Patch testing with fragrance mix II: results of the IVDK 2005–2008

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Background: The fragrance mix (FM I), established in 1977, detects the majority, but not all cases of contact allergy to fragrances. Based on European research 2002/2003, fragrance mix II (FM II) was developed to supplement FM I. In 2005, the German Contact Dermatitis Research Group (DKG) added FM II to their baseline series.

Objectives: To evaluate reactions to FM II and its constituents in routine patch testing.


Results: A total of 35 633 patients were patch tested with FM II as part of the DKG baseline series. Of these, 1742 (4.9%) reacted positively. Concomitant reactions to FM I were observed in 41.9% of the patients reacting to FM II. In 367 FM II-positive patients, a full breakdown test of the mix was performed. Of these, 47.7% reacted to hydroxyisohexyl 3-cyclohexene carboxaldehyde, 16.1% to citral, 11.4% to farnesol, 3.8% to hexyl cinnamal, 2.7% to coumarin, and 2.5% to citronellol.

Conclusions: FM II is an important screening and diagnostic tool to detect fragrance allergy. Hydroxyisohexyl 3-cyclohexene carboxaldehyde is the most important fragrance allergen in FM II.

Key words: citral; citronellol; coumarin; farnesol; fragrance allergy; fragrance mix II; hexyl cinnamal; hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC); patch test. © John Wiley & Sons A/S, 2010.

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In 1977, the fragrance mix (FM I), consisting of eight common constituents of perfumes, was introduced for patch testing by Larsen (1). Although FM I is still the most important patch test preparation for diagnosing fragrance contact allergy, not all cases of contact allergy to fragrances are detected by this mix. It has been estimated that at least 15% are missed (2). To supplement FM I, 14 additional frequently used fragrance substances were tested in a large European multicentre trial at the end of the last century (3). As a result of this study, fragrance mix II (FM II) was established, comprising 6 out of these 14 fragrance chemicals with the highest frequencies of sensitization (4, 5). FM II consists of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), citral, farnesol, hexyl cinnamal (α-hexyl cinnamic aldehyde), coumarin, and citronellol. Of the different patch test concentrations studied, FM II 14% in petrolatum (pet.) was the most suitable, when considering the balance of sensitivity, specificity, and the frequency of doubtful reactions. However, for patch testing with the single constituents, test concentrations were recommended that are twice as high as in FM II 14% pet. (4, 5). In 2008, Bruze et al. on behalf of the European Society of Contact Dermatitis (ESCD) and the European Environmental Contact Dermatitis Research Group (EEDCRG) reviewed patch test experience with
FM II and HICC and recommended to include both, FM II and its constituent HICC, in the European baseline patch test series (6). Some European patch test clinics had already added FM II and/or HICC to their baseline series in 2005 or 2006, and yielded positive reactions to FM II in 1.8–5.5% and to HICC in 0.5–2.7% of the patients tested (7). The German Contact Dermatitis Research Group (DKG) added FM II 14% pet. in the German patch test baseline series in January 2005 (8), while HICC 5% pet. had been included in January 2002 (9). As detailed analyses of large-scale routine patch test data with these fragrance materials are yet lacking, we analysed the results of the Information Network of Departments of Dermatology (IVDK).

Patients and Methods

The multicentre project IVDK, established in 1989, is an instrument for epidemiological surveillance of contact allergy in Germany, Switzerland, and Austria, and has been described in detail elsewhere (10). Briefly, patients with suspected allergic contact dermatitis attending one of the participating centres are patch tested according to the guidelines of the DKG (11) and current international recommendations (12). Patch test material is obtained from Almirall Hermal, Reinbek, Germany, and applied for 24 or 48 hr, according to standards in the respective centres. Readings are taken until at least day 3 (D3). Patch test data along with a standardized set of demographic and clinical data of the patients are transmitted twice yearly in an anonymous format to the IVDK data centre in Göttingen and are quality controlled (13). Data analysis is performed according to international guidelines (14) using SAS™ software (version 9.2, SAS Institute, Cary, NC, USA).

