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Validation of a questionnaire algorithm based on repeated open application testing with the constituents of fragrance mix I

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Summary

Background In a European study on contact allergy in the general population, it was 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 and algorithm. The secondary aim was to investigate whether there was any association between the outcome of the repeated open application test (ROAT) and the patch test reactivity.
Methods In total, 109 patients with and without contact allergy to fragrance mix (FM) I were recruited. Volunteers from six European dermatology clinics participated in the study including a patch test and a ROAT.
Results Positive ROAT reactions were noted in 26 of the 44 volunteers with contact allergy to FM I. None of the volunteers reacted to the vehicle ($P < 0{\cdot}001$). More individuals with a positive algorithm had positive ROATs than those with a negative algorithm. However, the difference was not statistically significant. The lower the patch test concentration eliciting a positive test reaction, the more likely 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 substantiated in this ROAT set-up. 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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Conflicts of interest
M.B. is a member of an expert panel for fragrance safety (http://fragrancesafetypanel.org).

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What's already known about this topic?

- To the best of our knowledge, a scientifically designed and conducted repeated open application test (ROAT) has never been performed before to validate a diagnosis of allergic contact dermatitis partly based on a questionnaire.

What does this study add?

- This is the largest controlled, randomized and blinded ROAT performed to date.
- Higher patch test reactivity to fragrance mix I indicated a greater likelihood of a positive ROAT.

What are the clinical implications of this work?

- Further refinement of the questions is required in order to diagnose allergic contact dermatitis from fragrances based on a questionnaire.

Questionnaires are useful tools to study the prevalence of diseases in various populations. These tools can be used to determine the prevalences of various skin diseases,[1] but there are presently no criteria of universal acceptance on how to establish a diagnosis of allergic contact dermatitis based on questionnaire answers. In a recent European study on contact allergy in the general population,[2–6] it was hypothesized 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.[2,3]

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 fragrance-hypersensitive individuals is equivalent to allergic contact dermatitis from fragrances. The secondary aim was to investigate whether there was any association between the outcome of the repeated open application test (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 (FM) I (8% in petrolatum) according to the previous study on 'The prevalence of fragrance contact allergy in the general population of five European countries: a cross-sectional study', with testing completed in 2011[3] (Fig. 1).

Study design

Before the start of the study, a training course for the dermatologists, nurses and technicians from the participating clinics was arranged in Malmö. The course contained a theoretical part and a practical part. There was information and discussion on the design of the study, as well as general background information on ROATs including information on how to read a ROAT. The information on how to read a ROAT was practised on volunteers with ongoing ROATs with fragrances and preservatives at the Malmö department. In this way, the various ROAT reactions in the range from negative to strong positive were used to exemplify the reading and classification of ROATs, as well as to calibrate the reading among dermatologists from the participating centres. The way to apply and distribute a fixed dose of the ROAT solution on the test area was demonstrated and practised. At this Malmö course there was also a refresher course on how to read a patch test.[7]

The patch test was performed (i) to evaluate the actual degree of reactivity to FM I and the ROAT solution and (ii) to confirm that the volunteers with no contact allergy to FM I based on the patch testing in 2011 still did not have allergy to FM I. It was decided before the start of the study that the individuals who had a change in reactivity from a negative reaction to FM I in 2011 to a positive reaction in 2014 should participate in the study as test individuals defined as having contact allergy to FM I.

A procedure was used to ensure that individuals with contact allergy to FM I and/or FM II were representative of volunteers with and without a positive algorithm. This procedure was determined in detail (Table 1).

