

Further important sensitizers in patients sensitive to fragrances*

I. Reactivity to 14 frequently used chemicals

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The aim of this study was to determine the frequency of responses to selected fragrance materials in consecutive patients patch tested in 6 dermatological centres in Europe. 1855 patients were evaluated with the 8% fragrance mix (FM) and 14 other frequently used well-defined fragrance chemicals (series I). Each patient was classified regarding a history of adverse reactions to fragrances: certain, probable, questionable, none. Reactions to FM occurred in 11.3% of the subjects. The 6 substances with the highest reactivity following FM were Lyrall® (2.7%), citral (1.1%), farnesol P (0.5%), citronellol (0.4%), hexyl cinnamic aldehyde (0.3%), and coumarin (0.3%). 41 (2.2%) of the patients reacted only to materials of series I and not to FM. 6.6% of 1855 patients gave a history of adverse reactions to fragrances which was classified as certain. This group reacted to FM only in 41.1%, to series I and FM in 12.0% and to series I only in 7.2%. 74.3% of the 39 patients reacting to both FM and 1 of the materials of series I had any type of positive fragrance history, which was significantly higher in comparison to those with isolated reactions to series I (53.6% of 41), $p = 0.04$. The study identified further sensitizers relevant for patch testing of patients with contact dermatitis, of which Lyrall® is the most important single chemical.

Key words: contact allergy; fragrance mix; fragrances; history of fragrance sensitivity; Lyrall®; patch testing. © Blackwell Munksgaard, 2002.

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Positive patch test reactions to the fragrance mix (FM) have steadily increased over recent years and range from 5.5% to 11.4% in most published studies on consecutive dermatological patients (1, 2). As the FM contains only 8, though still widely used, perfume constituents, this diagnostic tool cannot identify all fragrance-sensitive individuals. De Groot et al. (3) estimate that about 15% of relevant cases are not detected by the FM. In our recent project on fragrances funded by the EU Commission we have therefore focused on identifying new sensitizers. Based on literature reports and own experience we have selected 14 defined chemi-

cals for patch testing in 6 dermatological centres of Europe (series I). 4- (4-hydroxy-4 methyl-pentyl)-3-cyclohexene carboxaldehyde (Lyrall®, International Flavors & Fragrances, New York, NY, USA; Kovanol®, Takasago International Corp., Tokyo, Japan) turned out to be of special importance and was therefore reported in a separate publication (4). In this paper we describe the results obtained with the other materials of series I. In the next paper we will report the reactivity to selected essential oils.

Materials and Methods

Test materials

All materials were obtained from leading manufacturers of the perfume industry. Purity grades varied but were usually in the range of 95–99% as

*Dedicated to Professor Detlef Petzoldt, Director of the Department of Dermatology, University of Heidelberg (Germany) on the occasion of his 65th birthday.

Table 1. Test concentrations

Test fragrance	Chemical name	CAS Reg. No.	Test concentration
1. Lyrall®	4-(4-hydroxy-4 methylpentyl)-3-cyclohexene carboxaldehyde	31906-04-4	5%
2. Citral (1:2 mixture of <i>cis</i> β -citral and <i>trans</i> α -citral)	3,7-dimethyl-2,6-octadien-1-al	5392-40-5	2%
3. Farnesol P (4 isomers)	3, 7, 11-trimethyl-2, 6, 10-dodecatrien-1-ol	4602-84-0	5%
4. DL-citronellol	3,7-dimethyl-6-octen-1-ol	106-22-9	1%
5. α -Hexyl cinnamic aldehyde (<i>trans</i> - and <i>cis</i> -)	2-(phenylmethylene)-1-octanal	101-86-0	10%
6. Coumarin	2H-benzopyran-2-one	91-64-5	5%
7. Lilial®	2-methyl-3- (4-tert-butylphenyl)propanal	80-54-6	10%
8. Linalool acetate	3,7-dimethyl-1,6-octadiene-3-yl acetate	115-95-7	10%
9. Dimethylbenzyl carbiny acetate (DMBCA)	2-methyl-1-phenylprop-2-yl acetate		5%
10. Cyclacet® (greenyl acetate)	4,7-methano-3a,4,5,6,7,7a-hexahydro-5 (or 6)-indenyl acetate	2500-83-6 (or 5413-60-5)	5%
11. Amyl salicylate	2-hydroxy benzoic acid, pentyl ester	2050-08-0	5%
12. Vertofix® (acetyl cedrene)	[3R (3a,3ab,7b,8aa)]-1-(2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one	68867-57-2	5%
13. Galaxolide®	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(gamma)-2-benzopyrane	1222-05-5	10%
14. ISO E Super®	2,3,8,8-tetramethyl-1,2,3,4,5,6,7,8-octahydro-2-naphthalenyl methyl ketone	54464-57-2	5%

