

Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix

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The currently used 8% fragrance mix (FM I) does not identify all patients with a positive history of adverse reactions to fragrances. A new FM II with 6 frequently used chemicals was evaluated in 1701 consecutive patients patch tested in 6 dermatological centres in Europe. FM II was tested in 3 concentrations – 28% FM II contained 5% hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall[®]), 2% citral, 5% farnesol, 5% coumarin, 1% citronellol and 10% α -hexyl-cinnamic aldehyde; in 14% FM II, the single constituents' concentration was lowered to 50% and in 2.8% FM II to 10%. Each patient was classified regarding a history of adverse reactions to fragrances: certain, probable, questionable, none. Positive reactions to FM I occurred in 6.5% of the patients. Positive reactions to FM II were dose-dependent and increased from 1.3% (2.8% FM II), through 2.9% (14% FM II) to 4.1% (28% FM II). Reactions classified as doubtful or irritant varied considerably between the 6 centres, with a mean value of 7.2% for FM I and means ranging from 1.8% to 10.6% for FM II. 8.7% of the tested patients had a certain fragrance history. Of these, 25.2% were positive to FM I; reactivity to FM II was again dose-dependent and ranged from 8.1% to 17.6% in this subgroup. Comparing 2 groups of history – certain and none – values for sensitivity and specificity were calculated: sensitivity: FM I, 25.2%; 2.8% FM II, 8.1%; 14% FM II, 13.5%; 28% FM II, 17.6%; specificity: FM I, 96.5%; 2.8% FM II, 99.5%; 14% FM II, 98.8%; 28% FM II, 98.1%. 31/70 patients (44.3%) positive to 28% FM II were negative to FM I, with 14% FM II this proportion being 16/50 (32%). In the group of patients with a certain history, a total of 7 patients were found reacting to FM II only. Conversely, in the group of patients without any fragrance history, there were significantly more positive reactions to FM I than to any concentration of FM II. In conclusion, the new FM II detects additional patients sensitive to fragrances missed by FM I; the number of false-positive reactions is lower with FM II than with FM I. Considering sensitivity, specificity and the frequency of doubtful reactions, the medium concentration, 14% FM II, seems to be the most appropriate diagnostic screening tool.

Key words: citral; citronellol; contact allergy; coumarin; farnesol; fragrance mix; fragrances; Lyrall[®]; patch testing; α -hexyl-cinnamic aldehyde. © Blackwell Munksgaard, 2005.

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The currently used fragrance mix (FM I), as introduced by Larsen in 1977 (1), contains only 8, albeit still major constituents, of perfumes. In a perfume, there may be over 100 different chemicals and many of these have a sensitizing poten-

tial [reviewed by de Groot and Frosch (2)]. It has been estimated that at least 15% of relevant cases of perfume allergy are not recognized by patch testing with the FM I (3). Recently, hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall[®]) has

been identified as an important sensitizer in patients sensitive to fragrances (4, 5). Furthermore, in a European multicentre trial with 14 frequently used chemicals the 'top 6' sensitizers within this series were Lyrall[®], citral, farnesol, citronellol, α -hexyl-cinnamic aldehyde (AHCA) and coumarin (6). The aim of the present study was to evaluate a new mix, FM II, containing these materials. Key questions were: Does the FM II identify additional patients with a positive fragrance history missed by FM I? Is it necessary to add FM II to the European standard series?

Materials and Methods

Test materials

FM II was prepared in 3 test concentrations (Table 1). The highest concentration of the mix (28%) resulted from the cumulative concentrations of the 'top 6' as used in the previous study (6). In order to reduce the frequency of possible irritant reactions, 2 lower test concentrations (14% and 2.8%) were evaluated and found to be suitable in pilot studies performed in Dortmund (270 patients) and Copenhagen (330 patients). The constituents were purchased from major manufacturers: *dl*-Citronellol (CAS 106-22-9), citral (CAS 5392-40-5), coumarin (CAS 91-64-5) and farnesol (CAS 4602-84-0) from Dragoco/Symrise (Holzminden, Germany); Lyrall[®] (CAS 31906-04-4) and AHCA (CAS 101-86-0) from International Flavors and Fragrances (New York, NY, USA). High-purity chemicals as used for the production of fine perfumes and eaux de toilettes were used. Petrolatum was chosen as the vehicle. In order to prevent phase separation, especially with 28% FM II and 14% FM II, hard paraffin was added. Stability of the mix and the constituents was followed for a total of 24 months. At a temperature of 6°C, stability of both the mixes in all 3 concentrations and the single constituents (SC) was excellent; i.e. the concentration was still more than 90% after 24 months. Because of phase separation at 25°C, sta-