It was further stressed that two dermatologists from each department had to participate, one reading the patch tests and the other responsible for all ROAT readings (Fig. 1 and Table 2). 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

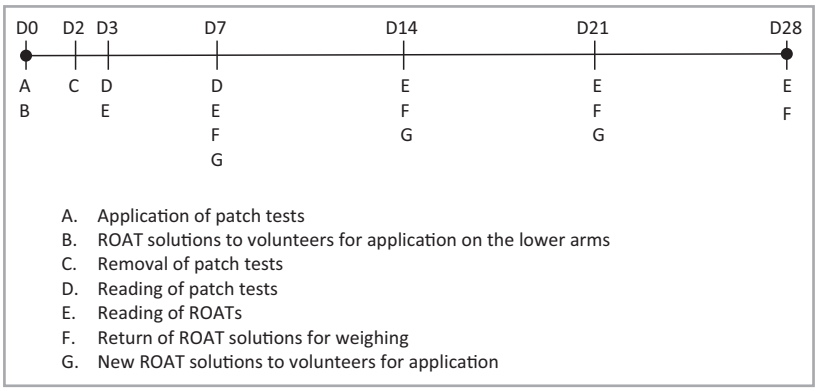

Fig 1. Time course of patch testing and repeated open application testing (ROAT). D, day.

Table 1 Suggested recruitment of individuals to the repeated open application test (ROAT) at the six participating clinics in 2014

	2011			Recruitment		
Centre	FM I pos (relevance)	FM II pos (relevance)	FM I pos + FM II pos (relevance)	FM I pos (relevance)	FM II pos (relevance)	Controls (relevance)
Heidelberg	13 (4)	12 (6)	4 (3)	7 (3)	9 (6)	4 (2)
Jena	8 (4)	11 (7)	4 (2)	6 (3)	8 (5)	6 (3)
Bergamo	11 (5)	5 (1)	2 (0)	7 (2)	6 (1)	7 (3)
Groningen	8 (4)	3 (1)	2 (2)	6 (3)	5 (3)	9 (5)
Coimbra	14 (9)	7 (2)	1 (1)	7 (5)	6 (2)	7 (3)
Malmö	12 (6)	6 (2)	3 (2)	7 (4)	6 (3)	7 (4)
Total	66 (32)	44 (19)	16 (10)	40 (20)	40 (20)	40 (20)

The 2011 data represent the numbers of individuals with contact allergy to fragrance mix I (FM I pos) and fragrance mix II (FM II pos) based on the patch testing in 2011. For all columns the figures within parentheses (relevance) represent the numbers of allergic individuals with a positive algorithm defined as fragrance contact allergy at patch testing combined with a questionnaire-based history indicating dermatitis at exposure and subsequent avoidance of scented products. All individuals with and without contact allergy to FM I and/or FM II, as well as the controls, were part of the patch tested cohort of approximately 3000 individuals from the general population in 2011. The controls should not have any contact allergy to any test preparation with FM I, FM II, separate ingredients of FM I and FM II, *Myroxylon pereirae* or colophony when tested in 2011.

Table 2 Instructions on how to recruit volunteers and 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 sex and age of those enrolled (hypersensitive to FM I and/or FM II) to the coordination centre in Malmö
5. The coordination 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, *Myroxylon pereirae* or colophony
6. In case one centre cannot recruit the necessary number of individuals, the coordination 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 three 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 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 (days 0, 7, 14 and 21). The used ROAT solutions must be returned to the clinic for weighing (days 7, 14, 21 and 28).
11. The reader of the patch tests **MUST NOT** know to which group the volunteer belongs, or the results of the ROATs
12. The reader of the ROATs **MUST NOT** know to which group the volunteer belongs, or the result of the patch testing
13. There **MUST** be two different readers of the patch tests and ROATs and they **MUST NOT** communicate any results of the testing between themselves during the study period

FM, fragrance mix.

or any other topic related to the study. 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 I (Table 2).

Patients

In total, 109 patients from the patch tested cohort in 2011 were recruited. The 2011 cohort consisted of approximately 3000 individuals in the European Dermato-Epidemiology Network (EDEN) Fragrance Study.[2–6] Volunteers from six European dermatology clinics participated: Coimbra, Portugal (n = 14); Bergamo, Italy (n = 14); Groningen, the Netherlands (n = 18); Heidelberg, Germany (n = 22); Jena, Germany (n = 18) and Malmö, Sweden (n = 23). Each clinic recruited volunteers from all groups. The guidelines on recruitment of volunteers are shown in Table 2.

