

Patch testing with a new fragrance mix – reactivity to the individual constituents and chemical detection in relevant cosmetic products

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A new fragrance mix (FM II), with 6 frequently used chemicals not present in the currently used fragrance mix (FM I), was evaluated in 6 dermatological centres in Europe, as previously reported. In this publication, test results with the individual constituents and after repeated open application test (ROAT) of FM II are described. Furthermore, cosmetic products which had caused a contact dermatitis in patients were analysed for the presence of the individual constituents. In 1701 patients, the individual constituents of the medium (14%) and the highest (28%) concentration of FM II were simultaneously applied with the new mix at 3 concentrations (break-down testing for the lowest concentration of FM II (2.8%) was performed only if the mix was positive). ROAT was performed with the concentration of the FM II which had produced a positive or doubtful (+ or ?+) patch test reaction. Patients' products were analysed for the 6 target compounds by gas chromatography-mass spectrometry (GC-MS). Results: 50 patients (2.9%) showed a positive reaction to 14% FM II and 70 patients (4.1%) to 28% FM II. 24/50 (48%) produced a positive reaction to 1 or more of the individual constituents of 14% FM II and 38/70 (54.3%) to 28% FM II, respectively. If doubtful reactions to individual constituents are included, the break-down testing was positive in 74% and 70%, respectively. Patients with a positive reaction to 14% FM II showed a higher rate of reactions to the individual constituent of the 28% FM II: 36/50 (72%). Positive reactions to individual constituents in patients negative to FM II were exceedingly rare. If doubtful reactions are regarded as negative, the sensitivity, specificity, positive predictive value and negative predictive value for the medium concentration of FM II towards at least 1 individual constituent was 92.3% (exact 95% confidence interval 74.9–99.1%), 98.4% (97.7–99.0%), 48% (33.7–62.6%) and 99.9% (99.6–100.0%), respectively. For the high concentration, the figures were very similar. The frequency of positive reactions to the individual constituents in descending order was the same for both FM II concentrations: hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall[®]) > citral > farnesol > citronellol > α -hexyl-cinnamic aldehyde (AHCA). No unequivocally positive reaction to coumarin was observed. Lyrall[®] was the dominant individual constituent, with positive reactions in 36% of patients reacting to 14% FM II and 37.1% to 28% FM II. 5/11 patients developed a positive ROAT after a median of 7 days (range 2–10). The 5 patients with a doubtful or negative reaction to 28% FM II were all ROAT negative except 1. There were 7 patients with a certain fragrance history and a positive reaction to either 28% or 14% FM II but a negative reaction to FM I. Analysis with GC-MS in a total of 24 products obtained from 12 patients showed at least 1–5 individual constituents per product: Lyrall[®] (79.2%), citronellol (87.5%), AHCA (58.3%), citral (50%) and coumarin (50%). The patients were patch test positive to Lyrall[®], citral and AHCA. In conclusion, patients with a certain fragrance history and a negative reaction to FM I can be identified by FM II. Testing with individual constituents is positive in about 50% of cases reacting to either 14% or 28% FM II.

Key words: α -hexyl-cinnamic aldehyde; citral; farnesol; hydroxyisohexyl 3-cyclohexene carboxaldehyde; individual constituents; Lyrall[®]; new fragrance mix; product analysis; repeated open application test (ROAT). © Blackwell Munksgaard, 2005.

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Patch test results obtained with a new fragrance mix (FM II) in 6 dermatological centres in Europe have been reported previously (1). FM II contains 6 frequently used constituents of perfumes: hydroxyisohexyl 3-cyclohexene carboxaldehyde [INCI, chemically 4-(4-hydroxy-4-methyl-pentyl)-3-cyclohexene carboxaldehyde (Lyrall[®])], citral, farnesol, citronellol, coumarin and α -hexyl-cinnamic aldehyde (AHCA). Positive reactions to FM II in 1701 consecutively tested patients were dose-dependent and increased from 1.3% (2.8% FM II), through 2.9% (14% FM II) to 4.1% (28% FM II). As the break-down testing with individual constituents of the current 8% FM I of Larsen (2) is often negative, it was of special interest to evaluate the performance of FM II with this respect.