bility of 28% FM II was followed for 6 months only. Stability at 25°C for 14% FM II was analysed for only 9 months, because the concentration of Lyrall[®] in the mix at that time was less than 80% of the original 2.5% concentration. All materials were kindly provided and prepared by Hermal (Reinbek, Germany). Hermal also performed the quality control investigations (data on file).

For comparison with the current FM a new batch of the standard 8% (in petrolatum) was used in each test centre, as provided by Hermal (Reinbek, Germany).

Patients and test centres

Consecutive patients attending contact dermatitis clinics at 6 dermatology departments were tested between October 2002 and June 2003 (Dortmund, Copenhagen, Malmö, Odense, London and Leuven). In addition to the standard series, all 3-concentrations of FM II and the SC of 28% FM II and 14% FM II were applied to the skin of the back for 2 days. In all centres, Finn ChambersTM on Scanpor[®] tape (Epitest, Tuusula, Finland) were used. Readings were taken at most centres on day 2 and 4. The second reading, usually at day 3 or 4, was used for the overall evaluation of positive test results. The reactions were categorized according to published guidelines (7).

History of fragrance sensitivity

Before patch testing, each patient was placed in 1 of 4 categories according to history of adverse reactions to perfumed products as follows: (i) certain: has reacted with an itching dermatitis to at least one fine perfume or aftershave and has reacted to other perfumed products; (ii) probable: has reacted to one or more perfumed products (e.g. deodorant), but a certain perfume has not been identified as the cause of a clinical reaction; (iii) questionable: has reacted to various cosmetics with or without fragrances; materials other than fragrance constituents may be the cause of reaction; and (iv) none: has never reacted to a perfumed material.

Further patient's data

Further information on each patient included age, sex, location of eczema, clinical diagnosis, atopic dermatitis (current, previously, never), hay fever and asthma.

Statistics

Data was entered from the case record form into an MS AccessTM database utilizing plausibility

Table 1. The compositions of the fragrance mix II (FM II) at 3 different concentrations

	FM II (%)	FM II (%)	FM II (%)
Citronellol	1	0.5	0.1
Citral	2	1	0.2
Coumarin	5	2.5	0.5
Lyrall [®]	5	2.5	0.5
Farnesol	5	2.5	0.5
α -Hexyl-cinnamic aldehyde	10	5	1
Total	28	14	2.8

Vehicle petrolatum.

checks to enhance data quality. Additionally, data was double entered and compared; possible inconsistencies were corrected based on the original case record form. Beyond data entry, management and analysis of validated data was performed using the SASTM software package (version 8.2, SAS Institute, Cary, NC, USA).

Descriptive statistical analyses included Cohen's kappa as a standard measure to quantify agreement beyond chance. Differences in the pattern of dichotomized patch test results (negative versus positive) were statistically analysed with the exact McNemar test; quadratic contingency tables larger than 2×2 with the Bowker test. *P*-values < 0.05 were considered significant. In view of an exploratory approach to analysis, *P*-values were not adjusted for multiple testing.

Results

In the 6 centres, 1701 patients were included in the study (range 234–375 per centre). 2 patients were not tested with FM I. Important demographic characteristics are shown in Table 2.

In all centres, the main location of eczema was the hands (approximately 40%). In London, the largest proportion of patients with no fragrance history was observed. Patch test results obtained with both types of FM are shown in Table 3 and in Fig. 1. Positive reactions to the FM II (14%) varied significantly with the anatomical site of dermatitis ($P = 0.023$, 99% CI: 0.019–0.027, Monte Carlo estimate of exact likelihood ratio chi-square test) with an above average frequency of positive reactions in generalized, axillary and face dermatitis.

