Fragrance allergy: assessing the safety of washed fabrics

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Background: Previously, a quantitative risk assessment suggested there was no risk of induction of fragrance allergy from minor residues of fragrance chemicals on washed fabrics.

Objective: To investigate whether there was any risk of the elicitation of contact allergy from fragrance chemical residues on fabric in individuals who were already sensitized.

Methods: Thirty-six subjects with a positive patch test to isoeugenol (n = 19) or hydroxyisohexyl 3-cyclohexene carboxaldehyde (n = 17) were recruited. Dose–response and fabric patch tests were performed, respectively, with filter paper and a cotton sample loaded with fragrance in ethanol–diethylphthalate (DEP) and applied in a Finn Chamber® or a Hill Top Chamber®.

Results: Only two subjects reacted to an isoeugenol patch test concentration of 0.01% (>20× the estimated likely skin exposure level), none reacted to lower concentrations. Of 36 subjects, 18 reacted to the fabric patch treated with ethanol–DEP vehicle alone and 20 to the fragrance-chemical-treated fabric patch. These were only minor non-specific skin reactions. They were also quite evenly distributed between the two fragrance chemical allergic groups.

Conclusions: On the basis of the examples studied, fragrance chemical residues present on fabric do not appear to present a risk of the elicitation of immediate or delayed allergic skin reactions on individuals already sensitized.

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Allergic skin reactions to fragrance chemicals have been reported as an increasing consumer problem in recent years (1–4). One potential source of exposure is presented by fabric washing products and by fragrance residues on washed fabrics, such that it has been conjectured this might present a risk to consumers (5). In a previous publication, an assessment of the likelihood that fragrances in washed and
conditioned fabrics might induce fragrance allergy was undertaken and it was concluded that it was improbable; a general absence of evidence of diagnosed fragrance allergy being associated with a clinical picture of clothing dermatitis was consistent with this conclusion (6). However, it was noted that the risk assessment conclusion and the absence of evidence left open the possibility that an individual who was already fragrance allergic from other sources of exposure might be sufficiently sensitive to experience an elicitation of their allergy by contact with fragrance residues on their clothing. Consequently, in the present work, the objective was to investigate whether there was any risk of the elicitation of allergy from fragrance chemical residues on fabric in individuals who were already sensitized. The allergens were selected on the basis that they represent two of the most common fragrance allergens, are widely used in laundry products and are among the more substantive fragrance chemicals.

**Materials and Methods**

**Panellist recruitment**

Individuals aged from 22 to 75 years and in generally good health but with an allergic sensitivity to either isoeugenol \((n = 19)\) or hydroxyisohexyl 3-cyclohexene carboxaldehyde \((n = 17)\) diagnosed via a positive patch test history \((4 \text{ at } 3+, \ 31 \text{ at } 2+ \text{ and } 1 \text{ at } 1+)\) were recruited in eight French and Belgian clinical centres. Eighty-one per cent of the panel of 36 was female. Each panellist gave fully informed written consent for their participation in the study. They were compensated for their necessary expenses associated with study participation. The study protocol was submitted to the Ile de France III Comité Consultatifs de Protection des Personnes se prêtant à des Recherches Biomédicales (CCPPRB) independent ethics committee at Tarnier-Cochin Hospital and was approved on 17 October 2006. The study was performed in compliance with good clinical practice and under the auspices of France’s Huriet-Sérusclat Act (relating to the protection of persons participating in biomedical research). An appropriate insurance policy was taken out.

**Study materials**

Isoeugenol and hydroxyisohexyl 3-cyclohexene carboxaldehyde were obtained from Firmenich®, Geneva, Switzerland. These materials were fresh samples of the quality normally used in fragrances in consumer products. To eliminate the need for analytical dose confirmation, all dilutions of these allergens were prepared under Good Laboratory Practice (GLP) conditions by a specialised company (CRID Pharma, Saint Gély du Fesc, France) immediately before dispatch and were transported and stored at approximately 4°C. For each test centre, this preparation was undertaken immediately before the start of the testing phase (itself driven by panellist availability) such that the materials were used within a week. The fragrance chemicals were diluted in ethanol/diethylphthalate (ethanol/DEP), 3:1, v/v. Twelve-millimetre Finn Chambers® with filter paper discs (supplied by Epitest Oy, Tuusula, Finland) were applied with Fixomull® tape (supplied by BSN Medical, Le Mans, France) for dose–response and control patch tests. Eleven-millimetre Hill Top Chambers® with cotton Webril® pad (Hill Top Companies, Cincinnati, OH, USA) were affixed to skin using Fixomull® tape for fabric and control patch tests. Cotton was selected because of the high deposition on this fabric type and its frequent, widespread use in clothing.