Hexyl cinnamic aldehyde was from Givaudan-Roure (Argenteuil, France) and all other test fragrances were from Dragoco (Holzminden, Germany).

indicated by the producer and used for the composition of fine perfumes. The materials were diluted with petrolatum (pet.) except for Galaxolide® (Dragoco, Holzminden, Germany)(50% isopropyl myristate). The fragrance material was carefully mixed with a small portion (about 1%) of the pet. using a pestle. After the material was homogeneously distributed the remaining pet. was added under continuous stirring. No emulsifier was added.

Test concentrations were based on literature reports (5) and pilot testing in 4 centres (Copenhagen, Dortmund, Malmö, London) (Table 1).

Patients and test centres

Consecutive patients of contact dermatitis clinics at 6 dermatology departments were tested (Dortmund, Copenhagen, Malmo, Odense, London and Leuven) in the time period between October 1997 and October 1998. In addition to the standard series, the 8% FM from the same source and batch (Hermal, Reinbek, Germany) was applied to the back skin for 2 days. Finn Chambers on Scanpor tape were used in all centres except Leuven (van der Bend chambers). Readings were taken at most centres on days (D) 2 and 4. The reading at D3 or D4 was used for overall evaluation of positive test results. Test reactions were categorized according to published guidelines (6).

History of fragrance sensitivity

Each patient was placed in 1 of 4 categories according to a history of adverse reactions to fragrances, as described previously (4): certain, probable, questionable and none. This history was taken before the patch testing procedure.

Results

Patch test results with series I and FM are shown in Table 2.

In the total population of 1855 patients the mean positivity rate to FM was 11.3% (range 8.2% – 23%). About 1/2 of the reactions were + in intensity. Doubtful reactions were observed in 6.3% of patients tested. Positive reactions to materials of series I were highest for Lyrall (2.7%), followed closely by citral (1.1%) and farnesol P (0.5%). The remaining substances caused reactions only rarely, the majority being of + type. Reactions classified as doubtful were observed at a much lower frequency compared to those obtained with FM (1.3% for citral and 0.3% for amyl salicylate).

Variations between the test centres are shown in Table 3. For FM and the top 6 of series I extraordinarily high values were observed in Leuven, the lowest figures being recorded in Gentofte. The ranking of the test materials was very similar in all centres.

Table 2. Results of series I obtained by testing 1855 consecutive patients. Listed are numbers of observed reactions

	Doubtful		Positive			Total	%
	n	%	+	++	+++		
FM 8%	116	6.3	110	80	20	210	11.3
Lyrall [®] 5%	20	1.1	23	23	4	50	2.7
citral 2%	25	1.3	12	8	1	21	1.1
farnesol P 5%	21	1.1	10	0	0	10	0.5
citronellol 1%	13	0.7	7	0	0	7	0.4
hexyl cinnamic aldehyde 10%	11	0.6	6	0	0	6	0.3
coumarin 5%	15	0.8	4	1	0	5	0.3
linalool acetate 10%	8	0.4	4	0	0	4	0.2
DMBCA 5%	12	0.6	3	0	0	3	0.2
Lilial [®] 10%	12	0.6	1	2	0	3	0.2
Iso E. Super [®] 5%	6	0.3	2	1	0	3	0.2
Vertofix [®] 5%	8	0.4	3	0	0	3	0.2
Galaxolide [®] 10% (in IPM)	6	0.3	3	0	0	3	0.2
amyl salicylate 5%	5	0.3	3	0	0	3	0.2
Cyclacet [®] 5%	7	0.4	1	0	0	1	0.1
Isopropyl myristate	0		1	0	0	1	0.1

