# Patch test results with fragrance markers of the baseline series – analysis of the European Surveillance System on Contact Allergies (ESSCA) network 2009–2012

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## Summary

**Background.** Contact allergy to fragrances is common, and impairs quality of life, particularly in young women.

**Objective.** To provide current results on the prevalences of sensitization to fragrance allergens used as markers in the baseline series of most European countries.

**Methods.** Data of patients consecutively patch tested between 2009 and 2012 in 12 European countries with fragrance allergens contained in the baseline series were collected by the European Surveillance System on Contact Allergies network and descriptively analysed. Four departments used the TRUE Test® system.

**Results.** The 'basic markers' were tested on 51 477 [fragrance mix II (FM II)] to 57 123 [*Myroxylon pereirae*, balsam of Peru] patients, and yielded positive reactions as follows: fragrance mix I 6.9%, *Myroxylon pereirae* 5.4%, FM II 3.8%, colophonium 2.6%, and hydroxyisohexyl 3-cyclohexene carboxaldehyde 1.7%, with some regional differences. Prevalences with TRUE Test® allergens were lower. Additional fragrances were tested on 3643 (trimethylbenzenepropanol) to 14 071 (oil of turpentine) patients, and yielded between 2.6% (*Cananga odorata*) and 0.7% (trimethylbenzenepropanol) positive reactions.

**Conclusions.** Contact allergy to fragrances is common throughout Europe, with regional variation probably being explained by patch test technique, and differences in exposure and referral patterns. The current basic markers of fragrance sensitivity in the baseline series should be supplemented with additional fragrance allergens.

**Key words:** contact allergy; contact sensitization; fragrances; patch testing; regional differences.

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Contact allergy to fragrances is quite frequent, affecting between 1.1% and 2.3% of the general population in Europe (1). Quality of life is considerably impaired in young women, and especially if sensitizations are multiple and of high degree (2). Cosmetics are the dominant causes of sensitization, but other exposures have also been documented: topical medications, household products, aromatherapy materials, and occupationally used materials (3–5). Recent studies have proven that fragrance mix I (FM I), fragrance mix II (FM II) and the widely used hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) are valuable screening tools for the detection of fragrance sensitivity (6–11). However, both mixes contain only a total of 14 substances, and, considering the wide array of materials used for perfuming, it is not surprising that not all cases of contact allergy to fragrances are identified by the two mixes. Therefore, an older marker, namely *Myroxylon pereirae* [balsam of Peru], is still present in the baseline series. Although *M. pereirae* is supposedly not used in perfumes, according to the recommendations of the Research Institute of Fragrance Materials, but only extracts and distillates thereof (12), this natural resin contains many chemicals that are constituents of perfumes and have sensitizing potential (e. g. cinnamon compounds, benzoic acid, and farnesol). Other materials, such as oil of turpentine and colophonium, have been shown to be associated with higher reactivity in fragrance-sensitive individuals, albeit to a lesser degree than *M. pereirae* (13, 14). Furthermore, studies with essential oils have shown that additional cases of fragrance sensitivity can be identified by testing with them (15, 16).

Following a previous study of the European Surveillance System on Contact Allergies (ESSCA) network (17), we extended the European baseline series with three essential oils and with the fragrance chemical trimethylbenzenepropanol (Majantol®) in some departments, based on recent reports on relevant sensitizations (18, 19). In the following, the results of a 4-year period are analysed, with the main focus on the prevalence of sensitization and regional differences throughout Europe.

## Methods

The retrospective analysis is based on data collected by the ESSCA (www.essca-dc.org) network. The ESSCA network has been described in previous publications (17). Briefly, clinical and demographic data, along with patch test results, of all patients patch tested in the departments participating in the ESSCA network for suspected allergic contact dermatitis are documented electronically in the local departments, with diverse data capture software and, partly, the multilingual software WINALLDAT/ESSCA provided by the ESSCA network (20). Patch testing was performed according to international recommendations (21). Data were pooled in the ESSCA network data centre in Erlangen for further analysis with R (version 3.1.1) software (22). Pertinent guidelines for the analysis of patch test data (23) were considered. Reactions designated as either +, ++ or +++ were classified as positive. The study period was January 2009 to December 2012, and the study included 12 European countries and, in total, 53 departments. A further description of this clinical sample can be found in (24).

