

Patch test concentrations (doses in mg/cm²) for the 12 non-mix fragrance substances regulated by European legislation

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Summary

Background. According to EU legislation, 26 fragrance substance allergens must be labelled on cosmetic products. For 12 of them, the optimal patch test concentration/dose has not been evaluated.

Objectives. To establish the optimal patch test doses in mg/cm² for the 12 fragrance substances that are not included in fragrance mix I or II in the European baseline patch test series.

Materials and Methods. Patch testing with the 12 fragrance substances was performed in a stepwise manner encompassing up to five rounds in at least 100 dermatitis patients for each round. Before patch testing, an individual maximum concentration/dose was determined for each fragrance substance.

Results. The predetermined maximum patch test concentrations/doses could be tested for all 12 fragrance substances, with no observable adverse reactions being noted.

Conclusions. For each fragrance substance investigated, it is recommended that half of the maximum patch test dose (mg/cm²) be used for aimed and screening patch testing.

Key words: active sensitization; adverse reaction; allergic contact dermatitis; contact allergy; fragrance substance; skin irritation.

The EU Cosmetics Directive requires that 26 sensitizing fragrance substances must be labelled on cosmetic products when present at >10 ppm in leave on or >100 ppm in rinse off products (1). This has facilitated the development of both primary and secondary preventive measures against the development of allergic contact dermatitis. To establish contact allergy, each fragrance

substance, or indeed any sensitizer, should be tested at the highest possible dose that will not induce patch test (active) sensitization and/or result in irritant reactions, particularly those with a morphology hard to distinguish from allergic reactions (false-positive) (2).

Although most of these fragrance substances are well-recognized contact sensitizers, data on irritation thresholds are often missing or insufficient. When skin irritation caused by a fragrance substance in petrolatum during patch testing is reported, it is usually expressed as a percentage and not as dose per unit area (3–7). Recently, active sensitization induced by patch testing with methyl 2-octynoate at 2% and 1% pet. was reported in 1 and 2 patients, respectively (8).

Currently, 14 of the 26 fragrance substances are ingredients of the two fragrance mixes in the European

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Conflicts of interest: Magnus Bruze is a member of REXPAN, an expert panel to the Research Institute of Fragrance Materials (RIFM).

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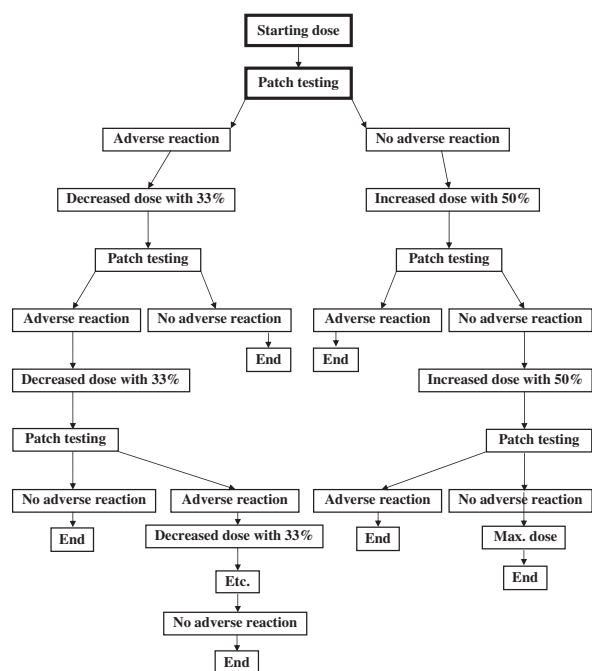


Fig. 1. Suggested step-by-step procedure with defined concentration/dose increments/decrements to find the optimal patch test concentration/dose in mg/cm^2 for any sensitizer.

baseline patch test series (9). The remaining 12 fragrance substances are less frequently tested, in part because of the lack of a systemically derived and recommended optimal patch test preparation.

Knowledge on the irritation threshold for a sensitizer is essential when recommending the patch test concentration (actually dose). The purpose of this study was to determine the patch test skin irritation threshold expressed as dose in mg/cm^2 skin for the 12 non-mix fragrance substances.

Materials and Methods

Study design

Figure 1 shows the study design. The start concentrations selected were the lowest concentrations that may induce patch test skin irritation, derived from information on skin irritation tests in humans supplied by the Research Institute of Fragrance Materials (RIFM, Anne Marie Api). At least 100 dermatitis patients at the eight participating clinics were patch-tested with the starting concentrations for the 12 fragrance substances. If testing at this concentration resulted in active sensitization or a substantial number of irritant reactions, the concentration was lowered by one-third for the next round of testing.

