

ORIGINAL ARTICLE

The significance of batch and patch test method in establishing contact allergy to fragrance mix I—EDEN Fragrance Study Group

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Background: A fragrance mix consisting of eight separate fragrance ingredients (fragrance mix I [FM I]) is present in most baseline patch test series. Patch testing with the TRUE Test technique is considered to detect less contact allergy to FM I than testing with the Finn Chamber technique.

Objective: To investigate the possible significance of batch and patch test method in establishing contact allergy to FM I.

Methods: Three thousand one hundred and nineteen individuals representing a sample of the general population were patch tested with two batches of FM I with two patch test techniques at six dermatology clinics in five European countries. The TRUE Test technique and the Finn Chamber technique with pet. preparations were used. McNemar's test was used for statistical calculations.

Results: The contact allergy prevalences varied between 0.7% and 2.6%. The patch tests with the mixes containing *Evernia prunastri* (oak moss) with a high content of chloratranol/atranol resulted in substantially more positive reactions than the corresponding tests with the mixes containing oak moss with a low content of chloratranol/atranol. The Finn Chamber technique detected significantly more contact allergic reactions than the TRUE Test technique ($P < 0.001$).

Conclusion: The Finn Chamber technique detects more contact allergy to FM I than the TRUE Test technique.

KEYWORDS

allergic contact dermatitis, chloratranol/atranol, delayed hypersensitivity, *Evernia prunastri*, general population, oak moss

1 | INTRODUCTION

Patch testing is the gold standard for the establishment of contact allergy. The highest possible concentration, actually dose/cm², of the sensitizer should be used with due consideration of adverse reactions, particularly patch test sensitization. Recently, a study on the prevalence of contact allergy and allergic contact dermatitis in the

European population was performed on the basis of a questionnaire answered by 12 377 citizens in five European countries. A subsample of 3119 individuals were patch tested with the European baseline series with a few modifications^{1–3} and with the separate fragrance ingredients in fragrance mix I (FM I) and fragrance mix II (FM II).^{4,5} For practical reasons, three TRUE Test panels were used supplemented with three pet. preparations tested with the Finn Chamber

technique.³ FM I is present in TRUE Test panel 1, but it detects less contact allergy to FM I than the Finn Chamber technique.^{6–9} The Finn Chamber technique was therefore also used to test FM I. Initially, FM I testing was intended to be performed with two test preparations based on the same batches of the eight fragrance ingredients, one with the TRUE Test technique and the other with the Finn Chamber technique. By misunderstanding, an oak moss (INCI: *Evernia prunastri*) extract with a low content of atranol and chloratranol was delivered to the Department of Occupational and Environmental Dermatology in Malmö, and from there to the manufacturer of TRUE Test panels in Denmark and the manufacturer of pet. preparations in Sweden. As oak moss has the same CAS numbers (90028-68-5, 9000-50-4, and 68917-10-2) independently of quality, that is, whether it has a high or low content of atranol and chloratranol, the mistake was not discovered until the material safety data sheet and the certificate of analysis belonging to the batch were scrutinized. Chemical analyses confirmed the low content of atranol and chloratranol. In this situation, it was decided to test FM I not only with two patch test techniques but also with two batches. In this article, we therefore present the results of patch testing with two batches of FM I tested with two patch test methods.

2 | MATERIALS AND METHODS

2.1 | Volunteers

Information on the randomization process and demographic characteristics of the 12 377 citizens answering the questionnaire and the individuals who were patch tested has been published elsewhere.^{1–5} In total, 3119 individuals (1405 males and 1712 females) were patch tested. The study was approved by the ethics committees of the six participating centres.

2.2 | Test preparations

The eight fragrance substances included in FM I and listed in Table 1 were forwarded to Malmö to be used to produce TRUE Test panel 1 and Nixema at Mekos (Hilleröd, Denmark), and to Chemotechnique Diagnostics (Vellinge, Sweden) for the production of pet. preparations. Nixema is a TRUE Test and a self-test; it is a medical plaster that incorporates two allergen patches with nickel sulfate and fragrance mix that was recently introduced onto the Swedish market for the detection of contact allergy to nickel and fragrance allergy, respectively.^{10,11} The oak moss with a low content of chloratranol/atranol was used for TRUE Test panel 1, and the oak moss with a high content of chloratranol/atranol¹² was used for the Nixema test. Two pet. preparations with the eight fragrance substances and the emulsifier sorbitan sesquioleate (one with a low content of chloratranol/atranol and one with a high content of chloratranol/atranol) were manufactured by Chemotechnique Diagnostics, using the same batches as Mekos. The concentration of the eight fragrance ingredients in the respective pet. preparations was 1.0% wt/wt and thus 8.0% wt/wt for the pet. mixes (Table 1). The concentrations of the fragrance ingredients were lower,¹³ but the same for FM I in TRUE Test panel 1 and