Test results with the TRUE Test® baseline series are presented separately, for better comparability. Altogether, 63 530 consultations have been registered. Patients not tested with the baseline series (for example, because of a recent, valid test shortly before) were not considered further in this analysis focusing on baseline series allergens. Altogether, 59 922 consultations involved the baseline series and at least one reading at days 3–5 after application of patch tests. Because of multiple consultations of some patients in the study period, all involving testing with the baseline series, this number is further slightly reduced to 59 728 single consultations of these patients (the most recent consultation was chosen for analysis).

### Petrolatum-based allergens

The fragrance allergens and test concentration are shown in Table 1. Only the first five materials are included in the European baseline series as tested in most European countries. Oil of turpentine has been included in the German baseline series for many years, according to the recommendation of the German Contact Dermatitis Research Group. The essential oils jasmine absolute, sandalwood oil and ylang-ylang oil were added to the German baseline series in 2010 after a multicentre study (16). Trimethylbenzenepropanol has been added to the 'monitor series' in some German departments, in order to obtain current data on sensitization, after two reports in the literature (18, 19). In this study, it was also tested in Switzerland, Spain, Finland, Lithuania and Poland at some points in the period of analysis. At present, the European baseline series does not include sorbitan sesquioleate, and very few local baseline series do so. Hence, positive reactions to FM I may, to a small extent, be attributable to contact allergy to sorbitan sesquioleate, which is used as an emulsifier for the mix (25, 26). A corresponding overestimation of the proportion of true positive reactions to FM I may therefore have occurred in the present analyses. All fragrance allergens were tested on consecutive patients. Stratification for departments and further details on the basic characteristics of this ESSCA network study are given elsewhere (24). For the analysis, only patch test readings that

were positive (≥1+) at day 3 (or days 4 or 5) were considered; non-positive results comprised negative, irritant and doubtful reactions.

### TRUE Test®

The vast majority of patients were tested with pet.-based allergens and investigator-loaded chamber systems. In only 2435 consultations of consecutive patients were patch tests with the TRUE Test® performed, in four departments altogether [most patients were tested in Groningen (n = 1468; the three available allergens were exclusively tested with the TRUE Test® in this department), followed by Madrid Princesa (n = 604), and some further patients in Amsterdam, Murcia, and León].

## Results

The overall results obtained with the 10 fragrance allergens in pet. are shown in Table 1, including stratification by sex and age (dichotomized). The proportion of males aged <40 years age was 13.1%, and that of females was 27.0%; the proportions of patients aged ≥ 40 years were 20.5% in males and 39.4% in females; for further details, see (24). FM I produced the highest prevalence of sensitization in 56 813 tested patients, in terms of 6.9% positive reactions (age-standardized and sex-standardized). *M. pereirae* followed closely (5.4%); the remaining three of the 'basic markers' produced lower prevalence rates, but still well over 1% (FM II 3.8%, colophonium 2.6%, and HICC 1.7%, respectively).

The other five materials were tested on smaller populations, ranging from 14 071 (oil of turpentine) to 3643 (trimethylbenzenepropanol). The prevalence was highest for ylang-ylang oil (*Cananga odorata*, 2.6%) and lowest for trimethylbenzenepropanol (0.7%). The distribution of reaction intensities is also shown in Table 1. In general, 1+ reactions were more frequent than ++ or +++ reactions, particularly in the case of *M. pereirae* and the essential oils. Irritant or doubtful reactions ranged around 2%, without any obvious pattern.

Table 2 provides data on the prevalence of sensitization to all 10 test materials stratified for the 12 European countries. Differences were marked, with the highest values being seen for FM I in Austria, Switzerland, and The Netherlands (range 13.6 to 11.1%), as compared with 3.8–4.9% in Lithuania, Italy, and Spain. However, in Lithuania only 865 patients were tested.

