

Fragrance allergy: assessing the safety of washed fabrics

David A Basketter¹, Annick Pons-Guiraud², Arian van Asten³, Catherine Laverdet⁴, Jean-Paul Marty^{†,5}, Ludovic Martin⁶, Daniel Berthod⁷, Sylvie Siest⁷, Françoise Giordano-Labadie⁸, Dominique Tennstedt⁹, Marie Baeck⁹, Martine Vigan¹⁰, Gérard Lainé¹¹, Christophe J Le Coz¹², Marie-Claude Jacobs¹³, Olivier Bayrou¹⁴, Marie-Anne Germaux¹⁵

¹DABMEB Consultancy Ltd, Sharnbrook, UK

²10 boulevard Malesherbes, 75008 Paris, France

³Unilever R&D Vlaardingen, Olivier van Noortlaan 120, 3130 AC, Vlaardingen, The Netherlands

⁴36 rue Bassano, 75008 Paris, France

⁵Faculté de Pharmacie, 92160 Châtenay-Malabry, France

⁶Service de dermatologie CHU Angers, 49933 Angers, France

⁷Unilever R&D, 92842 Rueil-Malmaison, France

⁸Service de dermatologie, Hôpital Purpan, TSA 40031, 31059 Toulouse cedex 9, France

⁹Cliniques Universitaires Saint-Luc Service de dermatologie, 1200 Bruxelles, Belgium

¹⁰CHU Saint Jacques Dpt de Dermatologie, 25030 Besançon cedex, France

¹¹41 bis rue Lacépède, 75005 Paris, France

¹²19 rue de l'Observatoire, 67000 Strasbourg, France

¹³279 rue François Gay, 1150 Woluwe-Saint-Pierre, Bruxelles, Belgium

¹⁴7 rue de l'Université, 75007 Paris, France

¹⁵7 Neerleest 24, 1020 Bruxelles, Belgium

Corresponding author: Dr David Basketter, 2 Normans Road, Sharnbrook, Bedfordshire MK44 1PR, UK

Telephone: 01234782944; **email:** david.basketter@ukonline.co.uk

Keywords: fabric washing; fragrance allergy; elicitation thresholds; risk assessment

Abstract

Background: Previously we undertook a quantitative risk assessment which demonstrated there was no risk of induction of fragrance allergy from minor residues on washed fabrics.

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

Patients/methods: 36 volunteers with a positive patch test to isoeugenol (n=19) or hydroxyisohexyl-3-cyclohexene carboxaldehyde (Lyrall[®]) (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 HillTop chamber.

Results: Only 2 volunteers reacted to an isoeugenol patch test concentration of 0.01 % (> 20 x the estimated likely skin exposure level), none reacted to lower concentrations. Respectively 18 and 20 of volunteers reacted to the fabric patch treated with ethanol-DEP vehicle alone and to the fragrance treated fabric patch with only minor non-specific skin reactions. These responses were fairly evenly distributed between the two fragrance allergic groups.

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

Introduction

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, we undertook an assessment of the likelihood that fragrances in washed and conditioned fabrics might induce fragrance allergy and concluded that it was highly improbable and that 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 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

Patient volunteer recruitment

Individuals aged from 22-75 years and in generally good health but with an allergic sensitivity to either isoeugenol (n=19) or hydroxyisohexyl-3-cyclohexene carboxaldehyde (Lyrall[®]) (n=17) diagnosed *via* a positive patch test history (4 at 3+, 31 at 2+ and 1 at 1+) were recruited in 8 French and Belgian clinical centres. There was no significant difference between the two groups in terms of the demographic characteristics for the ITT population. 81% of the panel of 36 was female. Each volunteer gave fully informed written consent for their participation in the study. The study protocol was submitted to the Ile de France III CCPPRB independent ethics committee at Tarnier-Cochin Hospital and was approved on October 17th 2006. The study was performed in compliance with Good Clinical Practice and under the auspices of France's Huriot-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 the perfumer 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 GLP conditions by a specialised company (Cridpharma, France) immediately before dispatch and were transported and stored at approximately 4°C. For each test centre, this preparation was undertaken immediately prior to the start of the testing phase (itself driven by panellist availability) such that the materials were used within a week. The fragrances were applied in ethanol/diethylphthalate, 3:1, v/v (ethanol/DEP). 8mm Finn chambers with filter paper

