

Fragrance allergy could be missed without patch testing with 26 individual fragrance allergens

Patnapa Vejanurug, Poohglin Tresukosol, Praneet Sajjachareonpong and Pailin Puangpet

Occupational and Contact Dermatitis Unit, Institute of Dermatology, 420/7 Rajavithi Road, Ratchathewi, Bangkok 10400, Thailand

doi:10.1111/cod.12522

Summary

Background: In 2003, the EU Cosmetics Directive stated that 26 fragrance substances must be listed on the cosmetic product ingredient labels. Not all of these 26 fragrance substances are detected by the usual screening markers comprising fragrance mix I, fragrance mix II, and *Myroxylon pereirae*.

Objectives: To evaluate the usefulness of testing with the 26 individual fragrance substances in addition to the standard fragrance screening markers.

Materials and Methods: Three hundred and twelve consecutive patients were patch tested with our baseline series and the 26 specific fragrance substances required to be declared on cosmetic product ingredient labels in accordance with the EU Cosmetics Directive.

Results: Positive reactions to at least either one of the 26 individual fragrance substances or the usual fragrance screening markers were seen in 84 of 312 patients (26.9%). Fifteen of these 84 patients (17.8%) reacted negatively to the fragrance screening markers. The most common individual fragrance allergens were cinnamyl alcohol (11.2%), cinnamal (9%), and hydroxycitronellal (3.8%). Sixty-two of 312 patients (19.8%) had at least one positive reaction to the fragrance screening markers.

Conclusion: Additional patch testing with the 26 individual fragrance allergens, or with the commonest fragrance allergens identified within these 26, should be performed to optimize the detection of fragrance allergy. Cinnamyl alcohol and cinnamal are important fragrance allergens in Thailand.

Key words: 26 labelled fragrances; allergic contact dermatitis; fragrances.

Fragrance substances are recognized as some of the most common and important causes of allergic contact dermatitis. These allergens constitute the second commonest global contact allergen group identified after nickel. The prevalence of fragrance contact allergy in the general population is 1.7–4.1% (1). At the Institute of Dermatology, Thailand, fragrance substances constitute the third commonest contact allergen group identified after nickel and methylchloroisothiazolinone/methylisothiazolinone, respectively. A previous study

in 323 Thai patients during 2004–2006 showed that 20.7% of patients reacted positively to fragrance mix I (FM I), whereas 10.7% reacted to *Myroxylon pereirae* (2).

Fragrances are ubiquitous in the personal environment, and are found in cosmetic products (including shampoos, conditioners, soaps, deodorants, and make-up), household goods (such as detergents, fabric softeners, air fresheners, cleaners, and polishes), topical medicaments, clothing, foods, and essential oils. Fragrances can even be found in cutting oil fluid (3). A recent survey found that, of 300 personal care and household products available in supermarkets, 89% contained fragrance chemicals (4).

Fragrance screening markers usually comprise the artificial mixes FM I, fragrance mix II (FM II), and the 'natural' mix *M. pereirae*. In 1977, FM I was first used as

Correspondence: E-mail: pailin.samutrapong@gmail.com

Conflicts of interest: The authors declare no conflict of interests

Accepted for publication 21 November 2015

a fragrance screening marker in the baseline series. FM I comprises eight ingredients, each at 1%, with sorbitan sesquileate as an emulsifier in petrolatum: *Evernia prunastri*, cinnamal, cinnamyl alcohol, α -amyl cinnamyl alcohol, geraniol, hydroxycitronellal, isoeugenol, and eugenol. However, at least 15% of fragrance-allergic patients were undetected with FM I (5). In 2005, FM II, containing hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), citral, farnesol, citronellol, hexyl cinnamal, and coumarin, was developed. The inclusion of FM II in the baseline series increased detection rates among patients who were not detected with FM I (6). Positive reactions to FM II were found in 2.9% of patients, and only 32% of these also had reactions to FM I (6). In the same year, the EU Cosmetics Directive required 26 fragrance substances to be labelled on the cosmetics product ingredient label if present at a concentration of > 0.001% (10 ppm) in a leave-on product or > 0.01% (100 ppm) in a rinse-off product. Fourteen of these fragrance substances are components of either FM I or FM II mix. However, several studies showed that patch testing with the standard fragrance markers could miss some fragrance-allergic patients. In 2011, a retrospective study of 1508 patients in Denmark showed that 11.7% of patients were undetected by screening with mixes alone, and would have been missed without patch testing with individual fragrance allergens (7).