For FM II, the figures were lower, generally being <10% (range 7.27 to 1.31%). Regional differences corresponded to those of FM I, with the exception of Lithuania (where the prevalence rates of FM I and FM II were nearly the same). HICC reached a prevalence of sensitization of >1% in all countries, thus justifying its place in the European baseline series; the distribution pattern among the participating countries was similar to that of FM II, which contains HICC at 2.5% as the predominant constituent upon breakdown testing. Concerning coupled reactivity between FM II and HICC, 43 074 patients were tested with both allergens. Of these, 578 reacted both to FM II and to HICC, 1192 only to FM II, and 181 only to HICC. This latter number corresponds to 0.42% of tested patients or 24% of all patients reacting to HICC.

The figures for *M. pereirae* and colophonium are in line with previous observations, with the exception of Lithuania, where colophonium had produced the highest value (5.6%) of all countries. Oil of turpentine reached prevalence values of 1.3–3.1% in the four countries testing it. The three essential oils were tested in three countries (Austria, Switzerland, and Germany) in considerable numbers of patients, and the highest range was seen for ylang-ylang oil (*C. odorata*; 2.4–4.5%); the figures for sandalwood oil (*Santalum album*) and jasmine absolute (*Jasminum grandiflorum*) were lower, but still >1%. Trimethylbenzenepropanol was tested on small groups of patients in six countries; only in Spain did the number tested exceed 1000 patients. The number of positive cases was low in Germany, Finland, and Lithuania, being just above 1%. If FM I, FM II and HICC are considered to be fragrance contact allergy markers, the overall reactivity (to pet.-based allergens) is 9.2%. If, additionally, *M. pereirae* resin and oil of turpentine are considered to be markers, the overall prevalence of positive patch test reactions to at least one of these allergens increases to 12.7%.

The results obtained with the three fragrance allergens in the TRUE Test® system are shown in Table 3. The prevalence was highest for FM I (3.9%), and lower for *M. pereirae* and colophonium (2.2% each). The distribution of reaction grades as compared with the pet.-based allergens was slightly different in terms of a lower proportion of irritant reactions/?+ and a higher share of + reactions for the three TRUE Test® allergen preparations.

## Discussion

The overall results show a rank order that is rather typical of fragrance markers: FM I, *M. pereirae*, FM II, HICC, and oil of turpentine (in descending frequency of positive reactions). In a recent analysis of the German IVDK network on over 130 000 patients (1999–2012), with overlap concerning about 13 500 patients also included in the present analysis, the rank order was very similar (FM I 8.7%, *M. pereirae* 8.4%, FM II 4.9%, and oil of turpentine 2.0%) (27). There is now abundant evidence