(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. 11mm HillTop chambers with cotton Webril pad (Hill Top Companies, Cincinnati, Ohio, USA) were affixed to skin using Fixomull tape for fabric and control patch tests. Cotton was selected due to the relatively 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 µg/g 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 (since the specific surface area of cotton is 20 cm²/g). Although similar data was 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 prior to 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 since application to skin would be under full occlusion for 48h.

For the dose-response patch tests, the concentration of allergens used was between 0.00001 and 0.01% (v/v) equivalent to 0.00045-0.00049 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 $\mu\text{g}/\text{cm}^2$ (6).

Study protocol

Figure 1 provides a pictorial overview of the protocol. Each volunteer received four dilutions of their allergen (0.00001%, 0.0001%, 0.001% and 0.01%; 20 μl) and an ethanol/DEP vehicle control in filter paper 8mm Finn chambers on the left hand side of their back as well as three 11mm HillTop chambers containing a cotton pad treated either with their allergen in ethanol/DEP vehicle (yielding 0.1 $\mu\text{g}/\text{cm}^2$ of allergen on the cotton; 150 μl), vehicle alone or a chamber simply containing the untreated cotton pad on the right hand side of their back. These patches (8 in all) were assessed at 1h for the presence of urticaria (without completely removing the patch), and then the patch fully reapplied. After 48h, the patches were removed and the skin sites assessed approximately 30 minutes later. The skin sites were then given a further assessment after 96h (or 72h if the volunteer could not attend on the last day).

Subjects were asked not to take a bath or shower during the 4 day study period.

The primary evaluation criterion was the occurrence of a contact dermatitis-type reaction which was scored according to the ICDRG guidelines (8). Digital photographs of local reactions in order to document better individual cases were taken for each reading. The study's secondary objective was the description of immediate or delayed local allergic phenomena occurring during or after the study (atopic eczema, dry skin, erythema, itching, vesicles, pigmentation, asthma, allergic rhinitis, episodes of wheezing). Statistical analysis of skin reactions used McNemar's test.

Results

Diagnostically, 31/36 individuals were moderately positive and 4/36 were strongly positive to their patch tests, indicating that the group represent a cohort with a significant degree of allergic reactivity.

In none of the volunteers 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 hour 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 expressed any type of skin response. 2/36 volunteers expressed 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 48 and 96 hour time points and a second volunteer who expressed a doubtful reaction only at the 96 hour time point (figure 2). Interestingly, neither of these were amongst the group of 4 volunteers with a 3+ diagnostic patch test history. None of the volunteers reacted to lower concentrations of either of the fragrance allergens or to the vehicle control applied in Finn chambers.

Concerning the responses to the HillTop chambers containing a cotton pad, 18/36 of the volunteers reacted to the fabric patch treated with ethanol/DEP vehicle alone, typically with

only minor skin reactions (nothing greater than +); 20/36 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 Figure 3, which shows clear responses in both test and fabric control patches at 48h which have essentially disappeared by 96h. 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. 13 of those volunteers, who showed any skin reaction, responded to both the fragrance 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 allergy. In 12/13 volunteers who reacted to both fragrance 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 to the fragrance allergen test chamber. The Hilltop chamber control which contained only an untreated cotton pad produced a very few scattered skin responses at the 48h time point (data not shown).

