinical relevance. For the 26 fragrance allergens with an absolute number of at least 10 positive patch test reactions during the study period,

Table 2. Consecutive patch test results for fragrance mix I (FM I), fragrance mix II (FM II) and the 26 EU-labelled fragrance substances from 2010 to 2015, N = 6004

Allergen (all in pet.)	% positive (95%CI) ^a	n (%)							
		+	++	+++	?+	IR	Negative	NT-S	NT
Fragrance mix I 8%	9.3 (8.5–10.0)	255 (4.3)	277 (4.6)	18 (0.30)	925 (15.4)	120 (2.0)	4349 (72.4)	53 (0.88)	7 (0.12)
Fragrance mix II 14%	4.4 (3.9–4.9)	103 (1.7)	155 (2.6)	4 (0.07)	535 (8.9)	72 (1.2)	5096 (84.9)	21 (0.35)	18 (0.30)
Hydroperoxides of linalool 1% ^b	3.9 (3.2–4.5)	101 (2.4)	52 (1.2)	2 (0.05)	878 (20.9)	301 (7.2)	2697 (64.3)	3 (0.07)	160 (3.8)
<i>Evernia furfuracea</i> 1%	3.0 (2.6–3.5)	79 (1.3)	95 (1.6)	3 (0.05)	141 (2.4)	18 (0.30)	5509 (91.8)	8 (0.13)	151 (2.5)
Hydroperoxides of limonene 0.3% ^b	2.5 (2.0–3.0)	64 (1.5)	34 (0.8)	4 (0.10)	574 (13.7)	245 (5.8)	3133 (74.7)	3 (0.07)	137 (3.3)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde 5% ^c	2.1 (1.7–2.5)	28 (0.47)	89 (1.5)	7 (0.12)	74 (1.2)	5 (0.08)	5772 (96.1)	13 (0.22)	16 (0.27)
Cinnamal 1% ^d	1.4 (1.1–1.7)	36 (0.60)	45 (0.75)	1 (0.02)	71 (1.2)	12 (0.20)	5683 (94.7)	5 (0.08)	151 (2.5)
<i>Evernia prunastri</i> 1% ^d	1.3 (1.0–1.6)	30 (0.50)	45 (0.75)	2 (0.03)	107 (1.8)	5 (0.08)	5655 (94.2)	11 (0.18)	149 (2.5)
Isoeugenol 1% ^d	1.1 (0.82–1.4)	22 (0.37)	41 (0.68)	1 (0.02)	73 (1.2)	7 (0.12)	5706 (95.0)	4 (0.07)	150 (2.5)
Hydroxycitronellal 1% ^d	0.92 (0.67–1.2)	28 (0.47)	25 (0.42)	1 (0.02)	66 (1.1)	4 (0.07)	5725 (95.4)	8 (0.13)	147 (2.5)
Farnesol 5% ^c	0.82 (0.58–1.1)	28 (0.47)	20 (0.33)	–	129 (2.2)	19 (0.32)	5654 (94.2)	3 (0.05)	151 (2.5)
Cinnamyl alcohol 1% ^d	0.64 (0.42–0.85)	22 (0.37)	15 (0.25)	–	81 (1.4)	9 (0.15)	5685 (94.7)	1 (0.02)	191 (3.2)
Hexyl cinnamal 10% ^c	0.45 (0.27–0.63)	15 (0.25)	11 (0.18)	–	55 (0.92)	4 (0.07)	5694 (94.8)	1 (0.02)	224 (3.7)
Citral 2% ^c	0.39 (0.22–0.56)	15 (0.25)	7 (0.12)	1 (0.02)	68 (1.1)	18 (0.30)	5724 (95.3)	3 (0.05)	168 (2.8)
Eugenol 1% ^d	0.36 (0.20–0.52)	12 (0.20)	8 (0.13)	1 (0.02)	59 (0.98)	7 (0.12)	5764 (96.0)	1 (0.02)	152 (2.5)
Butylphenyl methylpropional 10%	0.33 (0.17–0.48)	10 (0.17)	8 (0.13)	1 (0.02)	33 (0.55)	4 (0.07)	5759 (95.9)	–	189 (3.2)
Geraniol 1% ^d	0.26 (0.12–0.40)	13 (0.22)	2 (0.03)	–	140 (2.3)	16 (0.27)	5644 (94.0)	–	189 (3.2)
Sorbitan sesquileate 20% ^d	0.19 (0.07–0.31)	6 (0.10)	5 (0.08)	–	62 (1.0)	21 (0.35)	5778 (96.2)	1 (0.02)	131 (2.2)
Amyl cinnamyl alcohol 1%	0.14 (0.03–0.24)	4 (0.07)	4 (0.07)	–	35 (0.58)	4 (0.07)	5747 (95.7)	–	210 (3.5)
Coumarin 5% ^c	0.14 (0.03–0.24)	3 (0.05)	5 (0.08)	–	36 (0.60)	6 (0.10)	5762 (96.0)	2 (0.03)	190 (3.2)
Amyl cinnamal 1% ^d	0.14 (0.03–0.24)	5 (0.08)	3 (0.05)	–	76 (1.3)	4 (0.07)	5726 (95.4)	–	190 (3.2)
Benzyl alcohol 1%	0.09 (0.03–0.20)	3 (0.05)	2 (0.03)	–	17 (0.28)	2 (0.03)	5790 (96.4)	–	190 (3.2)
Citronellol 1% ^c	0.07 (0.02–0.18)	3 (0.05)	1 (0.02)	–	66 (1.1)	11 (0.18)	5732 (95.5)	–	191 (3.2)
Anise alcohol 1%	0.03 (0–0.12)	–	2 (0.03)	–	16 (0.27)	2 (0.03)	5790 (96.4)	–	194 (3.2)
α -Isomethylionone 1%	0.03 (0–0.12)	2 (0.03)	–	–	16 (0.27)	4 (0.07)	5790 (96.4)	–	192 (3.2)
Benzyl cinnamate 5%	0.02 (0–0.1)	1 (0.02)	–	–	21 (0.35)	3 (0.05)	5788 (96.4)	–	191 (3.2)
Benzyl salicylate 1%	0.02 (0–0.1)	1 (0.02)	–	–	30 (0.50)	3 (0.05)	5781 (96.3)	–	189 (3.2)
Benzyl benzoate 1%	–	–	–	–	38 (0.63)	10 (0.17)	5765 (96.0)	–	191 (3.2)

