

Identification of Lilial® as a fragrance sensitizer in a perfume by bioassay-guided chemical fractionation and structure-activity relationships

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Fragrance materials are among the most common causes of allergic contact dermatitis. The aim of this study was to identify in a perfume fragrance allergens not included in the fragrance mix, by use of bioassay-guided chemical fractionation and chemical analysis/structure-activity relationships (SARs). The basis for the investigation was a 45-year-old woman allergic to her own perfume. She had a negative patch test to the fragrance mix and agreed to participate in the study. Chemical fractionation of the perfume concentrate was used for repeated patch testing and/or repeated open application test on the pre-sensitized patient. The chemical composition of the fractions giving a positive patch-test response and repeated open application test reactions was obtained by gas chromatography-mass spectrometry. From the compounds identified, those that contained a "structural alert" in their chemical structure, indicating an ability to modify skin proteins and thus behave as a skin sensitizer, were tested on the patient. The patient reacted positively to the synthetic fragrance *p*-*t*-butyl- α -methylhydrocinnamic aldehyde (Lilial®), a widely used fragrance compound not present in the fragrance mix. The combination of bioassay-guided chemical fractionation and chemical analysis/structure-activity relationships seems to be a valuable tool for the investigation of contact allergy to fragrance materials.

Key words: *p*-*t*-butyl- α -methylhydrocinnamic aldehyde; Lilial®; Lilestral®; fragrance allergy; fragrance sensitizers; structure-activity relationships; gas-chromatography – mass-spectrometry analysis; bioassay-guided chemical fractionation. © Munksgaard, 2000.

Accepted for publication 8 May 2000

Sensitivity to fragrance ingredients is a common and clinically important problem in Europe. 8 to 15% of eczema patients, and about 1–2% of the general population, are contact allergic to standard indicators of fragrance chemical allergy (1, 2).

Fragrance materials are the leading cause of allergic contact dermatitis (ACD) from cosmetics (3), which cover a broad spectrum of daily-use products. Among these, perfumes and colognes consist entirely of highly complex mixtures of fragrance chemicals in appropriate solvent systems, with concentrations ranging from 5% to 30% in the finished formulation. Skin contact with leave-on products like perfumes or deodorants can ex-

tend to 12 h or more. Moreover, they are often applied to the skin repeatedly, and thus a cumulative effect of potential sensitizers is expected.

At present, there is only available symptomatic treatment of allergic contact dermatitis from fragrance hypersensitivity. In order to prevent fragrance allergy in both non-sensitized (primary prevention) and already-sensitized (secondary prevention) individuals, it is necessary to have sensitive and reliable methods for the identification of fragrance chemicals causing dermatitis in individual cases. Reliable tests allowing prospective recognition of contact allergens among the myriad of chemicals used in fragrance composition are of

high priority for the cosmetics industry, as well as for public health officials and dermatologists.

The principle behind structure-activity relationships (SARs) is that the properties of a chemical are inherent in its molecular structure, with respect to its mode of interaction with a defined system (e.g., a biological structure, a macromolecule such as a protein, etc.). SARs are developed by looking for links between structure and biological activity, based on mechanistic or empirical grounds. Recent progress in the understanding of the molecular basis of ACD has helped the development of SAR analysis for sensitizing chemicals (4, 5). SAR studies have also recently been used for the evaluation of the sensitizing potential of ingredients present in marketed deodorants and identified by gas chromatography-mass spectrometry (GC-MS) analysis (6). Modern analytical methods such as GC-MS and liquid chromatography-mass spectrometry (LC-MS) can give access to the composition of complex mixtures.

The aim of this study was to identify in a perfume fragrance allergens not included in the fragrance mix by use of bioassay-guided chemical fractionation, GC-MS analysis and SARs. For this purpose, we selected a perfume that gave a history of eczema and a positive patch test in a patient who had a negative patch test to the fragrance mix.

Case Report

A 45-year-old woman presented with a history of eczema after use of selected fine fragrances, along with recurrent facial and periorbital dermatitis. She had previously documented contact allergy to nickel sulfate and to colophonium in the European standard series, and a history of dermatitis under nickel-containing objects in contact with skin. She gave negative results to repeated patch testing with the fragrance mix. She was patch tested with 3 commercial perfumes and showed positive results with infiltrated itchy erythematous papular reactions. Subsequent repeated open application test (ROAT) with 1 of the perfumes was positive within 4 days, with the development of itchy, erythematous papular dermatitis on the application site on the right forearm, whereas simultaneous ROATs with the other 2 commercial products were negative after 8 days. The perfume which gave positive patch test and ROAT results was chosen for further analysis.

