

A review of 241 subjects who were patch tested twice: could fragrance mix I cause active sensitization?

J.M.L. White, J.P. McFadden and I.R. White

Department of Cutaneous Allergy, St John's Institute of Dermatology, St Thomas' Hospital, London SE1 7EH, U.K.

Summary

Correspondence

Jonathan White.

E-mail: jonathanmlwhite@hotmail.com;

jonathan.white@gstt.nhs.uk

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Key words

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Conflicts of interest

None declared.

Background Active patch test sensitization is an uncommon phenomenon which may have undesirable consequences for those undergoing this gold-standard investigation for contact allergy.

Objectives To perform a retrospective analysis of the results of 241 subjects who were patch tested twice in a monocentre evaluating approximately 1500 subjects per year.

Methods Positivity to 11 common allergens in the recommended Baseline Series of contact allergens (European) was analysed: nickel sulphate; Myroxylon pereirae; fragrance mix I; para-phenylenediamine; colophonium; epoxy resin; neomycin; quaternium-15; thiuram mix; sesquiterpene lactone mix; and para-tert-butylphenol resin.

Results Only fragrance mix I gave a statistically significant, increased rate of positivity on the second reading compared with the first ($P = 0.011$). This trend was maintained when separately analysing a subgroup of 42 subjects who had been repeat patch tested within 1 year; this analysis was done to minimize the potential confounding factor of increased usage of fragrances with a wide interval between both tests. To reduce the confounding effect of age on our data, we calculated expected frequencies of positivity to fragrance mix I based on previously published data from our centre. This showed a marked excess of observed cases over predicted ones, particularly in women in the age range 40–60 years.

Conclusions We suspect that active sensitization to fragrance mix I may occur. Similar published analysis from another large group using standard methodology supports our data.

Patch testing is the gold-standard investigation for suspected contact allergy. Approximately 1500 patients are patch tested per annum in the Department of Cutaneous Allergy at St John's Institute. International guidelines exist to ensure that patch testing is broadly reproducible between centres, to minimize undesirable irritant reactions and to maximize sensitivity and specificity of the test.¹ When patch testing in patients with eczema confirms a contact allergy, eczema severity tends to improve and, consequently, quality of life as well.²

Despite the benefits of patch testing, various adverse events have been reported, including uncomfortable irritant reactions; flare of dermatitis; postinflammatory hyper- or hypopigmentation of the test site; scarring after severe reactions; granulomata (testing with beryllium or zirconium); anaphylactoid reactions (testing with antibiotics); and bacterial infections at the test site.

The most important adverse consequence of patch testing is patch test (active) sensitization, where subjects previously not

allergic become sensitized to one or more of the test chemicals. Active sensitization is characterized by a negative reaction at the conventional time of reading (days 2 and 4), followed by a late patch test reaction at days 10–20. Repeat patch testing³ then shows a conventional patch test reaction at days 2–4. Repeat patch testing is mandatory, as some allergens tested typically show late reactions in the absence of active sensitization (e.g. gold).

Active sensitization is thought to be uncommon (approximately 0.1% of all reactions in a large series⁴). Certain allergens may be more likely to cause active sensitization. Recent controversies over para-phenylenediamine have stimulated the publication of estimates for active sensitization as low as 0.3% (St John's)⁵ and as high as 1.5% (IVDK).⁶ Other allergens reported as causing active sensitization include *Primula* extracts (< 0.7%⁷ to 25%⁸), isothiazolinones (< 1% depending on the concentration used⁹) and acrylates (up to 4.5%¹⁰). Regrettably, methodologies in these studies have not been uniform.

Some patients were not retested, hence allowing the possibility of a straightforward late patch test reaction, and sometimes the researchers relied on a patient's history of a late reaction, without even re-examining the patient. These factors may, at the very least, reduce estimates of the true incidence of active sensitization. Fragrance ingredients have not been considered as significant active sensitizers.

Subjects who have been patch tested twice are an interesting group. They may provide information about the occurrence of active sensitization. However, contact allergies observed on first testing may also disappear. This may be due to a number of factors, either external (temperature/season of patch testing, site on the body of patch tests etc.) or internal (true immunological loss of allergy, analogous to loss of type I allergy over time, e.g. to penicillin).

We therefore undertook to identify all patients patch tested more than once at our unit. We did not include those who had been tested previously in other centres. This study has the advantage of minimizing interobserver differences; we have been the main clinicians over the whole study period and have rigorous internal audit of positive results (all positive reactions will be reviewed by at least two of the clinicians and the patch test grade agreed upon).