**Table 1.** Overall results obtained with the 10 fragrance allergens patch tested in petrolatum

| Allergen | Concentration (%) | n tested | % + | % ++/+++ | % ?/IR | % positive | % pos. std. | 95%CI | % positive | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | males aged <40 years | males aged ≥40 years | females aged <40 years | females aged ≥40 years |
| *Myroxolon pereirae* resin (balsam of Peru) | 25 | 57 027 | 4.09 | 1.87 | 2.93 | 5.89 | 5.42 | 5.24–5.6 | 3.1 (2.7–3.5) | 7.6 (7.1–8.1) | 3.4 (3.1–3.7) | 7.5 (7.2–7.9) |
| Colophonium (colophony) | 20 | 57 123 | 1.47 | 1.26 | 1.58 | 2.76 | 2.64 | 2.51–2.77 | 2.1 (1.8–2.5) | 2.9 (2.6–3.2) | 2.2 (1.9–2.4) | 3.3 (3–3.5) |
| Fragrance mix I | 8 | 56 813 | 4.34 | 2.96 | 2.75 | 7.36 | 6.87 | 6.67–7.08 | 4 (3.6–4.5) | 7.5 (7–8) | 5.3 (5–5.7) | 9.7 (9.3–10.1) |
| Fragrance mix II | 14 | 51 477 | 2.66 | 1.30 | 2.60 | 4.04 | 3.81 | 3.64–3.97 | 2.3 (2–2.7) | 4.2 (3.8–4.6) | 3.3 (3–3.6) | 4.9 (4.6–5.2) |
| HICC | 5 | 50 675 | 1.09 | 0.66 | 1.20 | 1.79 | 1.71 | 1.59–1.82 | 0.9 (0.7–1.2) | 1.6 (1.3–1.8) | 1.7 (1.5–2) | 2.2 (2.0–2.4) |
| *Jasminum grandiflorum / officinale* extract (jasmine absolute) | 5 | 9601 | 1.51 | 0.37 | 2.53 | 1.89 | 1.58 | 1.34–1.83 | 0.7 (0.3–1.3) | 1.4 (1.0–1.9) | 1.2 (0.7–1.7) | 2.6 (2.2–3.1) |
| Trimethylbenzenepropanol | 5 | 3643 | 0.55 | 0.19 | 1.93 | 0.74 | 0.72 | 0.43–1.00 | 0.5 (0.1–1.8) | 0.6 (0.2–1.4) | 0.9 (0.4–1.8) | 0.7 (0.4–1.3) |
| Oil of turpentine | 10 | 14 071 | 1.71 | 0.51 | 2.52 | 2.22 | 2.00 | 1.76–2.23 | 1.3 (0.8–1.9) | 2.3 (1.8–2.8) | 1.7 (1.3–2.2) | 2.5 (2.2–3) |
| *Santalum album* extract (sandalwood oil) | 10 | 9598 | 1.23 | 0.34 | 2.82 | 1.57 | 1.46 | 1.21–1.71 | 0.7 (0.3–1.4) | 1.4 (1–1.9) | 1.7 (1.2–2.3) | 1.7 (1.3–2.1) |
| *Cananga odorata* extract (ylang-ylang oil) | 10 | 9596 | 2.09 | 0.74 | 2.5 | 2.83 | 2.64 | 2.3–2.97 | 1.7 (1–2.5) | 2.4 (1.9–3.1) | 2.9 (2.2–3.8) | 3.0 (2.5–3.5) |

95% CI, 95% confidence interval to the standardized prevalence; figures in parentheses, exact 95% confidence interval to the age- and sex-stratified prevalences; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; IR, irritant reaction; % pos. std., proportion of positives, directly age-standardized and sex-standardized.
All allergens in pet.