The large number of reactions classified as doubtful/irritant (?/IR) seen with FM II was analysed with regard to a change to positive at higher concentrations. This profile is shown in Fig. 2. At 2.8% FM II, a reversal from doubtful to positive at 14% FM II was observed in nearly 40% of cases, whereas the corresponding figure for the medium and highest concentration of FM II was only 10%. The majority of doubtful reactions (85%) seen at 14% FM II remained as such even when the test concentration was raised to 28% FM II. The cross tabulation of reactions to medium versus high concentration of FM II is shown in Table 4a. There was significant asymmetry (Bowker test), i.e. there were significantly more positive reactions with both higher concentrations, related to the lower one. The gain was particularly high for the comparison between 2.8% and 14% FM II.

Table 4b compares the reactions to FM II and I. There were significantly more positive

and doubtful reactions, respectively, to FM I than to FM II in both concentrations further considered (14% and 28%). If the doubtful reactions are counted as negative, a total of 31/70 (44.3%) patients positive to 28% FM II were negative to FM I; for 14% FM II this proportion was 16/50 (32%).

Table 5 shows the reactions to FM I and II in relation to the history of adverse reactions to fragrances. 111 patients were positive to FM I; of these 37 had a certain and 21 a probable history; of all patients with a certain and probable history, the FM I detected 25.2% and 15.3%, respectively. The corresponding figure for the patients reacting to FM I without any fragrance history (false positive) was 45 (3.5%). Based on the total number of FM I-positive patients, however, the proportion of patients without a fragrance history was quite high: 45/111 (40.54%).

Patients with a certain fragrance history reacted positively to FM II with increasing test concentration: 8.1%, through 13.5% to 17.6%. The corresponding figures for FM II-positive patients without a fragrance history were lower than with FM I (0.5% to 2.0%). In relation to the total number of positive reactions to FM II, the percentage of patients with no history was also lower than with FM I: 27.3% for 2.8%; 32% for 14%; and 35.7% for 28% FM II. If patients with questionable (doubtful) reactions are considered, the proportion with a certain fragrance history ranges between 7.5% (FM I) and 18.2% (FM II 28%).

In Table 6, the test results with both types of mixes are related to 2 distinctly different categories of history (certain and none), in order to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). For FM I, sensitivity is 25.2% and specificity 96.5%. The corresponding figures for FM II are concentration dependent and range from 8.1% to 17.6% (sensitivity) and 99.5% to 98.1% (specificity), respectively. PPV, i.e. the proportion of test-positive patients with a certain history of fragrance allergy, was highest for 2.8% FM II with 66.7%; NPV, i.e. the proportion of test-negative patients with no history of fragrance allergy, was lowest for the same test material with 90.4%.

In Table 7 a cross tabulation of FM I and II (all 3 concentrations) test results, stratified for 3 types of history of adverse reactions to fragrances, is presented. 'Any' type of history aggregates certain, probable and questionable into 1 group. FM I detects significantly more patients with a certain and 'any' type of fragrance history than FM II (McNemar test). However, this comparison also demonstrates that the proportion of positive reactors in the group with no history is

Table 2. Important patient characteristics, per centre and total

	Copenhagen (n = 375)	Dortmund (n = 237)	Leuven (n = 299)	London (n = 303)	Malmö (n = 234)	Odense (n = 253)	Total (n = 1701)
Female (%)	64.9	56.1	64.6	59.4	62.1	64.8	62.2
Age (min/median/max)	18/47/94	18/48/93	18/42/96	14/39/87	13/43/88	18/43/85	13/44/96
Location of eczema (%)							
Hands	38.6	39.7	30.4	36.3	47.2	48.6	39.6
Face	14.1	18.1	18.1	23.4	16.2	11.9	17.0
Axillae	1.3	0	1.7	2.6	1.7	0	1.3
Feet	2.4	3.8	6.7	3.6	2.6	4.4	3.9
Universal	25.8	8.0	13.4	19.5	15.3	17.8	17.4
‘Other’	17.8	30.4	29.8	14.5	17.0	17.4	20.9
History of atopy (%)							
Atopic eczema, current	12.8	26.2	18.1	23.1	23.4	11.5	18.7
Atopic eczema, previously	6.1	2.5	6.4	9.6	1.7	5.1	5.5
Hay fever, current	22.1	18.1	19.4	29.0	9.8	11.1	19.0
Hay fever, previously	0.5	0	2.3	0	0.4	1.6	0.8
Asthma, current	12.0	3.8	6.7	17.5	17.5	5.1	10.6
Asthma, previously	0.5	0	2.7	0	0	0.8	0.7
History of adverse reaction to fragrances (%)							
Certain	6.9	14.8	9.0	1.3	11.1	11.9	8.7
Probable	8.8	6.8	7.0	5.0	14.5	7.9	8.2
Questionable	14.1	0.8	8.7	6.6	4.7	8.3	7.8
None	70.2	77.6	75.3	87.1	69.8	71.9	75.3