Table 3. Top 6 of series I in 6 European centres

	Dortmund n = 281	Gentofte n = 303	London n = 475	Malmö n = 395	Leuven n = 70	Odense n = 331
FM	11.4%	8.2%	10.3%	13.9%	23.0%	9.9%
Lyrall [®]	1.4%	2.6%	1.2%	3.0%	17.2%	2.4%
Citral	1.1%	1.3%	0.6%	1.2%	5.7%	0.6%
Farnesol P [®]	0.0%	0.3%	0.6%	0.2%	5.7%	0.6%
Hexyl cinnamic aldehyde	0.0%	0.0%	0.4%	0.0%	5.7%	0.0%
Citronellol	0.0%	0.0%	0.6%	0.5%	2.8%	0.0%
Coumarin	0.7%	0.0%	0.2%	0.0%	2.8%	0.0%

Table 4. History of adverse reaction to fragrances of each patient related to positive patch tests results to fragrance mix and series I. The total of 1855 patients was separated into 4 groups of fragrance history

	History	FM positive only	Series I and FM positive	Only series I positive
Certain	124/1855 (6.6%)	51/124 (41.1%)	15/124 (12.0%)	9/124 (7.2%)
Probable	150/1855 (8.0%)	29/150 (19.3%)	7/150 (4.7%)	6/150 (4.0%)
Questionable	170/1855 (9.2%)	14/170 (8.2%)	7/170 (4.1%)	7/170 (3.5%)
None	1411/1855 (76.1%)	77/1411 (5.4%)	10/1411 (0.7%)	19/1411 (1.3%)

Tables 4 and 5 and Fig. 1 show the patch test results in relation to the history of adverse reactions to fragrances. In our population of 1855 consecutive patients, 124 (6.6%) reported a fragrance history which was considered certain (patient has reacted with an itching dermatitis to at least one fine perfume or aftershave and has reacted to other scented products). Of these, 41.1% were positive to FM only, a further 12% were positive to FM and series I. It is noteworthy that 7.2% showed a positive reaction only to one of the materials in series I and were negative to FM.

However, in 19 cases positive to series I materials only, no positive fragrance history was ascertained. For statistical comparison, the groups with a fragrance history that was certain, probable or questionable were pooled together as any type of his-

tory *versus* none. If this is applied to the 3 groups of patients listed in Table 4, a statistically significantly higher rate of any type of history was seen in the group positive to FM and series I, 29/39 (79.3%) than in patients positive only to series I, 22/41 (53.6%), $p = 0.04$, χ^2 test. The difference from those positive only to FM 94/171 (55.0%) was not statistically significant, $p = 0.09$, χ^2 test (Fig. 2).

In Table 6 the test results obtained with FM are compared to those of series I – for clarity reasons the data are shown only for positive reactions of the top 6 of series I. It is evident that with all fragrances of series I, except α -hexyl cinnamic aldehyde, FM was negative in individual cases (19–42%). The highest discrepancy was found for Lyrall (21 patients reacted positively to Lyrall but were negative to FM). For further details regarding his-

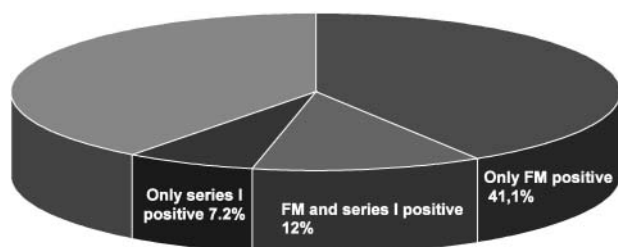


Fig. 1. History of adverse reactions to fragrance and benefit of series I. Test results of 124 patients with a certain history.

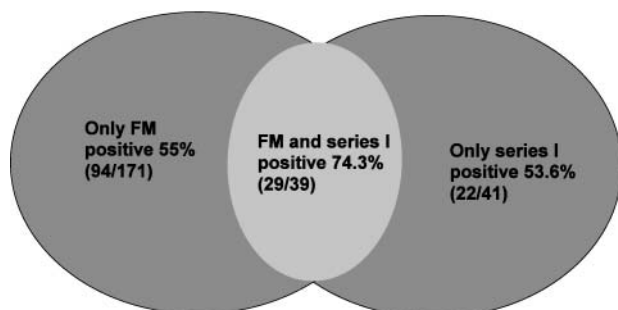


Fig. 2. Patients positive to fragrance mix and series I in relationship to any type of history of fragrance sensitivity (certain, probable, questionable). Patients with a reaction to FM and one of the materials of series I showed a higher proportion of a positive history than the group with isolated FM reactions ($p=0.09$) and the group with isolated series I reactions ($p=0.04$).

tory of fragrance sensitivity and causative products the reader is referred to the previous publication on Lyrall (4).

