

In vitro evaluation of acaricidal and fungicidal activity of the house dust mite acaricide, Allerbiocid

B. J. HART, B. GUÉRIN* and N. NOLARD†

Royal Agricultural College, Cirencester, Gloucestershire, U.K. *Laboratory Allerbio, 55270 Varennes-en-Argonne, France and †Institute of Hygiene and Epidemiology (IHEM), Mycology Section, Brussels, Belgium

Summary

The acaricidal and fungicidal activities of a new acaricide Allerbiocid, formulated for the control of house dust mites, were investigated. The components of the Allerbiocid formula are: 3% benzyl benzoate (acaricidal and fungicidal), 1% tannic acid (protein denaturant) and 0.5% Tween (surface active agent) in 70% isopropyl alcohol (antiseptic). At application rates of both 150 and 250 ml/m², the acaricidal activity appeared to work by both ingestion and contact and remained active upon contact with mites for up to 10 weeks. When Sabouraud agar was treated with Allerbiocid at a concentration of 5 ml/20 ml medium, the preparation was also found to have fungicidal and fungistatic activities on various species of fungi commonly found in house dust. Allerbiocid contains tannic acid which is a protein denaturant. Levels of *Der p I* in dust were found to be reduced by over 90% after treatment with Allerbiocid. The importance of denaturation of mite faecal allergens and allergens associated with dead fungal spores is discussed.

Clinical and Experimental Allergy, Vol. 22, pp. 923–928. Submitted 24 July 1991; revised 3 February 1992; accepted 16 March 1992.

Introduction

Allergy to domestic mites is a serious medical problem, the increasing incidence of which seems to correlate with higher housing standards, for example better insulation and fitted carpeting, which may enhance development of mite populations [1]. It is now clear from the work of Tovey *et al.* [2] that not only mites, but also their deposited faeces are important sources of allergens and thus for effective treatment both must be removed or neutralized from the environment of asthmatics who are sensitized to these allergens.

Domestic pyroglyphid mites, which live principally on shed human skin scales, are detritivores associated with man. They live primarily in mattresses and bedding where there is an abundant supply of food. During occupation, the temperature and humidity of the human body provide an ideal microclimate in the mattress for mites, and when unoccupied, mites are protected from extremes of ambient temperature and humidity [3]. In addition, mites inhabiting the deeper layers of this niche are protected

from the effects of acaricide treatments, due to difficulties in penetrating mattresses with acaricides.

Despite this problem, many acaricides against house dust mites are now commercially available. Most of these products have been shown to reduce mite numbers or allergen levels in the home, but the clinical benefit of this is still a subject of controversy. Problems lie in defining and measuring clinical benefits, including symptoms, consumption of medication and peak expiratory flow rates. In addition, difficulties arise in controlled studies when trying to compare groups of similar age, with similar allergic disease, exposed to similar levels of house dust allergens. Nevertheless, some of these acaricides have shown improvements in symptoms, peak expiratory flow rates and reduced airway responsiveness, particularly when an integrated mite control programme (including acaricide, rigorous cleaning, removal of carpets, etc.) is followed [4]. It seems likely therefore that acaricides, if used properly, may play an important role in preventing and controlling asthma associated with house dust mites. Recently acaricides incorporating tannic acid to denature faecal allergens have been suggested as being more effective as they not only kill the mites, but also denature faecal allergens [5].

Correspondence: Dr B. Guérin, Laboratory Allerbio, 55270 Varennes-en-Argonne, France.

The present study was designed as a preliminary investigation of the *in vitro* acaricidal activity of the acaricide Allerbiocid (Allerbio, Varennes-en-Argonne, France) which incorporates tannic acid. The fungicidal activity of this product was also studied, due to the suggested importance of fungi in mite development [6] and also the role of fungi themselves as a source of allergens [7]. Clinical studies using this product will be reported in a future publication.