FM I in Nixema (Table 1). The low concentrations of the FM I fragrance ingredients in TRUE Test panel 1 and Nixema are attributable to differences in bioavailability between FM I in pet. and the hydrophilic dried-in preparation.¹³

2.3 | Patch testing and statistics

The patch testing took place in Coimbra (Portugal), Bergamo (Italy), Groningen (The Netherlands), Jena (Germany), Heidelberg (Germany), and Malmö (Sweden).^{3–5} The TRUE Test panel 1, the Nixema test and the two pet. preparations, tested in Finn Chambers (Smart Practice, Phoenix, Arizona) with a diameter of 8 mm and mounted on Scanpor tape, were applied on the upper back. For the pet. preparations, 20 mg was applied onto the respective chamber.¹⁴ The tests were held under occlusion for 48 hours, and read on day (D) 3 according to ICDRG guidelines.¹⁵ The four test preparations with FM I are henceforth sometimes called Finn low, Finn high, TRUE low, and TRUE high. Finn and TRUE refer to Finn Chamber and TRUE Test. Low and high refer to the content of chloratranol/atranol.

McNemar's test, two-sided, was used for the pairwise comparisons of the contact allergy rates between the four patch test preparations with FM I.

3 | RESULTS

The results of patch testing with the four tests for FM I are shown in Table 2. The contact allergy prevalences varied between 0.7% and 2.6%. The patch tests with the mixes containing oak moss (*E. prunastri*) with a high content of chloratranol/atranol resulted in substantially more positive contact allergy reactions than the corresponding tests containing oak moss with a low content of chloratranol/atranol; 157% more for the TRUE Test technique, and 24% more for the Finn Chamber technique. When the patch test results for the respective FM I preparations (low and high content of chloratranol/atranol, respectively) were compared with the two methods used, the Finn Chamber technique was more sensitive, with 200% and 44% more cases of contact allergy being detected with the low-content and high-content oak moss, respectively. The pairwise comparisons between contact allergy rates resulted in highly statistically significant differences for all comparisons except for those between Finn low and Finn high ($P = 0.040$) and between TRUE high and Finn low ($P > 0.3$).

Figure 1A shows how those reacting with a doubtful, + or ++ reaction to TRUE high reacted to TRUE low. Figure 1B,C shows the same, but based on how those reacting with a doubtful, + or ++ reaction to Finn high reacted to Finn low (Figure 1B) or to TRUE high (Figure 1C).

4 | DISCUSSION

This study illustrates two important aspects of patch testing; namely, the significance of the batch and the significance of the patch test technique for the result.¹⁶ Contact sensitizers to be tested may

TABLE 1 Manufacturers/suppliers and concentrations of the fragrance mix I (FM I) ingredients in four test preparations

Ingredient	Manufacturer/supplier	Finn Chamber				TRUE Test	
		FM I low		FM I high		FM I low	FM I high
		Concentration (% wt/wt)	Concentration (mg/cm ²)	Concentration (% wt/wt)	Concentration (mg/cm ²)	Concentration (µg/cm ²)	Concentration (µg/cm ²)
Cinnamic alcohol	Bedoukian Danbury, Connecticut	1.0	0.4	1.0	0.4	78	78
Cinnamal	Bedoukian Danbury	1.0	0.4	1.0	0.4	49	49
Hydroxycitronellal	Firmenich, Plainsboro, New Jersey	1.0	0.4	1.0	0.4	78	78
α-Amyl cinnamal	International flavors and fragrances, Union Beach, New Jersey	1.0	0.4	1.0	0.4	21	21
Geraniol	International flavors and fragrances	1.0	0.4	1.0	0.4	100	100
Eugenol	Firmenich	1.0	0.4	1.0	0.4	49	49
Isoeugenol	International flavors and fragrances	1.0	0.4	1.0	0.4	21	21
<i>Evernia prunastri</i> (oak moss)	Robertet, Grasse, France	1.0	0.4	1.0	0.4	100	100
Sorbitan sesquioleate	Chemotechnique diagnostics, Vellinge, Sweden	5.0	2.0	5.0	2.0	0	0