The proof of the clinical relevance of an allergic patch test reaction is the presence of individual constituents in a specific product that the patient had used and which had caused a contact dermatitis. Therefore, chemical analysis of patients' products was undertaken to determine the presence of one of the 6 individual constituents of FM II suspected to have caused the sensitization(s).

Materials and Methods

Details of patients, patch test technique and of the FM II tested in 3 concentrations (2.8%, 14% and 28% in petrolatum) have been described previously (1). The individual constituents of 14% FM II and of 28% FM II were applied simultaneously with the mix. The single constituents of 2.8% FM II were tested only if there was a positive or doubtful (+ or ?) reaction to this concentration of the new mix.

Repeated open application test (ROAT)

According to the protocol, whenever possible a ROAT had to be performed on patients showing a + or ? reaction to 1 of the concentrations of FM II. 0.1 ml of FM II at the lowest concentration, which had produced such a patch test reaction was applied $\times 2$ daily onto an area of 3×3 cm of the volar aspect of the forearm. On the opposite arm, white petrolatum was applied as the control. Reactions were read as described elsewhere (3). The ROAT was considered as positive, if there was erythema of at least 25% of the test area and homogeneous infiltration or papules (no require-

ment as to the number of papules). Applications were then discontinued or after a maximum duration of 14 days.

Chemical analysis of patients' products

Chemical analysis of target fragrance compounds in the patients' products was performed as described previously (4, 5). Fragrance compounds from lotions and creams were extracted in methanol (4) and analysed by gas chromatography-mass spectrometry (GC-MS) (5). Fine perfumes, eaux de toilettes and deodorants were appropriately diluted in methanol (4) followed by GC-MS analysis (5). Identification of the fragrance compounds was performed in full-scan mode, and quantification was performed in selective ion mode.

Results

Individual constituents

Tables 1 and 2 show the test results obtained with the individual constituents of the highest and the medium concentration of FM II in the total population ($n = 1701$) and the patients with a positive reaction to 14% FM II and 28% FM II, respectively. Positive reactions to an individual constituent in patients negative to the mix were rare. Slight deviations were seen only with Lyrall[®] and citral. The frequency of positive reactions in descending order was the same for both concentrations: Lyrall[®] > citral > farnesol > citronellol > AHCA. No unequivocally positive reaction was observed to coumarin.

For 14% FM II, the separation was positive in 18/50 (36%) patients for Lyrall[®]; the remaining 5 substances resulted in 13 positive reactions (26%) – this amounts to a total of 31 positive reactions to individual constituents (some patients had multiple reactions). Altogether, 24/50 patients (48%) had at least 1 positive reaction to individual constituents; 13 had a doubtful reaction and 13 were completely negative.

For 28% FM II, the break-down testing was positive in 26/70 (37.1%) patients for Lyrall[®]; the remaining 5 substances resulted in 23 reactions. Altogether, 38/70 (54.3%) patients had a positive reaction to at least 1 individual constituent (11 doubtfuls and 21 negatives). The frequency of doubtful reactions was highest to Lyrall[®] (10/

Table 1. Reactions to constituents of the FM II in medium and high concentration (day 3/4 readings).

a) FM II, 14% (medium concentration)

	%	All patients			FM II positive (n = 50)		
		?/IR	+	++/+++	?/IR	+	++/+++
Lyrall [®]	2.5	24	11	9	10	9	9
Citral	1.0	14	4	2	4	4	2
Farnesol	2.5	20	4	0	4	4	0
Citronellol	0.5	18	2	0	2	2	0
AHCA	5.0	20	0	1	1	0	1
Coumarin	2.5	6	0	0	0	0	0

b) FM II, 28% (high concentration)

	%	All patients			FM II positive (n = 70)		
		?/IR	+	++/+++	?/IR	+	++/+++
Lyrall [®]	5.0	30	16	12	10	15	11
Citral	2.0	40	7	5	7	6	5
Farnesol	5.0	30	5	1	5	5	1
Citronellol	1.0	23	4	0	2	4	0
AHCA	10.0	16	1	1	2	1	1
Coumarin	5.0	7	0	0	1	0	0

Number of reactions exceeds number of patients due to multiple reactions in some patients.