The following 109 individuals were recruited based on the patch test results in 2011 (Fig. 2). (i) Forty individuals hypersensitive to FM I, 24 with and 16 without a positive history. Seven of these 40 volunteers had simultaneous contact allergy to FM II. (ii) Thirty individuals hypersensitive to FM II, 18 with and 12 without a positive history. Seven of these 30 had simultaneous contact allergy to FM I. (iii) Forty-six individuals without contact allergy to FM I, FM II, ingredients of FMs or *Myroxylon pereirae*; of these, 23 did and 23 did not have a positive history.

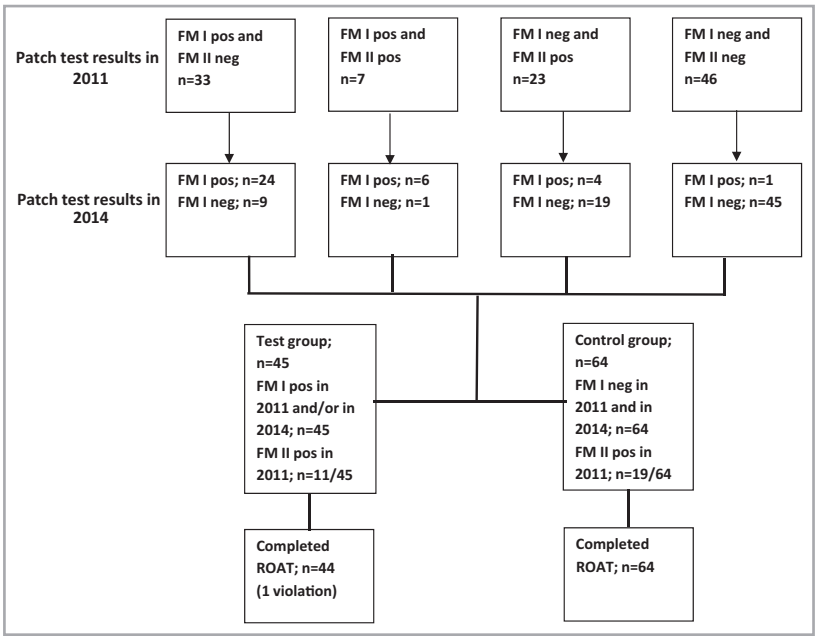

Fig 2. The number of volunteers without (neg) and with (pos) contact allergy to fragrance mix (FM) I and/or FM II, based on the patch testing in 2011 and in 2014, participating in the repeated open application test (ROAT) in 2014.

Chemicals and test preparations

The eight fragrance ingredients present in FM I, and the solvents used, are shown in Table 3, together with concentrations for patch test preparations and ROAT solutions. The same batches of the fragrance ingredients were used for the patch testing and ROAT solutions, and they had also been used for the patch testing performed in 2011 with petrolatum preparations. The fragrance ingredients were kept frozen in the period between 2011 and 2014. Ethanol 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 I and ROAT FM II, contained the ingredients of the respective FM at the highest possible concentrations based on the International Fragrance Association (IFRA) standards that were effective in 2011 when the patch testing was performed within the EDEN Fragrance Study. The concentrations used for the stock solution of FM I at 8% w/v (each ingredient at 1·0%) and for the ROAT FM I solution with varying concentrations of the FM I ingredients are given in Table 3. Ethanol–DEP 98/2 v/v was used as the vehicle for both the stock solution and the ROAT solution. Dilutions were made from the FM I stock solution at 2·5%, 0·8%, 0·25%, 0·08%, 0·025% and 0·008% 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. The ROAT solutions, the vehicle and dilutions of the two

Table 3 Manufacturers and suppliers, and concentrations of the fragrance mix (FM) I ingredients in the patch test preparation at 8·0% and the ROAT FM IA solution used for patch testing and the repeated open application test (ROAT)