With the Finn Chamber (diameter, 8 mm) technique, 20 mg of a pet. preparation was applied. FM I low indicates the mix containing oak moss with a low content of atranol and chloratranol. FM I high indicates the mix containing oak moss with a high content of atranol and chloratranol.

represent chemically defined compounds, such as cinnamal and isoeugenol, or chemicals such as oak moss (INCI: *E. prunastri*), which consists of an unknown number of compounds, many of which are unidentified. Both the synthesis of these compounds and the extraction processes may vary with the manufacturer; therefore, the test compound should be obtained from one manufacturer, to minimize the possibility of variation in numbers, types and concentrations of “contaminants” (undesired compounds).¹⁶ Furthermore, although the sensitizer is manufactured by one company, there might still be batch-dependent variation¹⁷ with possible significance for the patch test result, as shown in the current study, with 157% and 24% more contact allergy cases being detected with FM I containing oak moss with a high content of chloratranol/atranol than with FM I containing oak moss with a low content when testing was performed with the TRUE Test technique and the Finn Chamber technique, respectively. To the best of our knowledge, FM I containing oak moss with a high and a low content of chloratranol/atranol, respectively, from the same manufacturer has not been patch tested before, but oak moss itself has. Substantially

more contact allergy is detected with oak moss with a high content of chloratranol/atranol.^{12,18–20}

In this multicentre patch test study, there was a high level of standardization of the patch test methodology. An expert in the technique of patch testing visited each of the six sites to monitor the patch testing and to ensure that it was conducted in a uniform manner and according to the protocol used, based on consideration of 16 factors of possible significance for the patch test result in multicentre patch test studies.¹⁶ Besides listing the 16 factors, a scoring system is also suggested, with various scores based on the relative importance of each factor for the quality of the multicentre patch test study. In the current study, in which different batches and patch test techniques were used intentionally, the highest scores were obtained for all factors except for lack of control of occlusion after 48 hours and only one test reading on D3. In spite of this, the total score allows this study to be classified as a multicentre patch test study with excellent quality.¹⁶

As expected, patch testing with FM I with the Finn Chamber technique resulted in significantly more contact allergy than testing with

TABLE 2 Rates of contact allergy to fragrance mix I (FM I) when tested with two batches (one with a low content of atranol and chloratranol, and one with a high content) with two test methods (TRUE Test and Finn Chamber technique), as well as pairwise comparisons made by the use of McNemar's test, two-sided

			P-values			
			True Test		Finn Chamber	
Test method and batch	Number of individuals tested	Number (%) of positive reactors	FM I low	FM I high	FM I low	FM I high
TRUE Test						
FM I low	3119	23 (0.7)		<0.001	<0.001	<0.001
FM I high	2741	50 (1.8)	<0.001		>0.3	<0.001
Finn Chamber						
FM I low	3119	65 (2.1)	<0.001	>0.3		0.040
FM I high	3108	81 (2.6)	<0.001	<0.001	0.040	