**Table 2.** Results obtained with the 10 fragrance allergens patch tested in petrolatum, stratified for country

| Country | n tested | n positive | % positive | % pos. std. | 95%CI |
|---|---|---|---|---|---|
| (a) Fragrance mix, 8% pet. | | | | | |
| Austria | 1113 | 163 | 14.65 | 13.63 | 11.57–15.69 |
| Switzerland | 5001 | 586 | 11.72 | 11.05 | 10.16–11.94 |
| Germany | 7364 | 671 | 9.11 | 8.36 | 7.7–9.02 |
| Denmark | 2547 | 219 | 8.6 | 7.75 | 5.98–9.51 |
| Spain | 3405 | 170 | 4.99 | 4.9 | 4.15–5.65 |
| Finland | 1056 | 74 | 7.01 | 6.81 | 5.3–8.33 |
| Italy | 9025 | 434 | 4.81 | 4.75 | 4.31–5.19 |
| Lithuania | 865 | 44 | 5.09 | 3.79 | 2.65–4.94 |
| The Netherlands | 2821 | 373 | 13.22 | 12.72 | 11.5–13.94 |
| Poland | 2826 | 148 | 5.24 | 5.1 | 4.28–5.91 |
| Slovenia | 5183 | 269 | 5.19 | 4.93 | 4.34–5.51 |
| United Kingdom | 15 571 | 1028 | 6.6 | 6.23 | 5.85–6.6 |
| (b) Fragrance mix II, 14% pet. | | | | | |
| Austria | 1113 | 87 | 7.82 | 7.27 | 5.71–8.83 |
| Switzerland | 5000 | 290 | 5.8 | 5.37 | 4.73–6 |
| Germany | 7406 | 386 | 5.21 | 4.77 | 4.27–5.28 |
| Denmark | 2554 | 137 | 5.36 | 4.8 | 3.38–6.22 |
| Spain | 3993 | 95 | 2.38 | 2.32 | 1.83–2.8 |
| Finland | 1057 | 35 | 3.31 | 3.23 | 2.17–4.3 |
| Italy | 1591 | 23 | 1.45 | 1.31 | 0.77–1.86 |
| Lithuania | 865 | 37 | 4.28 | 3.64 | 2.38–4.9 |
| The Netherlands* | 4274 | 279 | 6.53 | 6.31 | 5.58–7.03 |
| Poland | 2827 | 117 | 4.14 | 4.02 | 3.29–4.74 |
| Slovenia | 5197 | 144 | 2.77 | 2.73 | 2.28–3.18 |
| United Kingdom | 15 579 | 447 | 2.87 | 2.76 | (2.5–3.02) |
| (c) Hydroxyisohexyl 3-cyclohexene carboxaldehyde, 5% pet. | | | | | |
| Austria | 1109 | 33 | 2.98 | 2.5 | 1.63–3.36 |
| Switzerland | 4968 | 113 | 2.27 | 2.14 | 1.73–2.56 |
| Germany | 7435 | 190 | 2.56 | 2.4 | 2.03–2.77 |
| Denmark | 2555 | 65 | 2.54 | 2.35 | 1.33–3.36 |
| Spain | 3500 | 36 | 1.03 | 1.02 | 0.67–1.37 |
| Finland | 544 | 12 | 2.21 | 2.06 | 0.87–3.25 |
| Italy | 6750 | 76 | 1.13 | 1.13 | 0.88–1.39 |
| Lithuania | 865 | 12 | 1.39 | 1.18 | 0.47–1.89 |
| The Netherlands | 4275 | 97 | 2.27 | 2.18 | 1.75–2.61 |
| Poland | 2825 | 58 | 2.05 | 1.98 | 1.47–2.49 |
| Slovenia | 5212 | 70 | 1.34 | 1.27 | 0.97–1.57 |
| United Kingdom | 10 616 | 145 | 1.37 | 1.33 | 1.11–1.55 |
| (d) *Myroxylon pereirae* resin (balsam of Peru), 25% pet. | | | | | |
| Austria | 1109 | 142 | 12.8 | 11.63 | 9.75–13.5 |
| Switzerland | 4984 | 489 | 9.81 | 8.99 | 8.18–9.79 |
| Germany | 7409 | 588 | 7.94 | 6.77 | 6.19–7.34 |
| Denmark | 2560 | 114 | 4.45 | 3.78 | 2.58–4.97 |
| Spain | 3361 | 166 | 4.94 | 4.75 | 4.02–5.49 |
| Finland | 1052 | 70 | 6.65 | 6.2 | 4.79–7.61 |
| Italy | 9248 | 336 | 3.63 | 3.63 | 3.25–4.01 |
| Lithuania | 865 | 51 | 5.9 | 5.79 | 4.08–7.5 |
| The Netherlands | 2820 | 229 | 8.12 | 7.76 | 6.78–8.74 |
| Poland | 2827 | 163 | 5.77 | 5.53 | 4.69–6.37 |
| Slovenia | 5182 | 206 | 3.98 | 3.8 | 3.28–4.32 |
| United Kingdom | 15 574 | 805 | 5.17 | 4.77 | 4.45–5.09 |
| (e) Colophonium (colophony), 20% pet. | | | | | |
| Austria | 1113 | 54 | 4.85 | 4.78 | 3.43–6.13 |
| Switzerland | 5002 | 165 | 3.3 | 3.26 | 2.74–3.78 |
| Germany | 7475 | 326 | 4.36 | 4.08 | 3.6–4.56 |