		Finn chamber and concentration			
		FM I, 8·0% w/v		ROAT FM IA, 17·67% w/v	
Ingredient	Manufacturer or supplier	% w/v	mg cm^{-2}	% w/v	mg cm^{-2}
Cinnamic alcohol	Bedoukian, Danbury, CT, U.S.A.	1·0	0·3	0·5	0·15
Cinnamal	Bedoukian	1·0	0·3	0·05	0·015
Hydroxycitronellal	Firmenich Inc., Plainsboro, NJ, U.S.A.	1·0	0·3	1·0	0·3
α-Amyl cinnamal	International Flavors and Fragrances, Union Beach, NJ, U.S.A.	1·0	0·3	10·5	3·15
Geraniol	International Flavors and Fragrances	1·0	0·3	5·0	1·5
Eugenol	Firmenich Inc.	1·0	0·3	0·5	0·15
Isoeugenol	International Flavors and Fragrances	1·0	0·3	0·02	0·006
Evernia prunastri (oak moss)	Robertet, Grasse, France	1·0	0·3	0·1	0·03

The vehicle 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.

FMs were tested. The test preparations were applied on the chambers immediately before application on the back to minimize evaporation.[8,9] Aliquots of 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 International Contact Dermatitis Research Group classification[10] on two occasions: day 3 and day 7. The ROAT areas on the arms were covered when the patch tests were read to avoid the possibility of biased reading.

Repeated open application test

Four test solutions were used, all using ethanol–DEP 98/2 v/v as the vehicle. (i) ROAT solution with the FM I ingredients at the highest possible concentrations. This solution is henceforth called ROAT FM IA. (ii) ROAT solution with the FM II ingredients at the highest possible concentrations. This ROAT solution was investigated separately. (iii) ROAT solution with only the vehicle. (iv) A second ROAT solution with only the vehicle.

The ROAT solutions were applied twice daily for 4 weeks on the volar aspects of the lower arms, two solutions on each arm, according to a Latin square table. Four areas of 3 × 3 cm each were used. The dose applied each time was two drops from a special propylene bottle, which gives a dose of approximately 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.

The ROAT test areas were scored five times. The first reading was on day 3 and the second on day 7, followed by readings at days 14, 21 and 28. To be considered a positive ROAT, ≥ 25% of the test area had 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 44 individuals with a positive patch test reaction to FM I in 2011 and/or 2014 (Fig. 2). In those with a positive ROAT, independently of patch test reactions to FM I, a comparison was made between the intraindividual reactions to the ROAT FM IA and the vehicle using McNemar's test, two sided. Another comparison was made for positive ROAT reactions to the ROAT FM IA solution between those with and those without contact allergy to FM I using Fisher's exact test, two sided. The associations between degree of reactivity and positive ROATs were investigated using Spearman's rank coefficient test. The degree of reactivity was defined as (i) the lowest patch test concentration in the series with dilutions of FM I resulting in at least a + reaction or (ii) the intensity of the patch test reaction to the ROAT FM I solution. The same statistical method was used to investigate a possible association between the 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

Participants with and without contact allergy to fragrance mix I

In total, 109 individuals were recruited and patch tested. Of these, 108 individuals completed the ROAT (41 male and 67 female). The mean age for these 108 individuals was 47·8 years (range 19–73).

Figure 2 shows the distribution of the 109 recruited volunteers with regard to patch test results to FM I and FM II in 2011, as well as the patch test results to FM I in 2014 (Table S1; see Supporting Information). Ten volunteers (25%) positive to FM I in 2011 did not test positive to FM I in 2014, while five (7%) who were negative to FM I in 2011 had become positive in 2014. Hence, the test group of individuals positive to FM I in 2011 and/or 2014 consisted of 45 individuals, while the control group with individuals negative to FM I in both 2011 and 2014 consisted of 64 volunteers, all of whom finished the study. One volunteer in the test group was excluded from the ROAT part of the study due to violation of the protocol. When a positive ROAT appeared on one test area after 1 week, all applications were incorrectly stopped in this volunteer.

Patch testing

None of the 35 individuals positive to FM I in 2014 reacted to the dilutions at 0·025% and lower. The lowest concentration eliciting a positive patch test reaction to the FM I dilutions was thus 0·08%, with reactions to this concentration in five individuals (Table S1; see Supporting Information). No one reacted to the vehicle. Positive patch test reactions to the ROAT FM IA solution were noted in 25 volunteers (Table S1). Twenty-two of these (88%) occurred in volunteers with simultaneous positive reactions to FM I. The remaining three positive reactors had negative patch test reactions to FM I in both 2011 and 2014 (22 of 44 vs. three of 64; $P < 0·001$, Fisher's exact test, two sided).