Materials and Methods

Bioassay-guided chemical fractionation

To identify the chemical(s) responsible for the patient's sensitivity to her own perfume, the perfume

concentrate from the commercial product was fractionated chemically, and the fractionation was combined with patch testing of the patient and/or ROAT with each fraction.

The perfume concentrate was obtained by concentrating the commercial perfume under reduced pressure at room temperature. The marketed product contained approximately 21% (w/w) of perfume concentrate. The concentrate was chemically fractionated by flash column chromatography on silica gel (MERCK; Geduran 60, 0.040–0.063 mm). The solvent for elution was a mixture of pentane and diethyl ether in an 8:2 proportion, respectively. The chemical fractionation was monitored by thin-layer chromatography (TLC). TLC was carried out on glass plates precoated with silica gel 60 F-254 (MERCK, 0.25 mm film thickness). The solvent for elution was a mixture of pentane and diethyl ether (8:2). TLC spots were visualized under a UV lamp at 254 nm. The plates were then sprayed with an anisaldehyde-sulfuric acid solution and charred on a hot plate. For each fraction obtained, the solvent was removed by evaporation under reduced pressure at room temperature.

Chemical fractionation of the perfume concentrate resulted in 6 fractions that were dissolved in ethanol and patch tested on the patient. Initially, the test material concentration was calculated so that it corresponded to the amount of the fraction present in the final formulation. Patch tests were performed using Finn Chambers on Scanpor tape, 2-day occlusion and reading on day (D) 3 and D7. ROATs with the fractions giving positive, or doubtful positive, patch test results were performed using 2 drops of an ethanolic solution of the test material, applied openly 2× a day, for 15 days, to a 4-cm² square on the flexor side of the forearm, just below the elbow flexure. In order to get more clear-cut results, and to compensate for a missing compound effect of many substances present in the test preparations, testing was also carried out with test material concentrations that corresponded to double the amount of the fraction present in the perfume. For this purpose, 6 new fractions of different composition, obtained from another chemical fractionation of the perfume concentrate (same conditions as above) were used. Patch tests and ROATs with these new fractions were performed as mentioned before.

The fractions that gave a positive patch test and a ROAT response were further fractionated by flash column chromatography on silica gel, as described above. A mixture of pentane and diethyl ether in a 9:1 proportion was used for the elution. The newly obtained fractions were subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis

The samples were analysed using a Varian 3400 gas chromatograph equipped with an on-column injector and a RTX5-MS column (60 m×0.25 DI×0.1 µm film thickness; Restek Corp., Evry, France). Helium was used as the carrier gas (32.5 cm/s). The following temperature program was used: 40°C (1 min), from 40°C to 300°C at 4°C/min and 300°C during 20 min. For GC-MS analysis, a Finnigan MAT TSQ 700 mass spectrometer coupled to the Varian 3400 gas chromatograph was used. The mass spectrometer was equipped with a Digital DEC Station 5000 and with a ICIS2 software. Mass spectra were obtained by electronic impact performed in the direct probe electron impact mode. Ionisation was achieved with a 70-eV electron beam and the ion source temperature was 180°C. Mass spectra of GC-peaks were compared with the reference mass spectra of the chemical substances present in the mass spectrum library. The match parameters chosen for the identification of a compound were spectrum-fit > 900 counts (out of 1000 counts for absolute fit) and purity > 800.

Target fragrance sensitizers

The evaluation of the sensitizing potential of the chemicals that were identified in the fractions analysed by GC-MS was based on the presence, in their molecular structure, of "structural alerts" as defined by Barratt et al. (4, 8), and currently used in the development of the expert system DEREK (deductive estimation of risk from existing knowledge) (7, 8). More than 50 rules or "structural alerts" have been identified so far and have been tested successfully with data-sets of chemicals that either do or do not classify as skin sensitizers. These rules are based on our current knowledge of the chemical basis of allergic contact dermatitis (9).

Among the many chemicals found in the GC-MS perfume concentrate analysed fractions, 9 compounds contained a "structural alert" in their chemical structure, indicating an ability to modify skin proteins and thus behave as a skin sensitizer. These compounds were patch tested on the patient at 1% and/or at 2% in petrolatum (pet.) or, if known sensitizers, at the concentration described in the literature (10).

