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ORIGINAL ARTICLE

Validation of questionnaire algorithm based on repeated open application testing with the constituents of fragrance mix II: the EDEN Fragrance Study

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Abstract

Background In a European study on contact allergy in the general population, it has been hypothesized that the combination of contact allergy to a fragrance together with a history indicating dermatitis at exposure and thereafter subsequent avoidance of scented products implied a diagnosis of allergic contact dermatitis.

Objectives The primary aim of this study was to validate this hypothesis/algorithm. The secondary aim was to investigate whether there was any association between the outcome of the recent repeated open application test (ROAT) and the patch test reactivity.

Methods One hundred nine subjects with and without contact allergy to fragrance mix II (FM II) were recruited. Volunteers from six European dermatology clinics participated in the study including a patch test and a ROAT.

Results Twenty-four positive ROAT reactions were noted in total including 20 of those 32 with contact allergy to FM II. None of the volunteers reacted to the vehicle ($P < 0.001$). More individuals with a positive algorithm had positive ROATs when compared with those with a negative algorithm. However, the difference was not statistically significant ($P = 0.12$). The lower the patch test concentration eliciting a positive test reaction, the more likely was a positive ROAT and the more likely that the positive ROAT appeared early during the investigative period.

Conclusions The algorithm used in this study was not validated but it was indicated in this ROAT setup. The stronger the patch test reactivity the more likely was a positive ROAT and the more likely it was that the positive ROAT appeared early during the application period.

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Conflict of interest

Magnus Bruze is a member of an expert panel for fragrance safety. http://fragrancesafetypanel.org/. The co-authors do not have any conflicts of interest related to this manuscript.

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Introduction

Contact allergy is not a disease but a reaction pattern. Contact allergy can be defined as an immunologically acquired delayed hypersensitivity. Allergic contact dermatitis is the disease which requires the presence of contact allergy. To establish a diagnosis of allergic contact dermatitis there has, besides the presence of contact allergy, to be exposure to the sensitizer, or possibly a cross-reacting substance, and demonstration that this exposure suffices to provide the necessary number of molecules of the sensitizer to explain the dermatitis with regard to the degree of

reactivity on the one hand and localization and course of the dermatitis on the other.[1]

Recently a European project in the general population was performed on skin diseases including allergic diseases with focus on contact allergy.[2–11] The major methods used were questionnaires and patch testing. Questionnaires can be used to get the prevalence for various skin diseases in various populations.[8] There are presently no criteria of universal acceptance on how to establish a diagnosis of allergic contact dermatitis based on questionnaire answers. It was hypothesized in the European project,[2–11] that the combination of contact allergy to a fragrance test preparation at patch testing together with a history indicating dermatitis at exposure and thereafter subsequent avoidance of scented products implied a diagnosis of allergic contact dermatitis.[4,5] When this algorithm was investigated in a recent repeated open application test (ROAT) with the ingredients of fragrance mix (FM) I, it was not substantiated in the experimental setup used.[12]

The primary aim of this study was to validate the hypothesis that a positive history defined as dermatitis at exposure to a scented product and thereafter avoidance of such products in individuals hypersensitive to FM II is equivalent with allergic contact dermatitis from fragrances. The secondary aim was to investigate whether there was any association between the outcome of the ROAT and the individual degree of patch test reactivity.

Materials and methods

The study consisted of a patch test and a ROAT in individuals with a previous positive or negative test to fragrance mix II (14% in petrolatum) according to the previous study in 2011 (Fig. 1).[4] The study procedure and elements are described briefly below and recently in detail elsewhere.[12]

Study design

A training course with a theoretical part and a practical part on patch testing and how to perform and read a ROAT was arranged in Malmö for dermatologists, nurses and technicians from participating clinics before the start of the study.[12,13]

The patch test (Fig. 1) was performed (i) to evaluate the actual degree of reactivity to FM II and the ROAT solution and (ii) to exclude that the volunteers recruited to be without contact allergy to FM II based on the patch testing in 2011 still were without allergy to FM II. The actual degree of reactivity to FM II and the ROAT solution was defined as the lowest concentration of FM II eliciting a positive patch test reaction and the intensity (+, ++, +++) of the patch test reaction to the ROAT solution, respectively. It was decided before the start of the study that the individuals who had changed their reactivity from a negative reaction to FM II in 2011 into a positive reaction in 2014 should participate in the study as test individuals, thus subjects with contact allergy to FM II.

The procedure on how to recruit individuals to get volunteers with contact allergy to FM II and/or FM I representing subjects with and without a positive algorithm was determined in detail and described elsewhere.[12]

It was further stressed that two dermatologists from each department had to participate, one reading the patch tests and the other one being responsible for all ROAT readings (Fig. 1 and Table 1). The dermatologists did not know to which group the volunteer belonged, and they were not allowed to communicate with the volunteers or each other on test results or any other topic related to the study (Table 1). It was also emphasized that whenever starting a group of individuals with the patch test and the ROAT, the group had to include both those with and those without contact allergy to FM II (Table 1).