Repeated open application test

The ROAT FM IA solution gave positive ROAT reactions in 26 of those 44 individuals (59%) with contact allergy to FM I in

2011 and/or 2014 (Table S1; see Supporting Information). None of these volunteers reacted to the vehicle ($P < 0{\cdot}001$). One of the 26 positive reactors had a negative FM I test in 2014. No positive ROAT at all was registered for the vehicle. Two of those 64 without contact allergy to FM I in 2011 and 2014 developed a positive ROAT (26 of 44 vs. 2 of 64; $P < 0{\cdot}001$) (Table S1).

Among the 44 volunteers with a positive patch test reaction to FM I in 2011 and/or 2014, a simultaneous positive patch reaction to the ROAT FM IA solution was noted in 22 individuals, and thus there was a negative patch test reaction to the ROAT FM IA solution in the another 22. In those 22 individuals with contact allergy to the FM IA solution, more positive ROATs were noted than in those without a simultaneous positive patch test reaction to the ROAT FM IA solution (20 of 22, 91%, vs. six of 22, 27%; $P < 0{\cdot}001$, Fisher's exact test, two sided).

Twenty-four of the 40 patients with contact allergy to FM I in 2011 had a positive algorithm according to the questionnaire and 16 had a negative algorithm (Table S1; see Supporting Information). Numerically more individuals (but not statistically significantly) with a positive algorithm had positive ROATs than those with a negative algorithm (15 of 24, 63%, vs. eight of 16, 50%; $P = 0{\cdot}52$).

In the group with contact allergy to FM I in 2011, 33 had allergy only to FM I. Of these, 17 developed a positive ROAT, compared with six in the subgroup with contact allergy to both FM I and FM II in 2011 (17 of 33 vs. six of seven; $P = 0{\cdot}21$). 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 IA in the respective subgroup (15 of 33 vs. six of seven; $P = 0{\cdot}09$).

Figures 3 and 4 show the associations between the dilutions of the ROAT FM IA and the degrees of patch test reactivity, respectively, and a positive ROAT. The lower the patch test concentration eliciting a positive test reaction, the more likely a positive ROAT ($P < 0{\cdot}001$). The same pattern is seen for the intensity of patch test reaction to ROAT FM IA and the outcome of the ROAT ($P < 0{\cdot}001$).

Figures 5 and 6 demonstrate the associations between the dilutions of the ROAT FM IA and the degree of patch test reactivity, respectively, and the first day of appearance of a positive ROAT. The lower the patch test concentration eliciting a positive test reaction, the more likely it is that a positive ROAT appears early ($P < 0{\cdot}001$). Furthermore, more intense reactions to the ROAT FM IA are more likely to appear early ($P < 0{\cdot}001$).

Discussion

When various usage tests including ROATs have been performed with fragrance sensitizers, positive reactions have been obtained in 0–100% of participants.[11–25] Major reasons for the great variation are the concentration, the actual dose per cm^2 of the applied usage or ROAT preparation, and the length of the application period. In the present study, positive ROAT reactions were obtained in 59% of those hypersensitive to FM I and in 3% of those without contact allergy to FM I. The difference is highly statistically significant, which rules out irritancy as the cause of the positive ROATs. Furthermore, the