?+, doubtful reaction, IR, irritant reaction; NT, not tested; NT-S, not tested – sensitized.

^aNT and NT-S were treated as missing.

^bImplemented in our fragrance series in 2012 (N = 4194).

^cConstituent of FM II.

^dConstituent of FM I.

clinical relevance was established in 59.4% (isoeugenol) to 83.1% (HICC).

FM I and single constituents

Of the total study population, 5772 (96.1%) were tested concomitantly with FM I and all of its single constituents, including the emulsifier SSO. Concomitant reactivities to FM I and its constituents were assessed among these, excluding patients (n = 10) sensitized to SSO 20% pet. Among 529 patients with contact allergy to FM I, 173 (32.7%) had a concomitant positive patch test reaction to at least one of the single fragrance constituents of

the mix. The distribution of patch test reactions to FM I among constituent-positive patients is shown in Table 4. In total, 173 of 188 patients (92.0%) with one or more positive patch test reactions to the single constituents of FM I had a concomitant positive patch test reaction to the mix. An additional 9 (4.8%) patients had a doubtful patch test reaction to FM I, and only 6 (3.2%) constituent-positive patients had a concomitant negative patch test result with FM I. Figure 1 shows age- and sex standardized yearly prevalence rates of contact allergy to FM I and its single constituents across the study period.