The chemicals tested were citronellol, geraniol, benzyl cinnamate and α -ionone purchased from Sigma-Aldrich (Saint Quentin Fallavier, France), benzyl salicylate purchased from Lancaster (Bischheim, France), methyl dihydrojasmonate, α -hexyl cinnamic aldehyde and α -isomethylionone

acquired from Interchim (Asnieres, France), and Lilial® which was obtained from Dragoco (Holzminden, Germany). Lilial® is the trade name of *p*-*t*-butyl- α -methylhydrocinnamic aldehyde (CAS [80-54-6]) manufactured by Givaudan-Roure (Argenteuil, France; also available as Lilestral® from BBA-Bush Boake Allen, London, UK).

Results

A first fractionation of the perfume concentrate gave 6 fractions (F1-F6), which were dissolved in ethanol and used for patch testing the patient. Initially, fractions F1-F6 were tested at a concentration equivalent to the present in the final formulation. Results are shown in Table 1. Weak patch test reactions to some of the fractions were observed. Only fraction F6 gave a clearly + patch test reaction. Use-test (ROAT) with this fraction, using ethanol as a control, revealed no adverse skin reaction. These results were therefore not conclusive for the identification of sensitizing fractions and another chemical fractionation of the perfume concentrate was thus performed. It gave 6 new fractions of different composition, which were dissolved in ethanol for patch testing the patient at a concentration equivalent to the double that present in the perfume. Results are shown in Table 2. Fraction F3' gave a positive patch test response only on D3, but fractions F2' and F6' elicited positive patch test reactions on D3 and doubtful on D7. Based on these results, ROATs were performed with fractions F2' and F6' (applied 2× daily for up to 15 days on both forearms) and weak reactions were observed. A transient slight oedema and erythema was observed from fraction F2' on day D8 and an itchy transient erythema from fraction F6' was observed on D13.

Subsequently, fractions F2' and F6' were frac-

Table 1. Patch test and ROAT results of the 1st fractionation of the perfume concentrate

Test material ^{a)}	Concentration ^{b)}	Patch test		ROAT
		D3	D7	
F1 (25%)	5.2%	—	—	NT
F2 (13%)	2.7%	?+	—	NT
F3 (7%)	1.5%	?+	—	transient erythema
F4 (12%)	2.5%	—	—	NT
F5 (14%)	2.9%	?+	—	—
F6 (18%)	3.8%	+	—	—
ethanol	as is	—	—	—

^{a)} % in the perfume concentrate of each fraction obtained in the fractionation.

^{b)} Test material concentration in ethanol. The concentrations were calculated so that they corresponded to the amount of the fraction present in the final formulation, which contained 21% (w/w) of perfume concentrate.

Table 2. Patch test and ROAT results of the 2nd fractionation of the perfume concentrate

Test material ^{a)}	Concentration ^{b)}	Patch test		ROAT
		D3	D7	
F1' (16%)	6.5%	?+	—	NT
F2' (28%)	11.7%	+	?+	transient erythema
F3' (17%)	7.1%	+	—	NT
F4' (13%)	5.4%	—	—	NT
F5' (10%)	4.3%	?+	—	NT
F6' (7%)	3.0%	+	?+	transient erythema
ethanol	as is	—	—	—

^{a)} % in the perfume concentrate of each fraction obtained in the fractionation.

^{b)} Test material concentration in ethanol. The concentrations were calculated so that they corresponded to the double of the amount of the fraction present in the perfume, which contained 21% (w/w) of perfume concentrate.

tionated chemically. 6 and 2 new fractions were obtained from fractions F2' (F2'.1-F2'.6) and F6' (F6'.1-F6'.2) respectively. These new fractions were analysed by GC-MS. Further fractionation was not considered suitable, because of the complexity to obtain more and more refined fractions by column chromatography. The GC-MS analysis of fractions F2'.1-F2'.6 and F6'.1-F6'.2 revealed that they contained many more substances than those identified by TLC. The fragrance substances and other substances identified in fractions F2'.1-F2'.6 and F6'.1-F6'.2 are described in Table 3. Not all GC-peaks were identified, the reason for this being either absence of reference mass-spectra, corresponding to the spectra of unidentified sub-

stances, in the MS-library; or a mixed GC-peak (containing more than 1 substance, and thus resulting in a mixed mass-spectrum). The identification of each substance was based on mass-spectrum fit >900 together with a purity parameter >800.