AHCA = α -hexyl-cinnamic aldehyde; ?/IR = doubtful irritant.

50 = 20% and 10/70 = 14.3% in the group of patients positive to 14% FM II and 28% FM II, respectively). The frequency of doubtful/irritant (?/IR) reactions in the total group of patients ranged between 2.4% (2% citral) and 0.4% (2.5% coumarin).

Table 2. Reactions to the fragrance mix (FM) II and its corresponding individual constituents (IC) (maximum reaction of at least 1 IC) in 1701 patients

		FM II, 14%		
		Negative	Doubtful	Positive
IC of FM II, 14%	Negative	1502	98	13*
	Doubtful	21	28	13
	Positive	0	2	24
		FM II, 14%		
		Negative	Doubtful	Positive
IC of FM II, 28%	Negative	1487	81	7*
	Doubtful	35	43	7
	Positive	1	4	36
		FM II, 28%		
		Negative	Doubtful	Positive
IC of FM II, 28%	Negative	1421	133	21*
	Doubtful	29	45	11
	Positive	1	2	38

*Significant asymmetry (Bowker test); i.e., significantly more patients react to the FM II than to any 1 individual constituent.

In the group of patients reacting to 14% FM II, the reactivity to the individual constituents rose from 48% to 72% (36/50) if the test concentration was doubled as present in the 28% FM II (Table 2).

2.8% FM II was positive in 22 of 1701 patients. Break-down testing was performed in 7 patients only; there was only 1 positive reaction (+) to 0.5% Lyrall[®].

As Table 2 summarizes, there is significant asymmetry (Bowker test), i.e. significantly more patients react to the FM II than to any 1 of the individual constituents in the 2 concentration levels. However, only 13/50 (26%) of patients positive to 14% FM II remain completely negative to the individual constituents; for 28% FM II this figure is 21/70 (30%). Hence, if doubtful reactions to individual constituents are added to the clearly positive reactions, the splitting is 'positive' in 74% for 14% FM II and in 70% for 28% FM II. If doubtful reactions are regarded as negative, the sensitivity, specificity, positive predictive value and negative predictive value for the medium concentration mix towards any 1 individual constituent is 92.3% (exact 95% CI: 74.9–99.1%), 98.4% (97.7–99.0%), 48% (33.7–62.6%) and 99.9% (99.6–100.0%), respectively. For the high concentration, the corresponding measures of diagnostic performance are 92.7% (80.1–98.5%), 98.1% (97.3–98.7%), 54.3% (41.9–66.3%) and 99.8% (99.5–100.0%), respectively.

A cross tabulation of reactions to medium versus high concentrations of individual constituents

revealed significant asymmetry (Bowker test) for Lyrall[®] and citral – there were 2 positive reactions each to the highest concentration when negative at the medium concentration. Furthermore, there were 19 doubtful reactions to LyrallTM and 33 to citral in the highest concentration in patients negative to the medium concentration, whereas the opposite was observed in 7 and 3 cases, respectively (data not shown).

In Table 3, the association between strength of test reaction to the mix and positive reactions to individual constituents is documented. It is evident that the yield of break-down testing increases with the strength of the reaction to the mix. For 14% FM II, there were a total of 9 reactions in 7 patients (of a total of 31 patients) showing a + reaction to the mix; the corresponding figures for ++ reactions were 15 reactions in 13 patients; +++ reactions: 7 reactions in 4 patients. Testing with the individual constituents at the highest concentration produced an increase in reactivity in the group of patients positive to 14% FM II from 24 to 36 patients (total of reactions of 31 and 47, respectively). For 28% FM II, the figures were as follows: + reaction, 14/14; ++ reaction, 29/21; and +++ reaction, 6/3.