Citral was the material with the next highest reactivity. Of the 21 patients with a positive reaction, 6 had a certain fragrance history; one of these was negative to FM. 4 patients were also positive to citronellol and 3 patients also to hydroxycitronel-

lal. These 3 patients were strongly positive to FM as well.

In London, Dortmund and Odense 3 cases with positive reactions to Lilial® (Dragoco) were observed. In the 2 with a ++ reaction, the FM was positive only in 1; this patient also had a certain fragrance history, while the other 2 patients had a questionable and no history, respectively. The 3 patients with positive reactions to Iso E super® (Dragoco) (London, Leuven) were also strongly positive to FM. However, all 3 denied a history of fragrance sensitivity.

Discussion

The most important result of this study is that the time-proven FM, as introduced by Larsen in 1977 (7), needs nowadays to be supplemented by further materials used by the fragrance industry. Lyrall is the clear 'winner' of series I, with a positivity rate of 2.7% in our population. As discussed previously, worldwide consumption of Lyrall was estimated to be 520 tons in 1996 (8) and it is not only used in fine perfumes but also in popular aftershaves, hand and body lotions and in deodorants for example (4). In a multicentre trial of the German Contact Dermatitis Group, 5% Lyrall (pet.) produced 1.9% positive reactions in 3230 patients (16/62 reacted only to Lyrall® and were negative to FM) and was therefore recommended for the German standard series as of June 2001 (9). A further candidate for the diagnostic 'arsenal' necessary to identify fragrance-sensitive individuals is citral. Allergic reactions were recorded in 1.1% of our patients, nearly 1/2 of them ++ reactions. In 25 cases the reaction was regarded as doubtful, showing erythema only. This may reflect irritancy or low levels of sensitization. In some cases the lower concentration of ci-

Table 5. Patients positive to fragrance mix and series I in relationship to their history

History	FM positive only (n = 171)	Series I and FM positive (n = 39)	Series I positive only (n = 41)
Certain	51/171 (29.8%)	15/39 (38.5%)	9/41 (21.9%)
Probable	29/171 (16.9%)	7/39 (17.9%)	6/41 (14.6%)
Questionable	14/171 (8.2%)	7/39 (17.9%)	7/41 (17.1%)
None	77/171 (45.0%)	10/39 (25.6%)	19/41 (46.3%)

Table 6. Reactions to FM in comparison to those of series I (top 6)

	Lyrall pos.	Citral pos.	Farnesol pos.	Citronellol pos.	Hexyl cinnamic aldehyde pos.	Coumarin pos.
FM positive	24	16	6	4	5	3
FM doubtful	5	1	0	0	1	0
FM negative	21	4	4	3	0	2
Total	50	21	10	7	6	5

tral (0.4% pet.) was tested and still elicited a reaction, though weaker than that obtained at 2%.

Citral is a known sensitizer, as recently demonstrated in the local lymph node assay (10). In comparison to strong sensitizers such as dinitrochlorobenzene (DNCB), citral was classified as a weak sensitizer (Human Class 3 based on the EC 3-value, i.e. estimated concentration required to produce a 3-fold increase in draining lymph cell proliferative activity). In the same class were other fragrances known to be relevant: eugenol, hydroxycitronellal and hexyl-cinnamic aldehyde. It is in line with clinical observations that isoeugenol and cinnamic aldehyde – 2 prominent constituents of the FM – were found to be more potent sensitizers and were in the same Human Class 2 as glutaraldehyde and tetramethylthiuram disulphide. Maltén et al. (11) noted as early as 1984 that citral may be relevant in patients with suspected sensitization to cosmetics: 2.6% of 182 patients were positive to citral 2% pet. Citral is widely used by industry as a 'safe' perfuming agent, based on the recommendation of IFRA that the sensitizing potential of citral will be reduced by the addition of limonene. Basketter (12) has recently reviewed the so-called quenching phenomenon and concludes that convincing experimental proof of this claim is still lacking. Medicine is evidence-based; the quenching hypothesis should now be expunged from the scientific literature.

