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Changes in Mite Allergen Levels in Homes using an Acaricide Combined with Cleaning Agents: A 3-Year Follow-Up Study

Key Words
Acaricides
Solidified benzyl benzoate
Mite avoidance
Mite allergen determinations
Der p I
Der f I

Abstract
Mite-infested furnishings from different houses were treated with an acaricide based on solidified benzyl benzoate combined with cleaning agents. Live mite populations, major mite allergen concentrations and guanine contents of dust collected from these furnishings were compared before and after treatment over a 3-year period. Significant short-term reductions in live mite numbers (95%, $p < 0.01$) and mite allergen concentrations (50%, $p < 0.01$) were observed. Mite eradication remained effective following long-term treatment, and allergen reduction reached 75% at the end of the 3rd year of the study. Measurement of guanine contents showed parallel results. Although these effects varied among the infested sites, long-term mite avoidance can be maintained by repeated treatments (2 per year).

Introduction

The role of the mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in allergic respiratory diseases induced by inhalation of house dust was discovered 20 years ago [1–3]. Since then, exposure to these mites and their allergens has been recognized as a risk factor for dust-sensitive patients [4–8]. Mite allergens are widely distributed in settled dust in the home environment, and the use of acaricides on different infested sites represents one of numerous mite avoidance measures. This procedure provides the advantage of killing mites, the active producers of allergens, as previously demonstrated with the use of such chemicals as pirimiphos-methyl [9], natamycin [10], benzyl alcohol [11] and benzoic acid esters [12, 13]. Acaricides only affect live mites, and their association with other agents has been proposed to improve removal of already present mite allergens. A spray composed of a mixture of benzyl alcohol and tannic acid as the allergen-denaturing agent was used to reduce mite allergenicity in dust from blankets and carpets [11]. Bischoff et al. [14] proposed using a combination of solidified benzyl benzoate (BB) and adsorbing/cleaning agents in two forms: foam or moist powder. The short-term effect of this combination on major mite allergen concentrations in house dust from carpets was recently reported [15].

This paper describes changes over a 3-year period in the levels of dust mite allergens harvested from different furnishings in homes where a combination of solidified BB and cleaning agents was applied. Mite allergen levels were assessed by measuring major group I allergens Der p I (from *D. pteronyssinus*) and Der f I (from *D. farinae*), and by determining guanine content, a marker of mite excretion. Mite eradication was checked by counting live mites.

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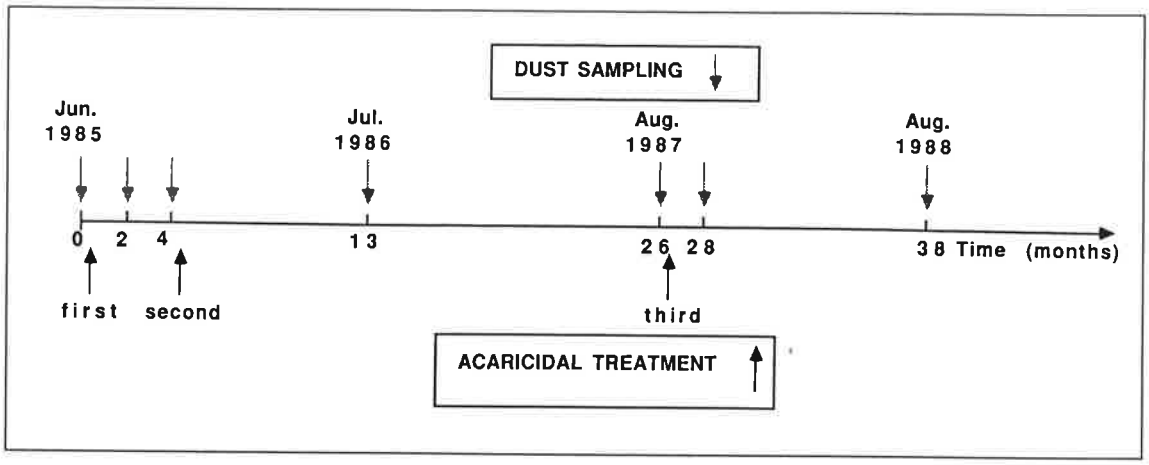

Fig. 1. Outline of dust sampling and acaricidal treatment schedules.