The patch test results to FM I and the ROAT solution with FM I as well as the ROAT results with the ROAT solution based on the FM I ingredients are reported elsewhere.[12]

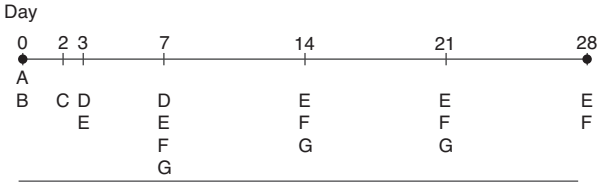

Figure 1 Time course of the patch testing and repeated open application testing (ROAT). A. Application of patch tests. B. ROAT solutions to volunteers for application on the lower arm. C. Removal of patch tests. D. Reading of patch tests. E. Reading of ROATs. F. Return of ROAT solutions for weighting. G. New ROAT solutions to volunteers for application.

Subjects

From the patch-tested cohort in 2011 with approximately 3000 individuals in the EDEN fragrance project, 109 subjects were recruited.[4–8,12] Volunteers from six European dermatology clinics participated, Bergamo Italy $n = 14$, Coimbra Portugal $n = 14$, Heidelberg, Germany $n = 22$, Jena Germany $n = 18$, Groningen The Netherlands $n = 18$ and Malmö Sweden $n = 23$. Each clinic recruited subjects from all groups. The following 109 individuals were recruited based on the patch test results in 2011 (Fig. 2):

- Thirty individuals hypersensitive to FM II, 18 with and 12 without a positive history. Seven of the 30 subjects had simultaneous contact allergy to FM I.
- Forty individuals hypersensitive to FM I, 24 with and 16 without a positive history. Seven of the 40 subjects had simultaneous contact allergy to FM II.
- Forty-six individuals without contact allergy to FM II, FM I, ingredients of FMs or *Myroxylon pereirae*, 23 with and 23 without a positive history.

Table 1 Instructions on how to recruit volunteers as well as on how to perform patch testing and the repeated open application test (ROAT)

1. Application to the board of ethics
2. After approval, recruitment of volunteers
3. Start the recruitment with those positive to FMs
4. Forward information on gender and age of those enrolled (hypersensitive to FM I and/or FM II) to the co-ordination centre in Malmö
5. The co-ordination centre will give each centre a list of randomized controls with the same coding approved in the previous multicentre study. These individuals will not have any contact allergy to any test preparation with FM I, FM II, separate ingredients of FM I and FM II, as well as *Myroxylon pereirae* and colophony
6. In case one, centre cannot recruit the necessary number of individuals, the co-ordination centre will suggest additional recruitment at another centre
7. The schedule for the patch tests and ROATs **MUST** be made up in such a way that individuals from the 3 groups of participants - (i) hypersensitive to FM I, (ii) hypersensitive to FM II and (iii) no hypersensitivity to fragrances - are mixed
8. The test solutions are made in Malmö and forwarded to participating centres. The solutions must be stored refrigerated before use and between the application on the volunteers
9. At patch testing, small Finn chambers with a diameter of 8 mm shall be used. A volume of 15 μL of each test solution shall be applied to each filter paper in the Finn chamber by a micropipette
10. The participant will get new ROAT solutions each week (D0, D7, D14 and D21). The used ROAT solutions must be returned to the clinic for weighing (D7, D14, D21 and D28)
11. The reader of the patch tests **MUST NOT** know to which group the volunteer belongs, neither the results of the ROATs
12. The reader of the ROATs **MUST NOT** know to which group the volunteer belongs, neither the result of the patch testing
13. There **MUST** be 2 different readers of the patch tests and ROATs and they **MUST NOT** communicate any results of the testing between themselves during the study period

D, day; FM, fragrance mix.

Chemicals and test preparations

The 6 fragrance ingredients present in FM II and solvents used are shown in Table 2 together with concentrations for patch test preparations and ROAT solutions. The same batches of the fragrance ingredients were used for patch testing and ROAT solutions and they had been used for the patch testing performed in 2011 with petrolatum (pet) preparations. The fragrance ingredients were kept frozen in the period between 2011 and 2014. Ethanol (eth) was purchased from CCS Healthcare AB, Borlänge, Sweden and diethyl phthalate (DEP) from Sigma Aldrich Chemie Gmbh, Steinheim, Germany.