In 15 patients, individual constituents of the high range were positive, while those of the medium range

were either negative ($n = 6$) or doubtful ($n = 9$); conversely, all patients positive to a medium range individual constituents were also positive to one of high range ($P < 0.001$, Bowker test). Of these 15 patients, 9 had a 'certain' and 5 a 'probable' relevance, while in 1 patient no relevance was found. Regarding reactions to the mixes, only 3 out of 2 were not positive to the FM II 14% (28%), but 7 to the FM I.

1 of the 2 patients with a positive reaction to 2.5% Lyrall[®] and a doubtful reaction to 14% FM II (Table 3) produced a positive (+) reaction to 28% FM II; the other 1 showed a doubtful reaction to both the 28% FM II and 5% Lyrall[®]. While the 1st patient had a fragrance history classified as certain, the 2nd patient had none.

Lyrall[®] has been identified as an important individual fragrance allergen earlier (6, 7) and has consequently been incorporated into the German standard series (8) to be tested along with the FM I. Thus, we analysed the diagnostic gain of testing the FM II in this setting (Table 4). 14 positive reactions to 14% FM II and 26–28% FM II were observed in those reacting negatively or doubtfully to FM I and Lyrall[®]; hence, a considerable benefit from testing the FM II is also evident in this situation.

In 867 patients, late readings at day 5 or 7 were recorded. For 2.5% Lyrall[®], there were 2 positive

Table 3. Association between intensity of test reaction to the mix and positive reactions to individual constituents

		FM II (14%)				
	%	Neg. ($n = 1523$)	? ($n = 128$)	+ ($n = 31$)	++ ($n = 15$)	+++ ($n = 4$)
Lyrall [®]	2.5	0	2	6	10	2
Citral	1.0	0	0	0	3	3
Farnesol	2.5	0	0	2	1	1
Citronellol	0.5	0	0	1	0	1
AHCA	5.0	0	0	0	1	0
Coumarin	2.5	0	0	0	0	0

		FM II (14%)				
	%	Neg. ($n = 1523$)	? ($n = 128$)	+ ($n = 31$)	++ ($n = 15$)	+++ ($n = 4$)
Lyrall [®]	5.0	1	2	11	12	2
Citral	2.0	0	2	2	5	3
Farnesol	5.0	0	0	4	1	1
Citronellol	1.0	0	0	1	1	2
AHCA	10.0	0	0	1	1	0
Coumarin	5.0	0	0	0	0	0

		FM II (28%)				
	%	Neg. ($n = 1451$)	? ($n = 180$)	+ ($n = 46$)	++ ($n = 21$)	+++ ($n = 3$)
Lyrall [®]	5.0	1	1	10	14	2
Citral	2.0	0	1	1	8	2
Farnesol	5.0	0	0	2	3	1
Citronellol	1.0	0	0	0	3	1
AHCA	10.0	0	0	1	1	0
Coumarin	5.0	0	0	0	0	0

AHCA = α -hexyl-cinnamic aldehyde; FM = fragrance mix.

Table 4. Comparison between reactions to fragrance mix (FM) I and Lyrall[®] as the most frequent allergen in the FM II, and FM II reactions in the respective high and medium concentration range, addressing the question of the additional benefit of the FM II if Lyrall[®] and the FM I are routinely tested as screening allergens

		Strongest reaction to Lyrall [®] or FM I		
		Positive	Doubtful	Negative
a) Medium concentration				
FM II	Positive	36	8	6
	Doubtful	22	44	62
	Negative	56	79	1388
b) High concentration				
FM II	Positive	44	11	15
	Doubtful	24	55	101
	Negative	50	68	1333

late reactions (1 negative and 1 doubtful at day 3/4 readings). For 1% citral, 1 doubtful reaction turned to a positive one at the late reading; the same was observed for 2% citral and 10% AHCA. For the other individual constituents, no late reactions were observed.

ROAT

Results of ROAT's performed on 11 patients are summarized in Table 5. A positive result was obtained in 5 patients after a median of 7 days (range 2–10). The most sensitive patient reacting with a ++ reaction to 2.8% FM II developed a positive ROAT already within 2 days. He also had a certain fragrance history. The 5 patients with a doubtful or negative reaction to 28% FM II were all negative except 1. (According to the study protocol, in patient no. 4507, the ROAT should have been performed with 14% FM II instead of 2.8% FM II)

History of adverse reactions to fragrances, reactions to FM II and FM I

Details of these aspects have been described previously (1). In addition, test results with individual constituents and ROAT are of interest.