Table 3. Patch test results with fragrance mixes (FMs), total and per centre, additionally stratified for sex. Outcome at D 3/4 reading

Centre	Female			Male			All		
	N-tested	+ to +++	?/IR	N-tested	+ to +++	?/IR	N-tested	+ to +++	?/IR
(a) FM I (8% pet.)									
Copenhagen	244	19	26	131	2	11	375	21	37
Dortmund	133	5	6	104	7	6	237	12	12
Leuven	193	17	3	106	4	0	299	21	3
London	180	7	3	122	8	2	302	15	5
Malmö	145	15	6	89	4	2	234	19	8
Odense	163	16	44	89	7	14	252	23	58
Total	1058	79 (7.5%)	88 (8.3%)	641	32 (5.0%)	35 (5.5%)	1699	111 (6.5%)	123 (7.2%)
(b) FM II (2.8% pet.)									
Copenhagen	244	2	10	132	0	2	376	2	12
Dortmund	133	1	0	104	1	1	237	2	1
Leuven	193	6	4	106	0	2	299	6	6
London	180	3	1	122	1	1	302	4	2
Malmö	146	2	2	89	1	0	235	3	2
Odense	163	4	5	89	1	3	252	5	8
Total	1059	18 (1.7%)	22 (2.1%)	642	4 (0.6%)	9 (1.4%)	1701	22 (1.3%)	31 (1.8%)
(c) FM II (14% pet.)									
Copenhagen	244	6	34	132	0	19	376	6	53
Dortmund	133	1	3	104	3	4	237	4	7
Leuven	193	13	4	106	2	2	299	15	6
London	180	4	5	122	3	2	302	7	7
Malmö	146	9	3	89	1	3	235	10	6
Odense	163	6	32	89	2	17	252	8	49
Total	1059	39 (3.7%)	81 (7.6%)	642	11 (1.7%)	47 (7.3%)	1701	50 (2.9%)	128 (7.5%)
(d) FM II (28% pet.)									
Copenhagen	244	7	50	132	0	25	376	7	75
Dortmund	133	3	6	104	4	8	237	7	14
Leuven	193	15	3	106	3	1	299	18	4
London	180	6	8	122	4	4	302	10	12
Malmö	146	13	9	89	3	4	235	16	13
Odense	163	10	37	89	2	25	252	12	62
Total	1059	54 (5.1%)	113 (10.7%)	642	16 (2.5%)	67 (10.4)	1701	70 (4.1%)	180 (10.6%)

?/IR, doubtful/irritant reaction.