Material and Methods

House Dust Samples

Selection of Mite-Infested Homes. Five homes in southern Bavaria were selected based on the presence of mite fauna and high guanine content in bedroom dust samples [16]. In each home, mattresses, upholstered furniture and bedroom or living room carpets were chosen as sampling sites.

Dust sampling. Dust was collected by vacuum cleaning the total area of each site (700 W, 2 min/m^2) at the beginning of the study (June 1985) and at short and long periods following acaricidal treatments (fig. 1). The dust was stored at 4 °C in sealed plastic bags until the raw dust was sieved through a 0.3-µm screen to obtain fine dust for analysis.

Acaricides

Composition. The foam acaricide was composed of benzoic acid ester in combination with polymers (7%), tensides (2%) and water (91%). The moist-powder acaricide was composed of benzoic acid esters combined with silicates (10%), cellulose fibers (41%), liquid paraffin (8%), inorganic salt (2%) and water (39%). Both products were provided by Werner and Mertz, Mainz, FRG (Acarosan®).

Application. Foam acaricide was rubbed in to mattresses and upholstered furniture (60 g/m^2) to allow penetration of the product. One week after foam application, furnishings were vacuum cleaned. The moist powder was brushed onto the carpets (75 g/m^2) and allowed to dry for 2–4 h. After complete drying, the carpet was carefully vacuumed until the surface was free of visible powder. Treatments took place at the beginning of the study (June 1985) and approximately 4 (October 1985) and 26 months (August 1987) later (fig. 1). All treatments were performed by the same operator. Between treatments, ordinary housecleaning was carried out.

Mite Counting

The number of live mites was assessed by a flotation technique, as previously described [17].

Guanine Determination

Semiquantitative and quantitative guanine determinations were performed on dust samples using the color paper test (Acarex-test®) [16] or a spectrophotometric method [18, 19], respectively. Acarex-test results were expressed in 4 upgrading classes (0–3) and the quantitative assay in milligrams of guanine per gram of fine dust.

Dust Extracts

200 mg of fine dust from each dust sample were extracted in 4 ml of borate-buffered saline. The supernatant obtained after centrifugation was filtered (0.45 µm). The filtrate constituted the dust extract. All dust samples were extracted at the same time and stored at −20 °C.

Measurements of Mite Group I Allergens

Measurements were performed on dust extracts by a two-site monoclonal antibody (mAb) ELISA. The mAb used in this study recognized specific and nonoverlapping epitopes on Der p I [20] and Der f I [21]. For the assay of Der p I, 10B9F6 mAb was coated onto microtiter plates, and 5H8C12 biotinylated mAb was used as the secondary antibody. In the Der f I assay, MAF 6 mAb was the catching antibody, and MAF 9 biotinylated mAb was the detecting antibody. The procedure described by Luczynska et al. [22] was performed with some modifications. Briefly, 100 µl of dust extract were incubated overnight with the coated wells. After extensive washing, 100 µl of the biotinylated mAb were added (8–12.5 ng/well). Bound mAb was detected by adding alkaline phosphatase conjugated to streptavidin (Amersham, UK), and the enzyme activity was determined in the presence of *p*-nitrophenyl phosphate (1 mg/ml) in diethanolamine buffer. Absorbance was read 20–30 min later, at 405 nm (Titertek Multiscan MCC340, Flow Laboratories). Reference curves were established for each allergen in the range of 0.5–62.5 ng/ml using standard solutions (International Solution 82/518 from the National Institute of Biological Standards and Control for Der p I, and purified Der f I [23] as internal reference for Der f I). Results were expressed as micrograms of Der p I and Der f I per gram of fine dust. The mean interassay coefficient of variation (CV) was 15% and the intra-assay CV was 5%.