Table 3. Proportions of clinical relevance in patients sensitized to the investigated allergens

Allergen-positive	Current (%)	Past (%)	Current and/or past (%)
Fragrance mix I 8% (n = 550)	65.8	33.5	79.5
Fragrance mix II 14% (n = 262)	67.9	35.1	82.4
Hydroperoxides of linalool 1% (n = 155)	63.2	26.5	76.1
<i>Evernia furfuracea</i> 1% (n = 177)	45.2	28.8	62.7
Hydroperoxides of limonene 0.3% (n = 102)	63.7	24.5	75.5
Hydroxyisohexyl 3-cyclohexene carboxaldehyde 5% (n = 124)	66.9	37.9	83.1
Cinnamal 1% (n = 82)	53.6	31.7	68.3
<i>Evernia prunastri</i> 1% (n = 77)	58.4	29.9	71.4
Isoeugenol 1% (n = 64)	39.1	31.3	59.4
Hydroxycitronellal 1% (n = 54)	50.0	40.7	70.4
Farnesol 5% (n = 48)	54.2	29.2	68.8
Cinnamyl alcohol 1% (n = 37)	59.5	35.1	73.0
Hexyl cinnamal 10% (n = 26)	61.5	30.8	76.9
Citral 2% (n = 23)	60.9	34.8	73.9
Eugenol 1% (n = 21)	38.1	33.3	66.7
Butylphenyl methylpropional 10% (n = 19)	68.4	10.5	73.7
Geraniol 1% (n = 15)	53.3	40.0	73.3
Sorbitan sesquiolate 20% (n = 11)	36.4	9.1	45.5
Amyl cinnamyl alcohol 1% (n = 8)	50.0	37.5	87.5
Coumarin 5% (n = 8)	75.0	62.5	87.5
Amyl cinnamal 1% (n = 8)	50.0	37.5	87.5
Benzyl alcohol 1% (n = 5)	40.0	40.0	60.0
Citronellol 1% (n = 4)	75.0	50.0	75.0
Anise alcohol 1% (n = 2)	50.0	50.0	100
α -Isomethylionone 1% (n = 2)	50.0	50.0	100
Benzyl cinnamate 5% (n = 1)	100	0	100
Benzyl salicylate 1% (n = 1)	0	0	0

In FM I-sensitized patients, stratifying on the strength of patch test reactions to FM I showed a stepwise increase in the frequency of patients with at least one positive patch test reaction to any of the single constituents: of 244 patients with a + reaction to FM I, 36 (14.8%) reacted to at least one of the constituents, increasing to 122 of 267 (45.7%) with a ++ reaction and 15 of 18 (83.3%) with a +++ reaction to FM I, respectively (Cochrane–Armitage trend test, $p < 0.0001$). Stratification of FM I-positive patients on MOAHLEA index variables and established clinical relevance of sensitization did not show any differences with regard to the proportion of patients with a concomitant positive patch test reaction to at least one single constituent (Table 5).

FM II and single constituents

A total of 5735 patients (95.5%) were tested concomitantly with FM II and all of its single constituents, and

were assessed for further analyses. Of 256 FM II-positive patients, 146 (57.0%) had a positive reaction to one or more of the single constituents during the study period, which was a significantly higher proportion than observed for FM I (χ^2 test, $p < 0.0001$). Concomitant reactivity to FM II in patients sensitized to the single constituents of the mix is shown in Table 4. A total of 146 of the 194 patients (75.3%) with a positive reaction to at least one constituent of FM II had a concomitant positive patch test reaction to the mix. Among constituent-positive patients, 24 (12.4%) had a doubtful concomitant patch test reaction to FM II, which was the same as the proportion of constituent-positive patients with a concomitant negative patch test result with FM II. The observed proportion of 'constituent-positive and mix-negative' patients was significantly higher than the observed proportion for FM I (χ^2 test, $p = 0.0008$). Regarding the single fragrance allergens, the highest proportion of concomitant negative patch test results with FM II was observed among farnesol-sensitized patients; 14 of 46 patients (30.4%) had negative test results with the mix. Figure 2 summarizes age- and sex-standardized yearly sensitization prevalences of contact allergy to FM II and its single constituents among patients tested concomitantly with these.