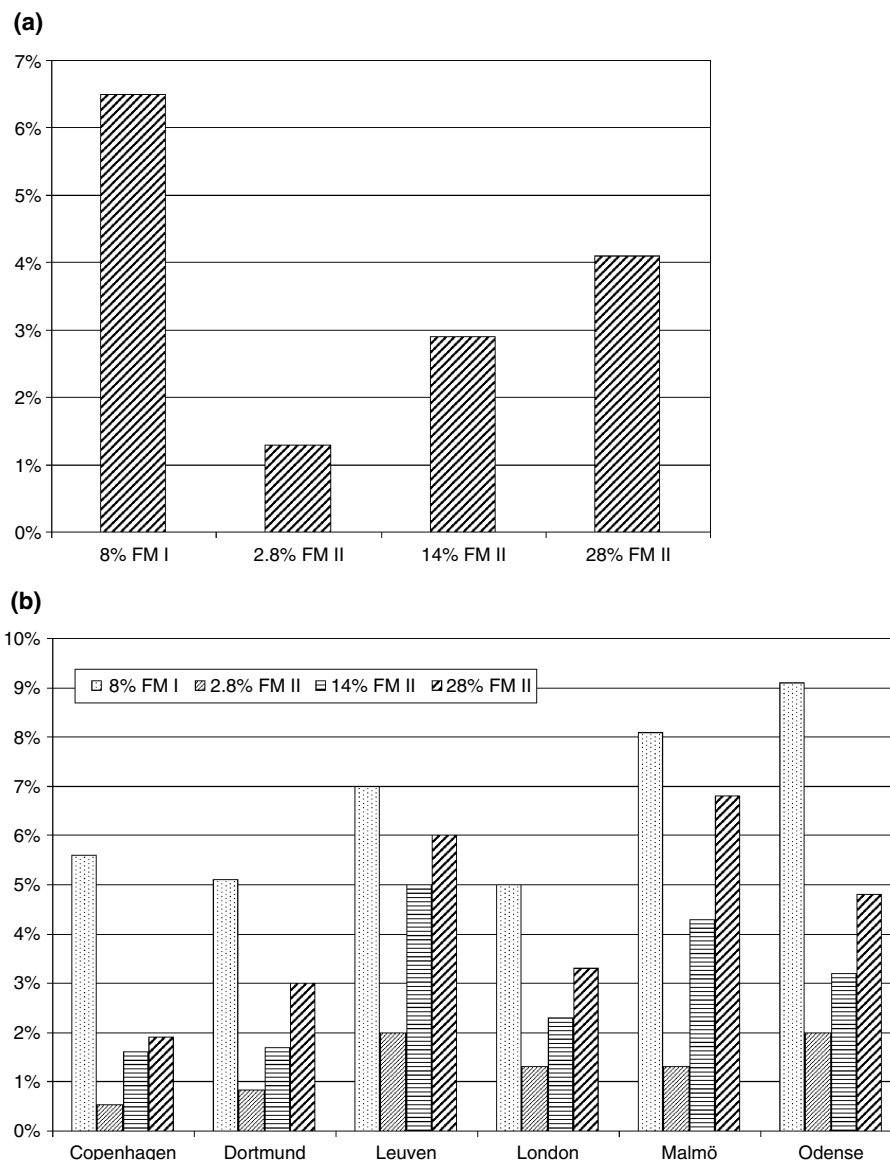


Fig. 1. (a) Positive reactions (in percentage) to fragrance mix I (FM I) and fragrance mix II (FM II) in 1701 patients of all 6 centres; (b) Positive reactions to FM I and FM II in the individual test centres.

significantly higher for FM I than for FM II (all 3 concentrations). Concordance between FM I and II is rather limited at the lowest FM II concentration (Kappa values ranging from 0.23 to 0.28) but considerably higher in the 2 subgroups with a history of fragrance allergy, though not in the subgroup without, when the FM II concentration is increased. In Table 7, it is also evident that there are patients with a certain fragrance history who are negative to FM I but positive to FM II namely 3, 5 and 7 in ascending test concentration (in relation to 148 patients with a certain history, the additional gain by testing with the FM II is 2.0%, 3.4% and 4.7%, respectively).

Discussion

These results show that the major aims of the study have been accomplished. The FM II detects additional patients with a certain history of adverse reactions to fragrances which would have been missed by the 'old' FM I. As the reactivity to FM II was above 1% for all 3 concentrations, it is justified to include this diagnostic tool into the European standard series. Although the proportion of sensitized patients detected beyond the FM I seems to be low (with 14% FM II, 4.5% of FM I-negative patients with a certain history and 2.6% of FM I-negative patients with 'any' fragrance history), this is of importance in the individual

Table 4a. Cross tabulation of reactions to medium versus high concentration of fragrance mix (FM) II at D 3/4

Low concentration		Next higher concentration		
		Negative	Doubtful	Positive
FM II, 2.8%	Negative	1521	111	16*
	Doubtful	2	17	12
	Positive	0	0	22
FM II, 14%	Negative	1445	70	8*
	Doubtful	6	109	13
	Positive	0	1	49

*Significant asymmetry (Bowker test), i.e. significantly more positive reactions with both higher concentrations, related to the lower one.

case. The 3rd question regarding the major sensitizers in the mix will be answered in a subsequent publication on the results of the break-down testing with the SC (9). Some aspects will be discussed in detail.