The two ROAT solutions, ROAT FM II and ROAT FM I, contained the ingredients of the respective FM at the highest possible concentrations based on the IFRA Standards being effective in 2011 when the patch testing was performed within the EDEN fragrance study. The concentrations used for the stock solution of FM II at 14% w/v and for the ROAT FM II solution with varying concentrations of the six FM II ingredients are given in Table 2. Eth/DEP 98/2 v/v was used as vehicle for both the stock solution and the ROAT solution. Dilutions were made from the FM II stock solution – 4.4%, 1.4%, 0.44%, 0.14%, 0.044% and 0.014% w/v. All test preparations were made at the Department of Occupational and Environmental Dermatology in Malmö.

Patch testing

When starting the ROAT, all volunteers were patch tested using the Finn chamber technique with small chambers, diameter 8 mm (SmartPractice, Phoenix, AZ, USA). The ROAT solutions, the vehicle and dilutions of the two FMs were tested. The test preparations were applied on the chambers immediately before the application on the back to minimize evaporation.[14,15] 15 μL of the solutions were applied on the chambers which remained on the back under occlusion for 48 h. The tests were scored according to the valid ICDRG classification[16] on two occasions, day 3 (D3) and D7. The ROAT areas on the arms were covered when the patch tests were read to avoid a possibly biased reading.

Repeated open application test

Four test solutions were used, all using eth/DEP 98/2 v/v as vehicle.

- ROAT solution with the FM II ingredients at highest possible concentrations (Table 3). This solution is henceforth called ROAT FM IIA.
- ROAT solution with the FM I ingredients at highest possible concentrations. This ROAT solution was separately investigated.
- ROAT solution with only the vehicle.
- ROAT solution with only the vehicle.

The ROAT solutions were applied on the volar aspects of each lower arm twice daily for 4 weeks, according to a Latin square table. Two areas with 3 × 3 cm each were used on each arm. The dose applied each time was two drops from a special propylene bottle which gives approximately a dose of 50 μL (5.6 μL/cm^2). The volunteers got new ROAT solutions every week when the used ROAT solutions for the previous week were given back to the respective department for weighing.

Scoring of the ROAT test areas was performed five times. The first reading was on D3, the second one on D7 followed by D14, D21 and D28. A positive ROAT required at least 25% of the test area to be erythematous with infiltration and/or papules. When a test area was judged positive, the application of the ROAT solution to this area was stopped while the other ROAT solutions continued to be applied until a positive reaction appeared or when the study was terminated after 4 weeks.

Statistical calculations

The number of positive ROATs was compared between those with a positive algorithm and those with a negative one among the 29 individuals with a positive patch test reaction to FM II in 2011 (Fig. 2). In those with a positive ROAT, independent of

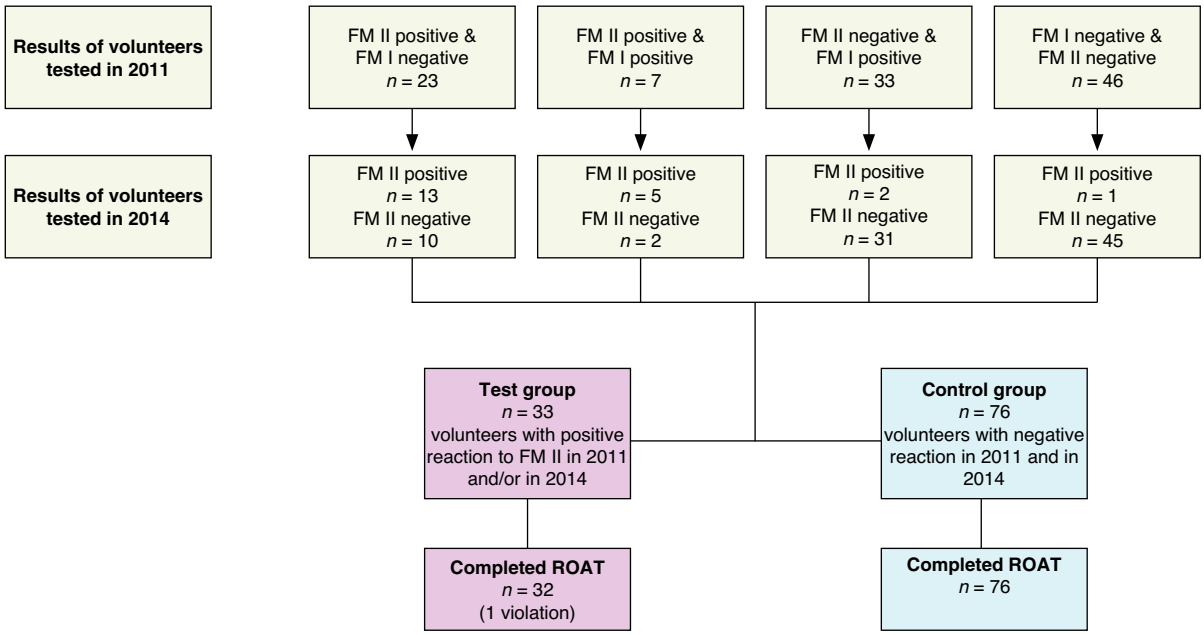

Figure 2 Flowchart showing patch test results of the volunteers on each occasion.