Materials and methods

Acaricide

The components of the Allerbiocid formula are: 3% benzyl benzoate (acaricidal and fungicidal), 1% tannic acid (protein denaturant) and 0.5% Tween (surface active agent) in 70% isopropyl alcohol (antiseptic). The mixture of these components disperses easily and forms a film that gives prolonged action.

Acaricidal activity

The acaricidal activity of Allerbiocid was measured by comparative counts, at regular intervals, of populations of *Dermatophagoides pteronyssinus* in treated and control samples. The effect of contact and ingestion of the acaricide was also investigated. Mites used in these studies were obtained from laboratory cultures maintained by one of the authors (B.J.H.) on a diet of 1:10 of beard shavings and dried yeast powder at 25°C, 75% relative humidity.

Acaricidal activity was measured in semi-natural conditions by infesting samples of carpet (5 × 10 cm) with 100 *D. pteronyssinus* (60 females, 20 males, 20 nymphs). Culture medium (0.025 g), consisting of a mixture of 1:10 beard shavings and dried yeast powder, was then added. The samples were then placed in optimal conditions for mite development (25°C, 75% relative humidity) for 24 hr to enable the mites to become established in the carpet fibres. After this time the samples were treated with a spray at the rate of 150 or 250 ml/m² of Allerbiocid (treated samples) or 70% isopropyl alcohol (positive control samples), or left untreated (negative control). The carpets were sprayed from a distance of 30 cm using a spray supplied by the manufacturer. The samples were returned to optimal conditions and left for 1, 7, 30, 60 and 90 days before live mites were counted. This was done by vacuuming the carpet pieces for 1 minute and transferring the dust collected to 70% ethanol for examination under a binocular microscope. A further 0.025 g culture medium was added to populations on carpet pieces after 30 and 60 days to ensure a sufficient food supply for their development. Each treatment was performed in triplicate.

The length of time that the acaricide remained active was also measured. Culture medium consisting of a mixture of 1:10 beard shavings and dried yeast powder was treated as before with a spray at the rate of 150 or 250 ml/m² of Allerbiocid (treated samples) or 70% isopropyl alcohol (positive control samples), or left untreated (negative control) and 0.001 g aliquots were placed into the wells of a microtitre plate. Ten adult female *D. pteronyssinus* were introduced into the wells which were then placed at 25°C and 75% RH for 24 hr after which time the dead and living mites were counted and removed. This procedure was repeated at weekly intervals to determine the persistence of the acaricide. A further test using a culture medium consisting of inert cellulose treated with acaricide as above and then added in equal parts with the beard shavings and yeast food mixture was done to separate the acaricidal activity due to contact from that due to ingestion. Each treatment consisted of 8–10 replicates. To further investigate the contact effects of the acaricide, 5 mm diameter filter paper discs (Whatman no. 541) were soaked with Allerbiocid, 70% isopropyl alcohol or left untreated. After drying, the discs were placed at the bottom of microtitre plate wells into which 10 adult female mites were introduced. After 24 hr at 25°C, 75% RH, the dead and living mites were recorded and removed. Each treatment consisted of 8–10 replicates and was repeated at weekly intervals to determine the persistence of the acaricide.

Fungicidal and fungistatic activity

In vitro studies The *in vitro* study consisted of comparing Petri dish cultures of fungal strains from the IHM collection after inoculation onto untreated Sabouraud medium and onto the same medium containing 5 ml Allerbiocid. Before treatment, the Allerbiocid solution was sterilized by filtration through a Millex 0.22 µm filter (Millipore). To determine whether any inhibitory effect of the acaricide was fungicidal or fungistatic, the inoculum implanted onto the plates containing Allerbiocid was reimplanted, after washing with physiological saline, onto Petri dishes containing untreated Sabouraud medium.

In situ studies The *in situ* studies made use of a fungus-contaminated synthetic fitted carpet removed from an office in Brussels. The office was on the second floor of a 50-year-old building which had been unoccupied for the past 5 years.