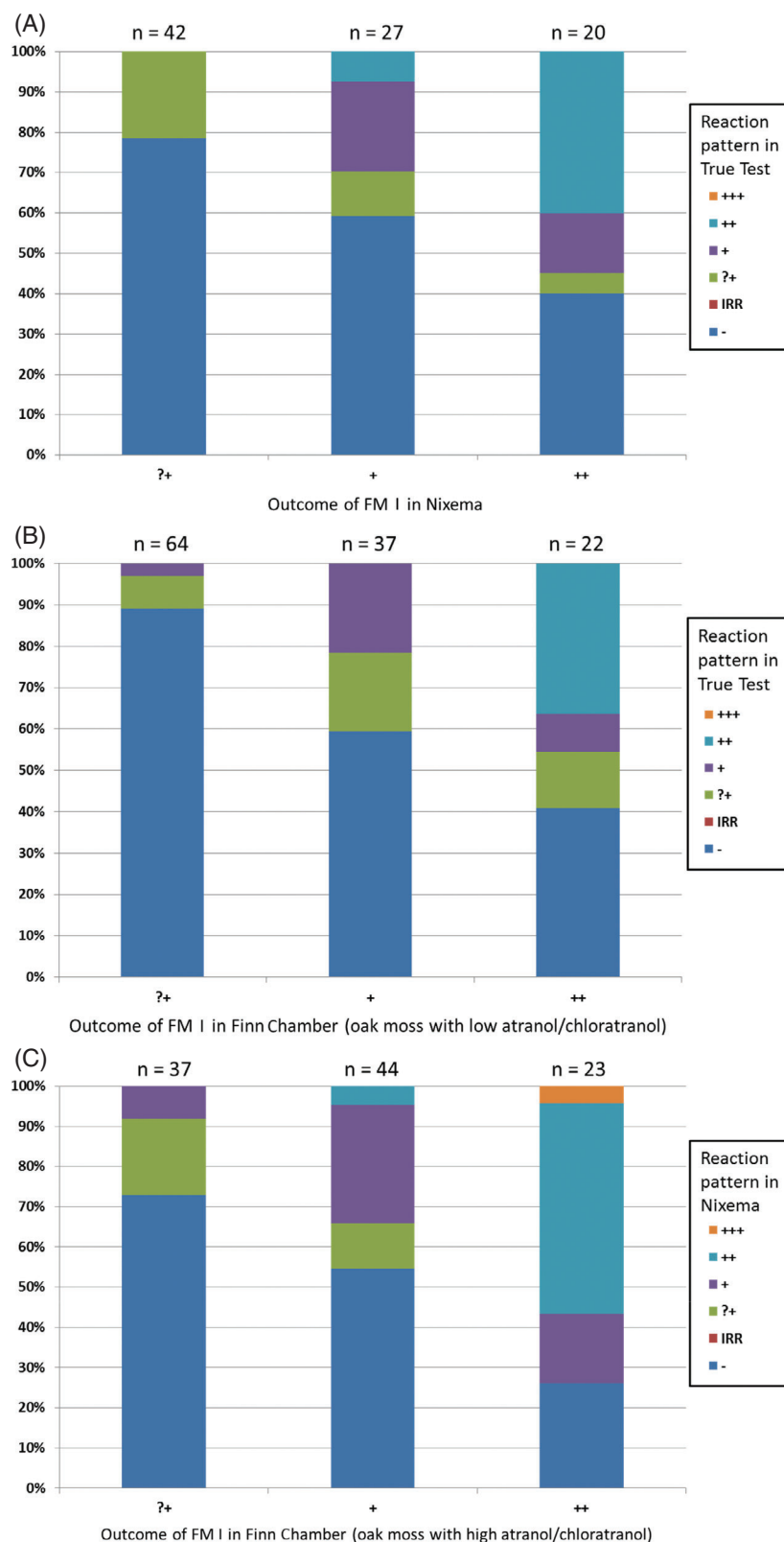


FIGURE 1 (A), Patch testing with fragrance mix I (FM I) with the TRUE Test technique (TRUE Test and Nixema); reaction pattern for FM I containing oak moss (*Evernia prunastri*) with a low content of chloratranol/atranol (TRUE Test) in those with doubtful (?+) reactions, weak (+) positive reactions and strong (++) positive reactions to FM I containing oak moss with a high content of chloratranol/atranol (Nixema). (B), Patch testing with FM I containing oak moss with a low content of chloratranol/atranol with the TRUE Test technique and the Finn Chamber technique; reaction pattern in TRUE Test for those reacting with doubtful (?+) reactions, weak (+) positive reactions and strong (++) positive reactions when tested with the Finn Chamber technique. (C), Patch testing with FM I containing oak moss with a high content of chloratranol/atranol with the TRUE Test technique and the Finn Chamber technique; reaction pattern in TRUE Test (Nixema) for those reacting with doubtful (?+) reactions, weak (+) positive reactions and strong (++) positive reactions when tested with the Finn Chamber technique. IRR, irritant reaction

the TRUE Test technique, independently of whether the oak moss contained a high or a low content of atranol and chloratranol (Table 2). The results of this study are in agreement with previous studies showing that both the Finn Chamber technique and the IQ chamber system detect more cases of contact allergy to FM I than the TRUE Test technique.^{6–9}