Materials and methods

All patch test data are stored electronically and list all patients patch tested from 1980 to the present day. The database was searched for those subjects who had been patch tested more than once. The clinical notes of all subjects identified were then reviewed manually. Any patient having been patch tested twice within 30 days was excluded from the study (as this scenario was often the result of technical difficulties with the first test, and hence may not be reliable). For a period of approximately 18 months during the test period (1984), fragrance mix I was used at 16% rather than 8%, and *para*-phenylenediamine hydrochloride was used instead of *para*-phenylenediamine free base. Again, for a brief period (until 1984), sorbitan sesquioleate was not included in fragrance mix I. Any subject who was tested on one occasion with either 16% fragrance mix, *para*-phenylenediamine hydrochloride or fragrance mix I without sorbitan sesquioleate was excluded from the analysis. Fragrance mix I 8% contains cinnamic alcohol, cinnamal, eugenol, α -amylcinnamal, hydroxycitronellal, geraniol, isoeugenol and *Evernia prunastri*, all at a concentration of 1%, with sorbitan sesquioleate at a concentration of 5%.

All subjects were tested using standard International Contact Dermatitis Research Group criteria,¹ FinnTM chambers (Epitest, Tuusula, Finland) and TrolabTM (Hermal, Reinbek, Germany) allergens, and readings were performed at days 2/3 and 4/5 by a dermatologist. There were no readings undertaken by the subjects themselves. The McNemar test for case-control studies was performed for each allergen using a web calculator (<http://www.graphpad.com>, last accessed on 19 September 2007). $P < 0.05$ was considered significant.

Table 1 Age at first patch test reading

Age range (years)	Total number in each age range at first patch test (n = 241), n (%)	Number in each age range of those repeat patch tested within a year (n = 42), n (%)
0–9	5 (2.1)	2 (4.8)
10–19	15 (6.2)	1 (2.4)
20–29	43 (17.8)	6 (14.3)
30–39	57 (23.7)	7 (16.7)
40–49	33 (13.7)	7 (16.7)
50–59	49 (20.3)	11 (26.2)
60–69	27 (11.2)	6 (14.3)
70–79	8 (3.3)	1 (2.4)
80–89	4 (1.7)	1 (2.4)
≥ 90	0 (0)	0 (0)

Results

We identified 241 individuals. Ages of these subjects ranged between 5 and 92 years, and showed a Gaussian distribution (Table 1). There were 81 males and 160 females in the study group (1 : 2), similar to the ratio seen in all those patch tested in our department (1 male : 1.5 females). Mean age difference between the two tests was 5.2 years (median 5).

Headline positivity (i.e. the total number of subjects having a positive patch test result for that allergen) was calculated on 11 common allergens (Table 2). Fragrance mix I showed the only statistically significant difference between the first and second readings, with a P-value calculated at 0.011 using the McNemar test. None of the other allergens showed a statistically significant difference between the first and second readings. As the second patch tests were necessarily after the first, it may have been that patients' use of fragrances (or other allergens) had changed in the time between the two tests. In

Table 2 Results of first/second test (n = 241)

	+/+	+/-	-/+	-/-	P-value
Nickel sulphate	32	11	4	194	0.1
Myroxylon pereirae	5	8	9	219	1.0
Fragrance mix I*	19	6	20	196	0.011
<i>para</i> -phenylenediamine	5	3	6	227	0.5
Colophonium	10	3	7	221	0.3
Epoxy resin	1	1	2	237	1.0
Neomycin	5	5	8	223	0.6
Quaternium-15	6	4	4	227	0.7
Thiuram mix	10	5	4	222	1.0
Sesquiterpene lactone	8	6	4	223	0.8
<i>para</i> -tert-butylphenol resin	1	2	3	235	1.0

*Statistically significant (nonsignificant results shown to one decimal place; significant result to three decimal places).