History of adverse reactions to fragrances

As commented upon in 2 previous papers of our group (6, 10), taking a history regarding fragrance reactions is associated with a degree of uncertainty. Not all patients with self-assessed 'certain history' have a real contact allergy to 1 or more fragrances. Reactions to a perfume in the past may have been due to a preservative, colour or fixative – irritancy is yet another possibility. However, most such patients will turn out to be allergic to a perfume ingredient, if studied intensively and followed for some time [e.g. repeated open application test for a minimum of 2 weeks (11–13)]. The frequency of patients who gave a certain history in this study (8.7%) is slightly higher than in 2 previous multicentre trials before [6.6% (6), 6.0% (10)]. As Table 1 demonstrates, there was considerable variation among the cen-

tres, which could reflect real regional differences or bias of the person taking the history.

A major criticism of Larsen's FM I is the relatively high number of 'false positives', i.e. a positive reaction is not linked to any history. This has also been found in our study, with a PPV of 45% for the FM I, i.e. 55% 'false-positive' reactions. As also discussed in detail previously (6), these patients often have a low-grade sensitization and do not react to commercial products with a relatively low concentration of perfume ingredients. If, however, such a patient is exposed repetitively over a longer period of time with the allergen in increasing concentrations, that individual might react with an allergic contact dermatitis – hence, the history may be regarded as false negative, and not the patch test result as false positive in these patients. These quantitative aspects have been neglected for a long time, but have increased our understanding of clinical observations considerably (14). Nevertheless, with regard to the FM II it is an advantage over the FM I that the number of positive reactions in patients without a fragrance history is significantly lower than those of FM I (Table 7).

Sensitivity and specificity

Although the history classification is not ideal, we have tried to use the 2 extremes – certain and none – as the basis for calculating sensitivity and specificity. Values for sensitivity seem very low (27.2% for FM I and 8.7%–21.5% for FM II). This is in sharp contrast to the experience of de Groot et al. (3), who estimated that only 15% of patients with a relevant contact allergy to fragrances were missed by the FM I. A major reason for this might be the change in perfume ingredients over time, as has been documented by a recent study (15). The 8 constituents of FM I are still widely used and represent the most frequent sensitizers in the fragrance group. Therefore, it is not surprising that FM I still identifies many patients with a certain history and is superior overall to FM II. The good concordance between reactions to FM I and II at the medium and highest concentration

Table 4b. Comparison of reactions to fragrance mix (FM) I (8% pet.) and FM II (14% and 28% pet., respectively) in 1699 patients (2 patients not tested with the FM I but FM II not considered).

	FM I, 8%			Total (FM II)
	Positive	Doubtful	Negative	
FM II, 14%				
Positive	34	4	12	50
Doubtful	21	41	65	127
Negative	56	78	1388	1522
FM II, 28%				
Positive	39	7	24	70
Doubtful	23	52	104	179
Negative	49	64	1337	1450
Total (FM I)	111	123	1465	1699

Significant asymmetry ($P < 0.0001$, Bowker test), i.e. significantly more doubtful and positive reactions, respectively, to the FM I than to the FM II in both concentrations.

Table 5. Reactions to the fragrance mix (FM) I and the FM II in its 3 concentrations related to the history of fragrance allergy (n, % of history category); 1701 patients, 2 patients not tested with FM I