Samples of carpet (25 cm²) were placed in square Petri dishes (25 cm²) onto which Allerbiocid was sprayed from a distance of 30 cm using a spray supplied by the manufacturer, until the surface appeared to the eye, to be

well wetted (approximately 50 ml/m²). The plates were then left open to the air for 24 h in a sterile environment, so that the fabric could dry naturally. Fungi in the carpet pieces were then cultured at 25°C (or 45°C for thermophilic moulds) on malt agar with chloramphenicol using the following two procedures. In the direct inoculation procedure the pieces of carpet were placed onto plates of nutrient agar for 24 h, agitating the fabric to facilitate sedimentation of fungal spores and other particles. After this time the carpets were removed, the plates were incubated and the number of colonies were counted after 7 and 15 days. In the dilution procedure, four smaller samples of carpet (9 cm²) were put into an Erlenmeyer flask containing 100 ml sterile physiological saline and agitated at a speed of 60 rev/min for 20 min. The suspension thus obtained was inoculated by the method of successive dilutions to 10⁻³.

Protein denaturing activity

To evaluate the denaturing activity of the acaricide on allergenic proteins, we tried to reproduce in the laboratory the method of application of Allerbiocid to samples of bedding dust in which the numbers of mites and the levels of *Der p* I were found to be high.

Samples of the selected dust (1g), which corresponds to samples of 2–3 m² of clean bedding, were incubated in 3 ml Allerbiocid (containing 1% tannic acid) or 3 ml of a 1% solution of various other tannic substances for 72 hr at ambient temperature, in a crystallizing dish (7 cm diameter) covered with aluminium foil. The various tannins used were: tannic acid (grade USP), tannins made from the products of condensation of sulphonic acids Basytan M (BASF, FRG); the tannin Basytan DLE (BASF, FRG), made from the products of condensation of phenol. These tests were done in triplicate.

The dust samples were extracted for 18 hr at 4°C, with magnetic mixing in a solution of 0.125 M ammonium bicarbonate (1/10 w/v). This was followed by centrifugation, collection of the supernatant and filtration through a 0.8 µm mesh. This extract (100 µl) was added to each well of a microtitre plate, prepared and coated by ALK (Denmark) for measurement of the *Der p* I allergen [8] and incubated for 24 hr at room temperature. After washing three times with PBS Tween buffer, 100 µl monoclonal anti-*Der p* I antibody was added to each well and incubated for 1.5 hr at room temperature. After washing three times in PBS Tween buffer, 100 µl of substrate (OPD) was then added to each well and incubated for 15 min. Finally, 100 µl of 1 M sulphuric acid was added to stop the reaction and after 30 min incubation in darkness, the plate was read at 490 nm using a microtitre plate counter and results were calculated

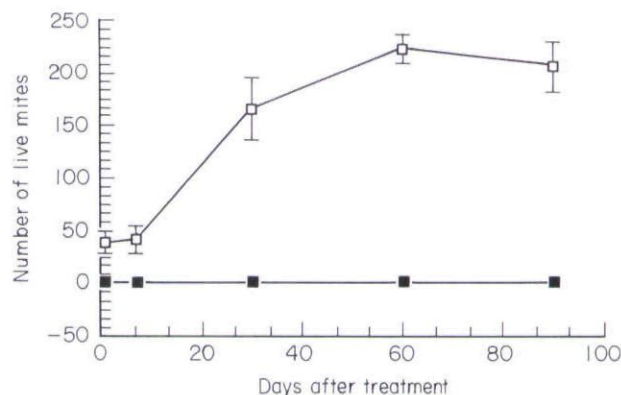


Fig. 1. Number of *D. pteronyssinus* mites surviving in carpets treated with Allerbiocid (150 or 250 ml/m²), 70% isopropyl alcohol (150 or 250 ml/m²) or without treatment. No mites survived any of the Allerbiocid or alcohol treatments and therefore results from these are represented by a single line (■). Results from untreated carpets are represented by □ symbols.

using a reference curve prepared in parallel with the experiment. Lind [8] determined the coefficient of variation (CV) for the assay as less than 20% when the *Der p* I allergen content of samples exceeded 1 µg/g dust. We considered this to be satisfactory since the majority of publications cite 2 µg *Der p* I/g dust as a risk factor for sensitization and development of asthma [9–10], although we recognize that some authors dispute this index [11].