This meant that lowering the concentration gave rise to a dilution series with incremental steps at 50%. If there was no active sensitization and there were, at most, a few irritant reactions, the test concentration was increased by 50% for the next round of testing, unless the starting concentration was already at the maximum selected.

When the test concentration was lowered, patch testing was continued until the concentration was reached at which no adverse reactions were noted. When the dose was increased, patch testing was continued up to a concentration that resulted in observable adverse reactions or until the chosen maximum concentration was reached.

The selected maximum patch test concentration was based on two criteria, one practical and one immunological. At concentrations above 30%, it is hard to ensure that the fragrance substance is dissolved and evenly distributed in pet. Furthermore, at these high concentrations, the pet. preparations become very liquid, making it harder to apply a defined dose to each patch unit. The immunological criterion concerns active sensitization, which is an unacceptable but occasionally unavoidable consequence of patch testing. The maximum test concentration was therefore based on either a fragrance substance's human no-effect level concerning sensitization, or a concentration five times higher than the maximum concentration used in scented products (RIFM information).

Patch test preparations

The 12 fragrance substances were supplied by seven companies. Amyl cinnamyl alcohol and α -isomethyl ionone were supplied by International Flavors and Fragrances (New York, USA). Anise alcohol, benzyl benzoate and benzyl cinnamate were supplied by Symrise GmbH and Co. (Holzminden, Germany). Benzyl alcohol and methyl 2-octynoate were supplied by Bedoukian Research (Danbury, CT, USA). Benzyl salicylate was supplied by Rhodia Brasil Ltda (Santo Andr , Brazil). Butylphenyl methylpropional was supplied by BASF Aktiengesellschaft (Ludwigshafen, Germany). *Evernia furfuracea* extract was supplied by Robertet (Grasse, France). D-Limonene and linalool were supplied by Millenium Speciality Chemicals (Jacksonville, FL, USA). The fragrance substance chemicals were kept refrigerated in their original packaging during periods between making the patch test samples at the various concentrations. All test preparations were made at the Department of Occupational and Environmental Dermatology in Malm .

Patients

For each round, 103–114 consecutive patients with suspected allergic contact dermatitis (varying between

Table 1. The predetermined starting and maximum patch test concentrations and other tested concentrations for the 12 fragrance substances when testing each fragrance substance one to five times. The concentration/dose increment for each testing round from the starting concentration/dose or tested concentration/dose is exactly/approximately 50% unless the predetermined maximum concentration necessitated a lower dose increment

	CAS	Maximum concentration (%) to be tested	Patch test concentration in % wt/wt in petrolatum*				
			Concentration (C) at start	$C \times 1.5^{\dagger}$	$C \times 1.5^2$	$C \times 1.5^3$	$C \times 1.5^4$
Amyl cinnamyl alcohol	101-85-9	15.0	8.0	12.0	15	—	—
Anise alcohol	105-13-5	25.0	5.0	7.5	12.0	18.0	25.0
Benzyl alcohol	100-51-6	30.0	20.0	30.0	—	—	—
Benzyl benzoate	120-51-4	30.0	20.0	30.0	—	—	—
Benzyl cinnamate	103-41-3	30.0	8.0	12.0	18.0	30.0	—
Benzyl salicylate	118-58-1	30.0	5.0	7.5	12.0	18.0	30.0
Butylphenyl methylpropional	80-54-6	22.5	10.0	15.0	22.5	—	—
<i>Evernia furfuracea</i>	90028-67-4	3.0	3.0	—	—	—	—
α -Isomethyl ionone	127-51-5	30.0	10.0	15.0	22.5	30.0	—
D-Limonene	5989-27-5	30.0	20.0	30.0	—	—	—
Linalool	78-70-6	30.0	30.0	—	—	—	—
Methyl 2-octynoate	111-12-6	0.5	0.5	—	—	—	—

*The vehicle Softisan™ was used instead of pet., for solubility reasons, for benzyl alcohol at 30.0% and anise alcohol at 25.0%.

[†]Concentration at start (C) multiplied by the factor 1.5.

10 and 15 patients for an individual department) were patch-tested with the preparations listed in Table 1.

Patch testing

Patch testing was performed with Finn Chambers® (diameter 8 mm) (Epitest OY, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway). In order to provide a defined amount of allergen, a 5-mm string of test material was applied to each patch unit. With the syringes used, this corresponded to a weight of 15 mg. The difference in weight between a 5-mm string from a syringe just taken out of the refrigerator and one from a syringe at room temperature was approximately 5%. The patches were applied to the back under occlusion for 2 days, as extra materials during the routine investigation of individuals with suspected allergic contact dermatitis reactions. Readings were performed in accordance with each clinic's routine, but with mandatory readings on day 3 or day 4.