Among 24 identified compounds, 9 were found to contain at least 1 "structural alert", which indicated an ability to modify skin proteins and thus behave as skin sensitizers (8). According to these results, 9 of the 24 identified compounds were patch tested on the patient in petrolatum. The type of chemical "structural alert" present in every compound along with the test concentrations and the results of the patch tests are shown in Table 4. The patient was only positive to Lilial® 1% pet. at D3. Clinical relevance was again identified by ROAT. The patient was positive on use testing to 1% Lilial® in ethanol after 8 days of application (application 15), showing red itching oedematous eczema in the elbow flexure on day 11, and was negative to ethanol. ROAT with Lilial® 1% pet. was also negative.

Lilial® was found in fraction F2'.4, together with α -hexyl cinnamic aldehyde and α -isomethylionone, as it is shown in Fig. 1. In this figure, only the most important GC-peaks are pointed out. As can be seen from the GC chromatogram, Lilial® was present in a small amount in the perfume concentrate compared to other fragrance materials (quantitative analysis not done). The presence of Lilial® in the perfume concentrate was confirmed

Table 3. Fragrances and other materials identified in the GC-MS analysis of fractions F2'.1-F2'.6 and F6'.1-F6'.2

Fraction F2'.1	Fraction F2'.2
►benzyl salicylate	►linalyl butyrate
►4,6-di- <i>tert</i> -butyl-2-methylphenol	►hinesol
	►oxacyclohexadecan-2-one
	►decahydro-4 α -methyl-1-methylene-7-(1-methylethylidene)-naphthalene
	►2 unidentified substances
Fraction F2'.3	Fraction F2'.4
►nerol acetate*	►Lilial®
►phenylethanol- α,α -dimethylacetate*	► α -hexyl cinnamic aldehyde
►3,7,7-trimethyl-bicyclo <4.1.0> heptane (<i>cis</i> -caran)*	► α -isomethylionone
►3,5-di- <i>tert</i> -butylphenol	►benzyl acetate
	► α -methylbenzyl acetate
	►1,6-octadien-3-ol-3,7-dimethylformate
	►propionic acid benzyl ester
	►5 unidentified substances
Fraction F2'.5	Fraction F2'.6
►benzyl cinnamate	► α -ionone
►1 unidentified substance	►caryophyllene oxide
	►1 unidentified substance
Fraction F6'.1	Fraction F6'.2
►methyl dihydrojasmonate	►citronellol
►diethyl phthalate	►geraniol
►1 unidentified substance	

* Also present in fraction F2'.4.

Table 4. Patch test results of the 9 compounds that were found to contain a "structural alert" in their chemical structure

Test material	Chemical structure	Structural alert ^{a), b)}		Conc. ^{c)}	D3 ^{d)}	D7 ^{e)}
citronellol		susceptible to being metabolized into an aldehyde		1%*	—	—
geraniol		susceptible to being metabolized into an α,β -unsaturated aldehyde		1%*	—	—
methyl dihydrojasmonate		ketone ester		1% 2%	— —	— —
benzyl salicylate		ester		1%*	—	—
Lilial [®]		aldehyde		1%*	+	—
α -hexyl cinnamic aldehyde		α,β -unsaturated aldehyde		2%*	—	—
α -isomethylionone		α,β -unsaturated ketone		1% 2%	— —	— —
benzyl cinnamate		α,β -unsaturated ketone ester		2%* 5%*	— —	— —
α -ionone		α,β -unsaturated ketone		1%* 2%*	— —	— —

^{a)} Chemical group in the molecule that indicated an ability to form covalent conjugates with skin proteins, as defined by Barratt et al. (8).

^{b)} R, R¹, R², R³, R⁴=alkyl, aryl.

^{c)} Patch test concentration in pet.

^{d)} Reading on day 3.

^{e)} Reading on day 7.