The first objective of this study was to evaluate the usefulness of testing with the 26 individual fragrance allergens in addition to the usual fragrance mix screens. The other objective was to investigate the frequency of positive patch test reactions to the 26 individual fragrance allergens within an Asian dermatitis population.

Materials and Methods

This prospective study was conducted at the occupational and contact dermatitis clinic, Institute of Dermatology, Bangkok, Thailand. A total of 312 consecutive dermatitis patients were enrolled in the study during December 2013 to August 2014. Patient data recorded at the first consultation included age, sex, occupation, the duration of dermatitis, the site of lesions, and exposure sources. The demographic data were recorded according to the MOALHEA index, which showed the percentage of each patient characteristic, including male sex, occupational dermatitis, atopic dermatitis, hand dermatitis, leg dermatitis, face dermatitis, and age > 40 years. All patients were patch tested with the baseline series, including fragrance screening markers (FM I, FM II, and *M. pereirae*) and 26 individual fragrance allergens. Sorbitan

sesquileate, an emulsifier in FM I, and trimethyl benzene-propanol (Majantol®) were also tested. Allergens (provided by Chemotechnique Diagnostics, Vellinge, Sweden) in Finn Chambers® (SmartPractice®, Phoenix, AZ, USA) were applied on the back. The results were read at day (D)2 and D4 according to ICDRG criteria.

Data analysis was performed with SPSS™. Chi-square tests were used for differences in characteristics. A *p*-value of < 0.05 was regarded as significant.

Results

Demographic data recorded by use of the MOALHEA index showed that fragrance-allergic patients were more likely to have hand dermatitis and to be aged > 40 years (Table 1). Of the 312 enrolled patients, 65 were males and 247 were females. The prevalence of fragrance allergy in females was higher than in males (28.7% versus 20.0%). Sixty-nine of 312 patients (22.1%) had a positive patch test reaction to at least one of the standard fragrance screening markers (FM I, FM II, and *M. pereirae*). Eighty-four of 312 patients (26.9%) reacted positively to any fragrance screening marker and/or 26 individual fragrance substances. These results showed that 15 of 84 patients (17.8%) were undetected when only patch testing with fragrance screening markers was performed. The sensitization rates of standard fragrance screening markers were 18.3% for FM I, 8.0% for FM II, and 6.8% for *M. pereirae* (Table 2).

Among 26 individual fragrance ingredients, the five most sensitizing allergens were cinnamyl alcohol (11.2%; 35/312), cinnamal (9%; 28/312), hydroxycitronellal (3.8%; 12/312), citral (3.2%; 10/312), and HICC (3.2%; 10/312) (Fig. 1). The six fragrance ingredients that did not give any positive reactions were hexyl cinnamal, benzyl alcohol, (unoxidized) linalool, anise alcohol, methyl-2-octynoate, and α -isomethyl ionone. Irritant reactions were found with cinnamal and α -isomethyl ionone, but only one reaction for each allergen occurred. Doubtful reactions were found with cinnamal, amyl cinnamyl alcohol, *E. prunastri*, eugenol, citral, (unoxidized) D-limonene, and (unoxidized) linalool.

Patients who had positive patch test reactions to the 26 individual fragrance ingredients showed a higher rate of concomitant reactions to FM I (118/146; 80.8%) than of concomitant reactions to FM II (54/146; 37%). The most common exposure sources were cosmetic products (29.5%), followed by toiletries (16.0%), deodorants (13.8%), household products (12.3%), perfumes (9.0%), and spa products (6.7%). Three of 312 patients (1%) had positive patch test reactions to trimethyl benzene-propanol. Two patients reacted positively to sorbitan sesquileate but had negative reactions to FM I.