The present analysis focuses retrospectively on patients who were tested between January 2005 and December 2008 with FM II as part of the DKG baseline series. Patch test reactions to FM II, concomitant reactions to FM I, HICC, and reactivity in those who underwent a full breakdown patch test of their baseline series. Patch test reactions to FM II, concomitant reactions to FM I, HICC, and reactivity in those who underwent a full breakdown patch test of their baseline series were employed.

### Results

#### Total test population

In all, there were 40 762 patients patch tested between January 2005 and December 2008 in 48 departments of dermatology comprising the IVDK. Of these, 37 651 were patch tested with the DKG baseline series. In 35 633 (94.6%) of these patients, FM II was tested. These patients are the basis for the present data analysis.

Patch test reactions to FM II are shown in Table 1. Positive reactions occurred in 1742 patients (4.9%). Reaction index [RI; (16)] was 0.28, positivity ratio [PR; (17)] was 74.9%. Analysis of patch test reactions by years showed no significant changes or any time trend (data not shown in detail).

Table 2 gives a description of total test population (n = 35 633) and those patients with negative (n = 32 914) or positive (+, +++, ++++) reactions (n = 1742) to FM II, respectively, according to the items of the MOAHLFA index. Compared with those without any reaction to FM II, there strikingly more patients aged 40 years or older among those reacting to FM II (78.7% versus 70.6%; P < 0.0001). In addition, there were significantly more females (P < 0.0001), patients with occupational dermatitis (P = 0.0149), atopic dermatitis (P = 0.0008), or hand dermatitis (P = 0.0063), among those with a positive reaction to FM II. In contrast, proportions of patients with leg or face dermatitis did not differ significantly. Axillae were mentioned as the main anatomical site in only 228 patients tested (0.6%). However, there was a significant (P < 0.0001) difference between those with a negative reaction to FM II (172 patients; 0.5%) and those with a positive reaction to FM II (45 patients; 2.6%), regarding this particular anatomical site. Suspected allergens are documented in categorized form in the IVDK. Up to three categories can be listed per case. Of the patients tested (n = 35 633), assumed allergen sources were ‘cosmetics, creams, sunscreens’ in 37.2% (n = 13 258),

### Table 1. Patch test reactions to fragrance mix II (FM II) (14% pet.) in the total test population

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Patient count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>32 914</td>
<td>92.4</td>
</tr>
<tr>
<td>?</td>
<td>755</td>
<td>2.1</td>
</tr>
<tr>
<td>+</td>
<td>1305</td>
<td>3.7</td>
</tr>
<tr>
<td>++</td>
<td>364</td>
<td>1.0</td>
</tr>
<tr>
<td>+++</td>
<td>73</td>
<td>0.2</td>
</tr>
<tr>
<td>Ir</td>
<td>222</td>
<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td>35 633</td>
<td>100.0</td>
</tr>
</tbody>
</table>
‘rinse-off body care products’ in 6.5% ($n = 2308$), and ‘perfume, deodorant, after shave, and so on’ in 3.7% ($n = 1328$). As there was a marked overlap of these subgroups, at least one of these categories was mentioned in 41.7% of the patients tested ($n = 14848$). Among these 14848 patients, the proportion of positive reactions to FM II was 5.8%. In the ‘cosmetics, creams, sunscreens’ group, it was 5.7%; in the ‘rinse-off body care products’ group, it was 4.9%; and in the ‘perfume, deodorant, after shave, and so on’ group, it was 9.9%.

As part of the DKG baseline series, both, FM I and FM II, were patch tested in 35490 patients. Of these, 7.2% reacted positive to FM I ($n = 2563$), and 4.8% to FM II ($n = 1717$). A total of 719 patients reacted to both of the mixes, representing 28.1% of those reacting to FM I and 41.9% of those reacting to FM II. In all, 3561 patients (10.0%) reacted to either of the mixes.

