

Optimal patch test concentration for three widely used sensitizing fragrance substances without mandatory labelling in cosmetics

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In 2012, a comprehensive review on the topic of fragrance contact allergens in cosmetics was published by the Scientific Committee on Consumer Safety of the European Commission.¹ It identified 82 fragrance substances as established contact allergens in humans, and an additional 18 fragrance substances were classified as contact allergens on the basis of animal data. Several of the 18 fragrance substances are among the top 100 fragrance compounds worldwide in terms of volume used, according to the International Fragrance Association (IFRA).¹ Among these high-volume fragrance substances, the IFRA has developed standards for the use levels of three of these compounds in scented consumer products,²⁻⁴ indicating that exposure is commonly occurring. In this study, we wanted to determine the optimal patch test concentration for these three fragrance substances (Figure 1), namely,

hexyl salicylate (CAS no. 6259-76-3), cyclamen aldehyde (CAS no. 103-95-7), and 2-methyl-3-(3,4-methylenedioxyphenyl)-propanal (MMDHCA/Helional) (CAS no. 1205-17-0), by using a protocol published by the European Society of Contact Dermatitis (ESCD).⁵

METHODS

Hexyl salicylate ($\geq 99\%$), cyclamen aldehyde ($\geq 95\%$) and MMDHCA ($\geq 98\%$) were purchased from Sigma-Aldrich (Saint Louis, Missouri) and stored at 5°C. Quality checks were performed with gas chromatography-mass spectrometry (GC-MS) analysis, which confirmed the three fragrance materials to be of high purity.

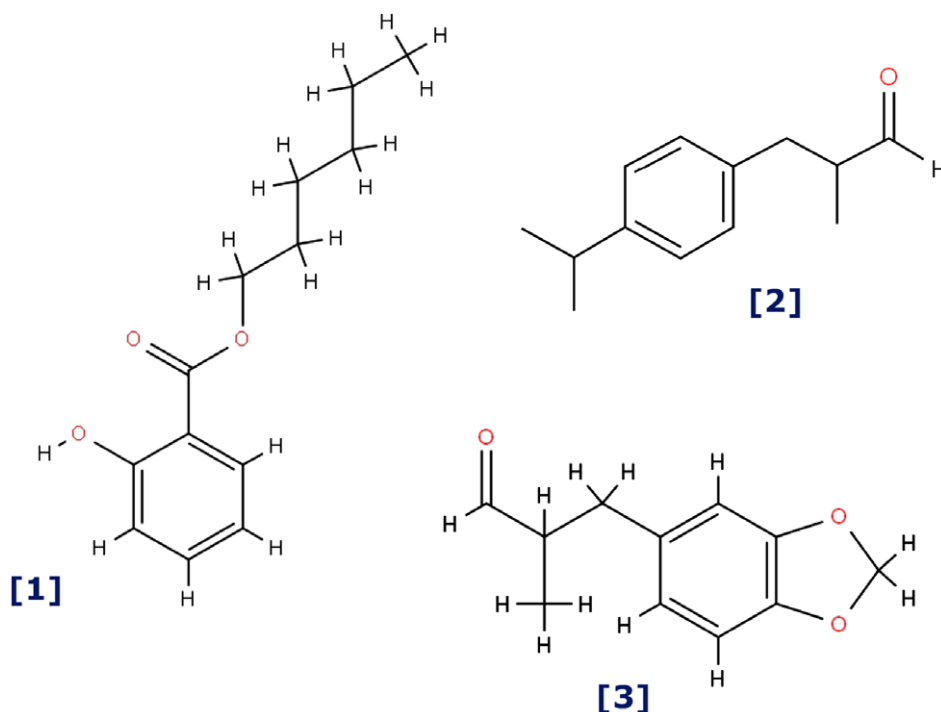


FIGURE 1 Chemical structures (www.chemspider.com, accessed December 16, 2018) of hexyl salicylate [1], cyclamen aldehyde [2], and 2-methyl-3-(3,4-methylenedioxyphenyl)-propanal (MMDHCA/Helional) [3]

TABLE 1 Local lymph node assay (LLNA) EC3 values, applied patch test concentrations (%) and observed reactions (n) for the investigated fragrance substances

Fragrance chemicals	CAS no.	LLNA EC3 ^a value (%)	Maximum allowed patch test concentration (%)	Patch test concentration in % wt/wt in pet. and observed reactions (n)									
				Concentration (C) at start (%), n = 100	C2 (%), n = 104	C3 (%), n = 103	C4 (%), n = 100	C5 (%), n = 87					
2-Methyl-3-(3,4-methylenedioxyphenyl)-propanal	1205-17-0	16.4	15.2	3.0	Pos.: 0	4.5 Pos.: 2	6.8 Pos.: 1	10.1 Pos.: 0	15.2 Pos.: 1				
					?+: 0	?+: 0	?+: 0	?+: 0	?+: 1				
					IR: 0	IR: 0	IR: 0	IR: 1	IR: 0				
Cyclamen aldehyde	103-95-7	22	5.1	1.0	Pos.: 0	1.5 Pos.: 1	2.3 Pos.: 0	3.4 Pos.: 0	5.1 Pos.: 0				
					?+: 1	?+: 0	?+: 0	?+: 0	?+: 1				
					IR: 0	IR: 0	IR: 0	IR: 1	IR: 0				
Hexyl salicylate	6259-76-3	0.18	30	5.0	Pos.: 0	7.5 Pos.: 0	11.3 Pos.: 0	16.9 Pos.: 0	25.3 Pos.: 0				
					?+: 2	?+: 0	?+: 0	?+: 1	?+: 1				
					IR: 0	IR: 0	IR: 0	IR: 0	IR: 0				

Abbreviations: IR, irritant reaction; Pos, positive reaction (+/++/+++); ?+, doubtful reaction.