Table 1. The MOALHFA index in fragrance patch test-positive and fragrance patch test-negative patients

MOALHFA	Fragrance-positive, n = 84		Fragrance-negative, n = 228		p-value*
	% (no.)	95%CI	% (no.)	95%CI	
Male	15.5 (13)	9.3–24.7	61.9 (52)	51.2–71.6	0.16
Occupational	17.9 (15)	11.1–27.4	82.1 (69)	72.6–88.9	0.17
Atopic dermatitis	42.9 (36)	32.8–53.5	57.1 (48)	46.5–67.2	0.70
Leg	25.0 (21)	17.0–35.2	75.0 (63)	64.8–83.0	0.89
Hand	53.6 (45)	43.0–63.9	46.4 (39)	36.2–57.0	0.015
Face	11.9 (10)	6.6–20.5	88.1 (74)	79.5–93.4	0.25
Age > 40 years	59.5 (50)	48.8–69.4	40.5 (34)	30.6–51.2	0.009

*Chi-square test.

Table 2. Number of patients with positive patch test reaction(s) to fragrance screening markers and to 26 individual fragrance substances (n = 312)

Substances	Positive reactions		Present relevance					
	n (%)	95%CI	n (%)	+?	IR	+	++	+++
Fragrance mix I	57 (18.3)	17.4–23.0	47 (82.5)	0	0	40	16	1
Fragrance mix II	25 (8.0)	5.5–11.6	21 (84.0)	0	0	22	3	0
<i>Myroxylon pereirae</i>	21 (6.8)	4.4–10.1	14 (66.7)	1	0	20	1	0
Total fragrance markers	69 (22.1)	17.8–26.9						
Cinnamyl alcohol	35 (11.2)	8.2–15.2	32 (91.4)	0	0	27	7	1
Cinnamal	28 (9.0)	6.3–12.7	25 (89.3)	1	1	22	6	0
Hydroxycitronellal	12 (3.8)	2.2–6.6	10 (83.3)	0	0	11	1	0
Citral	10 (3.2)	1.8–5.8	7 (70.0)	1	0	9	1	0
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	10 (3.2)	1.8–5.8	9 (90.0)	1	0	8	2	0
Geraniol	8 (2.6)	1.3–5.0	6 (75.0)	0	0	7	1	0
<i>Evernia furfuracea</i>	8 (2.6)	1.3–5.0	5 (62.5)	0	0	6	1	1
Isoeugenol	7 (2.2)	1.1–4.6	5 (71.4)	0	0	5	2	0
Farnesol	5 (1.6)	0.7–3.7	4 (80.0)	0	0	5	0	0
Benzyl salicylate	4 (1.3)	0.5–3.2	3 (75.0)	0	0	4	0	0
<i>Evernia prunastri</i>	3 (1.0)	0.3–2.8	2 (66.7)	1	0	3	0	0
Eugenol	3 (1.0)	0.3–2.8	2 (66.7)	1	0	3	0	0
Citronellol	3 (1.0)	0.3–2.8	3 (100.0)	0	0	3	0	0
Butylphenyl methylpropional	3 (1.0)	0.3–2.8	3 (100.0)	0	0	3	0	0
D-Limonene (unoxidized)	2 (0.7)	0.2–2.3	1 (50.0)	1	0	2	0	0
Amyl cinnamyl alcohol	1 (0.3)	0.1–1.8	1 (100.0)	1	0	1	0	0
Coumarin	1 (0.3)	0.1–1.8	1 (100.0)	0	0	1	0	0
Benzyl cinnamate	1 (0.3)	0.1–1.8	1 (100.0)	0	0	1	0	0
Benzyl benzoate	1 (0.3)	0.1–1.8	1 (100.0)	0	0	1	0	0
Amyl cinnamal	1 (0.3)	0.1–1.8	1 (100.0)	0	0	1	0	0
Hexyl cinnamal	0	–	0	0	0	0	0	0
Benzyl alcohol	0	–	0	0	0	0	0	0
Linalool (unoxidized)	0	–	0	1	0	0	0	0
Anise alcohol	0	–	0	0	0	0	0	0
Methyl-2-octynoate	0	–	0	0	0	0	0	0
α -Isomethyl ionone	0	–	0	0	1	0	0	0
Total for 26 individual fragrance substances	72 (23.1)	18.6–28.0						
Total for fragrance markers and 26 individual fragrance substances	84 (26.9)	22.2–32.1						

IR, irritant reaction.