As for FM I, after stratification on patch test reactivity to the mix, a significant trend was observed for the proportion of FM II-positive patients with at least one positive patch test reaction to a single constituent: of patients with a +, ++ and +++ reaction to FM II, 33 of 101 (32.7%), 109 of 151 (72.2%) and 4 of 4 (100%), respectively, had one or more positive reactions to the single mix constituents (Cochrane–Armitage exact trend test, $p < 0.0001$). Similarly to what was found for FM I, no differences were observed for the proportion of FM II-positive patients with a concomitant reaction to at least one constituent when stratifying for clinical characteristics and established clinical relevance (Table 5).

Non-mix fragrance allergens and concomitant reactivity to FM I and FM II

During the study period, a total of 5940 patients (98.9%) were tested concomitantly with at least FM I and FM II. For the four non-mix fragrance substances with at least 10 positive patch test reactions, the proportion of concomitant positive patch test reactions to either FM I or FM II (Table 6) ranged from 30.0% (Lim-OOH) to 73.7% (butylphenyl methylpropional/Lilial®). Figure 3 shows age- and sex standardized frequencies of sensitization by test year to the non-mix fragrance substances with at least 10 positive patch test reactions during the total study period. The number of patients patch tested

Table 4. Distribution of patch test reactivity to fragrance mix I (FM I) and fragrance mix II (FM II) in patients with a positive patch test reaction to the respective single mix constituents

FM I single constituent-positive	FM I-positive, n (%)	FM I-doubtful, n (%)	FM I-negative, n (%)
Cinnamal, n = 74	69 (93.2)	4 (5.4)	1 (1.4)
<i>Evernia prunastri</i> , n = 70	67 (95.7)	1 (1.4)	2 (2.9)
Isoeugenol, n = 55	50 (90.9)	5 (9.1)	0
Hydroxycitronellal, n = 44	42 (95.5)	1 (2.3)	1 (2.3)
Cinnamyl alcohol, n = 34	33 (97.1)	1 (2.9)	0
Eugenol, n = 17	16 (94.1)	0	1 (5.9)
Geraniol, n = 13	9 (69.2)	2 (15.4)	2 (15.4)
Amyl cinnamal, n = 8	6 (75.0)	1 (12.5)	1 (12.5)
At least one FM I constituent, n = 188	173 (92.0)	9 (4.8)	6 (3.2)
FM II single constituent-positive	FM II-positive, n (%)	FM II-doubtful, n (%)	FM II-negative, n (%)
HICC, n = 120	110 (91.7)	8 (6.7)	2 (1.7)
Farnesol, n = 46	23 (50.0)	9 (19.6)	14 (30.4)
Hexyl cinnamal, n = 26	19 (73.1)	3 (11.5)	4 (15.4)
Citral, n = 21	15 (71.4)	2 (9.5)	4 (19.1)
Coumarin, n = 7	4 (57.1)	2 (28.6)	1 (14.3)
Citronellol, n = 4	4 (100)	0	0
At least one FM II constituent, n = 194	146 (75.3)	24 (12.4)	24 (12.4)

HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.

with the single non-mix fragrances varied slightly for each year.