To help distinguish the morphology of irritant or allergic patch test reactions, a special protocol was used (Table 2). On the basis of the day 3 or day 4 reading, the dermatologist classified the reaction as either allergic, irritant, doubtful, or no reaction.

For classification as an allergic reaction, the morphology had to include erythema and infiltration covering the whole test area. Papules and vesicles were also considered to be allergic features in cases when they were present together with the mentioned minimum morphological features of an allergic reaction.

Table 2. Protocol used whenever there was a patch test reaction to any of the 12 fragrance substances)

Whenever there is a patch test reaction to anyone of the 26 fragrance substances tick the appropriate box/boxes.

	D2	D3/4	D7
Erythema covering the whole test area	☐	☐	☐
Erythema not covering the whole test area	☐	☐	☐
Infiltration/oedema covering the whole test area	☐	☐	☐
Infiltration/oedema not covering the whole test area	☐	☐	☐
Papule/s	☐	☐	☐
Vesicle/s	☐	☐	☐
Bulla/e	☐	☐	☐
Dry skin	☐	☐	☐
Scaling	☐	☐	☐
Pustule/s	☐	☐	☐
Erosion	☐	☐	☐
Necrosis	☐	☐	☐
Petechiae	☐	☐	☐
Shiny skin	☐	☐	☐
Silk paper structure	☐	☐	☐
Other morphology Specify.			
.....			
Assessment based on D3/4 reading:			
☐ Allergic reaction ☐ Irritant reaction ☐ Doubtful reaction			

Dry skin, scaling, pustules, erosions, necrosis, petechiae, shiny skin and skin with a silk-like (or cigarette paper-like) structure were considered to be signs of an irritant reaction.

All other combinations of erythema, infiltration, vesicles and papules were defined as examples of doubtful reactions.

Table 3. The distribution of patch test reactions to the 12 fragrance substances when tested with the one to five1 concentrations/doses specified in Table 1

Reaction type substance	C, n = 108			C × 1.5*, n = 103			C × 1.5 ² , n = 110			C × 1.5 ³ , n = 106			C × 1.5 ⁴ , n = 114		
	IR	DR	AR	IR	DR	AR	IR	DR	AR	IR	DR	AR	IR	DR	AR
Amyl cinnamyl alcohol	0	1	3	0	1	0	0	3	1	—	—	—	—	—	—
Anise alcohol	0	1	0	0	0	0	0	2	0	0	6	1	0	6	2
Benzyl alcohol	0	0	0	0	1	0	—	—	—	—	—	—	—	—	—
Benzyl benzoate	0	0	0	0	1	0	—	—	—	—	—	—	—	—	—
Benzyl cinnamate	0	0	0	0	0	0	0	2	0	0	0	1	—	—	—
Benzyl salicylate	0	1	0	0	0	0	0	1	0	0	0	1	0	5	3
Butylphenyl methylpropional	0	0	2	0	0	0	0	1	0	—	—	—	—	—	—
<i>Evernia furfuracea</i>	0	1	5	—	—	—	—	—	—	—	—	—	—	—	—
α -Isomethyl ionone	0	1	1	1	0	1	0	1	0	0	2	0	—	—	—
D-Limonene	0	1	1	0	0	0	—	—	—	—	—	—	—	—	—
Linalool	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—
Methyl-2-octynoate	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—

AR, allergic reaction; C, start concentration; DR, doubtful reaction; IR, irritant reaction; n, number of dermatitis patients tested for the given concentration.

*Start concentration (C) multiplied by the factor 1.5.

Results

The results are given in Table 3. Only one irritant reaction was observed, for α -isomethyl ionone at 15.0%; testing with 22.5% and 30.0% in 110 and 106 patients, respectively, did not elicit any irritant reactions. The predetermined maximum patch test concentrations were used for all 12 fragrance substances, without any evidence of active sensitization or irritant reactions. Allergic reactions were noted for 10 of the 12 fragrance substances, with the highest rate recorded for *E. furfuracea* (five reactions), followed by benzyl salicylate and amyl cinnamyl alcohol (both four reactions). The relative number of doubtful reactions increased with each test round (Table 3).