Discussion

Our study showed that fragrance-allergic patients were more likely to have hand dermatitis and be aged > 40 years. The suspected reason for the former is that

hands were commonly exposed to cosmetics, household products, and perfumes, all of which typically contain fragrances. Moreover, the higher age of patients is in keeping with the general observation that the prevalence of

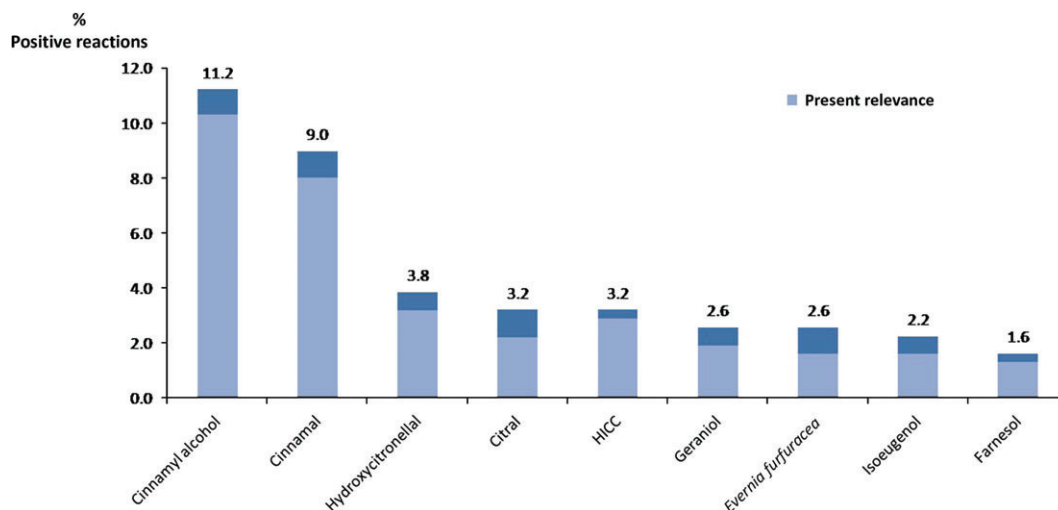


Fig. 1. Positive patch test reactions to individual fragrances and present relevance. HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.

contact allergy to cosmetic allergens increases with age, possibly for immunological reasons (loss of regulatory function) (8).

FM I, FM II and *M. pereirae* (and HICC in some centres) are currently used as fragrance screening markers. Nonetheless, our study showed that 15 of 84 fragrance-allergic patients (17.8%) were undetected without patch testing with individual fragrance substances. In 2011, a study of 1508 patients reported that 11.6% of fragrance-allergic patients were missed when patch testing with individual fragrance ingredients was not performed, meaning that patch testing with individual fragrance ingredients increased the incidence of fragrance positive reactions approximately 1.5% of the dermatitis population (7). However, the percentage of undetected patients in this study is lower than ours, probably because HICC is not included in our baseline series. Several recent studies have highlighted false-negative results in patients who are screened for fragrance allergy with the mixes alone (9–12). These findings were confirmed by a retrospective study on 1951 patients during 2011–2012. Only 117 of 203 patients (57.6%), who had positive reactions to any of 26 fragrance ingredients reacted positively to a fragrance marker in the baseline series (13).

The commonest individual fragrance allergens identified were cinnamyl alcohol (11.2%), cinnamal (9%), hydroxycitronellal (3.8%), citral (3.2%), and HICC (3.2%) (Fig. 1). Cinnamyl alcohol was also the most frequent individual fragrance allergen identified in a study in London (UK), followed by *Evernia furfuracea* and isoeugenol (13). Additional screening patch tests with the above common fragrance allergens instead of all 26 fragrance substances

may be more suitable for the economic situation in Thailand.