As Table 5 summarizes the 5 patients with a positive ROAT had a certain history in 3 cases, 1 was questionable and 1 had none. The FM I was negative or doubtful in 3 cases. Individual constituents were positive in 3 cases (Lyrall[®] 2 and farnesol 1).

Table 6 summarizes the details of 7 patients with a certain history, a positive reaction to either 14% FM II or 28% FM II and a negative reaction to FM I. All patients reacting to 14% FM II were also positive to 28% FM II, 3 patients also reacted to 2.8% FM II and 2 patients showed a doubtful reaction to this concentration. Break-down testing was positive for Lyrall[®] (2 positives and 1 recorded as IR), farnesol (3) and AHCA (1).

At 28% FM II, there were 2 more patients with a certain history and a negative reaction to FM I. Both tested negatively to the individual constituents had a + reaction to 28% FM II and a doubtful one to 14% FM II.

Product analysis

Table 7a summarizes the results of the analysis for the 6 target substances in a total of 24 products obtained from 12 patients. The majority of

Table 5. Repeated open application test (ROAT) – results of 11 patients and their reactions to fragrance mix (FM) I, FM II and individual constituents of FM II

Patient	History	FM II 28%	FM II 14%	FM II 2.8%	Reaction to IC	FM I 8%	ROAT conc. %	Pos./neg. (day)
6411	Certain	++	++	++	Lyrall [®] ++	++	2.8%	pos. (2)
5309	Certain	+	+	?	Farnesol +	?	2.8%	pos. (8)
5407	Certain	++	+	+	Lyrall [®] +	++	2.8%	pos. (7)
6292	None	+	?	–	–	–	28%	pos. (10)
508	Question	?	?	–	Lyrall [®] ?	–	14%	pos. (8)
4507	None	+	?	–	–	–	2.8%	neg. (13)
8401	None	+	+	?	Lyrall [®] +	–	2.8%	neg. (14)
474	Question	?	–	–	–	?	28%	neg. (14)
443	None	–	?	–	citral ?	?	14%	neg. (14)
451	Certain	?	?	–	–	–	14%	neg. (14)
476	None	–	–	?	Lyrall [®] +	–	2.8%	neg. (14)

Table 6. Patients with a certain history of adverse reactions to fragrances and a positive reaction to fragrance mix (FM II, but negative to FM I)

Patient (centre)	28% FM II	14% FM II	2.8% FM II	Reaction to IC
2896 (Copenhagen)	+	+	?	2.5% Lyr [®] ? 5% Lyr [®] IR
6258 (Dortmund)	++	+	+	2.5% Farnesol ? 5% Farnesol ++
6410 (Dortmund)	+++	+++	++	2.5% Lyr [®] ++ 5% Lyr [®] +++ 2.5% Farnesol + 5% Farnesol + 10% AHCA ?
67 (Leuven)	+	+	+	2.5% Lyr [®] ? 5% Lyr [®] +
94 (Leuven)	+	+	?	5% Farnesol +
27 (Leuven)	+	?	–	neg.
277 (Leuven)	+	?	–	neg.

patients had positive patch test reactions to Lyr[®] and citral, but there was also 1 to AHCA. These materials were identified by chemical analysis in 19 products (Lyr[®]), 4 products (citral) and 1 product (AHCA). Concentrations varied from 0.017 to 3.832% for Lyr[®], 0.011–0.142% for citral and 0.010–1.087% for AHCA. All patients had used their respective products and reported adverse reactions compatible with an allergic contact dermatitis. This was particularly evident in the patient who had used the deodorant containing AHCA and Lyr[®], respectively.

Table 7b summarizes the results of the analysis for all 6 individual constituents which could be identified in the 24 products. Citronellol was found in 87.5%, closely followed by Lyr[®] (79.2%) and AHCA (58.3%). While citral and coumarin were present in ½ of the patients' products, farnesol was not detected in any of the products (detection limit approximately 5 p.p.m. for all 3 isomers combined).