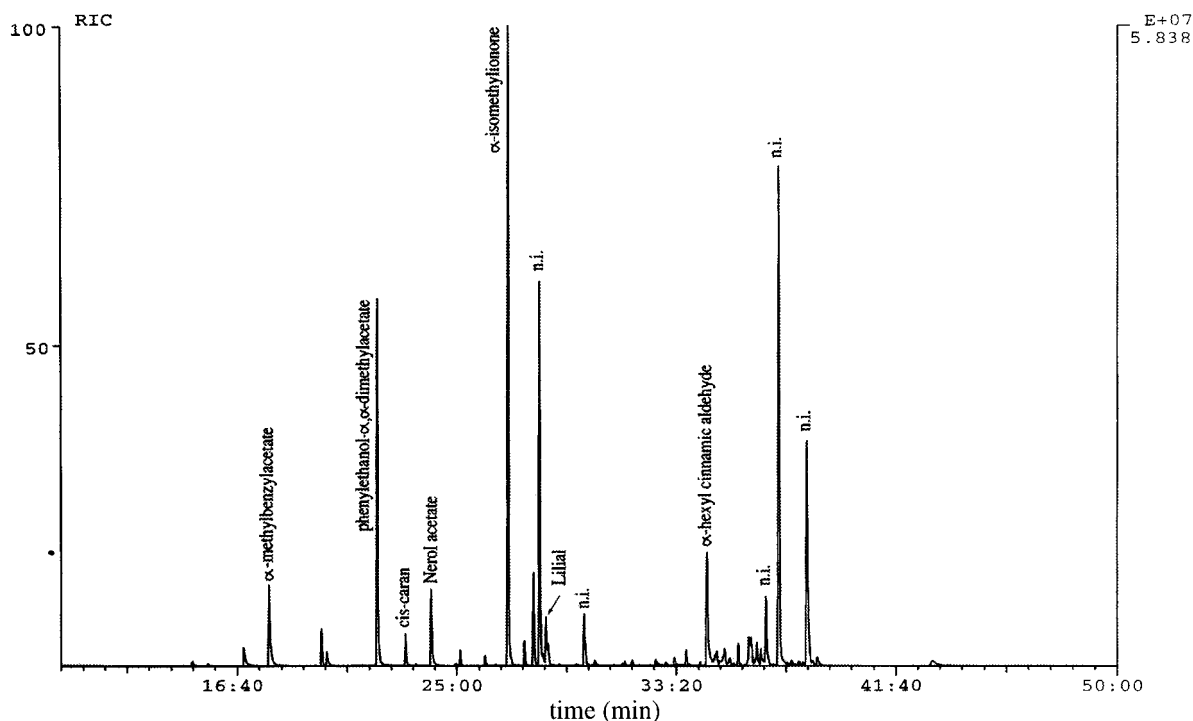
* According to A. de Groot (10).

by a gas chromatography co-injection of a Lilial[®] reference, and by comparison of the obtained mass spectra. Fraction F2'.4 also contained some of the compounds of fraction F2'.3 such as nerol acetate, *cis*-caran and phenylethanol- α,α -dimethylacetate, because of the difficulty of isolating them in separate fractions.

Multiple consultations were needed from the onset of the disease to the identification of Lilial[®] as a fragrance sensitizer in the perfume.

Discussion

A major preventive step in the field of fragrance allergy is the detection and identification of offending chemical(s) in perfumed products present in the patient's environment. This can be an extremely arduous task, since more than 2000 fragrance ingredients are available to the perfumer for compounding a fragrance formula, which may consist of 10 to 300 or more different ingredients.



n.i.=not identified (absence of reference mass-spectra or GC-peak containing more than 1 substance resulting in a mixed mass-spectrum).

Fig. 1. Characteristic peak of Lillal® in the GC chromatogram of fraction F2'4.

The aim of our work was to identify in a complex mixture such as a perfume fragrance allergens that are not included in the fragrance mix. In this study, bioassay-guided chemical fractionation combined with GC-MS analysis and SAR studies allowed the identification of Lillal® as a sensitizer, in a patient sensitive to her own perfume but with negative patch test results to the fragrance mix.

Normally, little information is available on the content of fragrance allergens other than the constituents of the fragrance mix in the products used by the patient. For many years it has been the policy of the fragrance industry not to disclose the fragrance formula. In the most recent edition of the International Fragrance Association (IFRA)'s code of practice, fragrance manufacturers are required to collaborate with dermatologists to discover the causative agents of clinical dermatitis in individual patients. Although progress has been made in the relationship between fragrance manufacturers and dermatologists, it is still difficult to obtain relevant information from manufacturers. Moreover, correspondence with industry delays the investigations of the patient's disease. Testing with coded fractions of the perfume in question is an alternative to ingredient information. In our study, bioassay-guided chemical fractionation of the perfume concentrate permitted the identification of the sensitizing fractions, which were

further submitted to GC-MS analysis in order to obtain the chemical composition.