Analysis of variance, using Scheffe's multiple range test for significance was performed on results using Apple Macintosh Statview software.

Results

Acaricidal activity

Figure 1 shows that the mites were killed within 24 hr of application of the acaricide to the carpet samples and that the mite population was unable to re-establish over a period of 90 days. This was also true for the positive control in which the carpet pieces were sprayed with 70% alcohol. Application rates of 150 and 250 ml/m² gave identical results. The negative control in which carpet samples were not given any spray treatment, showed an increase in the mite population, reaching a peak after 60 days.

Mites introduced at weekly intervals onto acaricide treated culture medium did not survive until approximately 76 ± 2 (s.e) days after the start of the experiment, indicating the persistence of the acaricide with respect to reinfestation. When inert cellulose treated with Allerbiocid was mixed with the culture medium (contact experiment), reinfestation was possible in a significantly shorter time 45 ± 2 days ($P < 0.01$). However, in experiments where mites were added at weekly intervals to acaricide treated filter paper, the acaricidal activity of 84 ± 2 days

Table 1. Fungistatic and fungicidal activity of Allerbiocid *in vitro*. Figures show mean values $\pm$ standard error (in mm growth) for a series of five Petri dishes after incubation for 7 and 10 days at 25°C

Reference strain	7 days		10 days		
	SAB	SAB + Allerbio	SAB	SAB + Allerbio	Repeat culture*
2646 <i>A. versicolor</i>	17 $\pm$ 2	0	20 $\pm$ 2	0	10 $\pm$ 2
4173 <i>S. atra</i>	27 $\pm$ 3	0	40 $\pm$ 2	0	0
3721 <i>C. cladosporoides</i>	39 $\pm$ 3	0	55 $\pm$ 3	0	0
3222 <i>C. sphaerospermum</i>	24 $\pm$ 2	0	34 $\pm$ 2	0	10 $\pm$ 2
3486 <i>A. murorum</i>	28 $\pm$ 2	0	37 $\pm$ 2	0	12 $\pm$ 2
1689 <i>C. cocloides</i>	60 $\pm$ 4	0	90 $\pm$ 4	0	0
2075 <i>U. botrytis</i>	68 $\pm$ 3	0	90 $\pm$ 5	0	0

* New culture of the fragment initially cultured on Sabouraud medium (SAB) + Allerbiocid, subsequently transferred onto SAB. Results after 10 days. SAB, Sabouraud medium. Allerbio, Allerbiocid.

Table 2. Effect of Allerbiocid *in situ*: Mesophilic (M), Xerophilic (X) and Thermophilic (T) moulds in carpets. Figures show the number of colonies observed on single Petri dishes

Mould type	Non-treated			Treated		
	M	X	T	M	X	T
<i>Direct inoculation</i>						
<i>Aspergillus fumigatus</i>	0	0	5	0	0	0
<i>A. versicolor</i>	50	50	0	0	0	0
<i>Penicillium sp.</i>	100	100	0	0	0	0
<i>By dilution</i>						
10 ⁻¹						
<i>A. fumigatus</i>	0	0	2	0	0	0
<i>A. versicolor</i>	C	C	0	0	0	0
<i>Penicillium sp.</i>	C	C	0	0	0	0
<i>Paecilomyces varotii</i>	0	0	1	0	0	0
10 ⁻²						
<i>A. fumigatus</i>	0	0	1	0	0	0
<i>A. versicolor</i>	C	C	0	0	0	0
<i>Penicillium sp.</i>	C	C	0	0	0	0
10 ⁻³						
<i>A. fumigatus</i>	0	0	0	0	0	0
<i>A. versicolor</i>	42	35	0	0	0	0
<i>Penicillium sp.</i>	3	2	0	0	0	0

C, colonies too numerous to count.

was not significantly different to that found when the acaricide was ingested. When culture medium, inert cellulose or filter paper was treated with 70% alcohol or left untreated, no acaricidal effect was observed.