		History			
		Certain	Probable	Questionable	None
FM I 8%	Positive	37	21	8	45
		25.3	15.3	6.0	3.5
	Doubtful	11	17	20	75
		7.5	12.4	15.0	5.9
	Negative	99	99	105	1162
		67.4	72.3	79.0	90.6
FM II 2.8%	Positive	12	4	0	6
		8.1	2.9	0.00	0.5
	Doubtful	10	7	1	13
		6.8	5.1	0.8	1.0
	Negative	126	126	132	1264
		85.1	92.0	99.3	98.5
FM II 14%	Positive	20	13	1	16
		13.5	9.5	0.8	1.3
	Doubtful	22	14	17	75
		14.9	10.2	12.8	5.9
	Negative	106	110	115	1192
		71.6	80.3	86.5	92.9
FM II 28%	Positive	26	15	4	25
		17.6	11.0	3.0	2.0
	Doubtful	27	15	22	116
		18.2	11.0	16.5	9.0
	Negative	95	107	107	1142
		64.2	78.1	80.5	89.0

(Table 7), observed only in the subgroup of patients with 'any' or 'certain' history, may be indicative of a co-exposure to ingredients of both mixes in fragrance materials that have caused sensitization. This supports the concept of testing with both the FM I and the FM II.

However, a certain group of patients has isolated sensitizations to 1 of the constituents of the new mix and does not react to FM I. As will be shown in detail in our subsequent publication, Lyrall[®], citral and farnesol are the most frequently found sensitizers when the mix is split. At that time, the

7 patients with a certain history, positive reaction to FM II and no reaction to FM I will also be discussed in detail.

Doubtful patch test reactions

As Table 3 shows, the number of reactions classified as ?/IR varied considerably among the centres. In Odense and Copenhagen, this proportion was consistently highest and ranged between 10% and 25% for FM I and II. In these departments, anything different from normal skin at the patch test site is recorded: slight erythema (diffuse or spotty) with or without papules are scored as 'follicular' or 'doubtful'. In other departments, these might be ignored ('negative') or read also as ?/IR. The 2 Danish departments are extremely restrictive regarding the requirements for a + reaction: in addition to erythema, there must be homogeneous palpable infiltration. Erythema and a few papules would not be sufficient for a + reaction. This practice is not followed by the other investigators of this study and probably explains most of the between-centre variation, i.e. why the frequencies of positive reactions are slightly higher and the number of ?/IR much lower in these centres. The original ICDRG reading scale defines a + reaction as a 'weak (non-vesicular) reaction' (16). Fregert's modification (17) is more specific: 'erythema, infiltration, possibly papules'; but according to him 'homogeneous palpable infiltration' is not required.

The criterion 'palpable infiltration' is a difficult matter to standardize and will always be a source of individual variation and dispute. Although considerable progress has been made with bio-engineering techniques (transepidermal water loss and blood flow), these have not yet reached sufficient reliability and validity for routine use (18, 19).

As the cross tabulation of reactions to medium versus high concentration of FM II shows

Table 6. Diagnostic properties of the fragrance mix (FM) I and FM II in its 3 concentrations, assessed towards 'certain' versus 'no' history of fragrance allergy as gold standard.

		History = certain		History = none				
	FM+	FM–	FM+	FM–	Sens (95% CI)	Spec (95% CI)	PPV (95% CI)	NPV (95% CI)
FM I (8%)	37	110	45	1237	25.2% (18.4–33.0%)	96.5% (95.3–97.4%)	45.1% (34.1–56.5%)	91.8% (90.2–93.2%)
FM II (2.8%)	12	136	6	1277	8.1% (4.3–13.7%)	99.5% (99.0–99.8%)	66.7% (41.0–86.7%)	90.4% (88.7–91.9%)
FM II (14%)	20	128	16	1267	13.5% (8.5–20.1%)	98.8% (98.0–99.3%)	55.6% (38.1–72.1%)	90.8% (89.2–92.3%)
FM II (28%)	26	122	25	1258	17.6% (11.8–24.7%)	98.1% (97.1–98.7%)	51.0% (36.6–65.2%)	91.2% (89.5–92.6%)

Sens = Sensitivity; Spec = Specificity; PPV = positive predictive value; NPV = negative predictive value.

Doubtful reactions regarded as negative (patients with 'probable' or 'questionable' history excluded). Proportions supplemented with an exact 95% confidence interval (CI)

Table 7. Additional diagnostic value of the fragrance mix (FM) II in its 3 concentrations in relation to FM I reactivity, stratified for 'certain' and 'any', respectively, versus 'no' history of fragrance allergy.