Table 2 Manufacturers and suppliers, and concentrations of the fragrance mix (FM II) ingredients in the patch test preparation at 14.0% and the ROAT FM IIA solution at 28.3% used for both patch testing and the repeated open application test (ROAT)

Ingredient	Manufacturer or supplier	FM II % w/v	14.0% w/v mg/cm^2	FM IIA % w/v	28.3% w/v mg/cm^2
Citral	Firmenich Inc., Plainsboro, NJ, USA	1.0	0.3	0.6	0.18
Citronellol	Bedoukian, Danbury, CT, USA	0.5	0.15	13.0	3.9
Coumarin	Rhodia Opérations, Lyon, France	2.5	0.75	1.5	0.45
Farnesol	Symrise GmbH & Co. KG, Holzminden, Germany	2.5	0.75	1.2	0.36
Hexyl cinnamal	Firmenich Inc.	5.0	1.5	10.5	3.15
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	International Flavours and Fragrances, Union Beach, NJ, USA	2.5	0.75	1.5	0.45

The vehicle used for both preparations was ethanol/diethyl phthalate 98/2 v/v. With the Finn chamber technique (diameter 8 mm), 15 μL of each solution was applied.

patch test reactions to FM II, a comparison was made between the intra-individual reactions to the ROAT FM IIA and the vehicle using McNemar's test, two-sided. Another comparison was made concerning positive ROAT reactions to the ROAT FM IIA solution between those with and without contact allergy to FM II using Fisher's exact test, two-sided. The association between degree of reactivity, defined as (i) the lowest patch test concentration in the series with dilutions of FM II resulting in at least a + reaction or (ii) intensity of patch test reaction to the ROAT FM II solution, and positive ROATs were investigated using Spearman's rank coefficient test in those with a positive reaction to FM II in 2011 and/or 2014. The same statistical method was used to investigate a possible association between degree of reactivity as defined above and the outcome of the ROAT defined as the reading day when a positive ROAT was observed for the first time.

Ethics committees

Approval was obtained from the ethics committees in the participating countries. The study was performed in accordance with the Declaration of Helsinki.

Results

The patch test results to FM I and the ROAT solution with FM I as well as the ROAT results with this ROAT solution are reported elsewhere.[12]

Table 3 Patch test and ROAT results for all participants

ID	Relevance algorithm	Patch test results							ROAT results				
		patch test 2011†	14%	4.4%	1.4%	0.44%	0.14–0.044% and vehicle	ROAT FM IIA	D3	D7	D14	D21	D28
1	pos	FM II	++	++	++	+	neg	+	+++				
2	pos	FM II	++	+	+	+	neg	+	+++				
3	pos	FM II	++	++	++	neg	neg	++	neg	+++			
4	neg	FM II	++	++	+	neg	neg	++	neg	+++			
5	pos	FM II	+	+	neg	neg	neg	+	neg	neg	++		
6	pos	FM II	+++	++	neg	neg	neg	++	neg	+++			
7	pos	FM II	+	+	neg	neg	neg	+	neg	neg	neg	neg	++
8	neg	FM II	+	+	neg	neg	neg	neg	neg	neg	++		
9	neg	FM II	++	+	neg	neg	neg	+	neg	neg	neg	neg	neg
10	pos	FM II	++	+	neg	neg	neg	+	neg	neg	++		
11	neg	FM II	+	neg	neg	neg	neg	neg	neg	neg	neg	+	
12	neg	FM II	++	neg	neg	neg	neg	+	neg	+++			
13	pos	FM II	neg	neg	neg	neg	neg	neg	neg	neg	+++		
14	neg	FM II	neg	neg	neg	neg	neg	neg	neg	+++			
15–19	neg	FM II	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
20–22	pos	FM II	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
23	pos	FM II+I	++	++	++	+	neg	++	++				
24	pos	FM II+I	++	+++	++	neg	neg	++	neg	++			
25	pos	FM II+I	++	++	+	neg	neg	++	++				
26	pos	FM II+I	++	++	neg	neg	neg	++	neg	+++			
27	pos	FM II+I	+	+	neg	neg	neg	+	+++				
28	pos	FM II+I	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
29	neg	FM II+I	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
30	neg	FM I	+	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
31	pos	FM I	+	neg	neg	neg	neg	++	++				
32	neg	FM I	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	+
33	pos	FM I	neg	neg	neg	neg	neg	neg	neg	neg	neg	+	
34	pos	FM I	neg	neg	neg	neg	neg	neg	neg	+			
35–47	neg	FM I	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
48–62	pos	FM I	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
63	neg	neg	+	+	+	neg	neg	+	neg	+			
64	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	+		
65–85	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
86–108	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Ethanol/diethyl phthalate 98/2 v/v was used as vehicle for all test solutions except for the patch test with FM II and/or FM I in 2011 when petrolatum was used as vehicle.
†FM II and/or FM I indicate a positive patch test reaction.