Table 3 Subjects patch tested twice within 1 year: results of first/second test (n = 42)

	+/+	+/-	-/+	-/-	P-value
Nickel sulphate	3	3	2	34	1.0
Myroxylon pereirae	1	3	2	36	1.0
Fragrance mix I	1	0	4	37	0.1
para-phenylenediamine	2	1	2	37	1.0
Colophonium	3	1	1	37	0.5
Epoxy resin	2	0	0	40	0.5
Neomycin	0	1	0	41	1.0
Quaternium-15	0	0	0	42	—
Thiuram mix	2	2	2	36	0.6
Sesquiterpene lactone	1	2	0	39	0.5
para-tert-butylphenol resin	1	0	1	40	1.0

None of the results is significant, although McNemar's test is only approximate and may not be reliable with the small numbers as described here.

this regard, subjects re-tested within 12 months are perhaps less likely to have changed their use of (exposure to) allergens than those patch tested decades apart. Forty-two subjects were, therefore, identified from this subgroup (Table 3) and, again, fragrance mix I showed a marked increase in headline positivity at the second reading compared with the first, although this did not reach statistical significance ($P = 0.1$). The other allergens had P-values ranging between 0.5 and 1.

Given that the frequency of fragrance allergy in patch-tested patients increases with their age,¹¹ we stratified the whole group according to age and gender and used the data of Buckley *et al.* (also generated from our unit) to calculate expected numbers of fragrance mix I positivity for any given age range (Table 4). Although the numbers are small and not suitable for statistical analysis, there is a higher number of positives at the second reading than we had expected from previous data. This is particularly marked in women aged 40–60 years.

Table 4 Actual fragrance mix positives according to age and sex (with predicted values from Buckley *et al.*¹¹ in brackets)

Age range (years)	Men, first reading (predicted in brackets)	Women, first reading (predicted in brackets)	Men, second reading (predicted in brackets)	Women, second reading (predicted in brackets)
0–9	0 (0)	0 (0)	0 (0)	0 (0)
10–19	0 (0)	1 (0)	0 (0)	1 (0)
20–29	2 (0) ^a	2 (2)	0 (0)	3 (1) ^a
30–39	3 (1) ^a	5 (3) ^a	1 (1)	3 (2)
40–49	0 (1)	2 (2)	4 (1) ^a	8 (4) ^a
50–59	2 (2)	2 (3)	2 (2)	7 (4) ^a
60–69	0 (1)	4 (2) ^a	1 (1)	5 (3) ^a
70–79	0 (1)	1 (0)	2 (2)	1 (1)
80–89	0 (0)	1 (0)	0 (0)	1 (0)
≥ 90	N/A	N/A	N/A	0 (0)

^aFigure higher than anticipated (but overall numbers are too small for statistical analysis). N/A, not applicable.

Discussion

Although numbers are small, the data are from a monocentre with tight audit/quality control, which minimizes interobserver variability. Our data show a statistically significant increase in headline positivity to fragrance mix I at the second patch test compared with the first, unlike 10 other common allergens. There was also an increase when a subgroup of subjects patch tested within 1 year (to reduce the impact of possible usage changes over a long time interval between tests) was subanalysed; however (due in part to low overall numbers), this did not reach statistical significance. Even controlling for the other important variable of age, the increased probability of a positive patch test to fragrance mix I at the second test is maintained, particularly for the age group 40–60 years in women. This may have arisen by chance, or alternatively there may be unknown susceptibility factors at play in this age group.

There are various explanations for these observations. The number of subjects is relatively small and the skewed distribution may have occurred by chance. We have not taken environmental factors into account; doing so would be difficult and any effect could never be adequately factored in to a retrospective study like ours. Personal usage of fragrances may have changed in the individuals, being a possible explanation of our results. Given the wide range of consumer products containing fragrances, retrospective estimation of fragrance use in individuals would only be very approximate and might be misleading. Finally, active sensitization to fragrance mix I could explain our data. This mix has not typically been thought to be a problem in terms of active sensitization; however, given that active sensitization is uncommon, and that few patients are patch tested twice, such data might not be apparent unless specifically sought.

Only occasional series looking at active sensitization have been described in the literature. Nine hundred and seventy-seven subjects were patch tested at least twice in a large series¹² reported by Carlsen *et al.* In this series, fragrance mix I and Myroxylon pereirae were the two most common allergens

tested to show a marked increase in net gain of positivity on the second test compared with the first. Another, smaller study,¹³ to the contrary, did not find any increase in fragrance positivity at the second test compared with the first. However, this study used the TRUE test™ system (Pharmacia, Hillerød, Denmark). Therefore, results may not be comparable with those of Carlsen *et al.*, nor our own. We hence hope that other groups will analyse their data to see if they support or reject our hypothesis that active sensitization to fragrance mix I may account for the discrepancy in positivity rates between patch tests, despite controlling for age and exposure. Significant active patch test sensitization may cause clinically relevant allergic contact dermatitis, which would contravene a guiding principle in medicine: 'Primum non nocere'.

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