**Table 2.** continued

| Country | n tested | n positive | % positive | % pos. std. | 95%CI |
|---|---|---|---|---|---|
| Denmark | 2552 | 85 | 3.33 | 3.31 | 2.06–4.57 |
| Spain | 3361 | 55 | 1.64 | 1.57 | 1.14–1.99 |
| Finland | 1052 | 45 | 4.28 | 4.03 | 2.87–5.19 |
| Italy | 9244 | 111 | 1.2 | 1.19 | 0.97–1.41 |
| Lithuania | 865 | 55 | 6.36 | 5.61 | 4.05–7.18 |
| The Netherlands | 2821 | 122 | 4.32 | 4.13 | 3.41–4.86 |
| Poland | 2827 | 50 | 1.77 | 1.68 | 1.21–2.15 |
| Slovenia | 5206 | 83 | 1.59 | 1.49 | 1.17–1.82 |
| United Kingdom | 15 569 | 423 | 2.72 | 2.67 | 2.41–2.92 |
| (f) Oil of turpentine, 10% pet. | | | | | |
| Austria | 1109 | 36 | 3.25 | 3.14 | 2.09–4.19 |
| Switzerland | 4972 | 85 | 1.71 | 1.62 | 1.26–1.98 |
| Germany | 7437 | 184 | 2.47 | 2.22 | 1.87–2.57 |
| Finland | 553 | 7 | 1.27 | 1.25 | 0.29–2.2 |
| (g) Trimethylbenzenepropanol, 10% pet. | | | | | |
| Switzerland | 424 | 4 | 0.94 | 0.81 | 0–1.69 |
| Germany | 981 | 9 | 0.92 | 1.12 | 0.35–1.9 |
| Spain | 1316 | 4 | 0.3 | 0.33 | 0–0.66 |
| Finland | 490 | 7 | 1.43 | 1.19 | 0.29–2.09 |
| Lithuania | 130 | 2 | 1.54 | 1.37 | 0–3.3 |
| Poland | 302 | 1 | 0.33 | 0.28 | 0–0.84 |
| (h) *Cananga odorata* extract (ylang-ylang oil), 10% pet. | | | | | |
| Austria | 1094 | 49 | 4.48 | 4.45 | 3.16–5.74 |
| Switzerland | 2962 | 83 | 2.8 | 2.87 | 2.21–3.53 |
| Germany | 5540 | 140 | 2.53 | 2.35 | 1.93–2.77 |
| (i) *Santalum album* extract (sandalwood oil), 10% pet. | | | | | |
| Austria | 1094 | 32 | 2.93 | 2.9 | 1.85–3.95 |
| Switzerland | 2964 | 42 | 1.42 | 1.34 | 0.91–1.78 |
| Germany | 5540 | 77 | 1.39 | 1.36 | 1.03–1.69 |
| (j) *Jasminum* spp. extract (jasmine absolute), 5% pet. | | | | | |
| Austria | 1094 | 33 | 3.02 | 2.56 | 1.67–3.45 |
| Switzerland | 2963 | 47 | 1.59 | 1.4 | 0.98–1.82 |
| Germany | 5544 | 101 | 1.82 | 1.61 | 1.28–1.95 |

95% CI, 95% confidence interval to the standardized prevalence; % pos. std., proportion of positives, directly age-standardized and sex-standardized.
*Groningen, 6.3%; Amsterdam-VU, 6.7% (crude) positive ($p = 0.66$, $\chi^2$ test).

**Table 3.** Overall results obtained with the three fragrance allergens available in the TRUE Test®

| Allergen | Concentration (mg/cm$^2$) | n tested | % + | % ++/+++ | % ?+/IR | % positive | % pos. std. | 95%CI |
|---|---|---|---|---|---|---|---|---|
| Fragrance mix | 0.43 | 2362 | 2.96 | 0.97 | 0.94 | 3.94 | 3.72 | 2.97–4.48 |
| *Myroxolon pereirae* resin (balsam of Peru) | 0.8 | 2362 | 1.78 | 0.47 | 0.67 | 2.24 | 2.18 | 1.59–2.78 |
| Colophonium (colophony) | 0.85 | 2360 | 1.86 | 0.59 | 0.46 | 2.46 | 2.25 | 1.67–2.84 |

95% CI, 95% confidence interval to the standardized prevalence; IR, irritant reaction; % pos.std., proportion of positives, directly age-standardized and sex-standardized.

that HICC is a relevant fragrance contact allergen, with prevalence figures ranging from 1.5% to 2.6% (25). The proportion of patients with a low degree of sensitization seems to have increased – those patients may only react to HICC 5% as tested separately in the European baseline series, and may remain negative to FM II, which contains HICC at the lower 2.5% concentration; the proportion of patients with this pattern of reactivity increased significantly, from 13.5% in 2005–2006 to 22.0% in 2013 (25). In the present analysis, 0.42% of all patients reacted only to HICC 5% pet., corresponding to 24% of all patients reacting to HICC, thus confirming the above finding.