It has sometimes been argued that a lower prevalence of contact allergy to FM I, as observed with the TRUE Test technique, is not invalid, as the other patch test techniques more often cause irritant reactions that are misinterpreted as allergic reactions, and are thus false-positive reactions. In this study, the fragrance ingredients used to produce panel 1 and the Nixema test (TRUE low and TRUE high, respectively) with the TRUE Test technique and for the production of the pet. preparations (Finn low and Finn high, respectively) originate from the same batches (Table 1). Within the respective test systems, the TRUE Test technique and the Finn Chamber technique with pet. preparations, the concentrations are almost the same (Table 1), as the only difference is a few hundreds of ppm of chloratranol/atranol in FM I between the oak moss preparations with a low and a high content of chloratranol/atranol. As chloratranol and atranol are the major sensitizers in oak moss,^{21,22} a lower rate of contact allergy detection is expected, as found for oak moss with a low content of chloratranol/atranol (Table 2). Figure 1A shows the difference in sensitivity when three types of reaction to TRUE high are examined. For doubtful, + and ++ reactions, the simultaneous reactions to TRUE low could either be reactions with the same reactivity or reactions with higher or lower reactivity. The difference in sensitivity is obvious, as there is a tendency for there to be a higher proportion of reactions with lower reactivity for all three types of reaction shown in Figure 1A.

The distribution of reactions to FM I tested with the TRUE Test technique, independently of whether it is FM I containing oak moss with a low content of chloratranol/atranol or FM I containing oak moss with a high content of chloratranol/atranol, in those with doubtful, + or ++ reactions to the corresponding pet. preparations tested with the Finn Chamber technique (Figure 1B,C) is the same as the distribution in Figure 1A, which strongly indicates that the higher contact allergy frequency with the Finn Chamber technique is explained by higher sensitivity of the Finn Chamber technique. For the types of reaction to the pet. preparations tested with the Finn Chamber technique shown in Figure 1B,C, the reactions to the corresponding test with the TRUE Test technique clearly show that the proportion of TRUE Test reactions with a lower degree of reactivity exceeds the proportion of reactions with a higher degree of reactivity for all reaction types, supporting the interpretation that the Finn Chamber technique is more sensitive, or, alternatively, that there is a quantitative difference in sensitivity.

However, there might also be a qualitative difference in sensitivity between the TRUE Test technique and the Finn Chamber technique with pet. preparations. The TRUE Test technique may, in another situation, be more sensitive than the Finn Chamber technique; for example, if the distribution of contact allergy to the eight fragrance ingredients in FM I in the tested population is different from the distribution today, and that the respective FM I, TRUE Test technique and pet. preparation with the Finn Chamber technique, differ in

their capacity to test positively depending on which fragrance ingredient the contact allergy is directed. The concentration of fragrance ingredient in the pet. preparation is 1.0% for all ingredients, but differs by a factor of 5 for the same eight fragrance ingredients in the TRUE Test (Table 1).

Currently, there are “two major types” of oak moss available, one with a low content of atranol and chloratranol, and another with a high content of atranol and chloratranol. As patch testing is a means to establish contact allergy rather than allergic contact dermatitis, the highest possible concentration, actually dose/cm², of the sensitizer should be used with due consideration of adverse reactions, particularly patch test sensitization.²³ Thus, the oak moss in FM I in the European baseline series should continue to be a chemical with a high content of atranol and chloratranol.²⁴ Furthermore, whenever prospective multicentre patch test studies are performed, the same batches of the sensitizers should be used unless the study is aimed at investigating the significance of batch for the patch test result.

CONFLICTS OF INTERESTS

Magnus Bruze is a member of the Expert Panel for Fragrance Safety (<http://fragrancesafetypanel.org/>).