FM II and its component HICC (5% pet.) were patch tested in parallel as part of the DKG baseline series in 35582 patients. Results are shown in Table 3. For this cross-tabulation, all positive reactions (+, ++, ++++) and negative, doubtful, and irritant reactions (neg, ?, ir) were summarized, respectively. A total of 1734 patients (4.9%) reacted to FM II, 836 (2.4%) reacted to HICC, and 728 reacted to both allergen preparations. These 728 patients are 42.0% of those reacting to FM II and 87.1% of those reacting to HICC. By patch testing with HICC in addition to FM II as part of the baseline series, 108 patients sensitized to HICC were diagnosed, who would have been missed, if only FM II had been tested. These 108 patients represent 12.9% of those sensitized to HICC, and 0.3% of the total test population, respectively.

**Patients having undergone a full breakdown test**

In 2217 patients (6.2% of the total test population), a full breakdown test of the FM II was performed. This was either performed when a positive reaction to FM II occurred or – right from the start – because of suspected fragrance allergy. This is reflected by the assumed allergen sources. ‘Cosmetics, creams, sunscreens’ was mentioned in 46.2% of the patients ($n = 1024$), ‘rinse-off body care products’ in 10.2% ($n = 227$), and ‘perfume, deodorant, after shave, and so on’ in 14.5% ($n = 321$), with an overlap of these subgroups. In 57.6% of the patients, at least one of these three categories was mentioned ($n = 1278$). As was to be expected, the group of patients who had undergone the full breakdown test differed from the total test population. MOAHLFA index of these 2217 patients was as follows: Males differed from the total test population. MOAHLFA index of these 2217 patients was as follows: Males represented 12.9% of those sensitized to HICC, and Occupational dermatitis 17.9%, Atopic dermatitis 26.2%, Hand dermatitis 29.5%, Leg dermatitis 10.4%, Face dermatitis 20.4%, Age 40 of years or more 73.3%. The axillae were documented as the main anatomical site in 2.4%.

A total of 367 out of 2217 patients (16.6%) reacted positively to FM II (RI = 0.71, PR = 68.1%).

Results of the breakdown test in patients reacting to FM II are given in Figures 1 and 2. Figure 1 shows the count of single mix constituents to which the patients reacted, classified by reaction intensity. It can be seen that in 42.8% of the patients with a + reaction to FM II, the breakdown test was completely negative, and that in 52.4%, there was a reaction to one single component of the mix. Among those patients with a strong reaction (+++, ++++) to the mix, the breakdown test was completely negative in only 8.5%, while 66.7% reacted to only one component. Additional data analysis showed that in 1755 out of 1789 patients (98.1%) with negative reaction to the mix, the breakdown test remained negative. The same applied to 53 out of 61 patients (86.9%) with doubtful or irritant reactions to the mix. In Figure 2, the percentage of reactions to

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**Table 2. MOAHLFA index of the total test population and of patients reacting negative or positive (+, ++, +++ to fragrance mix II (FM II))**

<table>
<thead>
<tr>
<th>Patients tested with FM II ($n = 35633$) (%)</th>
<th>FM II negative ($n = 32914$) (%)</th>
<th>FM II positive ($n = 1742$) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male M</td>
<td>38.8</td>
<td>38.9</td>
</tr>
<tr>
<td>Occupational dermatitis O</td>
<td>14.7</td>
<td>14.5</td>
</tr>
<tr>
<td>Atopic dermatitis A</td>
<td>18.5</td>
<td>18.3</td>
</tr>
<tr>
<td>Hand dermatitis H</td>
<td>27.2</td>
<td>27.1</td>
</tr>
<tr>
<td>Leg dermatitis L</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Face dermatitis F</td>
<td>15.3</td>
<td>15.2</td>
</tr>
<tr>
<td>Age ≥40 years</td>
<td>71.2</td>
<td>70.6</td>
</tr>
</tbody>
</table>