Patients who are sensitive to *M. pereirae* are older and suffer more frequently from leg dermatitis – topical medications and remedies of various types containing

natural resins are still, in some regions, the primary causes of sensitization rather than cosmetics or fine perfumes, which currently do not contain *M. pereirae*, although they do containt distillates and extracts thereof (12). *M. pereirae*-sensitive patients have often no positive fragrance history, and show little overlap with patients reacting positively to FM I, and even less overlap with patients reacting positively to FM II (14); moreover, they are substantially older than patients reacting positively to the other fragrance markers (27), which may point to (partly) historical sources of sensitization, as mentioned above.

Combined positive reactions between the fragrance markers (except for FM II and HICC) have not been analysed in this study, but have been reported by several authors (11, 13, 25, 27). The degree of positive associations between the two classic markers (FM I and FM II, respectively) and the natural resin mixtures decreases in the order *M. pereirae*, colophonium, and oil of turpentine. As with *M. pereirae*, sources other than fragrances are frequent causes of sensitization, that is, occupational materials (glues, tapes, paints and varnishes, cooling fluids, etc.) and non-occupational products (household, hobbies, etc.).

These factors influencing sensitization have to be considered in explaining the regional differences in the prevalence data. For FM I, FM II, and HICC, the high prevalences in Austria, Switzerland and The Netherlands are probably attributable to the use of cosmetics, which have relatively high concentrations of sensitizing fragrances. On the other hand, in Italy and Spain, the prevalences for these allergens were relatively low. Ethnic factors cannot be fully excluded but, in all likelihood, differences in exposure are responsible for this. Conversely, in Spain, an unusually high rate of geraniol sensitivity has been traced to the use of a medicament containing geraniol (28).

The three essential oils were tested in only three countries, and all showed prevalences of >1%, qualifying them for inclusion in the baseline series. Ylang-ylang oil produced the most positive reactions, in agreement with previous studies (15, 16). The role of trimethylbenzenepropanol as a fragrance sensitizer is still unclear, because it was tested on a relatively low number of consecutive patients, producing positive reactions in between 0.3% and 1.4%. In a recent study from the United Kingdom on 1951 patients, trimethylbenzenepropanol was positive in 0.8% (29). Of the 15 positive cases, 7 also reacted to FM I and 4 reacted to FM II. Furthermore, in this study, the baseline series fragrance markers were extended with the 26 fragrance substances for which labelling is mandatory in the EU. The constituents of FM I were tested at the double concentration as present in the mix (except for *Evernia furfuracea* and cinnamal). The prevalence rates of positive reactions were 6.4% for FM I, 3.3% for FM II, 3.5% for *M. pereirae*, 1.3% for HICC, and 2.7% for colophonium. The last of these was not considered to be a baseline fragrance marker by the authors. The single fragrance allergens with the greatest contact allergy frequencies were cinnamyl alcohol, *E. furfuracea*, and isoeugenol. Of the 203 patients who reacted to any of the 26 fragrance marker in the baseline series, only 57.6% also reacted to a fragrance marker in the baseline series: 52.7% reacted to either FM I or FM II, 13.8% reacted to *M. pereirae*, and 6.4% reacted to HICC. These findings show that the baseline fragrance markers should be supplemented with other substances used by the fragrance industry. However, so far, no further candidate among the 26 substances to be labelled according to the present regulation seems to qualify for inclusion in the baseline series. Further patch test data from large multicentre studies, confirmation of positive reactions by repeated open application tests and information on sources of sensitization are needed. In addition, recent findings have indicated that oxidized forms of certain fragrances, such as limonene and linalool, have a higher sensitizing potential and should be preferred for diagnostic patch testing (30).