Associations with Myroxylon pereirae resin and colophonium

The data were analysed to consider whether reactions to FM I and FM II correlate with sensitivity to Myroxylon pereirae resin and colophonium. Every patient had been tested with the European standard series, which contains Myroxylon pereirae resin and colophonium. If there was a positive reaction to these materials, they were recorded on the case report form (?/IR reactions were counted as negative).

There was a strong association between FM I and Myroxylon pereirae resin (odds ratio 15.9,

exact 95% confidence interval (CI): 8.0–31.1). The association between Myroxylon pereirae resin and FM II was weaker: OR 4.6, 95% CI 1.3–12.4 (14%) and OR 4.8, 95% CI 1.7–11.4 (28%). In contrast, no significant association between colophonium and either FM I or FM II was found.

Discussion

The major outcome of this study is that the results of break-down testing of the new mix are in a range of 48% to 54% for 14% FM II and 28% FM II, respectively (74% and 70%, respectively, if doubtful reactions to individual constituents are included). If the test concentration of the individual constituents is doubled, the yield may reach 72%, as the results with the group of patients positive to 14% FM II have shown. Conversely, a positive reaction to 1 of the individual constituents is nearly always associated with a positive reaction to the FM II. In other words, the FM II rarely gives a false-negative reaction, if a contact allergy to one of the individual constituents is present. This is not always the case and is a major criticism of mixes, as reported for the thiuram and mercapto mixes (9).

The problem of testing with the individual constituents of FM I has been the subject of several studies (10–14). Taken together, about 50% of the FM-allergic patients reacted to at least 1 of the constituents. On the basis of 4900 patients evaluated in the German network, it became evident that the positivity rate of individual constituents is dependent on the strength of the patch test reaction to 8% FM I (14). In this study, reactions to individual constituents were as

Table 7a. Chemical analysis of patients' products and results of patch testing with the individual constituents of FM II

Centre (patients)	Products (type)	Analysis positive (concentration %)	Patch test positive (patients)
Copenhagen 4	6 products (2 D, 1 EdT, 3 L)	Lyrall [®] 5 (0.017–0.78) cital 2 (0.011–0.015)	Lyrall [®] 4 cital 2
Leuven 3	7 products (4 FP, 2 EdT, 1 L)	Lyrall [®] 6 (0.065–3.677)	Lyrall [®] 3
London 2	2 products (2 D)	AHCA 1 (0.027) Lyrall [®] 1 (0.057)	AHCA 1 Lyrall [®] 1
Dortmund 3	9 products (6 EdT, 3 FP)	Lyrall [®] 7 (0.083–3.832) cital 2 (0.028–0.044)	Lyrall [®] 3 cital 1
Total 12	24 products	Lyrall [®] 19 cital 4 AHCA 1	Lyrall [®] 11 cital 3 AHCA 1

Product type: D = deodorant; EdT = Eau de Toilette; FP = fine perfume; L = lotion, cream.

follows: 39% for + reactions to FM I, 69% for ++ reactions and 93% for +++ reactions. In our study, these proportions were 7/31, 13/15 and 4/4 for 14% FM II and 14/46, 21/21 and 3/3 for 28% FM II, respectively. Hence, a similar relationship was found, but with a higher proportion of positive individual constituent reactions in the case of ++ or +++ FM reactions.

As has been discussed by others, allergens in combination may have additive or synergistic effects on the elicitation response (15, 16). Johansen et al. (16) demonstrated that, if a patient is highly sensitized to hapten A and moderately sensitized to hapten B, reactivity to the combined application of the allergens will result in a much stronger biological response compared to those obtained by separate testing. In cases of low levels of sensitization to 2 or even more constituents of the mix, this can amount to a weak-positive reaction to the mix. If the individual constituents are then tested separately, they may be completely negative or rated as ?/IR. The problems of patch test reading in this difficult 'grey zone' have been extensively discussed previously (1).