Participants with and without contact allergy to FM II

One hundred nine individuals were recruited and patch tested. There were 108 individuals completing the ROAT, 67 females and 41 males. The mean age for these 108 individuals was 47.8 years with the range 19–73 years.

Figure 2 shows to which groups the 109 recruited volunteers belonged with regard to patch test results to pet preparations of FM II and FM I in 2011 as well as the patch test results to eth/DEP solutions of FM II in 2014 (Fig. 2, Table 3). Twelve (41%) subjects positive to FM II in 2011 did not test positively to FM II in 2014 while three (3.8%) individuals negative to FM II in 2011 had become positive in 2014. Hence, the test group of individuals positive to FM II in 2011 and/or 2014 consisted of 32 individuals while the control group with individuals negative to FM II in both 2011 and 2014 consisted of 76 subjects who all finished the study. One volunteer in the test group was excluded concerning the ROAT part due to violation of the protocol. When a positive ROAT appeared at one test area after 1 week, all applications were incorrectly stopped in this volunteer.

Patch testing

None of the 29 individuals positive to FM II in eth/DEP in 2014 reacted to the dilutions at 0.14% and lower. The lowest FM II

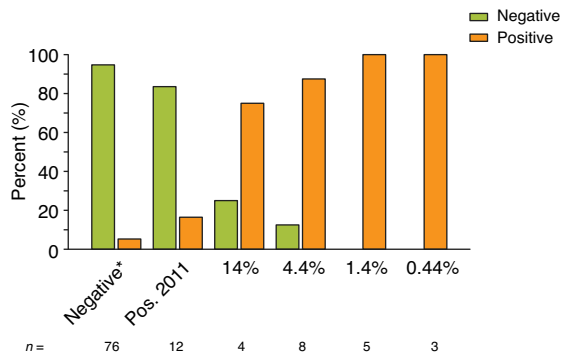

Figure 3 Outcome of repeated open application test (ROAT) with ROAT FM II in ethanol/diethyl phthalate, based on fragrance mix II (FM II) ingredients, in volunteers patch tested with FM II in both 2011 and 2014. Volunteers are stratified by the reactivity to dilutions of FM II in ethanol/diethyl phthalate at patch testing in 2014. *'Negative' indicates negative patch test reactions to FM II in both 2011 (vehicle petrolatum) and 2014 (vehicle ethanol/diethyl phthalate). 'Pos. 2011' indicates positive patch test reactions to FM II in 2011 and a negative one in 2014. The 14%, 4.4%, 1.4% and 0.44% indicate the lowest patch test concentration eliciting a positive reaction to FM II in 2014. When all volunteers within a reactivity group have developed a positive ROAT, the red bar peaks at 100%.

dilutions eliciting a positive patch test reaction was thus 0.44% to which reactions were noted in 3 subjects (Table 3). No one reacted to the eth/DEP vehicle.

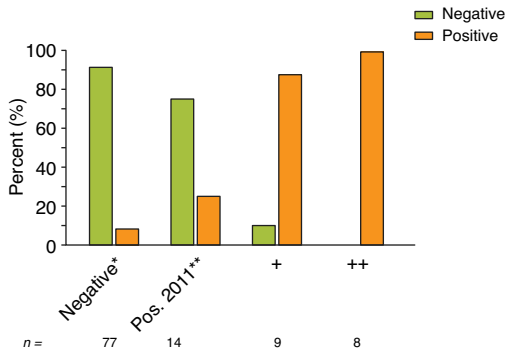

Figure 4 Outcome of repeated open application test (ROAT) with ROAT FM IIA in ethanol/diethyl phthalate, based on fragrance mix (FM) II ingredients, in volunteers with various intensities of test reactions to ROAT FM IIA in ethanol/diethyl phthalate at patch testing in 2014. *'Negative' indicates negative patch test reactions to FM II in both 2011 (vehicle petrolatum) and 2014 (vehicle ethanol/diethyl phthalate) as well as a negative patch test reaction to ROAT FM IIA in 2014. **'Positive in 2011' indicates positive patch test reactions to FM II in 2011 and negative ones to both FM II and ROAT FM IIA in 2014. '+ and ++' indicate positive patch test reactions to ROAT FM IIA in 2014. When all volunteers within an intensity group have developed a positive ROAT, the red bar peaks at 100%.

Positive patch test reactions to the ROAT FM IIA solution in eth/DEP was noted in 17 volunteers who all showed simultaneous positive reactions to FM II in eth/DEP (Table 3).