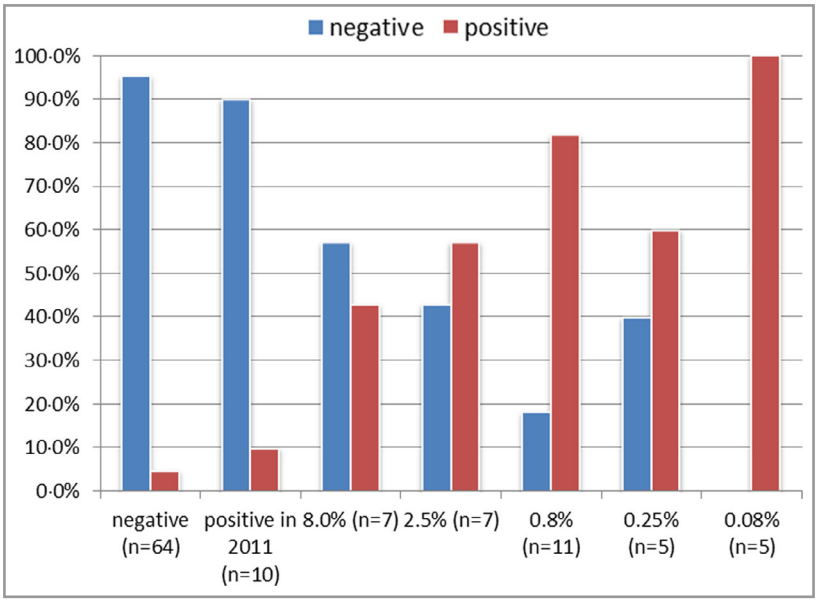

Fig 3. Outcome of the repeated open application test (ROAT) with ROAT FM IA, based on fragrance mix (FM) I ingredients, in volunteers patch tested with FM I in both 2011 and 2014. Volunteers are stratified by the reactivity to dilutions of FM I at patch testing in 2014. 'Negative' indicates negative patch test reactions to FM I in both 2011 and 2014. 'Positive in 2011' indicates positive patch test reaction to FM I in 2011 and a negative one in 2014. The 8%, 2·5%, 0·8%, 0·25% and 0·08% concentrations indicate the lowest patch test concentration eliciting a positive reaction to FM I in 2014. When all volunteers within a reactivity group have developed a positive ROAT, the red bar peaks at 100%.

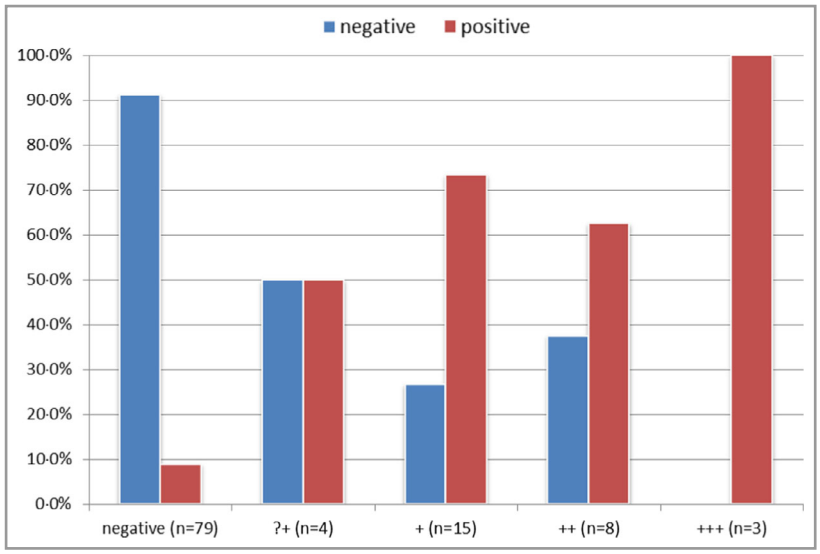

Fig 4. Outcome of repeated open application test (ROATs) with ROAT FM IA, based on fragrance mix (FM) I ingredients, in volunteers with various intensities of test reactions to ROAT FM IA at patch testing in 2014. When all volunteers within an intensity group have developed a positive ROAT, the red bar peaks at 100%.