With regard to structure-activity relationship (SAR) and sensitization, the structural alert type 3 (α,β unsaturated aldehydes, amides, esters and ketones) was found for citral and was present in more than 1/2 of 71 analysed deodorants of the European market (13). Citral was found in 25% of 59 domestic and occupational products at a concentration range of 48–1088 ppm (14).

Farnesol P is the 3rd material which should to be added to the fragrance series for patch testing. Although it produced only 10 + reactions in our group there is no question that this substance is a sensitizer. There are several clinical reports (15–18). Deodorants frequently contain farnesol P because of its antimicrobial activity (19). Farnesol P is a weak sensitizer according to guinea pig studies (20). It can be detected in balsam of Peru (*Myroxylon Pereirae* resin) and in poplar buds. Patients sensitized by propolis ointments may react to a deodorant containing farnesol P.

The next 3 substances, citronellol, hexyl cinnamic aldehyde and coumarin, showed much lower patch test reactivity. Some of the reactions recorded as doubtful may represent low grades of sensitization, as discussed previously (4). Allergic reactions to coumarin are well-documented. Maltén et al. (11) in his series of 182 patients found

6.8% positive to coumarin 8% pet. In another clinical study from the Netherlands, involving approximately 1400 patients tested between 1978 and 1997, 58 patients had positive reactions to coumarin 5% pet (21). 38 of these had also positive reactions to other fragrance markers, but 20 reacted to coumarin alone.

In a worldwide multicentre investigation on 167 subjects with proven sensitization to fragrance materials (by previous patch tests or on historical and clinical grounds), 1.2% of the reactions to 5% coumarin were interpreted as allergic in the final evaluation (23). In a bioassay-guided fractionation of a perfume, coumarin was identified as a relevant sensitizer in a patient sensitive to her own perfume but negative to the fragrance mix (23).

In experimental procedures, purified coumarin was found to be neither a contact nor a photosensitizer (24, 25). However, as we have discussed previously, these assays have failed more than once in identifying sensitizers, if the chemical has been widely used in humans (4). With regard to coumarin it may be that an as yet unidentified derivative or impurity is the sensitizer. Another explanation is cross-reactivity with coumarin in patients sensitive to other fragrance chemicals or mixtures.

The sensitizing potential of hexyl cinnamic aldehyde cannot be doubted as it is recommended as a positive control in animal sensitization assays (26). It has been shown to be clinically relevant by de Groot et al. (27).

Citronellol has been reported to give a positive reaction in subjects allergic to citronella oil (28) and was considered relevant in patients with cosmetic dermatitis (29). It is found in about 70 essential oils, at particularly high concentrations in Bulgarian rose oil. It is synthesized from geraniol-nerol or citral. Citronellol is relatively stable in comparison to geraniol, which contains one or more double bonds. In a recently performed worldwide multicentre study on 178 patients with proven sensitization to fragrances, citronellol (5% pet.) was positive in 5.6% (30). FM was positive in 78.7% of this cohort, which is attributable to the inclusion criteria used by the authors (positive reaction to FM or other fragrance allergens).

The remaining 8 chemicals seem to be less important as diagnostic screening agents. However, they may be of great relevance in individual cases. Lilial and Iso E super elicited strong patch test reactions in 2 patients of this group, although some remained unclear as to relevance. Unfortunately, none of the patient's products suspected as the cause of sensitization was available for chemical analysis. Lilial was recently found to be the sole cause of allergic contact dermatitis after SAR-orientated fractionation of the perfume of a pa-

tient (31). She was also negative to FM. In a Japanese series of 685 patients suspected of fragrance sensitivity, 10% Lillal (lily aldehyde) produced a positivity rate of 0.44% (19). Larsen (32) reported a case of axillary dermatitis in a 22-year-old man who reacted severely to 1% Lillal® and less intensely to geraniol.

