

Original articles

Concentrations of the domestic house dust mite allergen *Der p* I after treatment with solidified benzyl benzoate (Acarosan) or liquid nitrogen

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Abstract

Background Various methods of killing the house dust mite to reduce exposure to allergen are being promoted even though complete data on their effects on allergen concentrations are not available. A study was designed to measure the concentrations of the main house dust mite allergen *Der p* I in homes treated with either solidified benzyl benzoate (Acarosan) or liquid nitrogen.

Methods *Der p* I concentrations were measured in dust collected from mattresses, bedroom carpets, and living room carpets in 10 houses treated with Acarosan and 10 houses treated with liquid nitrogen. Samples were collected before the treatment (in July 1990) and three and six months afterwards (October 1990 and January 1991). Forty untreated houses were concurrently sampled as controls.

Results *Der p* I concentrations were similar in the three groups at baseline. No significant fall was seen in either of the two treated groups three or six months after treatment. Concentrations in the control houses increased significantly—twofold to threefold in dust sampled from mattresses and bedroom carpets between baseline and October 1990. This increase was not seen in either of the treated groups of houses, but there was no significant difference in the *Der p* I concentrations in these houses and the control houses from any site at any time point.

Conclusions Neither Acarosan nor liquid nitrogen reduced the concentrations of *Der p* I for as long as six months after application. A small effect was probably present as the rise seen in control houses in the three month samples was not found in the treated houses. This effect, however, is likely to be of little clinical importance and also to be transient as the trend was lost by six months.

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The house dust mite, *Dermatophagoides pteronyssinus*, and its allergens are increasingly recognised as important factors in the pathogenesis of allergic asthma.¹ This recognition has prompted the use of various methods for reducing exposure to allergen. These have included intensive cleaning, barriers inter-

posed between patient and mattress, control of indoor humidity, and the use of various chemical acaricides.²⁻⁴ Acaricides kill the house dust mite but have no direct effect on *Der p* I, the main faecal glycoprotein allergen, and there are few data available on changes in allergen concentrations after the use of these agents.

Several, mainly uncontrolled, studies have examined the effects of various acaricides on asthma.⁵⁻⁷ A recent study failed to show a significant fall in *Der p* I concentrations after the use of a solidified benzyl benzoate containing acaricide⁸ but Dorward *et al* reported a reduction in symptoms and in inhalation of bronchodilator after the use of liquid nitrogen.⁵ Mite numbers decreased significantly but allergen concentrations were not measured. Concentrations of mites and of *Der p* I may, however, be dissociated because *Der p* I concentrations may remain raised for months after a fall in mite numbers.⁹ This persistence of allergen makes the clinical benefits of acaricides difficult to assess, especially as recolonisation with mites occurs rapidly.

We have measured *Der p* I concentrations over six months in two groups of houses: one was treated with solidified benzyl benzoate (Acarosan: Crawford Chemicals, Milton Keynes) and in the other mites were killed by freezing with liquid nitrogen.

Methods

We assayed *Der p* I concentrations in dust collected from the homes of 20 randomly selected patients with asthma in South Manchester. The selection was made from a random starting point in the skin test records for 1989, as described in an earlier paper.¹⁰ The houses were randomly assigned to be treated with either Acarosan or liquid nitrogen. In each house we treated the carpets and soft furnishings in the living room and the carpet and mattresses in the bedroom used by the patient.

As part of a parallel allergen surveillance study we studied 40 similar houses in the same area, which received no special treatment.¹⁰ These had dust samples collected at the same time as the 20 treated houses and they served as controls.

TREATMENT OF FURNISHINGS AND MATTRESSES

Application of Acarosan

Moist powder was used for carpets and foam for mattresses and soft furnishings. The application was performed in accordance with

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the manufacturer's instructions. Powder was liberally applied and worked into carpets with a stiff brush. It was allowed to remain undisturbed for six hours and then removed by vacuuming. Foam was rubbed into the mattress and upholstery fabric with a dampened applicator cloth and allowed to dry. One application was made at the start of the study in July 1990.

Application of liquid nitrogen

Liquid nitrogen (British Oxygen Company, England) was sprayed, with specially designed equipment, on to carpets, other furnishings, and mattresses in quantities sufficient to soak them and was then allowed to evaporate. The mattresses and carpets were vacuumed the next day to remove the killed mites. As with Acarosan, only one application was made, in July 1990.