**Table 3. Concomitant reactions to fragrance mix II (FM II; 14% pet.) and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC; 5% pet.)**

<table>
<thead>
<tr>
<th>HICC</th>
<th>Neg. ?, ir.</th>
<th>Pos.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM II</td>
<td>33740</td>
<td>108</td>
<td>33848</td>
</tr>
<tr>
<td></td>
<td>1006</td>
<td>728</td>
<td>1734</td>
</tr>
<tr>
<td>Total</td>
<td>34746</td>
<td>836</td>
<td>35582</td>
</tr>
</tbody>
</table>
the respective mix constituents in patients reacting positive to the mix is depicted. It can be seen that HICC is by far the most frequent allergen. Remarkably, among those with a negative reaction to FM II \((n=1789)\), farnesol (15 patients; 0.8%) and citral (11 patients; 0.6%) were more frequently positive than HICC (4 patients; 0.3%). The same applied to those 61 patients with doubtful or irritant reactions to the mix, of whom 4 (6.6%) reacted to farnesol, 2 (3.3%) to citral, but only 1 (1.6%) to HICC.

We analysed data of subgroups of patients defined by the assumed allergen source categories mentioned above to find out if some of the FM II fragrances were connected to special allergen exposures. As mentioned, there was a large overlap between the three subgroups. Therefore, we only considered those patients, in whom one of these categories was mentioned, but not the respective two other ones. Thus, sample size was rather small, and we had only 124 FM II-positive patients with a full breakdown test and suspected allergen source ‘cosmetics, creams, sunscreens’, 14 FM II-positive patients with a full breakdown test and suspected allergen source ‘rinse-off body care products’, and 25 FM II-positive patients with a full breakdown test and suspected allergen source ‘perfume, deodorant, after shave, and so on’. In all of the three groups, ranking of the most frequent fragrance allergens was the same as in the total breakdown test group, that is HICC leading by far, followed by citral and farnesol (data not shown in further detail).

**Discussion**

Our analysis of large-scale patch test data on FM II shows that the test preparation FM II 14%
pet. has good diagnostic properties (RI = 0.28, PR = 74.9%) and thus is suitable for patch test screening of fragrance allergy from this point of view. With a rather constant reaction frequency of 4.9% positive reactions when patch tested in consecutive patients in Germany, Switzerland, and Austria, its inclusion in the baseline series is well justified. The prevalence observed here is considerably higher than the 2.9% positive reactions observed in 1701 consecutive patients in the initial European multicentre trial and dose–response study (4, 5). Nardelli et al. reported patch test results from Leuven, Belgium. They found positive reactions to FM II in 7 out of 335 patients tested with the baseline series in 2005 (18). Recently, data from the European Surveillance System on Contact Allergies (ESSCA) 2005/2006 have been published (7). In several of the participating centres, FM II and HICC had already been included in the baseline series at that time. The percentage of positive reactions to FM II ranged from 1.8% in southern Europe to 5.5% in central Europe. Of those 10 centres forming the latter region, 8 are members of the IVDK, and their data are also part of this data analysis. Any explanation of the different proportions of patients reacting to FM II remains speculative. Most probably, test populations differed, as far as age distribution, anatomical site of skin disease, and indication for patch testing is concerned. Furthermore, one might speculate that common fragrance compositions of body care products may differ between European regions. In addition, the composition of fragrances has probably changed since the obligatory labelling of 26 fragrances in the EU in 2005, covering also the ingredients of the FM II, as shown before (19). However, although reaction frequencies differ in other European countries, our data strongly support the decision to add FM II to the European baseline series (6).