Chemicals identified by this procedure were screened using SAR methods for their potential sensitization properties. Despite the complexity and poor understanding of some metabolic processes which lead to skin sensitization, it is possible, with chemical knowledge, to describe relationships between chemical structures and the ability to form covalent conjugates with proteins. This knowledge, relating chemical structure to a specific toxic end-point like skin sensitization, can be broken down into a series of chemical rules (i.e. "structural alerts") which have previously been found to correlate with the skin sensitization capacity (8).

Consideration of the chemical properties of a wide variety of known sensitizers and comparison with non-sensitizers has shown that, in most cases, the binding of the sensitizer to the protein takes place by the protein acting as a nucleophile and the sensitizer acting as an electrophile (11). Therefore, the presence of an electrophilic chemical function in the allergen molecule can be considered as a "structural alert". Furthermore, many chemical functions can be biochemically transformed (metabolized) into electrophilic chemical functions and must also be considered as "structural alerts". The "structural alerts" that are typically used for

the evaluation of the sensitizing potential of chemicals have been listed elsewhere (6).

Based on the idea that the identification of one or more chemically reactive sites in the molecule structure indicates a hazard, we evaluated their presence in the compounds identified by GC-MS analysis of fractions F2'.1-F2'.6 and F6'.1-F6'.2. Among the 9 molecules found to contain at least 1 "structural alert", citronellol and geraniol (one of the constituents of the fragrance mix) are susceptible to being metabolized into the electrophilic chemical groups of aldehydes and α,β -unsaturated aldehydes, respectively. Other electrophilic molecular substructures found to be considered as "structural alerts" were ketones, α,β -unsaturated ketones and esters. Aldehydes are known to be chemically reactive with proteins. Ketones and esters are less reactive than aldehydes and a lower sensitizing potential is expected. α,β -Unsaturated aldehydes or ketones, or molecules susceptible to being metabolized into α,β -unsaturated aldehydes, should be regarded as potential skin sensitizers (9).

The 9 compounds were patch tested on the patient. Lilial[®], which contains an aldehydic chemical function in its chemical structure, was the only compound eliciting a positive patch test reaction (1% pet.). The normal contact with the allergen at the time of the use of the commercial product was simulated in a ROAT performed with 1% dilutions of Lilial[®] in petrolatum and in ethanol. Only the 1% ethanolic solution gave a positive ROAT. This finding shows the importance of the vehicle to allergen release when performing a ROAT. It is well known that in many cases the diagnosis of allergic contact dermatitis could be improved by the judicious choice of an appropriate vehicle. However, the influence of the vehicle on use test experiments has not been investigated systematically, and should be the subject of future studies.

Lilial[®] is a trade name of *p*-*t*-butyl- α -methylhydrocinnamic aldehyde. It is a synthetic fragrance that has not been reported to occur in nature. It is a colourless to pale-yellow liquid with a powerful, floral-fresh odour, and has been in public use since the 1960s. It has already been reported that repeated contact with Lilial[®] may cause allergic contact dermatitis. In a worldwide multicentre investigation, 167 patients suspected of fragrance sensitivity were patch tested with Lilial[®] 5%, and 2 patients (1.2%) gave an allergic reaction (2). Contact allergy to Lilial[®] from a deodorant has also been reported in a young man who developed axillary dermatitis after using a new roll-on antiperspirant. He patch tested positive to the deodorant and the perfume from the deodorant. Fractionation and subsequent patch testing of the fractions

showed that the offending allergen was Lilial[®] (1%) (12). Moreover, 0.44% of 685 consecutive Japanese patients with eczema had an allergic reaction to Lilial[®] 10%. Patch tests were performed with 0%, 2%, 5% and 10% Lilial[®] in the 685 patients with contact dermatitis. Only 1 irritant reaction was found to each concentration and 10% Lilial[®] was recommended for patch testing (13). However, De Groot (10) recommends 1% Lilial[®] for patch testing.

Thus, Lilial[®] is a known contact allergen and in principle the diagnosis could have been made by screening with a patch test series of fragrance allergens. The problem is that about 100 fragrance substances have been identified as contact allergens and optimal test concentrations are known only for a few of these (14).

Lilial[®] was present in fraction F2'.4. We did not succeed in identifying the allergen in fraction F6' and other allergenic components or compound effects may therefore be suspected. For practical reasons and capacity, the investigations had to be limited to only 1 of the fractions.