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REFERENCES

1. Rossi M, Coenraads PJ, Diepgen T, et al. Design and feasibility of an international study assessing the prevalence of contact allergy to fragrances in the general population: the European dermatology epidemiology network fragrance study. *Dermatology*. 2010;221:267-275.
2. Naldi L, Cazzaniga S, Gonçalves M, et al. Prevalence of self-reported skin complaints and avoidance of common daily life consumer products in selected European regions. *JAMA Dermatol*. 2014;150:154-163.
3. Diepgen TL, Ofenloch R, Bruze M, et al. Prevalence of contact allergy in the European general population. *Br J Dermatol*. 2016;174:319-329.
4. Diepgen TL, Ofenloch R, Bruze M, et al. Prevalence of fragrance contact allergy in the general population of five European countries. *Br J Dermatol*. 2015;173:1411-1419.
5. Ofenloch R, Diepgen TL, Bruze M, et al. Colophony as marker for fragrance allergy in the general European population. *Br J Dermatol*. 2016;174:695-696.
6. Suneja T, Belsito DV. Comparative study of Finn chambers and T.R.U.E. test methodologies in detecting the relevant allergens inducing contact dermatitis. *J Am Acad Dermatol*. 2001;45:836-839.
7. Lazarov A, David M, Abraham D, Trattner A. Comparison of reactivity to allergens using the True test and IQ chamber system. *Contact Dermatitis*. 2007;56:140-145.
8. Mortz CG, Andersen KE. Fragrance mix I patch test reactions in 5006 consecutive dermatitis patients tested with True Test[®] and Trolab[®] test material. *Contact Dermatitis*. 2010;63:248-253.
9. Frosch PJ, Duus Johansen J, Schuttelaar ML, et al. ESSCA network. Patch test results with fragrance markers of the baseline series—analysis of the European Surveillance System on Contact Allergies

- (ESSCA) network 2009-2012. *Contact Dermatitis*. 2015;73:163-171.
10. Josefson A, Svensson Å, Färm G, Engfeldt M, Meding B. Validation of self-testing as a method to estimate the prevalence of nickel allergy. *Acta Derm Venereol*. 2011;91:526-530.
 11. Elsner P, Bjarnøe H, Aberer W, Brasch J. Evaluation of a self-test device used in allergic contact dermatitis. *Skin Pharmacol Physiol*. 2015;28:257-263.
 12. Mowitz M, Zimerson E, Svedman C, Bruze M. Patch testing with serial dilutions and thin-layer chromatograms of oak moss absolutes containing high and low levels of atranol and chloroatranol. *Contact Dermatitis*. 2013;69:342-349.
 13. Bruze M, Andersen KE, Goossens A, on behalf of the ESCD and EECDRG. Recommendation to include fragrance mix 2 and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall[®]) in the European baseline patch test series. *Contact Dermatitis*. 2008;58:129-133.
 14. Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis*. 2007;56:281-285.
 15. Fregert S. *Manual of Contact Dermatitis*. 2nd ed. Copenhagen, Denmark: Munksgaard; 1981.
 16. Bruze M. Thoughts on how to improve the quality of multicentre patch test studies. *Contact Dermatitis*. 2016;74:168-174.
 17. Fregert S. Batch-consciousness in dermatologic management. *Acta Derm Venereol Suppl (Stockh)*. 1979;59:63-65.
 18. Nardelli A, Giménez-Arnau E, Bernard G, Lepoittevin JP, Goossens A. Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals? *Contact Dermatitis*. 2009;60:91-95.
 19. Mowitz M, Svedman C, Zimerson E, Bruze M. Usage tests of oak moss absolutes containing high and low levels of atranol and chloroatranol. *Acta Derm Venereol*. 2014;94:398-402.
 20. Andersen F, Andersen KH, Bernois A, et al. Reduced content of chloroatranol and atranol in oak moss absolute significantly reduces the elicitation potential of this fragrance material. *Contact Dermatitis*. 2015;72:75-83.
 21. Bernard G, Giménez-Arnau E, Rastogi SC, et al. Contact allergy to oak moss: search for sensitizing molecules using combined bioassay-guided chemical fractionation, GC-MS, and structure-activity relationship analysis. *Arch Dermatol Res*. 2003;295:229-235.
 22. Johansen JD, Bernard G, Giménez-Arnau E, Lepoittevin JP, Bruze M, Andersen KE. Comparison of elicitation potential of chloroatranol and atranol—2 allergens in oak moss absolute. *Contact Dermatitis*. 2006;54:192-195.
 23. Bruze M, Condé-Salazar L, Goossens A, Kanerva L, White IR. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis*. 1999;41:241-250.
 24. Bruze M, Goossens A, Isaksson M. Recommendation to increase the test concentration of methylchloroisothiazolinone/methylisothiazolinone in the European baseline patch test series—on behalf of the European Society of Contact Dermatitis and the European Environmental and Contact Dermatitis Research Group. *Contact Dermatitis*. 2014;71:35-40.

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