The results obtained with the TRUE Test® point towards an important technical detail. The prevalence of positive reactions was relatively low for all three allergens available in this system. Considering that the majority of patients were tested in Groningen (The Netherlands), the differences from the results obtained with the pet.-based FM I and *M. pereirae* are striking. The second test centre in The Netherlands was the Free University department in Amsterdam, and provided almost exclusively data obtained with pet.-based allergens; it may therefore be responsible for the high figures (for FM I, 12.7% of 2821 patients positive). In the two departments, FM II was tested in pet. on 4274 patients, as it is not available in the TRUE Test®. Interestingly, with this pet.-based allergen, the sensitization prevalence rates were very similar (see footnote to Table 2). This may point to FM I of the TRUE Test® having lower sensitivity for detecting contact allergy than the pet.-based test system (31), although other explanations may also be valid. In a study from Israel on 207 patients, the concordance between various allergens tested with the TRUE Test® and with investigator-loaded IQ Chambers™ was studied: high concordance was found for methylchoroisothiazolinone (MCI)/methylisothiazolinone (MI), nickel sulfate, formaldehyde, and *p*-phenylenediamine (81.5% to 72.7%), moderate concordance was found for quaternium-15, potassium dichromate, and fragrance

mix (66.7 to 58.1%), and low concordance was found for cobalt chloride and *M. pereirae* (27.6 to 18.2%) (32). In this study, among a group of 18 patients reacting to FM I and 'with demonstrated exposure' (i.e. clinical relevance to fragrances), 50% reacted to both IQ Chambers™ and the TRUE Test®, 44% reacted only to FM I IQ Chambers™, and 6% reacted only to FM I TRUE Test®. In another study on 167 patients in the United States, the Finn Chambers® system was found to be superior in detecting clinically relevant allergies to FM I, *M. pereirae*, and thiuram mix, whereas the TRUE Test® was found to be more sensitive for nickel, neomycin, and MCI/MI (33). Therefore, patch test results may differ with the test system used; for FM I in general, the TRUE Test® yields lower reactivity with fewer irritant reactions, which is also apparent in this study (and slightly better reproducibility), but with the drawback of relevant contact allergies being missed (see above) (31). Together, these findings indicate that the TRUE Test® sensitivity for screening fragrance contact allergy is definitely lower than that of the pet.-based chamber system. The test concentration of FM I in the TRUE Test® system has not been changed since its inauguration, and needs to be re-evaluated.

As mentioned in 'Methods', the emulsifier sorbitan sesquioleate was not separately tested at 20% in the European baseline series. Therefore, the test results for FM I (containing 5% sorbitan sesquioleate) may have to be corrected by the exclusion of sorbitan sesquioleate-sensitive patients. According to a recent study on 2952 patients reacting positively to FM I, 5.4% reacted positively to 20% sorbitan sesquioleate; the majority of positive reactions to FM I in sorbitan sesquioleate-positive patients were not attributable to fragrance constituents after breakdown testing (25). In a previous multicentre study on 709 consecutive patients, the frequency of reactivity to 20% sorbitan sesquioleate was only 0.7% (26). In order to validate a positive reaction to FM I, it is therefore necessary to test the emulsifier either initially as addition to the European baseline series, or later when the breakdown test with the single constituents is performed. However, the overestimation of sensitization prevalence introduced into the present analysis can be considered to be minor.

In conclusion, our data show that the basic fragrance markers FM I, FM II and HICC yield positive reactions in 6.9 to 1.7% of consecutively tested patients, showing that fragrance allergy is a common problem across Europe. The three essential oils ylang-ylang oil, jasmine absolute and sandalwood oil also yielded a rate of positive reactions of >1%, and thus qualify for inclusion in the European baseline series. Labelling for these essential oils and for a number of other important fragrance allergens, as suggested by a recent SCCS opinion (5), is still lacking. Hence, clinical relevance is difficult to evaluate, and allergen avoidance is difficult to achieve. Regional differences in the prevalence of sensitivity among European countries exist, and are most likely attributable to differences in exposure and test populations.

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