Repeated open application test

Positive ROAT reactions The ROAT FM IIA solution in eth/DEP gave positive ROAT reactions in 20 of those 32 (63%) with contact allergy to FM II in 2011 (in pet) and/or 2014 (in eth/DEP; Table 3). None of these volunteers reacted to the eth/DEP vehicle ($P < 0.001$). Four of the 20 positive ROAT reactors had a negative patch test to FM II test in 2014. No positive ROAT at all was registered for the vehicle. Four of those 76 (5%) without contact allergy to FM II in 2011 and 2014 developed a positive ROAT (20/32 vs. 4/76; $P < 0.001$; Table 3).

Positive patch test reactions to FM II and ROAT FM IIA solution vs. a positive ROAT Among the 32 volunteers with a positive patch test reaction to FM II in 2011 (in pet) and/or 2014 (in eth/DEP), a simultaneous positive patch reaction to the ROAT FM IIA solution in eth/DEP was noted in 17 individuals and thus a negative patch test reaction to the ROAT FM IIA solution in another 15 individuals. In those 17 individuals with contact allergy to the ROAT FM IIA solution, more positive ROATs were noted as compared to those without a simultaneous positive patch test reaction to the ROAT FM IIA solution (16/17, 94%, vs. 4/15, 27%; $P < 0.001$, Fisher's exact test, two-sided).

A positive algorithm vs. a positive ROAT Seventeen of the 29 subjects with contact allergy to FM II in pet in 2011 had a positive algorithm according to the questionnaire and 12 a negative algorithm (Table 3). Numerically more, but not statistically significant, individuals with a positive algorithm had positive ROATs when compared with those with a negative algorithm (13 of 17, 76%, vs. 5 of 12, 42%; $P = 0.12$).

Exclusive contact allergy to FM II vs. a positive ROAT In the group with contact allergy to FM II in pet in 2011, 22 had only allergy to FM II and of these 13 developed a positive ROAT as compared to 5 in the subgroup of contact allergy to both pet preparations with FM I and FM II in 2011 (13/22 vs. 5/7; $P = 0.68$). A similar difference in the number of ROAT reactions in these subgroups was noted when also requiring a simultaneous positive patch test reaction to ROAT FM IIA in the respective subgroup (9/22 vs. 5/7; $P = 0.21$).

Degree of patch test reactivity vs. a positive ROAT Figures 3 and 4 show the association between degree of patch test reactivity to the dilutions of FM II and the ROAT FM IIA, respectively, and a positive ROAT. The lower the patch test concentration of FM II in eth/DEP eliciting a positive test reaction, the more likely a positive ROAT ($P < 0.001$). The same pattern is seen for

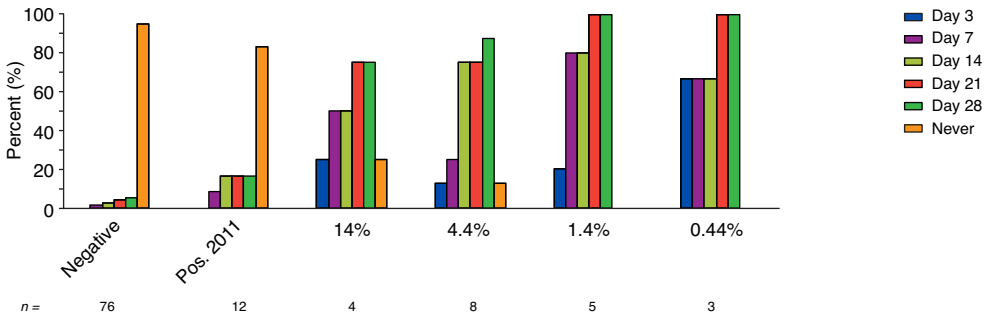

Figure 5 Cumulative positive reactors in volunteers to the repeated open application test (ROAT) with ROAT FM IIA in ethanol/diethyl phthalate, based on fragrance mix (FM) II ingredients, on days 3–28. Volunteers are stratified by the reactivity to dilutions of FM II in ethanol/diethyl phthalate at patch testing in 2014. 'Negative' indicates negative patch test reactions to FM II in both 2011 (vehicle petrolatum) and 2014 (vehicle ethanol/diethyl phthalate). 'Pos. 2011' indicates positive patch test reactions to FM II in 2011 and a negative one in 2014. The 14%, 4.4%, 1.4% and 0.44% concentrations indicate the lowest patch test concentration eliciting a positive reaction to FM II in 2014. When all volunteers within a reactivity group have developed a positive ROAT on a reading day, the coloured bar peaks at 100%.

intensity of patch test reaction to ROAT FM IIA in eth/DEP and outcome of ROAT ($P < 0.001$). Figures 5 and 6 demonstrate the association between degree of patch test reactivity to the dilutions of FM II and the ROAT FM IIA, respectively, and first day of appearance of a positive ROAT. The lower the patch test concentration of FM II eliciting a positive test reaction, the more likely a positive ROAT appears early ($P < 0.001$). The same pattern is seen for intensity of patch test reaction to ROAT FM IIA and outcome of ROAT with regard to the first day of appearance of a positive ROAT ($P < 0.001$).