The higher gain of break-down testing with individual constituents at doubled concentration, as observed in our study, confirms the concept of

synergistic effects in a mix (15, 16). It may be advisable in general to test the individual constituents of a mix at a higher concentration than those present in its mix. Dose-response studies in an even more elaborate way than those performed here may be necessary to determine the appropriate test concentration of a new mix and its individual constituents. Break-down testing of the Larsen mix FM I is usually performed with the individual constituents at 1% in petrolatum – the concentration is the same as in the mix. The mix contains sorbitan sesquioleate as an emulsifier. Addition of sorbitan sesquioleate to the individual constituents increases significantly the yield of positive break-down testing (38.3% versus 54.8%), but also frequently produces irritant or doubtful reactions, probably due to penetration-enhancing effects (10). The American Contact Dermatitis Group uses higher concentrations of the individual constituents of FM I in order to avoid false-negative results (4% to 5% with the exception of cinnamal at 1%) (17). This concept has been promoted already by de Groot et al. (18) on the basis of a study in the Netherlands. The new FM II does not contain sorbitan sesquioleate, but hard paraffin has been added for improved dispersion and stability. All individual constituents were dissolved in petrolatum only. This aspect might have influenced the test results. However, we think that our data provide enough evidence that the new fragrance mix should be tested at 14%, and the break-down be performed with the individual constituents of 28% FM II. Because of its pre-eminent role as a leading perfume allergen, we recommend testing 5% Lyrall[®] in addition to the 14% FM II, because a small proportion of patients might be missed with 2.5% Lyrall[®] as present in the mix (2 patients positive to 5% Lyrall[®] showed a doubtful reaction to 14% FM II, and 1 patient was negative). Monitoring of data on an international scale will show

Table 7b. Individual constituents of fragrance mix (FM) II identified in 24 products (detection limit of all individual constituents except farnesol 2 p.p.m.; detection limit of farnesol (3 isomers combined) 5 p.p.m.; n.d., not detected)

	Identified (%)	Concentration (range)
Lyrall [®]	19/24 (79.2%)	0.017–3.832
Citronellol	21/24 (87.5%)	0.031–1.022
AHCA	14/24 (58.3%)	0.010–1.087
Cital	12/24 (50.0%)	0.011–0.142
Coumarin	12/24 (50.0%)	0.002–0.140
Farnesol	n.d.	n.d.

AHCA = α -hexyl-cinnamic aldehyde.
All products contained at least 1–5 IC.

whether this procedure is necessary or can be simplified by testing only the 14% FM II for screening.

The results of the ROAT are particularly helpful in this context. If there is a strong ++ reaction to 28% FM II and at least a + reaction to 14% FM II, it is very likely that the ROAT will be positive. If reactions to FM II are doubtful even at the highest test concentrations, it is unlikely that open application will result in an allergic contact dermatitis. Although we could include only 11 patients in the ROAT study and conclusions therefore remain limited, this view is confirmed by findings with FM I and individual allergens (10, 19, 20).

As Johansen et al. (7) have recently demonstrated with Lyrall[®] and Svedman et al. (20) with hydroxycitronellal, there is a clear dose–response relationship between the reactivity on patch testing and after repeated open application.

While oak moss (*Evernia prunastri* extract) and isoeugenol are the major individual constituents contributing to a positive reaction for FM I, Lyrall[®] is the dominant constituent of FM II. Reactions to Lyrall[®] were more frequent than those obtained with the 5 remaining individual constituents. While Lyrall[®], citral, farnesol and AHCA caused + and ++ reactions, citronellol produced only a few + reactions. Coumarin remained completely negative except for a few doubtful reactions. This is somewhat surprising because coumarin has been reported as an allergen (21, 22) and in a recent study on 658 patients with hand eczema, it was found to be positive in 0.5% (23). These authors suggested coumarin to be a candidate for a special ‘hand eczema series’; in this study, the most common reactions to fragrances not included in the FM I were to citral (4.3%), Lyrall[®] (2.1%) and oxidized L-limonene (0.9%). Citral was tested at 3 concentrations (2%, 1% and 0.5%) and showed a clear dose–response relationship. Citral may cause allergic and irritant reactions in this concentration range. If patients had a definitely positive reaction to citral ($\geq +$), they showed a significantly higher reactivity to other fragrances as compared to those patients with ?/IR reactions to citral (24). Therefore, reactions to citral must be interpreted with great caution. Only if there is a proven exposure to citral and a convincing link by history (elicitation or aggravation of a contact dermatitis), can a patient then be regarded as hypersensitive to citral. A positive ROAT and/or the reproduction of a positive patch test after some weeks can be of further support.