		History = 'certain'			History = 'none'			History = 'any'		
		FM I positive	FM I negative	Kappa (95% CI)	FM I positive	FM I negative	Kappa (95% CI)	FM I positive	FM I negative	Kappa (95% CI)
FM II	positive	9	3	0.28	6	0	0.23	13	3	0.27
2.8%	negative	28*	107	(0.11–0.45)	39*	1237	(0.08–0.38)	53*	348	(0.15–0.40)
FM II	positive	15	5	0.42	9	7	0.28	25	9	0.44
14%	negative	22*	105	(0.25–0.60)	36*	1230	(0.13–0.43)	41*	342	(0.31–0.57)
FM II	positive	19	7	0.50	9	16	0.24	30	15	0.47
28%	negative	18*	103	(0.33–0.67)	36*	1221	(0.10–0.37)	36*	336	(0.35–0.60)

*Significantly more positive reactions to the FM I (asymmetry according to McNemar test, $P < 0.05$, 2-sided).

Doubtful reactions regarded as negative. Cohen's kappa value given as chance-corrected measure of association on the premise that both FMs aim at diagnosing 'fragrance allergy'

(Table 4a) there is a significant increase in positivity at the higher test concentration. A reaction read as doubtful at 2.8% FM II might turn to a + at 14% FM II and ++ at 28% FM II. Such patterns have been observed in several cases, and also with other allergens on threshold testing (14). This proportion is apparently not large, because the majority of doubtful reactions remained of this type despite an increase in concentration (Fig. 2). This might be due to the relatively high number of doubtful reactions recorded in the 2 Danish centres. On the other hand, this might indicate 'true irritancy' and not low levels of sensitization, especially when comparing doubtful reactions to 14% and 28%.

This discussion was a necessary prerequisite for the decision on the most appropriate test concentration of FM II. We suggest the medium concentration of 14% for routine use. At 14% FM II, the number of doubtful reactions was below 3% in 4 centres, and in the 2 Danish centres, the values were similar to those of FM I. The frequency of doubtful reactions to 28% FM II was

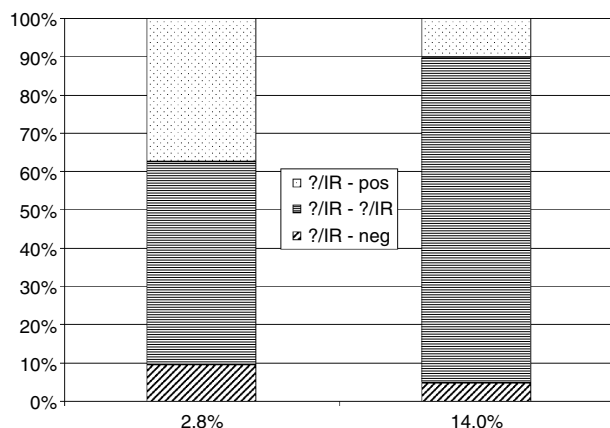


Fig. 2. Change of type of reactions with regard to concentration of FM II: % of reactions (neg., doubtful, pos.) to next higher concentration of the FM II; doubtful reactions to lower concentration = 100%.

higher than those of FM I in all centres. Only 2 of the 7 patients with a certain history and a negative reaction to FM I had a doubtful reaction to 14% FM II; these turned clearly positive at 28% FM II (Table 7). Therefore, if 14% FM II only was used for routine testing, these patients would have remained unclear with regard to proof of their contact allergy to fragrances. Further diagnostic procedures such as the repeated open application test (ROAT) are indicated in such a case and might confirm the suspected sensitization. If 28% FM II were to be used routinely, we would be concerned about the high number of doubtful reactions, which lacked a clear clinical correlate and might therefore discredit the test. We stress, however, that a few cases of relevant sensitization might be missed by the medium concentration of the mix. This is not a unique phenomenon of fragrances or mixes, but has frequently been observed with other allergens tested at the border of irritancy (20, 21). The recommended test concentration for patch testing is nearly always a compromise: there is no ideal concentration for patch testing due to the wide individual variability and further factors influencing the biologic response. Patch testing with 14% FM II on a large scale in different countries will provide the data pool which enables a firm judgement on the additional diagnostic value of the new fragrance mix compared to the original FM I.

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