Patch test reactivity to series I would have been much higher if aimed testing of patients with suspected or proven fragrance allergy, instead of consecutive testing of patients attending a patch test clinic, had been performed, as the results of Larsen et al. demonstrate (22, 30). Furthermore, regional differences have to be taken in account. The department of Leuven University recorded the highest figures for all the materials. This has been due to the relatively low number of patients tested ($n = 70$). The use of the van der Bend chamber is a methodological difference to the other centres. In addition, the high figures may be explained by the fact that this department is a special reference centre for Belgium, attracting many difficult cases with multiple sensitivities. This would correlate with the observation that in Leuven 25.7% of the patients tested had a fragrance history classified as certain or probable, in contrast to lower corresponding figures of other centres (range 13.2–16.8%). Furthermore, the high rates of sensitization may be associated with the frequent use of one or more popular products containing a potential sensitizer or particular habits in the prescription of doctors (e.g. 'natural remedies' with essential oils).

Finally, our data regarding the history of fragrance sensitivity in relationship to the patch test results are of special interest. It is the largest data pool on this issue ever reported. In most patch test studies with fragrance materials this aspect has never been elucidated to such an extent. Johansen et al. (33) have found a significant correlation between a positive reaction to FM and self-reported adverse reactions (visible skin symptoms) to scented products (no such relationship could be established to balsam of Peru). We classified our population of 1855 patients into 4 categories of fragrance sensitivity by history: 6.6% certain, 8.0% probable, 9.2% questionable and 76.1% none. We are fully aware of the possibility that not all of those with a self-assessed certain history have a real contact allergy to one 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, based on our own clinical experience, most such patients will turn out to be allergic to a perfume ingredient, if studied intensively and followed for some time. Therefore, we have confidence in these figures and estimate that at least 5% of consecutively tested

patients of a patch test clinic in Central Europe do have a real problem with fragrances.

In a Danish study on 567 unselected persons of the general population, the FM (TRUE Test system) was positive in 1.1% (34). In a follow-up study 8 years later, the risk of contact sensitivity to the cosmetic-related haptens had increased significantly from 2.4% in 1990 to 5.8% in 1998 (35); women in the age group of 15–22 years were positive to FM in 2.9% (men 2.6%). Schnuch et al. has estimated the absolute number of patients with contact dermatitis, based on the sales figures for patch test preparations of the standard series in Germany (36). With regard to fragrances, the data of the IVDK over a 9-year period of 78,067 patients were used. In these patients FM was positive in 11.7%, taking together all types of positive reactions (+ to +++), and in 4.0% if only strong reactions (++ and +++) were considered. It was estimated that in the population of Germany the FM positivity rate would be 4.1% and 1.4%, respectively, (3.36 and 1.15 million of the total population of 82 million). Even if this worst case scenario is ameliorated, the frequency of unequivocal allergic reactions (++, +++) in the general population is estimated still to be 656,000 subjects.

It has been documented that patients with strong reactions to FM have a high degree of certain fragrance history and frequently react to exposure tests such as the repeated open application test (ROAT) (37). It should not need to be said that manufacturers of fragrances can no longer dispute these figures, and claim that fragrances are safe or that they cause contact allergies only in rare cases!

As shown in Table 4 and Fig. 1 it is noteworthy that of this group with a certain history, only 53% have reacted to the currently used FM; 12% were positive to FM and 1 of the materials of series I. A further 7.2% were only positive to series I, which was actually only to Lyrall. In other words, the benefit or gain in testing for true cases of contact allergy to fragrances lies primarily in testing with Lyrall and this will identify another 7% of cases. However, as the data with no fragrance history show, we still have a considerable portion of patch test positives to FM, series I and Lyrall without 'a history of fragrance problem'. These may be false-positive reactions, i.e. positive reactions in the absence of allergy, caused by irritancy. On the other hand, a low level of sensitization resulting in a weak erythematous reaction must be considered. We think that this may be more frequent than previously considered, because exposure to fragrance ingredients is now ubiquitous, having increased considerably over recent years (household products of any kind, the booming fine perfume market including perfumes for babies and toy sets for

children, various cosmetics, hand creams, sunscreens, and, last but not least, aromatherapy). In this context, it is important that patients with hand eczema have a significantly increased risk of being fragrance sensitive (38). In a multifactorial analysis on the association between occupation and contact allergy to FM, Uter et al. (39) showed that masseurs, physiotherapists and geriatric nurses had the highest occupational risk of acquiring this type of sensitivity. Further non-occupational influencing factors were atopy, female sex, increasing age and certain locations, particularly the axillae. Therefore, patients with chronic eczema, particularly of the hands, may not be aware of their fragrance sensitivity.