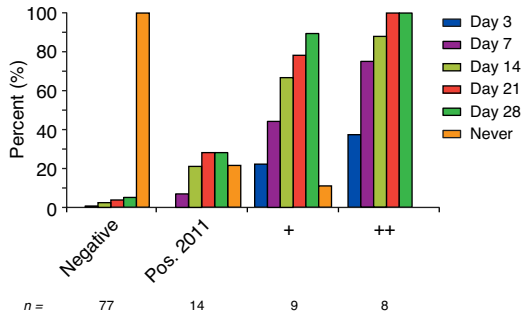

Figure 6 Cumulative positive reactors in volunteers to the repeated open application test (ROAT) with ROAT FM IIA in ethanol/diethyl phthalate, based on fragrance mix (FM) II ingredients, on days 3–28. Volunteers are stratified by the patch test reactivity to ROAT FM IIA in ethanol/diethyl phthalate, in 2014. 'Negative' indicates negative patch test reactions to FM II in both 2011 (vehicle petrolatum) and 2014 (vehicle ethanol/diethyl phthalate) as well as a negative patch test reaction to ROAT FM IIA in 2014. 'Pos. 2011' indicates positive patch test reactions to FM II in 2011 and negative ones to both FM II and ROAT FM IIA in 2014. '+ and ++' indicate positive patch test reactions to ROAT FM IIA in 2014. When all volunteers within an intensity group have developed a positive ROAT, the coloured bar peaks at 100%.

Discussion

Many usage tests have been performed with fragrance sensitizers. The proportion of positive reactions varies between 0 and 100%.[17–31] Concentration, actually dose/cm², of the applied usage/ROAT preparation and length of application period are major reasons for the great variation. In the previous study on FM I based on the same material and design, positive ROAT reactions were obtained in 59% of those being hypersensitive to FM I and in 3% of those without contact allergy to FM I.[12] In the present study on FM II, the corresponding figures were similar, 63% and 5%, respectively (Table 3). This difference in the present study is highly statistically significant which rules out irritancy as the cause of the positive ROATs. Furthermore, the lack of reactions to the vehicle when applied for 4 weeks in all individuals demonstrates that the positive ROATs are manifestations of allergic contact dermatitis from FM II ingredients. The fact that 37% of the volunteers hypersensitive to FM II did not develop a positive ROAT indicates that they can use scented products containing the FM II materials on non-damaged skin without getting skin problems, particularly if the products are used less frequently than in this study. Furthermore, the maximum concentrations of some of the FM II ingredients have been lowered since 2011 according to the IFRA standards. However, despite a lower exposure, the situation may be different if products such as scented moisturizers are applied on skin with an existing dermatitis.[32] There were 12 subjects with a positive patch test reaction to FM II in 2011 who had a negative reaction

in 2014. False-positive reactions in 2011 may be one explanation or a difference in the number of FM II molecules penetrating the skin as petrolatum was the FM II vehicle in 2011 and eth/DEP in 2014. However, the development of a positive ROAT in two of these individuals (17%) indicates that the non-positive reactions in 2014 instead may have been false negative.

The four positive ROATs in those without contact allergy to FM II may be explained by (i) a false-negative reaction to FM II in eth/DEP in 2014. These four volunteers also patch-tested negatively to the ROAT FM IIA solution in eth/DEP. It is therefore possible that the contact allergy is directed towards the fragrance materials being present at lower concentrations in the ROAT FM II solution as compared to the concentrations in the pet preparation with FM II. For four of the six ingredients in the ROAT FM IIA solution, the concentrations are lower than the corresponding concentrations in the test preparation with FM II at 14% (Table 2). Repeated exposure to the ROAT FM IIA solution might still help accumulate the sufficient number of molecules in the skin to elicit a positive ROAT. (ii) Irritant contact dermatitis indistinguishable from an allergic contact dermatitis and (iii) sensitization to a fragrance material during the ROAT are other explanations. This possibility (iii) would have been substantially strengthened if a patch test with FM II and ROAT FM IIA solution performed after the termination had resulted in a positive test. However, such a test was not performed.

Among the 32 volunteers with a positive patch test reaction to FM II in 2011 and/or 2014, more positive ROATs were noted in those with a positive patch test reaction to the ROAT FM IIA as to compared to those without ($P < 0.001$).