Discussion

The current study investigated sensitization in recent years to 25 of the 26 fragrance allergens with mandatory labelling in cosmetics and household detergents among consecutively patch tested dermatitis patients from a single university clinic. During the 6-year study period, 15.7% of patients had a positive patch test reaction to FM I, FM II, or one of the 25 fragrance substances. Of the individual fragrance allergens, Lin-OOH showed the highest prevalences of sensitization, with 3.9% positive patch test reactions, followed by *E. furfuracea* (3.0%), Lim-OOH (2.5%), and HICC (2.1%). Among FM I-positive patients, 32.7% had a concomitant positive patch test reaction to at least one mix constituent, and 3.2% of the patients with a positive patch test reaction to a FM I constituent had a concomitant negative patch test result to the mix. Among FM II-positive patients, 57.0% had a concomitant positive patch test reaction to at least one mix constituent, and 12.4% of patients sensitized to at least one FM II constituent had a concomitant negative patch test result to the mix. Both the proportion of 'mix-positive and constituent-positive' patients and the proportion of 'mix-negative and constituent-positive' patients were significantly higher for FM II than for FM I.

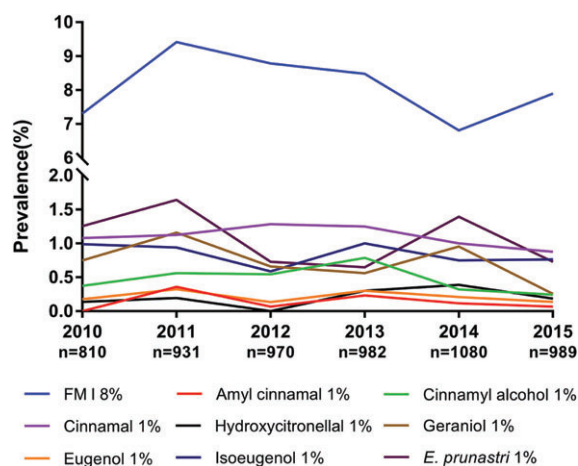


Fig. 1. Age- and sex-standardized yearly prevalences of contact allergy to fragrance mix I (FM I) and its single constituents, in patients (n = 5762) tested concomitantly with these (excluding 10 patients sensitized to the emulsifier sorbitan sesquioleate).

Unlike previous studies reporting on consecutive patch testing with the 26 fragrance allergens (23–27), we included patch test results for Lin-OOH and Lim-OOH from 2012 onwards, when consecutive patch testing with the oxidized terpenes was implemented at our department. Beyond the oxidized terpenes, the ranking of the single constituents most often causing contact allergy observed in the current study was comparable to

Table 5. Associations (odds ratios) for subgroups of fragrance mix I (FM I)-positive (n = 529) and fragrance mix II (FM II)-positive (n = 256) patients with regard to a concomitant positive patch test reaction to at least one constituent of the respective mix

Subgroups of FM I/FM II-positive patients	Odds ratio (95%CI)	
	FM I constituent-positive	FM II constituent-positive
Male	1.28 (0.83–1.97)	1.15 (0.63–2.09)
Occupational relevance (reference: no occupational relevance)	1.22 (0.77–1.93)	1.45 (0.78–2.72)
Atopic dermatitis	1.17 (0.75–1.84)	0.79 (0.44–1.42)
Hand eczema (reference: no hand eczema)	1.07 (0.74–1.56)	1.00 (0.61–1.64)
Leg eczema (reference: no leg eczema)	1.29 (0.41–4.02)	0.74 (0.21–2.64)
Face eczema (reference: no face eczema)	1.11 (0.76–1.62)	0.81 (0.48–1.36)
Aged ≥40 years	1.22 (0.78–1.90)	0.74 (0.42–1.31)
Established current relevance (reference: unknown)	0.97 (0.66–1.43)	1.26 (0.74–2.14)
Established past relevance (reference: unknown)	1.07 (0.73–1.57)	1.45 (0.86–2.46)
Established current and/or past relevance (reference: unknown)	1.04 (0.65–1.66)	1.87 (0.96–3.61)