Discussion

Fragrance allergens in laundry products have been suspected to be a significant cause of skin sensitisation/ACD in the consumer (e.g. 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). So, 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 which provides 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 fully in previous publications, quantitative risk assessment leads to the conclusion that the level of fragrance 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 volunteers 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 10x the maximum level of their allergen that was likely 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 48h 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 to 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 to examine what is known about the thresholds for the elicitation of isoeugenol and hydroxyisohexyl-3-cyclohexene carboxaldehyde allergies from past clinical studies in sensitised volunteers. For both of these allergens, data suggest that in a standard repeated open application test, the elicitation threshold would be approximately 0.2 $\mu\text{g}/\text{cm}^2$ 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 $\mu\text{g}/\text{cm}^2$ 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 clinical study in our view provides reassurance that there will not be a problem of elicitation of allergy to a specific fragrance residue on washed fabric, but leaves open, apparently, 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 relatively 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 clinically, there has been no evidence of a bias toward reactivity in those suffering from atopic dermatitis (18). Again, this is also consistent with the fact that atopics are not generally more likely to be fragrance allergic than non-atopics (19, 20).

Given the above considerations, it is concluded that the fragrance 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

This study was fully funded by Unilever.

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Table 1. Results overview – 1. Isoeugenol

Volunteer	Diagnostic patch test grade	Diagnostic patch test threshold	Fabric patch control results*	Fabric patch with isoeugenol
1	+++	>0.01%	?/0	?/0
2	+++	>0.01%	?/Soap effect	0/0
3	+++	>0.01%	+/Soap effect	0/0
4	+++	>0.01%	0/0	?/0
5	++	>0.01%	0/0	0/0
6	++	>0.01%	0/0	0/0
7	++	>0.01%	0/0	0/0
8	++	>0.01%	0/0	0/0
9	++	>0.01%	0/0	0/0
10	++	>0.01%	0/0	?/?
11	++	>0.01%	?/0	0/0
12	++	>0.01%	0/0	0/0
13	++	>0.01%	0/IR	0/IR
14	++	>0.01%	0/0	+/IR
15	++	>0.01%	0/IR	0/IR
16	++	>0.01%	?/IR	?/IR
17	++	>0.01%	0/0	0/IR
18	++	0.01%	0/+	0/+
19	++	0.01%	+/0	+/0
Total reactors			9/19	10/19

*Results presented as a 48h/96h score; IR = irritant reaction; ? = a doubtful reaction

Table 2. Results overview – 2. Hydroxyisohexyl-3-cyclohexene carboxaldehyde

Volunteer	Diagnostic patch test grade	Diagnostic patch test threshold	Fabric patch control results*	Fabric patch with Lyral [®]
20	++	>0.01%	+/+	0/0
21	++	>0.01%	0/0	0/0
22	++	>0.01%	0/0	0/0
23	++	>0.01%	+/IR	0/0
24	++	>0.01%	0/0	0/IR
25	++	>0.01%	0/IR	0/IR
26	++	>0.01%	0/0	?/IR
27	++	>0.01%	0/0	0/0
29	++	>0.01%	0/IR	0/IR
29	++	>0.01%	0/IR	0/IR
30	++	>0.01%	0/?	0/?
31	++	>0.01%	?/?	?/0
32	++	>0.01%	0/0	0/0
33	++	>0.01%	0/IR	0/IR
34	++	>0.01%	0/0	0/0
35	++	>0.01%	+/+	0/+
36	+	>0.01%	0/0	0/?
Total reactors			9/16	10/16

*Results presented as a 48h/96h score; IR = irritant reaction; ? = a doubtful reaction