^a EC3: the estimated concentration of a chemical necessary to give a 3-fold increase in local lymph node proliferative activity as compared with vehicle-treated controls.

According to the ESCD protocol, the starting patch test concentration for a novel fragrance substance is the lowest concentration that might induce patch test skin irritation. Maximum patch test concentrations are based on a concentration up to five times higher than used in scented leave-on cosmetic products, as long as this does not exceed the human no-observed-effect level (NOEL) from human repeated insult patch test (HRIPT) studies.⁵ For this study, the Research Institute for Fragrance Materials (RIFM) provided summarized irritancy data from HRIPT studies for the three fragrance substances (personal communication with the RIFM, 2016). For hexyl salicylate, the starting concentration was based on a previous clinical report on 218 fragrance-sensitized volunteers patch tested at a 5% concentration without any positive reactions.⁶ The NOEL concentrations and maximum use concentrations for the three fragrance substances were available from the IFRA standards.²⁻⁴

After informed consent had been obtained, we included consecutive dermatitis patients, aged ≥ 18 years, referred to our department for patch testing. Exclusion criteria were the same as those normally causing postponement of patch testing.⁷ Approximately 100 consecutive patients were tested with the predetermined starting concentrations. Interim evaluations of the patch test results for these determined whether the individual concentrations were increased (by 50%) or decreased (by 33%) in the next series of ~ 100 patients. The concentration was decreased if active sensitization was expected or more than a few irritant reactions were registered. This procedure was repeated up to a maximum of four times. For the patch test procedure, 20 mg of test material suspended in pet. was applied to the upper back in Finn Chambers (8 mm; SmartPractice, Phoenix, Arizona), with an occlusion time of 2 days. Patch test readings were performed on day (D) 2, D3 and D4, and D5 and D7, and scored according to guidelines.⁷ To detect any possible cases of active sensitization, patients were told to contact the clinic if any patch test reactions developed after their final visit.

RESULTS

The patch test concentrations applied throughout the study and the observed reactions are shown in Table 1. No cases of active sensitization were suspected, and the patch test concentration was increased for each subsequent round for all three fragrances, with only 87 patients being patch tested in the final round, owing to practical issues. Among all 494 consecutive patients, four (0.8%, 95% confidence interval [CI]: 0.3-2.1%) had positive patch test reactions to MMDHCA, and one (0.2%, 95%CI: 0.04-1.1%) had a positive patch test to cyclamen aldehyde; no positive reactions to hexyl salicylate were seen. One patient already had ++ reactions to both cyclamen aldehyde 1.5% pet. and MMDHCA 4.5% pet. in the second round of patch testing (Figure 2). This patient also had a + reaction to *Myroxylon pereirae* (balsam of Peru) 25% pet., and a + reaction to fragrance mix II (FM II) 14% pet. The remaining three patients with contact allergy to MMDHCA (all ++ reactions) also had ++ or +++ patch test reactions to both fragrance mix I 8% pet. and FM II 14% pet.

DISCUSSION

Overall, it was feasible to apply the ESCD protocol⁵ for establishing optimal patch test concentrations for novel sensitizing fragrance substances in a clinical setting. We observed clearly allergic positive patch test reactions to the two fragrance substances MMDHCA and cyclamen aldehyde, which are both categorized as moderate sensitizers on the basis of animal data.¹ MMDHCA is a synthetic fragrance chemical with the molecular formula $C_{11}H_{12}O_3$, and is used in concentrations of up to 5.3% in leave-on cosmetic products.⁴ Its scent is described as "green, floral (cyclamen) with top notes of ozone and new mown hay."⁸ Cyclamen aldehyde is also a synthetic fragrance chemical, with the molecular formula $C_{13}H_{18}O$, and is used in concentrations up to 2.7% in leave-on cosmetics.² It has a floral scent of cyclamen or lily-of-the-valley.⁹ In the



FIGURE 2 Strong positive (++) patch test reactions to both cyclamen aldehyde 1.5% pet. (marked "1") and 2-methyl-3-(3,4-methylenedioxyphenyl)-propanal 4.5% pet. (marked "3") on reading day 3 (D3). Both reactions had been scored as doubtful (?+) on day 2

current study, we saw no positive patch test reactions to hexyl salicylate patch tested up to a concentration of 25%. On the basis of animal data, hexyl salicylate with an EC3 value of 0.18% is categorized as an extreme sensitizer.¹⁰ As indicated in the IFRA standard,³ hexyl salicylate is widely used in cosmetics such as perfumes and aftershaves up to a concentration of 16% in the finished product. Although we cannot exclude the possibility that contact allergy to hexyl salicylate can occur, it seems unlikely that the fragrance chemical is an extreme sensitizer in humans.

This investigation provides further evidence that labelling of all fragrance substances with sensitizing potential should be mandatory for cosmetics and household detergents, in terms of both improving the diagnosis of fragrance contact allergy, and enabling avoidance of future exposure in those sensitized. As the ESCD protocol⁵ recommends that half of the maximum tolerated concentration investigated be used for screening purposes, we recommend patch testing MMDHCA at 7.5% pet. (3.0 mg/cm²), cyclamen aldehyde at 2.5% pet. (1.0 mg/cm²), and hexyl salicylate at 12.5% pet. (5.0 mg/cm²).

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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