that reported by Heisterberg et al. from the same patch test population between 2008 and 2010 (24, 25). The prevalences of sensitization to the natural extracts *E. furfuracea* (4.1% versus 3.0%) and *E. prunastri* (2.5% versus 1.3%) were lower in the current study than in the period 2008–2010. It is of note that the 2-year study period from 2008 to 2010 included a 6-month interval during which patch testing with the 26 fragrances was only performed in patients suspected of having fragrance allergy, which could potentially cause an increase in the observed proportion of positive patch test reactions (24). The differences in sensitization to the two natural extracts could also be an indication of an actual reduced frequency of sensitization among tested patients. Between 1997 and 2007, a large reduction was reported in the total tonnage of both processed treemoss (*E. furfuracea*) and oakmoss (*E. prunastri*) used by the cosmetics industry, which could indicate a general decrease in exposure (35). In the current study, we did observe a decrease in sensitization to *E. furfuracea* from 2010 to 2015, whereas changes in sensitization to *E. prunastri* across patch test years were more heterogeneous. Depending on the processing of the natural extracts, both of these can contain the potent contact sensitizers atranol and chloroatranol (35, 36). The Scientific Committee on

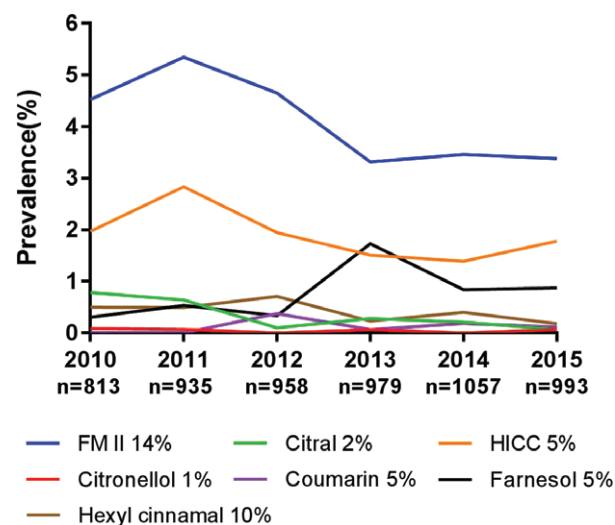


Fig. 2. Age- and sex standardized yearly prevalences of contact allergy to fragrance mix II (FM II) and its single constituents, in patients (n = 5735) tested concomitantly with these. HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Table 6. Concomitant reactivity to non-mix fragrance substances and fragrance mix I (FM I) and fragrance mix II (FM II)

Allergen-positive	Concomitant positive reactions		
	FM I (%)	FM II (%)	FM I and/or FM II (%)
Hydroperoxides of linalool (n = 152)	25.7	15.1	31.6
<i>Evernia furfuracea</i> (n = 169)	47.3	18.9	50.3
Hydroperoxides of limonene (n = 100)	26.0	13.0	30.0
Butylphenyl methylpropional/Lilial® (n = 19)	63.2	73.7	73.7
Amyl cinnamyl alcohol (n = 8)	62.5	62.5	62.5
Benzyl alcohol (n = 5)	40.0	40.0	40.0
Anise alcohol (n = 2)	100	100	100
α-Isomethylionone (n = 1)	0	0	0
Benzyl cinnamate (n = 1)	0	0	0
Benzyl salicylate (n = 1)	100	100	100

Consumer Products, advising the European Commission, has stated that both atranol and chloroatranol should not be present in cosmetic products, owing to their high sensitizing potency (37). Commercial attempts have been made to reduce the content of atranol and chloroatranol in oakmoss absolute for its continued use in scented consumer products; however, manufacturers can only guarantee <100 ppm in the subsequent product (38). In the current study, 96% of consecutive dermatitis