Fungistatic and fungicidal activity in vitro

The fungal strains from the IHEM used in this study were *Chaetomium cocloides*, *Ulocladium botrytis*, *Aspergillus versicolor*, *Cladosporium sphaerospermum*, *C. cladosporoides*, *Acremonium murorum* and *Stachybotrys atra*, IHEM numbers 1689, 2075, 2646, 3222, 3721, 3486 and 4137 respectively. These species were chosen as they are commonly isolated from the homes of patients with type I hypersensitivity to moulds.

Table 1 shows that the viability of the fungal strains grown on Sabouraud agar is greatly reduced by treating with Allerbiocid at a concentration of 5 ml/20 ml medium. The effect of the acaricide when used at various dilutions was fungicidal for some strains of fungus (*Chaetomium cocloides*, *Ulocladium botrytis*, *Cladosporium cladosporoides*, *Stachybotrys atra*) and fungistatic for others (*Aspergillus versicolor*, *Cladosporium sphaerospermum*, *Acremonium murorum*).

In pieces of synthetic carpet impregnated with Allerbiocid, mesophilic, xerophilic and thermophilic fungi were destroyed (Table 2).

Protein denaturing activity

The various tannins tested were found to be extremely efficient protein denaturants under the experimental conditions used (Table 3). The reduction of *Der p* I ranged from 2.5 to 18.5% of the original value (4 µg). Tannic acid

Table 3. Allergenic denaturing activity of Allerbiocid. Samples of dust (1 g) containing 4 µg *Der p I* were incubated for 72 hr with 3 ml of a 1% tannin solution at ambient temperature. Figures show the mean weights and mean % of the original level of *Der p I* ($\pm$ standard error) remaining in the samples after this treatment

	Weight of <i>Der p I</i> (µg)	% of original value
Tannic acid USP	0.12 $\pm$ 0.01	3.16 $\pm$ 0.36
Basytan DLE	0.22 $\pm$ 0.04	5.58 $\pm$ 1.04
Basytan M	0.53 $\pm$ 0.12	13.25 $\pm$ 3.03
Allerbiocid	0.13 $\pm$ 0.01	3.41 $\pm$ 0.22

and Allerbiocid showed the greatest reduction in *Der p I* in both mean weight and percentage; these values were significantly lower than those for Basytan M ($P < 0.05$).

Discussion

In the present study we have investigated the acaricidal and fungicidal activities of a new acaricide Allerbiocid, formulated for the control of house dust mites. When carpet pieces were infested with mites and then sprayed with 70% alcohol, similar results to those with Allerbiocid were found, suggesting that the acaricide had little more killing power than 70% alcohol. Nevertheless, if filter paper, culture medium or inert cellulose was sprayed and dried before addition of mites, the mites survived the 70% alcohol treatment. It was evident from this that the wetting action of the spray had a deleterious effect on the mites and that mites could survive on the various substrates whenever the alcohol had dried. This was in sharp contrast to the long-term effects found with the acaricide.

The acaricidal activity appeared to work by both ingestion and contact, although the use of Allerbiocid treated inert cellulose (contact) killed mites over a shorter period of time. This may be explained by a dilution effect. The treated inert cellulose was mixed with equal parts of untreated culture medium and this dilution, resulting in less contact of the mites with the acaricide, may account for the lower acaricidal activity of this treatment. Nevertheless, overall the preparation was found to rapidly kill both mites and fungi and therefore represents a promising candidate for control of house dust mites and fungi and thus allergen levels in homes. Extrapolation of *in vitro* results to the home situation may, however, be misleading due to a number of problems associated with the use of acaricides in the home.