This might contribute to the relatively high figure of patients with positive reactions to either FM or series I and a negative history. In other words, the proportion of false-positive reactions might be smaller than these figures suggest. Realizing the importance of this aspect, our group is currently studying fragrance constituents in various household products and their role in causing/aggravating hand eczema (14).

Quantitative aspects may be highly relevant to the fragrance issue. We have shown recently that there is a wide individual variation in the degree of perfume sensitivity. Repeated open application tests may have to be performed for 4 weeks to obtain a positive reaction in a subject with low sensitivity (40, 41). Such a patient may not react to a product with a relatively low concentration of the same fragrance material in a formulated cosmetic product, particularly if the dose is low or exposure very short (e.g. in rinse-off products). This phenomenon is not restricted to fragrances but has been increasingly recognized with important occupational sensitizers such as metals and preservatives (42).

In this context we would like to emphasize that the test concentrations used in this and the following study (series II) were based on literature reports and pilot testing. These concentrations may not be optimal and may have to be revised after careful dose-response studies in sensitive individuals.

In conclusion, this study has shed more light on the growing problem of fragrance allergy and has identified important sensitizers for diagnostic patch testing which should be used in addition to the FM of Larsen. At present we are studying the efficacy of a new mix containing the top 6 materials of series I.

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References

1. De Groot A C, Frosch P J. Adverse reactions to fragrances. *Contact Dermatitis* 1997; 36: 57–86.
2. Johansen J D, Menné T. The fragrance mix and its constituents: a 14-year material. *Contact Dermatitis* 1995; 32: 18–23.
3. De Groot A C, van der Kley A M J, Bruynzeel D P et al. Frequency of false-negative reactions to the fragrance mix. *Contact Dermatitis* 1993; 28: 139–140.
4. Frosch P J, Johansen J D, Menné T et al. Lyrall® is an important sensitizer in patients sensitive to fragrances. *Br J Dermatol* 1999; 141: 1076–1083.
5. De Groot A C, Weyland J W, Nater J P. *Unwanted Effects of Cosmetics and Drugs Used in Dermatology*, 3rd edn. Amsterdam: Elsevier, 1994.
6. Wahlberg J E. Patch testing. In: Rycroft R J G, Menné T, Frosch P J, Lepoittevin J P, eds. *Textbook of Contact Dermatitis*, 3rd edn. Heidelberg: Springer, 2001: 435–468.
7. Larsen W G. Perfume dermatitis. A study of 20 patients. *Arch Dermatol* 1977; 113: 623–626.
8. Somogyi L. The flavour and fragrance industry serving a global market. *Chem Ind* 1996; 170–173.
9. Geier J, Brasch J, Schnuch A, Lessmann H, Pirker C, Frosch P J. Lyrall® is included in the patch test standard series in Germany. *Contact Dermatitis*, in press.
10. Basketter D A, Blaikie L, Dearman R J et al. Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Dermatitis* 2000; 42: 344–348.
11. Maltén K E, van Ketel W G, Nater J P, Liem D H. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984; 11: 1–10.
12. Basketter D. Quenching: fact or fiction? *Contact Dermatitis* 2000; 43: 253–258.
13. Rastogi S C, Lepoittevin J P, Johansen J D et al. Fragrances and other materials in deodorants: search for potentially sensitizing molecules using combined GC-MS and structure activity relationship (SAR) analysis. *Contact Dermatitis* 1998; 39: 293–303.
14. Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001; 45: 221–225.
15. Hemmer W, Focke M, Leitner B, Götz M, Jarisch R. Axillary dermatitis from farnesol in a deodorant. *Contact Dermatitis* 2000; 42: 168–169.
16. Goossens A, Merckx L. Allergic contact dermatitis from farnesol in a deodorant. *Contact Dermatitis* 1997; 37: 179–180.
17. De Groot A C. Contact allergy to cosmetics: causative ingredients. *Contact Dermatitis* 1987; 17: 26–34.
18. Sugai T. Group study IV – farnesol and lily aldehyde. *Environ Dermatol* 1994; 1: 213–214.
19. Hausteiner U F, Herrmann J, Hoppe U, Engel W, Saueremann G. Wachstums- hemmung coryneformer Bakterien durch ein naturidentisches Dreistoff- gemisch (Farnesol, Glycerinmonolaurat, Phenoxyethanol: HGQ). *Akt Dermatol* 1991; 17: 359–361.
20. Hausen B M, Evers P, Stüwe H T, König W A, Wollenweber E. Propolis allergy (IV). Studies with further sensitizers from propolis and constituents common to propolis, poplar buds and balsam of Peru. *Contact Dermatitis* 1992; 26: 34–44.
21. Kunkeler A C M, Wejland J W, Bruynzeel D P. The role of coumarin in patch testing. *Contact Dermatitis* 1998; 39: 327–328.
22. Larsen W, Nakayama H, Lindberg M et al. Fragrance contact dermatitis: a worldwide multicenter investigation (Part I). *Am J Contact Dermatitis* 1996; 7: 77–83.