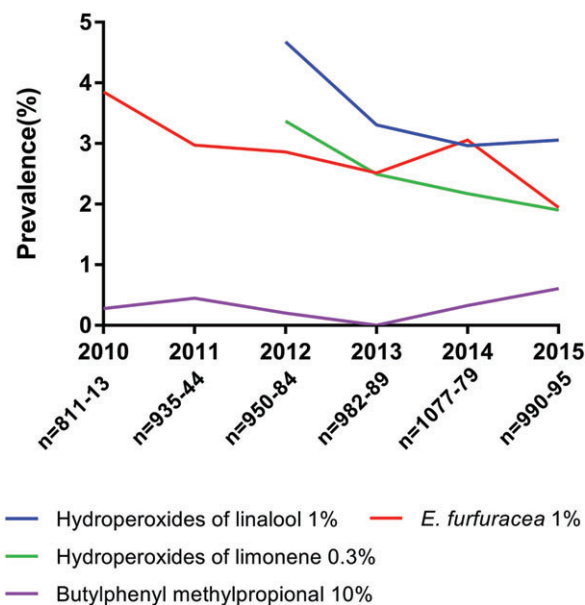


Fig. 3. Age- and sex standardized yearly prevalences of contact allergy to non-mix fragrance substances (only fragrances with at least 10 sensitized individuals during the study period are shown, for improved clarity).

patients sensitized to *E. prunastri* had a concomitant positive patch test reaction to FM I, and would hence have been diagnosed with fragrance allergy if they had been patch tested with only the European baseline series. For *E. furfuracea*, however, only 50% of sensitized patients had a concomitant positive patch test reaction to FM I and/or FM II. It has previously been established that at least two subgroups of *E. furfuracea*-sensitized patients can be defined: one is sensitized to common constituents found in both treemoss and oakmoss, such as atranol and chloroatranol, and the other is sensitized to (contaminating) resin acids, as indicated by a concomitant positive patch test reaction to colophonium (39). As we did not include results on contact allergy to colophonium in the current study, we do not know the additional proportion of patients sensitized to *E. furfuracea* who would have been otherwise detected as having a possible fragrance allergy on the basis of colophonium sensitization.

It was expected that both oxidized linalool and oxidized R-limonene would be among the single fragrance allergens with the highest sensitization prevalences in the current study, on the basis of previous clinical investigations reporting high frequencies of contact allergy to these (19–22). In the current study, a high proportion of patients had a doubtful patch test reaction to Lin-OOH (20.9%) and, to a lesser extent, to Lim-OOH (13.7%). In the previous multicentre trials on the oxidized terpenes,

using the same patch test concentrations as in the current study, the frequencies of doubtful patch test reactions varied from none to 24.5% for Lim-OOH and none to 36.2% for Lin-OOH. These variations were attributed to differences in scoring practice between participating departments (20, 21). In a previous dose–response study on oxidized linalool (40), 62% of patients with a doubtful patch test reaction to a lower dose of the oxidized terpene had a weak positive patch test reaction when tested concomitantly with a twofold increased patch test concentration. This could indicate that at least some of the observed doubtful patch test reactions to Lin-OOH and Lim-OOH in the current study do represent contact allergy to the oxidized fragrance terpenes. Further investigations in this patient subgroup could be a future research area of interest. For both oxidized terpenes, the highest prevalence of sensitization was observed in 2012 following their introduction into our baseline patch test series, with a close to parallel decline in sensitization frequencies in the following years. However, both fragrance allergens, which are ubiquitously found in scented consumer products, have remained well above the proposed lower limit of 0.5–1% positive patch test reactions for inclusion in baseline testing of consecutive dermatitis patients (7).

The current study showed several interesting aspects with regard to breakdown testing of FM I and FM II in consecutive patients. We were able to reproduce the findings reported by Geier et al. (15) in selected FM I-positive patients, showing a significant trend in the proportion of FM I-positive and FM II-positive patients with a concomitant positive patch test reaction to at least one mix constituent, after stratification on the strength of reaction to the relevant mix. Additionally, we showed that clinical characteristics, such as age, sex, and a history of atopic dermatitis, as well as established clinical relevance of contact allergy to the relevant mix, was not related to the proportion of mix-positive patients reacting to at least one single mix constituent.