**Rationale for fabric dose selection**

The dose for isoeugenol was chosen on the following basis: the fabric washing and conditioning products giving the highest deposition of fragrance on to fabric were measured, delivering 0.1 and 0.13 μg/g of cotton, respectively (Unilever, unpublished data). This combined dose of 0.23 μg/g cotton represents a surface area dose of 0.0115 μg/cm² (because the specific surface area of cotton is 20 cm²/g). Although similar data were not available for hydroxyisohexyl 3-cyclohexene carboxaldehyde, worst case estimation showed that the fabric dose from combined product use would not exceed 0.16 μg/cm² before rinsing and drying, during which it is estimated that at least 90% of the dose would be lost (7). From these considerations, it was determined that a fabric dose of 0.1 μg/cm² (0.63 μg/ml) would provide a suitable exaggeration of exposure, particularly as application to skin would be under full occlusion for 2 days.

For the dose–response patch tests, the concentration of allergens used was between 0.00001 and 0.01% (v/v) equivalent to 0.000045–0.00049 to 0.45–0.49 μg/cm² which is for the latter more than 20-fold higher than the likely skin exposure levels associated with a fabric washed with washing powder and a fabric softener. The likely exposure levels for isoeugenol and hydroxyisohexyl 3-cyclohexene carboxaldehyde are, respectively, estimated to 0.022 and 0.016 μg/cm² (6).

**Study protocol**

Figure 1 provides a pictorial overview of the protocol. Each panellist received four dilutions of their allergen \((0.00001\%, 0.0001\%, 0.001\% \text{ and } 0.01\%; 20 \mu l)\) and an ethanol/DEP vehicle control in filter paper 12-mm Finn Chambers® on the left-hand
Results

From their previous diagnostic history, 31 of 36 individuals were known to be moderately positive and 4 of 36 individuals were strongly positive to their patch tests, indicating that the group represents a cohort with a significant degree of allergic reactivity.

In none of the panellists on any of the eight patch test sites was there any evidence of the erythema, oedema or sensory effects that would be typically associated with contact urticaria at the 1-hr time point (data not shown). There were no reports of respiratory effects associated with exposure to either of the fragrance allergens nor was there any alteration in their skin condition on non-treatment sites.

Tables 1 and 2 contain an overview of the delayed hypersensitivity results obtained in this study and report the total number of individuals who showed any type of skin response. Of the 19 panellists, two showed a weak delayed allergic reaction to an isoeugenol patch test concentration of 0.01% in ethanol/DEP, comprising one with a positive reaction at both D2 and D4 time points and a second panellist who showed a doubtful reaction only at the D4 time point (Fig. 2). Interestingly, neither of these were among the group of four panellists with a 3+ diagnostic patch test history. None of the panellists reacted to lower concentrations of either the fragrance allergens or to the vehicle control applied in Finn Chambers®.

Concerning the responses to the Hill Top Chambers® containing a cotton pad, 18 of the 36 panellists reacted to the fabric patch treated

<table>
<thead>
<tr>
<th>Panellist</th>
<th>Diagnostic patch test (1%) grade*</th>
<th>Patch test threshold (%)</th>
<th>Fabric patch control results†</th>
<th>Fabric patch with isoeugenol‡</th>
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<tbody>
<tr>
<td>1</td>
<td>+++</td>
<td>&gt;0.01</td>
<td>?/N</td>
<td>?/N</td>
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<tr>
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<td>?/Soap effect N/N</td>
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<tr>
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<td>&gt;0.01</td>
<td>+/Soap effect N/N</td>
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<tr>
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<tr>
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<td>++</td>
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<td>Total reactors</td>
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<td>9/19</td>
<td>10/19</td>
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</table>

*, a doubtful reaction; IR, irritant reaction; N, negative.

*Response recorded to a diagnostic patch test to 1% isoeugenol conducted before this study.
†Concentration at which the subject was positive in the 12-mm Finn Chamber® dose response.
‡Results presented as a D2/D4 reading.
with ethanol/DEP vehicle alone, typically with only minor skin reactions (nothing greater than +); 20 of 36 volunteers reacted to the fragrance-treated fabric patch, again with minor responses and at the same frequency and intensity as those reacting to the untreated fabric. A typical panellist showing these minor reactions is presented in Fig. 3, which shows clear responses in both test and fabric control patches at D2 which have essentially disappeared by D4. Statistical analysis (McNemar’s test) confirmed that there was no significant difference in the response of the two groups ($P = 0.75$). The responses to the allergen-treated fabric patches were evenly distributed between the isoeugenol and hydroxyisohexyl 3-cyclohexene carboxaldehyde allergic groups. Thirteen of those panellists, who showed any skin reaction, responded to both the fragrance-chemical-treated and the vehicle-treated fabric patch. Many of the responses were classed as doubtful or irritant; only in two cases (volunteer numbers 20 and 35 who were from different clinic locations) were + grade reactions recorded at both scoring time points and both of these occurred in response to the fabric patch vehicle control and so were deemed not to be relevant in terms of fragrance chemical allergy. In 12 of 13 panellists who reacted to both fragrance-chemical-treated and vehicle-treated patches, the type and levels of reaction were identical. The exception (volunteer
31) gave a slightly higher reaction to the vehicle control patch compared with the fragrance allergen test chamber. The Hill Top Chamber® control that contained only an untreated cotton pad produced a very few scattered skin responses at the D2 time point (data not shown).