Discussion

Interestingly, it was possible to test all 12 fragrance substances at the predetermined maximum test concentrations without any adverse reactions, although previous reports in the literature had indicated that lower concentrations would give irritant reactions (5, 7). This discrepancy may be attributable to higher amounts of test preparation being used in previous studies and/or the tendency of the dermatologist to classify doubtful reactions as irritant ones. In this study, two reactions were initially classified as irritant reactions and one as allergic without the presence of the necessary morphological features to justify this classification according to Table 2, which is why these three reactions were reclassified as doubtful reactions.

The study design (Fig. 1) is based on previous studies that aimed to determine the optimal patch test concentration for inclusion in a baseline or additional patch test series (2). However, the use of a protocol that includes recording of morphological features to help in the classification of patch test reactions and the use of a defined dose when using pet. preparations are both unique features of this study. The knowledge of the significance of the applied dose is not new; this has meant that solvents other than pet. have been used as vehicles when the patch test reactivity has been investigated in humans or in sensitization studies in animals (10). However, for practical reasons, pet. is the preferred vehicle for most contact sensitizers tested routinely. Today, 20 mg of a pet. preparation is recommended for application to a small Finn Chamber® (diameter 8 mm) (11), whereas we used 15 mg in the present study. When the present study was planned, there was no recommendation for the amount of pet. preparation to apply to a Finn Chamber®. However, it is recommended that 15 μ l of liquid should be applied, and 15 mg was therefore selected, as this is the corresponding weight, provided that the density is 1 g/cm³.

However, almost no adverse reactions were obtained in the present study with the 15-mg pet. preparation. Although the purpose of the study was not to investigate the rates of contact allergy to the 12 fragrance substances, 24 allergic reactions were registered to 10 of the 12 substances. As expected, most allergic reactions were elicited by *E. furfuracea*. The two fragrance substances not eliciting any allergic reactions were benzyl alcohol and benzyl benzoate. Although non-oxidized limonene and linalool are considered to be non-sensitizers (12, 13),

Table 4. The maximum tested concentrations and doses in mg/cm² for the 12 fragrance substances when using a 15-mg pet. preparation on each Finn Chamber® and the recommended concentrations and doses when using a 20-mg pet. preparation

Fragrance	Maximum concentration (% wt/wt)	Tested maximum dose (mg/cm ²)	Recommended test dose (mg/cm ²)	Recommended test concentration (% wt/wt)
Amyl cinnamyl alcohol	15.0	4.5	2.25	5.0
Anise alcohol	25.0	7.5	3.75	10.0
Benzyl alcohol	30.0	9.0	4.5	10.0
Benzyl benzoate	30.0	9.0	4.5	10.0
Benzyl cinnamate	30.0	9.0	4.5	10.0
Benzyl salicylate	30.0	9.0	4.5	10.0
Butylphenyl methylpropional	22.5	6.75	3.38	10.0
<i>Evernia furfuracea</i>	3.0	0.9	0.45	1.0
α -Isomethyl ionone	30.0	9.0	4.5	10.0
D-Limonene	30.0	9.0	4.5	10.0
Linalool	30.0	9.0	4.5	10.0
Methyl-2-octynoate	0.5	0.125	0.075	0.2

two allergic reactions to these materials were recorded. Whether any oxidation products were formed during shipping, storage or the manufacture of test preparations remains unknown. Dermatologists still have no access to standardized patch test preparations with oxidized limonene and linalool.

On the basis of the results, it would seem that adverse reactions were not seen with the concentrations/doses tested and that the highest test concentrations/doses could be used for testing. Although 20 mg is the recommended amount of pet. preparation to be applied in a small Finn Chamber®, the concentration/dose to be recommended should allow a variation in the applied dose. The recommended dose is therefore half the maximum dose used in this study. Thus, considering that a 20-mg pet. preparation should be used instead of 15 mg, the recommended concentrations are 37.5% ($50 \times 15/20$) (here, rounded figures are used) of the maximum concentrations tested (Table 4). When these concentrations/doses are used for aimed or screening testing and there is a doubtful reaction, the same and the double concentration/dose can be tested in case it

is important to establish/rule out contact allergy. Such testing should not cause any problems, according to the results of this testing.

In summary, this study suggests a model with which to find the optimal patch test concentration/dose for a sensitizer. When the results of this study based on a limited sample size are applied to the 12 non-mix fragrance substances, the predetermined maximum patch test concentrations/doses can be used without expecting any adverse reactions. For aimed and screening patch testing, half the doses are recommended. As for other patch test preparations, these preparations should be subjected to constant review, as new reports on adverse reactions, particularly active sensitization, may become apparent over time (2).

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