The fragrance mix detects possibly 70 to 80% of fragrance sensitivity, leaving 20 to 30% undetected (15). Although progress has been made in improving the safety standards of fragrances, the figures on adverse effects reported by dermatologists worldwide suggest that the safety procedures employed are far from sufficient. Therefore, there is a need to identify more fully the nature of the main fragrance chemicals responsible for causing contact allergy in consumers, improve risk-assessment procedures and establish improved diagnostic tools for the screening of fragrance allergy.

The current case illustrates that the combination of analytical and structure-activity relationships methods is a valuable tool for the management of contact allergy to fragrance materials.

The presence of a "structural alert" in a structure is an indication of an ability to modify skin proteins and thus behave as a skin sensitizer. However, it is not sufficient to prove that a chemical is a potential skin sensitizer. The potential of a chemical to act as a contact allergen can be strongly dependent not only on its reactivity, but also on its hydrophobicity. Indeed, physicochemical properties of chemicals present in perfumes or deodorants (size, lipophilicity, etc.) combine to make most of them good skin penetrants, a prerequisite for skin sensitization. Rules in terms of molecular structures, lipophilicity, molecular weight and volume, and concentration, which could be used in an SAR approach to the identification of hazardous molecules in fragrances, should be also considered in future studies.

Acknowledgements

This study was supported by the EU-Biomed 2 Programme (contract no. BMH4-CT96-0877). The authors are very grateful to Estelle Motsch for her assistance during the GC-MS analysis of the fractions.

References

1. Frosch P J et al. (EECDRG). Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995; 33: 333–342.
2. Larsen W et al. Fragrance contact dermatitis. A worldwide multicentre investigation (Part I). *Am J Contact Dermatitis* 1996; 7: 77–83.
3. De Groot A C, Frosch P J. Fragrances as a cause of contact dermatitis in cosmetics: clinical aspects and epidemiological data. In: Frosch P J, Johansen J D, White I R (eds): *Fragrances: beneficial and adverse effects*. Berlin-Heidelberg: Springer-Verlag, 1998: 66–75.
4. Barratt M D, Basketter D A, Roberts D W. Structure-activity relationships for contact hypersensitivity. In: Lepoittevin J-P, Basketter D A, Goossens A, Karlberg A-T (eds): *Allergic contact dermatitis: the molecular basis*. Berlin-Heidelberg: Springer-Verlag, 1997: 129–154.
5. Roberts D W, York M, Basketter D A. Structure-activity relationships in the murine local lymph node assay for skin sensitization: α,β -diketones. *Contact Dermatitis* 1999; 41: 14–17.
6. Rastogi S C, Lepoittevin J-P, Johansen J D, Frosch P J, Menné T, Bruze M, Dreier B, Andersen K E, White I R. 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.
7. Sanderson D M, Earnshaw C G. Computer prediction of possible toxic action from chemical structure; the DEREK system. *Hum Exp Toxicol* 1991; 10: 261–273.
8. Barratt M D, Basketter D A, Chamberlain M, Admans G D, Langowski J J. An expert system rulebase for identifying contact allergens. *Toxicol in Vitro* 1994; 8: 1053–1060.
9. Lepoittevin J-P, Basketter D A, Goossens A, Karlberg A-T (eds): *Allergic contact dermatitis: the molecular basis*. Berlin, Heidelberg: Springer-Verlag, 1997.
10. De Groot A C (ed): *Test concentrations and vehicles for 3700 chemicals. Patch testing*. Amsterdam; Elsevier, 1994.
11. Roberts DW, Lepoittevin J-P. Hapten-protein interactions. In: Lepoittevin J-P, Basketter D A, Goossens A, Karlberg A-T (eds): *Allergic contact dermatitis: the molecular basis*. Berlin-Heidelberg: Springer-Verlag, 1997: 81–111.
12. Larsen W G. Allergic contact dermatitis to the fragrance material lilial. *Contact Dermatitis* 1983; 9: 158–159.
13. Sugai T. Group study IV-farnesol and lily aldehyde. *Environ Dermatol* 1994; 1: 213–214.
14. De Groot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997; 36: 57–86.
15. Johansen J D, Rastogi S C, Menné T. Contact allergy to popular perfumes; assessed by patch test, use test and chemical analysis. *Br J Dermatol* 1996; 135: 419–422.

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