Table 1. Short-term effects of the first application of powder or foam acaricide (June 1985) on carpets (C), upholstery (U) and mattresses (M)

Sites	Live mite numbers/g dust			Der p I + Der f I, µg/g dust		
	June 1985	after treatment		June 1985	after treatment	
		Aug. 1985	Oct. 1985		Aug. 1985	Oct. 1985
C1	45	0	0	81	44	25
C2	44	0	2	12	2.4	3.8
C3	28	0	2	11	3.8	4.4
C4	0	0	0	3.2	1.9	1.6
U1	85	10	23	25	12	14
U2	45	0	5	35	13.5	14
U3	9	0	n.d.	17.4	3.7	n.d.
U4	223	13	n.d.	14.3	14	n.d.
M1	208	15	0	20.4	n.d.	15.4
M2	60	0	0	28	15	23
M3	5	0	0	26	11.4	15.4
Mean reduction, %		95	95		51	49

n.d. = Not done, sample not obtained.

Data Analysis

The Wilcoxon signed rank test for paired samples was used to compare mite numbers and mite allergen levels in dust samples before and after acaricidal treatments. Significance was assumed at $p < 0.05$. Mean reductions in mite counts and allergen concentrations were calculated as follows: 100 × (mean result before treatment – mean result after treatment) / (mean result before treatment).

Results

Live mite populations and mite allergen levels in dust collected from 4 carpets (C1–C4), 4 types of upholstery (U1–U4) and 3 mattresses (M1–M3) were determined before and after acaricidal treatments over a 3-year period (June 1985 to August 1988). At the beginning of the study, all dust samples except one (C4) contained live mites whose numbers ranged from 5 to 213 per gram of dust, with *D. pteronyssinus* as the dominating species, followed by *D. farinae*. Mite group I allergen levels, measured by ELISA, were found to be higher than 10 µg of Der p I plus Der f I allergen per gram of dust except for the C4 sample (3.2 µg/g).

Short-Term Effects

Two and four months after the first treatment (June 1985), the number of live mites per sample decreased substantially (table 1). In many cases, no live mites were found, and mean reductions after both periods were similar (95%, $p < 0.01$). Results of mite group I allergen concentrations for each site are presented in the same table. In August and October 1985, there were significant mean reductions in the sums of Der p I plus Der f I (51% and 49%, respectively, $p < 0.01$). As a small amount of the acaricidal powder may remain in the carpets a short time after application, its interference with the mite allergen measurement was tested. A 20% (w/w) of dry acaricidal powder added to 6 dust samples led to a mean mite allergen reduction of 15.5%. Differences observed between titrations with and without powder were not significant ($p > 0.05$).

Long-Term Effects

Figures 2 and 3 illustrate individual results for live mites and Der p I plus Der f I allergen concentrations of dust samples collected more than 9 months after acaricidal treatments. M2 and U1 (sofa) could not be monitored during the whole period as they were removed by the inhabitants.

In July 1986, the mean reduction in live mites and group I allergen concentrations reached, respectively, 98% ($p < 0.01$) and 80% ($p < 0.01$) on sites which underwent a second treatment. For U3 and M3, treated only once in June 1985, the mite allergen level remained stable or increased. Between July 1986 and August 1987, only U1 and U4 received an additional treatment. At the end of this 2nd period, there was an occurrence of live mites