23. Mutterer V, Giménez Arnau E, Lepoittevin J P et al. Identification of coumarin as the sensitizer in a patient sensitive to her own perfume but negative to the fragrance mix. *Contact Dermatitis* 1999; 40: 196–199.
24. Hausen M B, Berger M. The sensitizing capacity of coumarins (III). *Contact Dermatitis* 1989; 21: 141–147.
25. Kaidbey K H, Kligman A M. Photomaximization test for identifying photoallergic contact sensitizers. *Contact Dermatitis* 1980; 6: 161–169.
26. Basketter D, Selbie E, Scholes E W et al. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. *Food Chem Toxicol* 1993; 31: 63–67.
27. De Groot A C, Bruynzeel D P, Bos J D et al. The allergens in cosmetics. *Arch Dermatol* 1988; 124: 1525–1529.
28. Keil H. Contact dermatitis due to oil of citronellal. *J Invest Dermatol* 1947; 8: 327.
29. De Groot A C. Adverse reactions to cosmetics. Thesis, University of Groningen, 1988.
30. Larsen W G, Nakayama H, Fisher T et al. Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis* 2001; 44: 344–346.
31. Arnau Giménez E, Andersen K E, Bruze M et al. Identification of Lilial® as a fragrance sensitizer in a perfume by bioassay-guided chemical fractionation and structure-activity relationships. *Contact Dermatitis* 2000; 43: 351–358.
32. Larsen W G. Allergic contact dermatitis to the fragrance material lilial. *Contact Dermatitis* 1983; 9: 158–159.
33. Johansen J D, Andersen T V, Veien N, Avnstorp C, Andersen K E, Menné T. Patch testing with markers of fragrance contact allergy. *Acta Derm Venereol (Stockh)* 1997; 77: 149–153.
34. Nielsen N H, Menné T. Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study. Denmark. *Acta Derm Venereol* 1992; 72: 456–460.
35. Nielsen N H, Linneberg A, Menné T et al. Allergic contact sensitization in an adult Danish population. Two cross-sectional survey eight years apart (The Copenhagen Allergy Study). *Acta Derm Venereol* 2001; 81: 31–34.
36. Schnuch A, Uter W, Geier J, Gefeller O for the IVDK study group. Epidemiology of contact allergy: an estimation of morbidity employing the clinical epidemiology and drug utilisation research (CE-DUR) approach. *Contact Dermatitis*, in press.
37. Frosch P J, Pilz B, Burrows D et al. Testing with fragrance mix. Is the addition of sorbitan sesquileate to the constituents useful? *Contact Dermatitis* 1995; 32: 266–272.
38. Christophersen J, Menné T, Tanghoj P et al. Clinical patch test data evaluated by multivariate analysis. Danish Contact Dermatitis Group. *Contact Dermatitis* 1989; 21: 291–299.
39. Uter W, Schnuch A, Geier J, Pfahlberg A, Gefeller O. Association between occupation and contact allergy to the fragrance mix: a multifactorial analysis of national surveillance data. *Occup Environ Med* 2001; 58: 392–398.
40. Andersen K E, Johansen J D, Bruze M et al. The time-dose-response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001; 170: 166–171.
41. Johansen J D, Bruze M, Andersen K E et al. The repeated open application test. Suggestions for a scale of evaluation. *Contact Dermatitis* 1997; 39: 95–96.
42. Menné T, Johansen J D et al. Allergic contact dermatitis in man – experimental and quantitative aspects. In: Rycroft R J G, Menné T, Frosch P J, Lepoittevin J P, eds. *Textbook of Contact Dermatitis*, 3rd edn. Heidelberg: Springer, 2001: 237–248.

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