All houses continued to be cleaned in their usual manner throughout the study period and no additional special cleaning methods were used in either group of treated houses, or in the control houses. No house was cleaned with a high efficiency filtration vacuum cleaner.

DUST COLLECTION

Dust was collected for *Der p 1* assay in July 1990 just before the houses were treated with Acarosan or liquid nitrogen and then three months and six months after the treatment, in October 1990 and January 1991. Dust in the 40 control houses was sampled concurrently. Each set of samples, from treated and control houses, was collected in the same month to eliminate the possibility of any variation due to seasonal effects on allergen concentrations.

The collection was made in a standardised manner by vacuuming for five minutes with a Medivac vacuum cleaner (Taylormaid, Macclesfield) on to preweighed filter paper (Whatman GFF, pore size 0.7 µm). Samples were taken from 2 m² of bedroom and living room carpet and from 1 m² of the upper surface at the head end of each patient's mattress; the same sites were sampled at each visit.

ASSAY OF *DER P 1*

Samples were coded and stored at 4°C until they were analysed. The dust was weighed after removing obvious "non-dust" matter. *Der p 1* was extracted (1:5 w/v) overnight at room temperature into phosphate buffered saline containing 0.05% Tween 20 and 0.2% bovine

serum albumin. Extracts were then vigorously homogenised and centrifuged at 3000 rev/min for 15 minutes and then stored at -20°C until they were analysed. *Der p 1* was assayed blind, in duplicate, by an enzyme linked immunosorbent assay technique (ALK Laboratories, Copenhagen) and results were expressed in ng per g of crude dust. Calibration curves were constructed on the basis of the manufacturer's reference samples and the spectrophotometer software was programmed to reject paired samples if they differed by more than 5%. This restricted intra-assay variation to this level. Batch to batch (interassay) variation, assessed by repeated assays on multiple house dust samples, was consistently 13-15%, less than the manufacturer's figure of 25% for crude dust *Der p 1*.

STATISTICAL METHODS

Data on *Der p 1* were normalised by log transformation before comparison. Changes within each group (Acarosan, liquid nitrogen, and control) during the six months were assessed with repeated measures of analysis of variance. Comparisons between groups and with time were made with two factor repeated measures of analysis of variance to assess both treatment and period effects. Significance was set at the 5% level.

Results

Ten houses were treated with Acarosan and 10 with liquid nitrogen. The treated and the control houses were similar in their location and their carpeting and in the proportions using central heating (nearly all in each group), and in these respects reflected the predominant pattern of current housing in Britain (personal communication, Manchester City Council).

Der p 1 concentrations were available from each of the three sampling sites in all 20 treated houses for July 1990 (before treatment), October 1990 (three months after treatment), and January 1991 (six months after treatment). Concurrent samples from 40 control houses were available for comparison.

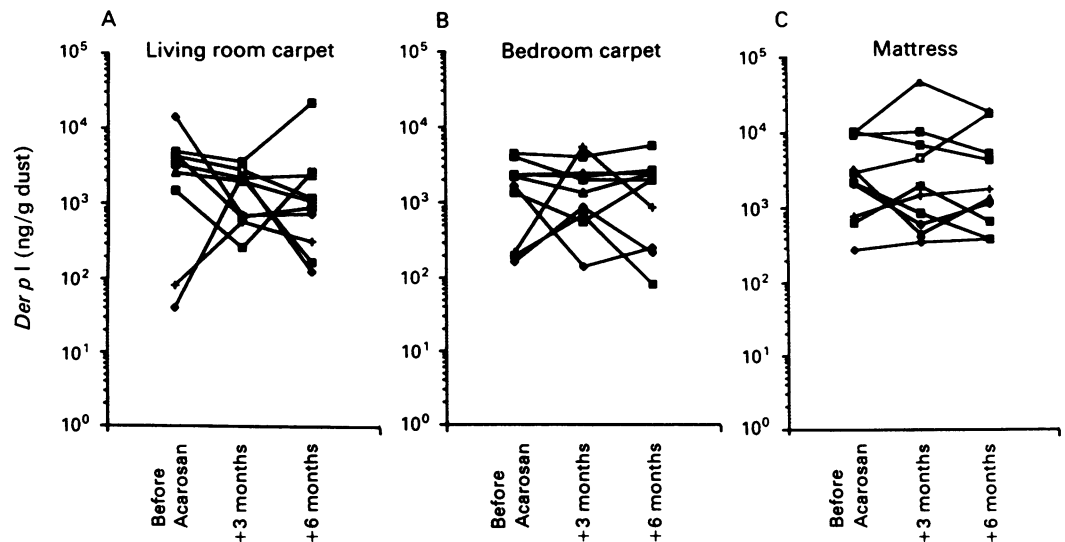
The table lists the geometric means and 95% confidence limits for the three groups at each sampling point. Although there was a trend towards lower concentrations in the living room carpet after treatment with Acarosan, we found no significant decrease in mean *Der p 1* concentrations either three months or six