Figure 1

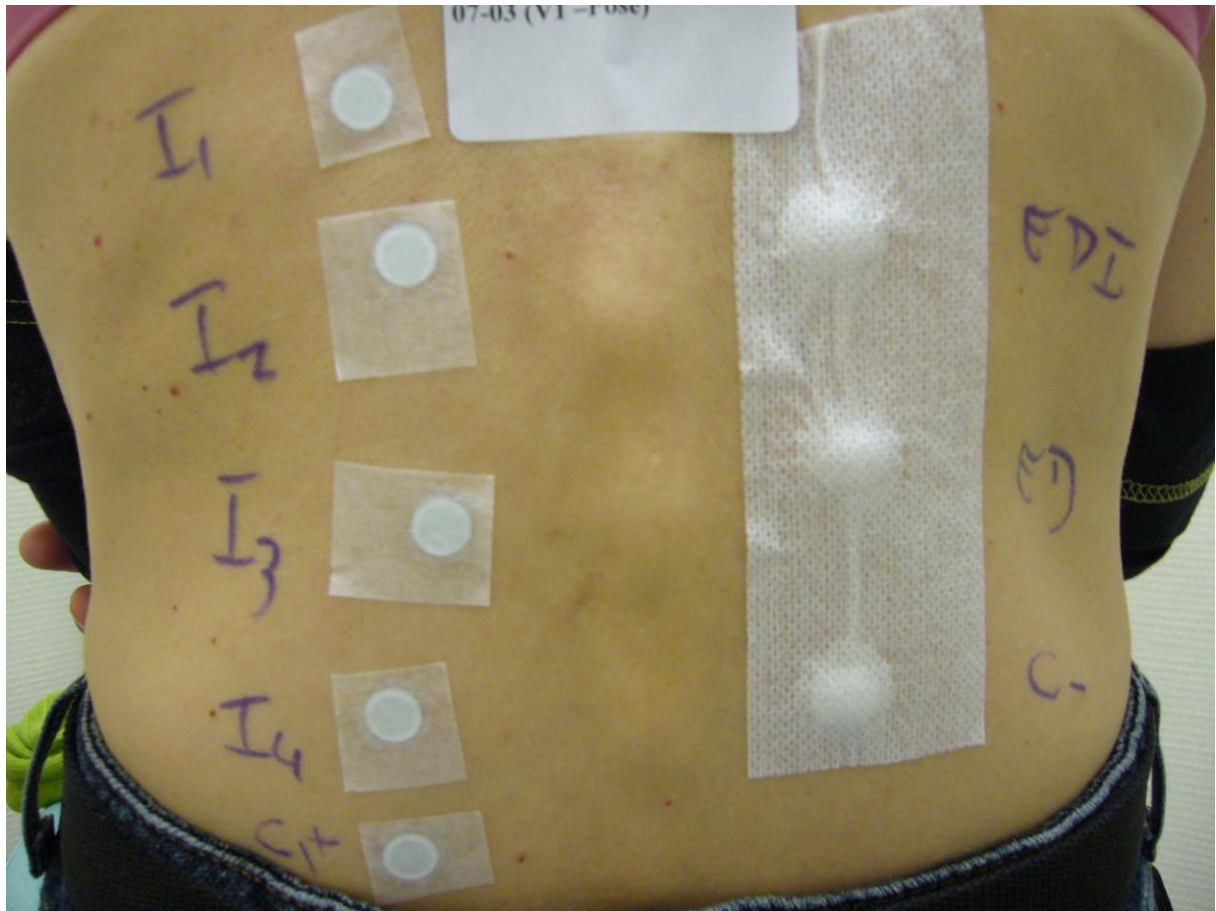


Figure 1 shows the placement of the eight test sites. On the left hand side are I₁-I₄ which are the four dilutions of isoeugenol (or Hydroxyisohexyl-3-cyclohexene carboxaldehyde) (Filter paper patch tests); below them is the filter paper Finn chamber vehicle control. On the right hand side are the 3 HillTop chambers, with the fragrance treated cotton pad at the top, the vehicle control in the middle and the blank chamber at the bottom.

Figure 2



Figure 2 shows a 2+ reaction to 0.01% isoeugenol at 96h. No reaction was recorded at any of the other test sites.

Figure 3



Figure 3a shows a subject with a selection of responses at 48h at the test sites (right hand side), with a similar level of reaction at the fabric patch control (C2+) and fabric patch with isoeugenol (EDI). Figure 3b shows the same panellist at 96h, where the level of response is very much less, suggesting the reactions are irritant in nature.