For example, we tested application rates of 150 and 250 ml/m² in an attempt to find a minimum effective dose. Since no difference in effect was found using these rates,

the minimum effective dose probably lies below 150 ml/m². Further studies are underway to determine more precisely the minimum effective dose of Allerbiocid, taking into consideration both the minimum dose required to kill mites and that required to denature mite allergens. This question also relates to concern over the potential long-term toxic effects to humans of the use of acaricides in the home. All of the components of Allerbiocid are, however, considered as non-toxic. Benzyl benzoate was granted GRAS status by FEMA (1965) and is approved by the FDA for food use (21 CFR 121.1164). The Council of Europe (1970) listed benzyl benzoate giving an ADI of 5 mg/kg. The food Chemical Codex (1972) has a monograph on benzyl benzoate. Tannic acid is used as a food additive, as a clarifying agent for beer and wine, in candy and chewing gum [12].

Many chemicals are available which will rapidly kill mites, but reinfestations of carpets and mattresses may result in the establishment of new populations within a short time. Our studies on the duration of the acaricidal activity of Allerbiocid have shown that the preparation remains active upon contact for up to 10 weeks. Thus in the home situation, it is estimated that applications of Allerbiocid would be required every three months to prevent reinfestation, although further *in vivo* studies are underway to confirm this in relation to the minimum effective dose.

Another problem encountered with the use of acaricides in the home is that allergens associated with mite faeces are not usually removed. Allerbiocid contains tannic acid which is a protein denaturant. It has been shown in the present study and in previous studies that tannic acid denatures the allergens in mite faeces, thus lowering the residual allergen level in homes treated with the acaricide [13,14]. This result is not surprising because of the known denaturing activity of phenols and aldehydes, particularly those which autopolymerize, e.g. formaldehyde. Phenols and aldehydes have been used for this property since the work on anatoxines by Ramon in 1923 [15] and Marsh *et al.* in 1970 [16] on allergenic extracts. Tannic acid was used by Naterman in 1941 [17] to reduce the allergenicity of pollen extracts. Multifunctional molecules such as glutaraldehyde, are also denaturants and have been used as tanning agents, as recommended by Paterson *et al.* in 1974 [18] for the production of allergoids. Currently it is unlikely that complete kill of mites or denaturation of *Der p I* will be achieved in the home, since acaricides do not completely penetrate mattresses or other thick furnishings. Nevertheless, clinical studies investigating clinical benefits of the use of acaricides containing tannic acid have been promising [5,19,20] and further such studies are underway using Allerbiocid.

We have also studied the effect of Allerbiocid on fungal allergens. Allerbiocid has a direct fungicidal or fungistatic activity on various common house dust moulds, but again, although the growth of fungi may be prevented by the use of this preparation, allergens associated with dead fungal spores remain in the dust and therefore remain a source of allergens. In this context the inclusion of tannic acid in Allerbiocid may be of importance and further studies into this question are required. Further, our studies on minimum effective dose will also investigate whether the minimum acaricidal dose of Allerbiocid has fungicidal or fungistatic activity.

The fungicidal activity of Allerbiocid may be important in the subsequent development of mite populations. Various authors have suggested the importance of fungi in mite development via their role in predigestion of lipids or as a source of various vitamins [21,22]. Allerbiocid may therefore inhibit development of mite populations indirectly as a result of its fungicidal activity. We have also found that tannic acid itself has an acaricidal activity (B. Hart, pers. comm.), which may render Allerbiocid particularly efficient because of the combination of benzyl benzoate and tannic acid with not only acaricidal, but also fungicidal and protein denaturing properties.

The present study has shown that in a semi-natural situation Allerbiocid efficiently kills mites and fungi and also denatures *Der p* I. Further tests are currently underway to investigate the efficacy, safety and practicality of this product in the home. The importance of such studies in not only determining the efficiency of acaricides in reducing mite allergens, but more importantly in assessing improvement in clinical symptoms, is increasingly being recognized [23].