Expectedly, there was an association between degrees of hypersensitivity and the outcome of the ROAT. The stronger reaction at patch testing, defined as the lowest FM II dilution eliciting a positive patch test or the intensity of the patch test reaction to ROAT FM IIA solution, the more likely was a positive ROAT, and the more likely it appeared early during the application period (Table 3 and Figs 3–6). All of those reacting positively at patch testing to the lowest FM II solutions (0.44% and 1.4%) and those with a ++ reaction to ROAT FM IIA developed a positive ROAT (Figs 3 and 4). For the latter group (++ reactions), all ROATs had appeared by the D7 reading (Fig. 6). The same time, 1 week, was needed for those five volunteers reacting down to 1.4% while only 3 days were needed for a positive ROAT for those three volunteers testing positively down to 1.4 (Fig. 5). This kind of relationship has previously been demonstrated for other fragrance sensitizers including isoeugenol[19,23], hydroxyisohexyl 3-cyclohexene carboxaldehyde[22,24], and oak moss[29,30] and was also reported in the ROAT study with FM I based on the same design and material as the present study.[12]

In the previous study, the algorithm that contact allergy to fragrances combined with skin problems and avoidance of scented products indicated a clinically relevant contact allergy could not be confirmed (OR: 1.6; 95% CI: 0.7–3.8).[12] Also the present study failed to validate the hypothesis that a positive history defined as dermatitis at exposure to a scented product and thereafter avoidance of such products in individuals hypersensitive to FM II is equivalent with allergic contact dermatitis from fragrances (OR: 2.0; 95% CI: 0.8–5.2). Possible reasons for the lack of association are lack of power and that insufficiently specific questions were used in the questionnaire. Another explanation is that a diagnosis of allergic contact dermatitis is difficult to establish. Sometimes it is easy when there is a known exposure to the sensitizer and a temporal relationship between the exposure and presence of dermatitis, maybe particularly the first time an allergic contact dermatitis appears. On the other hand, the exposure might not be expected[33] and therefore overlooked or the exposure assessment may require chemical investigations as for sensitizers such as formaldehyde and epoxy resins. Furthermore, a dermatitis might have a multifactorial background where the contribution of the allergic contact dermatitis may vary over time. It is unlikely that an individual with a currently unknown contact allergy to a sensitizer, for example formaldehyde or a fragrance material, will suspect the contribution of a low degree exposure to the sensitizer to be of any importance. However, such an exposure may still be clinically relevant, particularly when there also are other factors present such as endogenous factors and exposure to irritants. These situations constitute a challenge in constructing questions for a questionnaire-based diagnosis of allergic contact dermatitis but the result of the present study is encouraging.

This multicentre ROAT study was preceded by a course with participating dermatologists and testing personnel. This multicentre ROAT study was preceded by a course with participating dermatologists and testing personnel. At the course held at the Department of Occupational and Environmental Dermatology in Malmö, the design of the study and the definition of a positive ROAT were discussed. Live volunteers undergoing various types of ROATs in Malmö were used to practice the reading and to calibrate it. The testing personnel from participating European centres were taught about how to apply a fixed volume of the ROAT solutions evenly on the test areas in order to be able to instruct the participating volunteers at the various clinics. The ROAT was controlled and the various ROAT solutions were allocated to the four test areas in a randomized way based on a Latin square table. There were two independent dermatologists consistently reading either the patch test or the ROAT in the individual volunteer to avoid bias. The dermatologists did not know whether the volunteer was hypersensitive to FM II or not and where the various ROAT solutions had been applied. Communication concerning the study was not allowed between the reading dermatologists and the volunteers. Though, the study would have benefitted from monitoring including site visits of an independent dermatologist and it should obviously have been stressed further that termination of application of a particular

ROAT solution before the end of the investigative period was only allowed in case of a positive reaction for that particular solution.

Also the patch testing part of the study can be considered to be of high quality. Actually, according to a recent publication on 16 factors of possible significance for the quality of a multicentre study, this study is scored as a patch test study with excellent quality.[34] The only factor not obtaining the highest score was the lack of monitoring.

In conclusion, a ROAT with the FM II ingredients at the highest possible concentrations allowed at the time when the volunteers filled in the questionnaire was tested and used as a proxy for allergic contact dermatitis when positive. The algorithm used in this study assuming that contact allergy to FM II together with an itching dermatitis at any time during the life followed by avoidance of scented products was equivalent with an allergic contact dermatitis was not substantiated. It was demonstrated that 37% of those with contact allergy to FM II did not develop a positive ROAT while thus 63% developed a positive ROAT. The stronger the patch test reactivity, defined as the lowest FM II dilution eliciting a positive patch test reaction or intensity of patch test reaction to the ROAT FM IIA solution, the more likely was a positive ROAT and the more likely it was that the positive ROAT appeared early during the application period. Individuals with a previous positive patch test reaction followed by a negative reaction to FM II at the start of the ROAT may still develop a positive ROAT.

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