In 1989, the European Environmental and Contact Dermatitis Research Group had tested the ‘Hausen mix’ in 2455 consecutive patients and

found citral and dihydrocoumarin to be the most frequent positive individual constituents next to isoeugenol and cinnamal of the Larsen mix (0.53% and 0.45% positives, respectively) (25).

Farnesol is certainly of great diagnostic value and probably many cases of deodorant contact allergy have been missed because farnesol is not present in the FM I (26). It is added to many deodorants for its antimicrobial activity and often listed in these products as an ingredient next to ‘parfum’. In a German multicentre study on 2021 consecutive patients, 5% farnesol was positive in 1.1% of the patients (27). The decision of the European Commission to include farnesol into the list of 26 fragrance materials to be labelled in the near future for consumer protection (28) is therefore supported by our data as well as those of Schnuch et al. (27).

The results of chemical analysis of the patients’ products confirmed that some of the materials tested in the FM II were indeed present. Patients patch test positive to Lyrall[®], citral and alpha hexyl cinnamic aldehyde were exposed to these chemicals by cosmetics of various types and in all likelihood sensitized by those or similar products. The patient with a positive reaction to alpha hexyl cinnamic aldehyde had developed an axillary dermatitis to a deodorant [Superdrug anti-perspirant (Superdrug, Croydon, Surrey, UK)] which contained this chemical at a concentration of 0.027%. While this patient from London had reacted only to 10% hexyl-cinnamic aldehyde and not to 5%, the 2nd patient reacting to hexyl-cinnamic aldehyde was seen in Leuven with ++ reactions to both test concentrations. He had a certain fragrance history and also showed positive reactions (+) to citral and citronellol. We are therefore convinced that hexyl-cinnamic aldehyde may be an important perfume allergen in individual cases. The apparently high discrepancy between the concentration in the deodorant eliciting a dermatitis in the axilla and the much higher concentration in the patch test material producing a positive reaction on the back is well known from quantitative studies with other sensitizers and can be explained by a number of factors influencing the biologic response (7, 29).

Lyrall[®] has been described before as an important sensitizer chemically identified in aftershaves, eaux de toilettes and deodorants (6). It is therefore not surprising that we have confirmed this observation in a different group of patients in 4 European centres. The concentration of Lyrall[®] in consumer products was again found to be high (up to 3.8%). Manufacturers and the International Fragrance Research Association (IFRA) have accepted that these use concentrations are too high

and have therefore recently recommended usage levels below 1.5% in finished cosmetic products (30). The scientific advisory committee to the European Commission (SCC NFP) has recommended a maximum concentration of 0.02%, based upon available elicitation data (31).

It is frequently stated that Myroxylon pereirae resin should be a good marker of perfume sensitivity. Recent publications dispute this (32, 33). Our data confirm that there is a statistically significant relation between reactivity to Myroxylon pereirae resin and FM I, which may be explained by coexposure rather than cross-reactivity in a strict sense. In contrast, there was only a weak association Myroxylon pereirae resin – FM II. This is not surprising because the 6 single constituents of FM II are not found in Myroxylon pereirae resin except for farnesol in small amounts (34). Regarding colophonium, no significant association at all was found in contrast to the observations of Wöhrle et al. (11).

If more patients with a suspected contact allergy to fragrances are to be identified by patch testing, the search to identify chemicals as sensitizers must continue (35). The 14% FM II may be a major step forward but its performance will need to be monitored in all geographical areas. It is very likely that the FM II will need to be modified or supplemented by another mix in the near future. The fragrance industry is a dynamic, lucrative and competitive business – many new chemicals are developed and launched onto the consumer market with limited safety data (36).

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