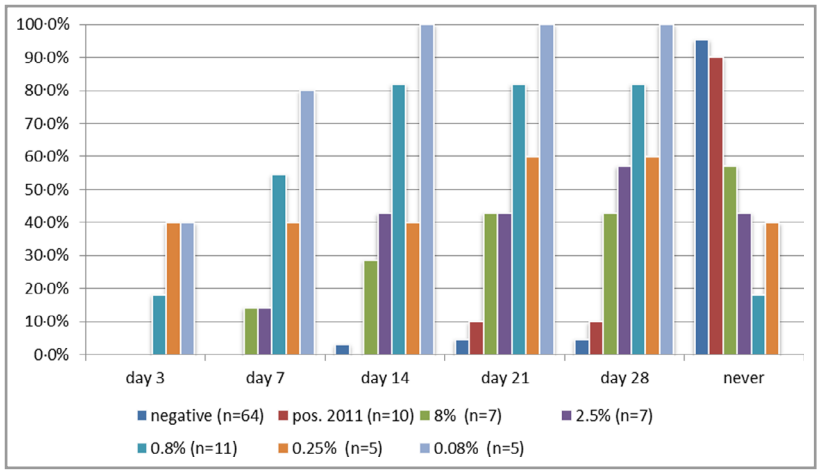

Fig 5. Cumulative positive reactors to the repeated open application test (ROAT) with ROAT FM IA, based on fragrance mix (FM) I ingredients, on days 3–28 in volunteers with various degrees of reactivity to dilutions of FM I at patch testing in 2014. 'Negative' indicates negative patch test reactions to FM I in both 2011 and 2014. 'Positive in 2011' indicates positive patch test reaction to FM I in 2011 and a negative one in 2014. The 8%, 2·5%, 0·8%, 0·25% and 0·08% concentrations indicate the lowest patch test concentration eliciting a positive reaction to FM I in 2014. When all volunteers within a reactivity group have developed a positive ROAT on a reading day, the coloured bar peaks at 100%.

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 I ingredients.

The fact that 41% of the volunteers hypersensitive to FM I did not develop a positive ROAT indicates that they can use scented products containing the FM I ingredients on nondamaged 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 I ingredients have been lowered since 2011 according to the IFRA standards. However, despite lower exposure, the situation may be different if products such as scented moisturizers are applied on skin with an existing dermatitis.[26] There were 10 participants with a positive patch test reaction to FM I in 2011 who in 2014 had a negative, irritant or doubtful reaction. One explanation may be false positive reactions in 2011 or a difference in the number of FM I molecules penetrating the skin, as petrolatum was the FM I vehicle in 2011 and ethanol–DEP in 2014. However, the development of a positive ROAT in one of these individuals indicates that the nonpositive reactions in 2014 instead may have been false negatives.

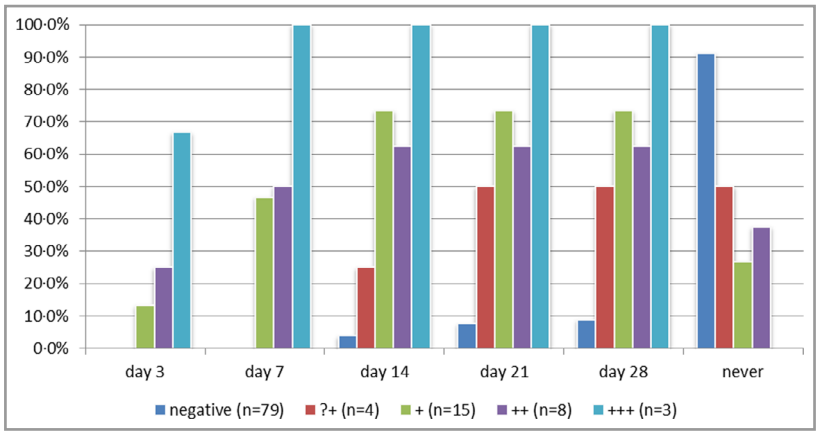

Fig 6. Cumulative positive reactors to the repeated open application test (ROAT) with ROAT FM IA, based on fragrance mix (FM) I ingredients, on days 3–28 in volunteers with various degrees of intensity of test reactions to ROAT FM IA at patch testing in 2014. When all volunteers within an intensity group have developed a positive ROAT, the coloured bar peaks at 100%.

The two positive ROATs in those without contact allergy to FM I may be explained by a false negative reaction to FM I in 2014. These two volunteers also patch tested negatively to the ROAT FM I solution. It is therefore possible that the contact allergy is directed towards the fragrance materials present at concentrations < 1% in the ROAT FM I solution. In the test preparation with FM I at 8%, each fragrance ingredient is present at 1%, while five fragrance ingredients are present at concentrations < 1% and two at higher concentrations in the ROAT FM I solution (Table 3). Repeated exposure to the ROAT FM IA solution might still help accumulate a sufficient number of molecules in the skin to elicit a positive ROAT. Other explanations are irritant contact dermatitis indistinguishable from an allergic contact dermatitis and sensitization to a fragrance material during the ROAT. This latter possibility would have been substantially strengthened if a patch test with FM I and ROAT FM IA performed after the termination had resulted in a positive test. However, such a test was not performed.