Geometric means (95% confidence intervals) of *Der p 1* levels at baseline and three and six months after the application of Acarosan and liquid nitrogen ($n = 10$ for each group; dust in 40 untreated (control) houses sampled concurrently)

	Living room carpet			Bedroom carpet			Mattress		
	Control	Acarosan	Liquid N ₂	Control	Acarosan	Liquid N ₂	Control	Acarosan	Liquid N ₂
July 1990 (before)	1126 (758-1698)	1669 (436-6546)	3449 (1683-7278)	1122 (832-1738)	1153 (457-2884)	2387 (977-5834)	1412 (1096-2188)	2432 (1000-5888)	2280 (1066-4864)
October 1990 (3 months after)	2630 (1412-4898)	1369 (724-2570)	2328 (1247-4345)	2884* (1778-4898)	1334 (616-2884)	2084 (1069-4074)	4073* (2188-7079)	2318 (759-7079)	1663 (891-3104)
January 1991 (6 months after)	2188 (1479-3236)	1000 (339-2884)	4017 (1352-11940)	1820 (1096-3020)	1039 (389-2818)	1581 (657-3801)	2399 (1698-3388)	2227 (794-6186)	1729 (955-3133)

* $p < 0.05$ by comparison with corresponding value at baseline (July 1990).

Figure 1 A–C—*Der p I* concentrations in mattresses and carpets in 10 houses before and six months after application of Acarosan. Although there were wide variations between individual houses there was no significant change from baseline in the group means (see table).



months after either of the treatments. Figure 1 shows the absence of significant changes in *Der p I* concentrations in dust collected from the three sampling sites in each house treated with Acarosan over the six months of the study. Figure 2 shows similar data for the houses treated with liquid nitrogen. The lack of efficacy of these two treatments is also apparent from the absence of a significant difference between the concentrations in the dust from the treated houses and the control houses.

Within the control houses there was an increase in *Der p I* from all three sampling sites in October—significant for mattresses and bedroom carpets ($p < 0.05$) but not for living room carpets ($p = 0.056$). This increase was not present in either of the treated groups of houses, suggesting that Acarosan and liquid nitrogen did have a minor effect, but this level of efficacy enabled them to reduce the rate at which new *Der p I* antigen was generated only to a level that was in equilibrium with the rate of removal. Treatment did not produce significantly lower concentrations than in the control houses at any time point or sampling site.

More striking was the wide variation in *Der p I* concentrations between houses. This 100–

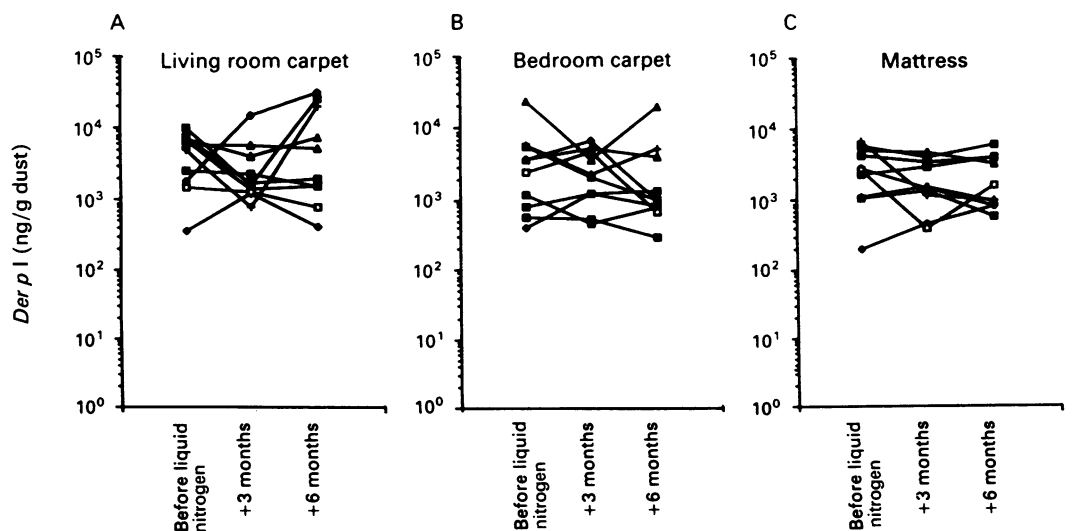
1000 fold variation far outweighs the 2–3 fold increase seen in October in the control houses as well as the even smaller effects of acaricide treatment in the two study groups.