The concomitant rate of reactions to the 26 individual fragrance substances with reactions to FM I was 80.8%, whereas the concomitant rate with reactions to FM II was only 37%. This finding was similar to those of a previous study, which showed a higher rate of concomitant reactions to FM I than of concomitant reactions to FM II (7). It was observed that the rate of concomitant reactions to FM I and its ingredients was higher than the rate of concomitant reactions to FM II and its components (14, 15).

In this study, the common exposure sources causing fragrance allergy were cosmetic products (29.5%), followed by toiletries (16.0%), deodorants (13.8%), household products (12.3%), perfumes (9.0%), and spa products (6.7%). A Danish study reporting on patch testing with 26 individual fragrances also showed that cosmetic products were the most frequent causes of fragrance allergic contact dermatitis (7). Fragrances were found in 88% of cosmetics and detergents in the market, such as shampoos, hair conditioners, liquid soaps, wet tissues, washing-up liquids, and multipurpose cleansers (16). At least one of the 26 individual fragrance allergens was identified in 50% of cosmetic products and 32% of detergents. The most frequent allergens were linalool (38%), hexylcinnamal (32%), butylphenyl methylpropional (29%), limonene (28%), and citronellol (20%) (16). However, deodorants are also important causes of allergic contact dermatitis, especially allergy detected by FM II and HICC patch testing (17).

Trimethyl benzenepropanol has recently been identified as another fragrance contact allergen with a high rate of sensitization. This fragrance allergen is not one of

the 26 fragrance ingredients, and is not a component of fragrance screening markers. The prevalence of contact allergy in the previous study was 0.5–5.4%. In our study, 3 of 312 patients (1%) reacted positively to 5% trimethyl benzenepropanol. Only one patient had concomitant reactions to FM I, FM II, *M. pereirae*, HICC, cinnamal, cinnamyl alcohol, and hydroxycitronellal. Additional patch testing with trimethyl benzenepropanol has been recommended in some studies when fragrance allergy is suspected (18–21).

HICC is another fragrance chemical that causes a high rate of allergy. HICC is the commonest sensitizing agent in FM II. A previous study reported that patch testing with 5% HICC can detect more patients than patch testing with FM II alone. Some centres in Europe have recommended that 5% HICC should be included in baseline series (22). However, a study on 10 010 patients in Sweden showed that only 0.2% of patients were undetected when they did not undergo additional testing with 5% HICC (23). Hence, the recommendation to include 5% HICC in baseline series is still controversial. Nevertheless, the frequency of HICC allergy detected in our study was 3.2%. Nine of 10 patients who had positive reactions to HICC also had concomitant reactions to FM II. Our data therefore showed that patch testing with FM II alone could miss ~10% of HICC-allergic patients.

Limonene and linalool are widely used fragrance compounds in cosmetics, personal care products, and household products. Only 2 of 312 patients (0.7%) had positive reactions to limonene. A doubtful reaction to limonene and a doubtful reaction to linalool were found in 1 case each. The low positive rate could be attributable to the use of non-oxidized commercial allergens in this study. Testing with hydroperoxide fractions (oxidized) and patients' products may give a better detection rate.

Conclusion

Patch testing with only standard fragrance mix screening markers would have missed 17.8% of fragrance-allergic patients in our cohort. Additional patch testing with 26 individual fragrance substances would increase the sensitivity of fragrance allergy screening by approximately one-fifth. Among 26 individual fragrance allergens, the most frequent contact allergens were cinnamyl alcohol, cinnamal, hydroxycitronellal, citral, and HICC, respectively. As the method for detection of contact allergy to fragrance substances improves, the overall prevalence increases. By using extensive screening methods, we have shown that 1 in 4 of our dermatitis patients have contact allergy to fragrance substances.