**Discussion**

Fragrance allergens in laundry products have been suspected to be a significant cause of skin sensitisation/allergic contact dermatitis in the consumer (5, 9). Quoting recently from the New Zealand Dermatology Society website giving advice to those with fragrance allergy: ‘Note that clothes washed in scented laundry detergent can be a problem with prolonged skin contact of the garment in the presence of moisture and heat. It would be best to use fragrance-free laundry detergent’ (9). Hence, such a perspective persists, despite the absence of clinical evidence that exposure to washed fabric can either induce fragrance allergy or elicit reactions in those already sensitized, an outcome which is consistent with risk assessments for fragrance allergens in this type of product (6, 10). However, the authors are not aware of published data that provide evidence of absence of an effect of fragrance on fabric in individuals with an existing fragrance allergy. Search on websites such as PubMed using the terms ‘fragrance’, ‘allergy’, ‘dermatitis’, ‘detergent’, ‘laundry’, ‘clothing’, ‘fabric’, ‘deposition’ and ‘residues’ in various combinations produces no hits of any significance.

As discussed in previous publications, quantitative risk assessment leads to the conclusion that the level of fragrance chemical residues on laundered fabric is not sufficient to induce allergic sensitivity (generally with a very substantial margin of safety) (6, 10). However, although such calculations show that the level of exposure is low, they do not address directly the question of safety for those already sensitised. Consequently, we elected to assess whether the risk of elicitation of skin allergy in fragrance allergic individuals represented a potential consumer problem. Taking a total of 36 subjects with a diagnosed history of allergy to isoeugenol or to hydroxyisohexyl 3-cyclohexene carboxaldehyde, we assessed the possibility of elicitation of their allergy by application of a cotton pad dosed with approximately 10× the maximum level of their allergen that was probably to remain as a residue on fabric after combined washing and conditioning processes. The outcome of the study was clear. Apart from minor non-specific reactions associated with the 2-day occluded application of a large chamber to the skin, the responses recorded with the fragrance allergen test patch were essentially identical in scale and scope compared with the vehicle-treated control patch.

The negative outcome of this study in terms of allergic response to fragrance is consistent with the theoretical considerations presented in a short review article (10) and in a generic industry risk assessment calculation for isoeugenol (11). This offers some reassurance, as does the fact that the occlusive fabric patches themselves were clearly borderline irritant, thus potentially enhancing any allergic reactivity. However, it is perhaps more reassuring to consider what is known about the thresholds for the elicitation of isoeugenol and hydroxyisohexyl-3-cyclohexene carboxaldehyde allergies from past clinical studies in sensitised individuals. For both of these allergens, data suggest that in a standard repeated open application test, the elicitation threshold would be approximately 0.2 μg/cm² whereas the single patch test threshold is somewhat lower (2, 12). Thus, it is reasonable to expect that half that dose level of 0.1 μg/cm² on a single occluded patch would not elicit any reaction. Furthermore, it should be remembered that the dose applied here to cotton fabric was many times higher than what would occur in reality and that in practice it has been estimated that perhaps as little as 1% of the applied dose actually is transferred to the skin (13). All of these factors combine to give confidence that fabric with fragrance residues will be safe, even for the sensitized consumer. Of course, this could be confirmed further by an extended study, involving more sensitized subjects, different fabric types and repeated exposure protocols.

The present study in our view provides reassurance that there will not be a problem of elicitation of allergy to a specific fragrance chemical residue on washed fabric but leaves open the question of whether allergens in combination or in the presence of irritants conspire to produce a problem which is greater than the sum of the individual parts. Research in this area is limited and suggests in reality that the effect is, at most, modest in scale (14–17). Consequently, it seems fair to conclude that the margins of safety are such that this potential issue is, in reality, of little or no importance, a view strengthened by the already mentioned absence of a history of clothing pattern dermatitis in association with fragrance allergy (6). Furthermore, where skin responses to detergents and/or their residues have been thoroughly investigated, there has been no evidence of a bias towards reactivity in those suffering from atopic dermatitis (18). Again, this is also consistent with the fact that atopic individuals are not generally more probably to be fragrance chemical allergic than non-atopics (19, 20).
Given the above considerations, it is concluded that the fragrance chemical residues deposited on laundered fabrics do not represent a significant risk of the elicitation of immediate or delayed allergic skin reactions on individuals already sensitized to fragrances.

Acknowledgements
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