Approximately one-third of FM I-positive patients had a concomitant positive patch test reaction to at least one mix constituent. In previous investigations on aimed breakdown testing in patients with an established contact allergy to FM I, the proportion of patients sensitized to at least one mix constituent has varied substantially, from 55% to 84% (11, 12, 14–16). However, in all of these investigations, some degree of bias in selecting FM I-positive patients for breakdown testing was present. Mann et al. (26) reported on 1951 consecutive eczema patients who were patch tested with the 26 fragrance allergens from 2011 to 2012 as a supplement to the baseline series. The single constituents of FM I were all

(except for cinnamal) tested at 2% pet. Among 124 FM I-positive patients, 54% had one or more concomitant positive patch test reactions to the single constituents. Additionally, 42% of patients with a positive patch test reaction to at least one FM I constituent did not have a concomitant positive patch test reaction to FM I, as compared with only 8% in the current study. Unfortunately, Mann et al. (26) did not report on the separate frequencies of irritant patch test reactions observed when patch testing was performed with the higher concentrations of the single FM I constituents. In the current study, none of the single FM I constituents tested at 1% could explain the observed variation in FM I sensitization across patch test years. We have recently published the results on contact allergy to FM I over time observed in the current study as part of a larger investigation on trends in FM I sensitization from 1986 to 2015 (5).

For FM II, the concentrations used for testing with the single constituents are twice as high as those in the mix. As compared with FM I, we observed a significantly increased proportion of both FM-II positive patients with a positive breakdown test result and a significantly increased proportion of FM-II constituent-positive patients with a concomitant negative reaction to the mix. In the current study, 57% of FM II-positive patients had a concomitant positive patch test reaction to at least one constituent. In selected FM II-positive patients, positive breakdown testing results have previously been reported in 64–72% of patients (10, 15). Interestingly, Mann et al. (26) reported that only 34% of 64 consecutive FM II-positive patients had a concomitant positive patch test reaction to one or more constituents. Regarding the differences from the results of the current study, possible explanations could be differences in patch test reading practice (inclusion of D7 readings in the current study) or differences in exposures. In the current study, 12.4% of constituent-positive patients had a concomitant negative patch test result to FM II; however Mann et al. (26) did not differentiate between doubtful and negative (including irritant) patch test reactions in their reporting.

HICC remains the most important fragrance allergen present in FM II. The observed prevalence of HICC

sensitization from 2010 to 2015 is comparable to the prevalence of 2.3% reported by Heisterberg et al. (24, 25) from 2008 to 2010 in the same patch test population. In 2012, the Scientific Committee on Consumer Safety concluded, in an extensive opinion on fragrance allergens in cosmetic products, that HICC should not be used in consumer products, because of an exceptionally high number of documented cases of contact allergy to this synthetic compound (17). We observed an age- and sex adjusted decrease in the frequency of contact allergy to HICC from 2.8% in 2011 to 1.4% in 2014; however, this was followed by an increase to 1.8% in 2015. Whether these changes in sensitization frequencies reflect a decrease in consumer exposure to HICC in recent years is unknown, and continued epidemiological surveillance of HICC sensitization in the coming years is of high interest. From the current study on consecutive patch tested dermatitis patients, it is, however, evident that sensitization to FM II is largely dependent on sensitization to HICC, with temporal trends in contact allergy to these paralleling each other closely.

In summary, several of the 26 fragrance allergens with mandatory labelling within the EU fulfil the criteria for inclusion in baseline patch testing of consecutive dermatitis patients, on the basis of high relative frequencies of sensitization. The single fragrances most often causing contact allergy in the current study were oxidized linalool and *R*-limonene, and the natural extract *E. furfuracea*. For these non-mix fragrance substances, only 30–50% of sensitized individuals are detected as fragrance-allergic when patch testing is performed with FM I and FM II. With regard to patch testing with the single constituents of FM I, results from the current and previous investigations seem to favour the use of higher patch test concentrations (except for cinnamal), as with FM II and its single constituents, in order to improve the diagnosis of fragrance contact allergy among dermatitis patients.

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