As can be seen from parallel patch tests, FM II detects a significant proportion of fragrance allergic patients beyond the FM I. Of the 3561 patients reacting to either of both, FM I or FM II, 998 (28.0%) would not have been detected without patch testing with FM II. Again, this is considerably more than in the European multicentre study from 2002/2003 (4, 5). In that study, 127 out of 1699 patients tested with both, FM I and FM II, reacted to either (7.5%). Of these, 16 patients (12.6%) would not have been diagnosed if FM II would have been missed. The reasons for the difference between the two studies are probably the same as mentioned earlier. Anyway, it underlines the importance of having both fragrance mixes in the patch test baseline series.

As was to be expected, we found higher proportions of positive reactions to FM II in patients with suspected allergen source in ‘cosmetics, creams, sunscreens’, even more pronounced in patients with suspected allergen source in ‘perfume, deodorant, after shave, and so on’. This is in line with the finding that, among patients reacting positive to FM II, there were more women, and more patients with hand dermatitis or axillary dermatitis. Although not surprising, this information (i) underlines the validity of the data and (ii) gives rise to the demand that HICC, citral, or farnesol should not be used in protective, cosmetic, or therapeutic hand creams, unless a safe concentration is established and is not exceeded (20). Heydorn et al. patch tested 658 patients with hand eczema in 2001/2002 with 14 fragrances selected for the criteria: (i) known human contact allergen, (ii) fragrances used in products associated with hand exposure, and (iii) quantities used in consumer products. These 14 fragrances included all components of the FM II except farnesol. In 67 patients (10.2%), a positive reaction to at least one of the 14 fragrance chemicals occurred. Proportions of positive reactions to FM II constituents were as follows: citral 28 (4.3%), HICC 14 (2.1%), coumarin and hexyl cinnamal 3 (0.5%) each, citronellol 2 (0.3%). The conclusion of this study was that fragrance allergy could be a common problem in patients with hand eczema. Interestingly, the frequency of positive reactions to the constituents of the FM II in this highly selected population was highest for citral, followed by HICC. This differs from all the other tested populations and might be related to the quantities of the different fragrances then incorporated into cosmetic products with hand exposure (21).

Parallel patch testing with FM II (14% pet.), which contains HICC at 2.5%, and HICC (5% pet.) showed that 12.9% of the patients allergic to HICC would have remained undiagnosed if HICC had not been tested. This is probably because of the higher test concentration of HICC, compared to its concentration in FM II. In a recent German multicentre patch test and Repeated Open Application Test (ROAT) study with HICC, 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC 5% pet. also reacted in the ROAT (20). Hence, one can assume that there are only very few false-positive patch test reactions to HICC 5% pet. HICC has been recognized as an important fragrance allergen for more than 10 years (22, 23). In a German multicentre study from 2000/2001, 62 of 3245 consecutive patch test patients (1.9%) reacted positively to HICC 5% pet., and the test preparation was regarded as a good diagnostic tool. Based on these results, the DKG added HICC 5% pet. to the German patch test baseline series from 2002 (9). Since then, HICC constantly elicits positive reactions in 2.2–2.5% in
the departments of dermatology of the IVDK (24; additional unpublished IVDK data). Nardelli et al. from Leuven, Belgium, reported positive patch test reactions to HICC as part of the baseline series in 2.1% (62 out of 2901) of consecutive patients tested between 2002 and 2005 (18). The Danish Contact Dermatitis Group observed sensitization to HICC in 2.1% of the patients tested in 2003 and in 2.8% of those tested in 2007 (25). These data closely match our results. In those European patch test clinics joining the ESSCA who had patch tested HICC as part of the baseline series in 2005/2006, positive reactions were noted in 0.5% in southern Europe and in 2.7% in central Europe (7), which may be indicative of geographic differences discussed earlier.