Acknowledgments

Dr B. J. Hart was funded by the National Asthma Campaign during this study.

References

- 1 Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease, and environmental control. *J Allergy Clin Immunol* 1987; 80:755–75.
- 2 Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289:592–3.
- 3 Koekkoek HHM, Van Bronswijk JEMH. Temperature requirements of a house-dust mite *Dermatophagoides pteronyssinus* compared with the climate in different habitats of houses. *Ent Exp Appl* 1972; 15:438–42.
- 4 Dorward AJ, Colloff MJ, MacKay NS, McSharry C, Thomson NC. Effect of house dust mite avoidance measures on adult atopic asthma. *Thorax* 1988; 43:98–102.
- 5 Green WF, Nicholas NR, Salome CM, Woolcock AJ. Reduction of house dust mites and mite allergens: effects of spraying carpets and blankets with Allersearch DMS, an acaricide combined with an allergen reducing agent. *Clin Exp Allergy* 1989; 19:203–7.
- 6 Lustgraaf B. Ecological relationships between Xerophilic fungi and house-dust mites (Acarida:Pyroglyphidae). *Oecologia (Berl.)* 1978; 33:351–9.
- 7 Salvaggio J, Aukrust L. Mold-induced asthma. *J Allergy Clin Immunol* 1981; 68:327–46.
- 8 Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae* and *D. microceras*. *Allergy* 1986; 41:442–51.
- 9 Platts-Mills TAE, Chapman MD, Pollart SM, Luczynska CM. Establishing health standards for indoor foreign proteins related to asthma: dust mite, cat and cockroach. *Toxicology and Industrial Health* 1990; 6:197–208.
- 10 Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house dust mite allergen (*Der p* I) and the development of asthma in childhood. *N Eng J Med* 1990; 323:502–7.
- 11 Price JA, Pollock I, Little SA, Longbottom JL, Warner JO. Measurement of airborne mite antigen in homes of asthmatic children. *Lancet* 1990; 6:895–7.
- 12 Leung AY. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. New York: John Wiley.
- 13 Miller JD, Miller A, Luczynska C, Rose G, Platts-Mills TAE. Effect of tannic acid spray on dust mite allergen levels in carpets. *J Allergy Clin Immunol* 1989; 83:262.
- 14 Thompson PJ, Stewart GA, Miller A, Miller J, Bird C. The effect of tannic acid on house dust mite allergen load in the home. *Aust N Z J Med* 1990; 20:542.
- 15 Ramon MG. Sur le pouvoir flocculant et sur les propriétés immunisantes d'une toxine diphtérique rendue atoxique (anatoxine). *Soc Bio* 1923; 88:167.
- 16 Marsh DG, Lichtenstein LM, Campbell DN. Studies on allergoids prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. *Immunology* 1970; 18:705.
- 17 Naterman HL. The treatment of hay fever by injection of suspended pollen tanate. *J Allergy* 1941; 12:378.
- 18 Patterson R, Suszko IM, Zeuiss CR. Polymerized ragweed antigen E. II. In vivo elimination studies and reactivity with IgE antibody systems. *J Immunol* 1973; 110:1413.
- 19 Price JA, Marchant JL, Warner JO. Allersearch DMS in the control of domestic allergens. *Clin Exp Allergy* 1990; 20 (suppl 1):65.
- 20 Green WF. Abolition of allergens by tannic acid. *Lancet* 1984; ii:160–1.
- 21 Bronswijk JEMH, Sinha RN. Role of fungi in the survival of *Dermatophagoides* (Acarina Pyroglyphidae) in house dust environment. *Environ Entomol* 1973; 2:1425.
- 22 Saint Georges-Grèdelet D de. Vitamin requirements of the European house dust mite *Dermatophagoides pteronyssinus* (Acar: Pyroglyphidae), in relation to its fungal association. *J Med Ent* 1987; 24:408–11.
- 23 Godfrey K. House dust mite avoidance—the way forward. *Clin Exp Allergy* 1991; 21:1–2.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.