Discussion

Very little information is available on how acaricides affect allergen concentrations. Despite this paucity of data, acaricides are being widely promoted as effective means of reducing exposure to allergen. Lau-Schadendorf *et al.*⁸ showed that Acarosan failed to reduce *Der p I* up to two months after application and, although total allergen (*Der p I* and the other important allergen, *Der f I*) was reduced in carpets, the magnitude of change was small and likely to be clinically insignificant. An earlier study showed that pirimphos methyl reduced *Der p I* by half in six weeks.⁶ *Der p I* is a very persistent allergen⁹ and this study failed to address the important question of how this rate of disappearance of the allergen was possible unless the acaricide either altered the allergen or in some way promoted its removal.

A recent paper reported an improvement in the symptoms of allergic rhinitis in patients

Figure 2 A–C—*Der p I* concentrations in mattresses and carpets in 10 houses before and three and six months after application of liquid nitrogen. As in the Acarosan group, there was no significant change in the group means up to six months after application (see table).



after treatment of their houses with Acarosan.¹¹ *Der p 1* loads were indirectly estimated by an assay of faecal guanine.¹² This method of measuring the allergen, the small size of the study, and important differences in characteristics between treated and control groups make the results less than conclusive.¹³ Other studies have assessed the clinical response to acaricide use without quantifying the allergen load and have produced mixed results.⁵⁻⁷

Measures to reduce exposure to house dust mite allergen over short periods must eliminate the allergen from the environment if they are to be effective. Eliminating the source of allergen alone can succeed only if (a) the source is completely eliminated and (b) the allergen does not persist for longer than it takes for the source to return. The reason for the absence of effect with the two methods we have tested is likely to be a combination of these two factors.

Acarosan produces an unpredictable mite kill rate, especially in mattresses, where it is applied only on the surface whereas the mites are concentrated deeper inside. This lack of complete acaricidal effect may allow sufficient mites to survive to maintain allergen concentrations. The increase in October seen in the control houses, possibly in response to changing environmental conditions, was abolished, which suggests that the numbers of mites did decrease after Acarosan treatment—but only to levels at which their production of allergen was counterbalanced by natural removal from the environment.

The reason for the unchanged concentrations with liquid nitrogen is probably linked more to the persistent nature of *Der p 1*. The low temperature (-195.8°C) produced by liquid nitrogen treatment does kill all mites present⁷ but does not alter or eliminate the allergen. As recolonisation with house dust mite is complete by six months *Der p 1* needs to persist for only the few months before new sources return. Available data suggest that *Der p 1* is stable and persists for several months after the numbers of mites decline.⁹ Possibly repeated applications are necessary to prevent this recolonisation and these may have to be combined with additional cleaning methods to eliminate the allergen once mites have been killed.⁵

We treated only two rooms in each house, a decision influenced by the cost and time required to treat entire houses. Whether the limited nature of the treatment accelerated the recolonisation of treated rooms because mites were carried in from adjoining areas remains unanswered. Possibly a more significant decrease in *Der p 1* concentrations would occur if entire houses were treated but this would make

the treatment more expensive and time consuming. We also did not ask patients to embark on rigorous cleaning regimens as these are impractical in terms of time and effort and unlikely to be complied with. All houses were cleaned with conventional vacuum cleaners throughout the study and the differences between treated and control houses are unlikely to be due to varying intensiveness in cleaning.

The absence of significant reductions in the concentrations of *Der p 1* after the use of Acarosan and liquid nitrogen raises the issue of whether acaricidal treatment is an appropriate strategy for avoiding the allergen. If the aim is to reduce exposure to *Der p 1* then attention must be directed towards methods that eliminate or alter the allergen—for example, use of tannic acid, barrier methods that separate the patient from the allergen or—perhaps most importantly—reducing the humidity of the indoor environment to make it less favourable to growth of the mites. These measures are more likely to reduce exposure to the allergen than elimination of the mites because *Der p 1* clearly persists for several months after acaricide treatment.

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