As part of the European multicentre study leading to the introduction of FM II into routine diagnostics, chemical analyses of 24 patients’ products (perfumes, deodorants, body care products) were performed (4, 5). HICC was detected in 19 out of 24 products, with concentrations of up to 3.8%. Because of the sensitization potential of HICC, in 2003 the International Fragrance Research Association (IFRA) recommended concentration levels below 1.5% in finished cosmetic products (leave on as well as rinse off) (26). However, none of the sensitization data mentioned earlier indicates any decreasing trend regarding sensitization to HICC since this IFRA recommendation was established. In the 43rd amendment of IFRA standards, published in July 2008, the recommendation of concentration limits for HICC were lowered to levels between 0.11% and 1.5% for most cosmetic products (26). This was even further reduced in the 44th amendment, published in November 2009: 0.02% for deodorants, lip products, and intimate wipes and 0.2% for all other leave-on and rinse-off cosmetics (26). The future will show if this limitation will indeed lead to a reduction of sensitization to HICC. Taking all this into consideration, it is still justified to patch test both, FM II and HICC, in the baseline series.

When analysing the results of breakdown tests, it was noteworthy to find that in 42.8% of the weak positive reactions to FM II, no reaction to any of its constituents occurred. With FM I, 61.0% (27) and 52.3% (28) of those with a weak positive reaction to the mix did not react to any of its constituents.

Thus, weak positive reactions to FM II seem to have a better positive predictive value [against reaction(s) to any one constituent as gold standard] than those to FM I, probably because of the fact that the sensitivity of testing with single constituents is increased by their doubled concentration, whereas single constituents of the FM I are tested in the same concentration as present in the mix. Two explanations for negative breakdown tests in patients weakly positive to the mix seem probable. First, these reactions to FM II are false-positive irritant reactions, presumably caused by the high total test concentration of 14% fragrances. Second, these are patients with a low-grade sensitization to one or more fragrances, leading to a positive patch test reaction only as expression of a compound allergy. However, the latter explanation seems less probable because test concentrations used in the breakdown test are twice as high as in the mix. Hence, one would expect positive patch test reactions to single fragrances even in less or weakly sensitized individuals, too. Sensitization to more than one of the mix constituents obviously is an uncommon event, occurring in only 4.8% of those with a + reaction to FM II, and in 24.8% of those with a ++ or +++ reaction to FM II. This is considerably less than with FM I, where 10.4% of those patients with a weak positive reaction and 32.0% of those with a strong positive reaction to the mix reacted to more than one of its components (27).

Ranking of the most frequently positive fragrances allergens contained in FM II was the same as found in the original European multicentre study that lead to the introduction of FM II into routine diagnostics (4, 5). In our data, HICC was by far the most important allergen, eliciting 39.6% positive reactions in patients with + reaction to FM II, and even 65.0% among patients with a strong reaction to the mix. Citral and farnesol took an intermediate rank with 16.4% and 11.4% positive reactions in all patients reacting positive to FM II. Compared with the other mix constituents, hexyl cinnamal, coumarin, and citronellol were of minor importance.

This ranking reflects reactivity in patients reacting positive to FM II. However, when the single components of FM II were tested in unselected consecutive patients, irrespective of their reactivity to FM II, a similar ranking was found. In 2003/2004, those 26 fragrance chemicals that have had to be labelled, according to European regulations since 2005, were patch tested in this manner in the IVDK, with positive reactions to HICC in 2.3%, to farnesol in 0.9%, to citral in 0.6%, to citronellol in 0.5%, to coumarin in 0.4%, and to hexyl cinnamal in 0.1% (24).

Positive test reactions to single fragrance chemicals of the mix hardly ever occurred when FM II was negative. However, in 13% of cases with doubtful or irritant FM II reactions, positive reactions to single constituents were observed, mainly to farnesol and citral. Most probably, there are cases of low-grade sensitization to these fragrance compounds, which are not unequivocally detected.
by the lower individual test concentration in the mix.

Conclusion

Our retrospective analysis of the patch test results with FM II in the baseline series as documented in the IVDK from 2005 to 2008 shows the following:

1. FM II is an important tool to detect additional fragrance allergies to FM I.
2. HICC is the most important single fragrance allergen in this mix, followed by citral and farnesol, while hexyl cinnamal, coumarin, and citronellol appear to be of less importance.

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