References

- Johansen JD. Fragrance contact allergy: a clinical review. *Am J Clin Dermatol* 2003; **4**: 789–798.
- Boonchai W, Iamtharachai P, Sunthonpalin P. Prevalence of allergic contact dermatitis in Thailand. *Dermatitis* 2008; **19**: 142–145.
- Arribas MP, Soro P, Silvestre JF. Allergic contact dermatitis to fragrances. Part 1. *Actas Dermosifiliogr* 2012; **103**: 874–879.
- Buckley D A. Fragrance ingredient labelling in products on sale in the UK. *Br J Dermatol* 2007; **157**: 295–300.
- Nardelli A, Carbonez A, Drieghe J, Goossens A. Results of patch testing with fragrance mix 1, fragrance mix 2, and their ingredients, and *Myroxylon pereirae* and colophonium, over a 21-year period. *Contact Dermatitis* 2013; **68**: 307–313.
- Frosch P J, Pirker C, Rastogi S C et al. Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. *Contact Dermatitis* 2005; **52**: 207–215.
- Heisterberg M V, Menné T, Johansen J D. Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. *Contact Dermatitis* 2011; **65**: 266–275.
- McFadden J P, White I R, Basketter D et al. The cosmetic allergy conundrum: inference of an immunoregulatory response to cosmetic allergens. *Contact Dermatitis* 2013; **69**: 129–137.
- Cuesta L, Silvestre J F, Toledo F et al. Fragrance contact allergy: a 4-year retrospective study. *Contact Dermatitis* 2010; **63**: 77–84.
- de Groot A C, van der Kley A M, Bruynzeel D P et al. Frequency of false-negative reactions to the fragrance mix. *Contact Dermatitis* 1993; **28**: 139–140.
- Katsarma G, Gawkrödger D J. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. *Contact Dermatitis* 1999; **41**: 193–197.
- Ponyai G, Nemeth I, Altmayer A et al. Patch tests with fragrance mix II and its components. *Dermatitis* 2012; **23**: 71–74.
- Mann J, McFadden J P, White J M et al. Baseline series fragrance markers fail to predict contact allergy. *Contact Dermatitis* 2014; **70**: 276–281.
- Krauthaim A, Uter W, Frosch P et al. Patch testing with fragrance mix II: results of the IVDK 2005–2008. *Contact Dermatitis* 2010; **63**: 262–269.
- Uter W, Geier J, Frosch P, Schnuch A. Contact allergy to fragrances: current patch test results (2005–2008) from the Information Network of Departments of Dermatology. *Contact Dermatitis* 2010; **63**: 254–261.
- Yazar K, Johnsson S, Lind M L et al. Preservatives and fragrances in selected consumer-available cosmetics and detergents. *Contact Dermatitis* 2011; **64**: 265–272.
- Heisterberg M V, Menné T, Andersen K E et al. Deodorants are the leading cause of allergic contact dermatitis to fragrance ingredients. *Contact Dermatitis* 2011; **64**: 258–264.
- Larsen W, Nakayama H, Lindberg M et al. Fragrance contact dermatitis: a worldwide multicenter investigation (Part I). *Am J Contact Dermat* 1996; **7**: 77–83.
- Larsen W, Nakayama H, Fischer T et al. Fragrance contact dermatitis – a worldwide multicenter investigation (Part

- III). *Contact Dermatitis* 2002; **46**: 141–144.
- 20 Schnuch A, Geier J, Uter W, Frosch P J. Majantol – a new important fragrance allergen. *Contact Dermatitis* 2007; **57**: 48–50.
- 21 Heisterberg M V, Johansen J D. Contact allergy to trimethyl-benzenepropanol (Majantol). *Contact Dermatitis* 2009; **61**: 360–361.
- 22 Bruze M, Andersen K E, Goossens A. Recommendation to include fragrance mix 2 and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall) in the European baseline patch test series. *Contact Dermatitis* 2008; **58**: 129–133.
- 23 Isaksson M, Inerot A, Lidén C et al. Multicentre patch testing with fragrance mix II and hydroxyisohexyl 3-cyclohexene carboxaldehyde by the Swedish Contact Dermatitis Research Group. *Contact Dermatitis* 2014; **70**: 187–189.