Among the 44 volunteers with a positive patch test reaction to FM I in 2011 and/or 2014, more positive ROATs were noted in those with a positive patch test reaction to the ROAT FM IA than in those without ($P = 0{\cdot}014$). In a way this result is expected, as those hypersensitive to FM I but without patch test reactions to ROAT FM IA may react to those five fragrance materials that are present in the ROAT FM IA at lower concentrations than in FM I (Table 3).

Expectedly, there was an association between the degree of hypersensitivity and the outcome of the ROAT. The stronger the reaction at patch testing – defined as the lowest FM I dilution eliciting a positive patch test, or the intensity of the patch test reaction to the ROAT FM IA solution – the more likely was a positive ROAT, and the more likely it was to appear early during the application period (Figs 3–6; and Table S1; see Supporting Information). All of those reacting positively at patch testing to the lowest FM I solution (0·08%) and those with a +++ reaction to ROAT FM IA developed a positive ROAT (Figs 3 and 4). For the latter group (+++ reactions), all ROATs had appeared by the day 7 reading (Fig. 6), while it took one more week until all ROATs were positive for those reacting down to 0·08% (Fig. 5). This kind of relationship has been demonstrated for other fragrance sensitizers including isoeugenol,[11,13] hydroxyisohexyl-3-cyclohexenecarboxaldehyde[16,18] and oak moss.[23,24]

There was an indication that it was the subgroup of volunteers hypersensitive to both FM I and FM II who developed a positive ROAT. One explanation could be a higher degree of reactivity to FM I at the initial patch testing in the subgroup with contact allergy to both FMs, but this was not supported statistically. Again, differences in the pattern of contact allergy to the eight fragrance materials in FM I, with more contact allergy to the ingredients present at concentrations > 1% in the ROAT FM IA, could be another explanation in the subgroup with contact allergy to both FMs.

Unfortunately, the algorithm predicting that contact allergy to fragrances combined with skin problems and avoidance of scented products indicated a clinically relevant contact allergy could not be confirmed, and there was no indication ($P = 0{\cdot}52$). One possible reason for the lack of association is that insufficient questions were used in the questionnaire. Another explanation, and maybe more likely, is that a diagnosis of allergic contact dermatitis on the whole 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 the presence of dermatitis – maybe particularly the first time an allergic contact dermatitis appears. On the other hand, the exposure might be unexpected[27] 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

that the contribution of a low-degree exposure to the sensitizer is 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. For these situations, it might be hard or virtually impossible to construct questions that might constitute a questionnaire-based diagnosis of allergic contact dermatitis.

To the best of our knowledge, this study is the largest ROAT, defined as the ROAT with most participants, performed so far. Furthermore, efforts were made to give the study a high quality. 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 practise the reading and to calibrate it. The testing personnel from participating European centres were taught 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 I 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. However, the study would have benefited from monitoring, including site visits by 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 allowed only in case of a positive reaction to that particular solution.

The patch testing part of the study can be considered to be of high quality. 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.[28] The only factor not obtaining the highest score was the lack of monitoring.

In conclusion, a ROAT with the FM I 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 assumed that contact allergy to FM I together with an itching dermatitis at any time during the life followed by avoidance of scented products was equivalent to an allergic contact dermatitis. However, this algorithm was not substantiated in this experimental set-up. On the other hand, it was demonstrated that 41% of those with contact allergy to FM I did not develop a positive ROAT, while 59% thus developed a positive ROAT. The stronger the patch test reactivity, defined as the lowest FM I dilution eliciting a positive patch test reaction or intensity of patch test reaction to the ROAT FM IA 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 I at the start of the ROAT may still develop a positive ROAT.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Detailed study results for each participant.