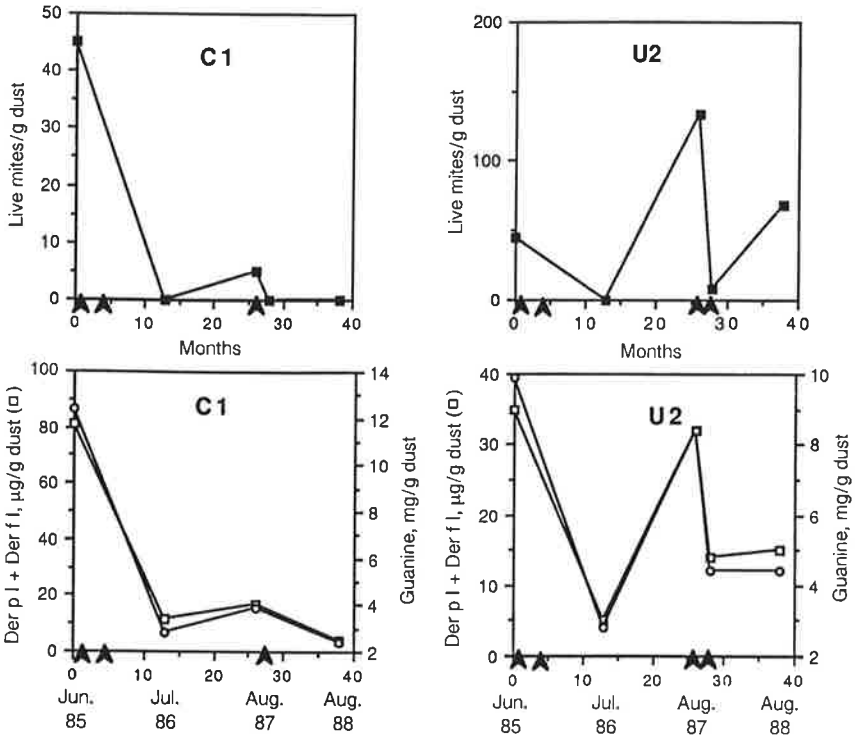

Fig. 2. Comparative changes in live mite population (■), mite allergen concentration (Der p I + Der f I) (□) and guanine content (○) in 1 carpet (C1) and 1 upholstered chair (U2) after acaricidal treatments (arrowheads) over a 3-year period (June 1985 to August 1988).

and an increase in mite allergen levels in many samples. The 3rd treatment performed on all sites led to a further decrease in the number of live mites. In August 1988, the mean reduction in the Der p I plus Der f I concentration was 75% ($p < 0.01$).

The guanine contents of dust samples collected at each year end of this study were tested by the semiquantitative method (Acarex-test) (table 2). The number of sites with high and moderate guanine levels (Acarex class > 2) was markedly reduced after acaricidal treatments, i.e. in July 1986 and August 1988. In contrast, the guanine levels increased in half of the sites in August 1987. The parallel changes found in results of ELISA assays and the Acarex-test were confirmed by quantitative guanine determinations, as shown by representative results for 1 carpet (C1) and 1 upholstered chair (U2) (fig. 2).

Discussion

The present work reports on the acaricidal and allergen-reducing properties of solidified BB combined with cleaning agents when used on mite-infested sites in homes.

This longitudinal study was performed over a 3-year period, from June 1985 to August 1988. Although the experiments were performed in an open trial, the findings were very informative. Up to now, effects of acaricides had not been monitored and reported over such a long period. Moreover, live mite populations, mite allergen levels and guanine contents of dust samples collected from different sites, such as carpets, upholstered furniture and mattresses, were followed in this study. Dust samples were collected at the same time each year over consecutive years to avoid seasonal variations in mite populations and mite allergen levels [24]. The procedure for collecting settled dust samples was standardized (time/surface area, operator) in order to eliminate other variations.

The results show that the acaricidal applications induce both a pronounced destruction of the mite population and a significant decrease in mite allergen levels in house dust from most of the sites.

The decrease in the number of live mites found in dust samples after treatments shows that acaricidal powder or foam can penetrate the furnishings to reach live mites. The 1st application reduced the number of live mites by 80–100%, depending on the type of furnishings. These results, obtained using a conventional mite detection

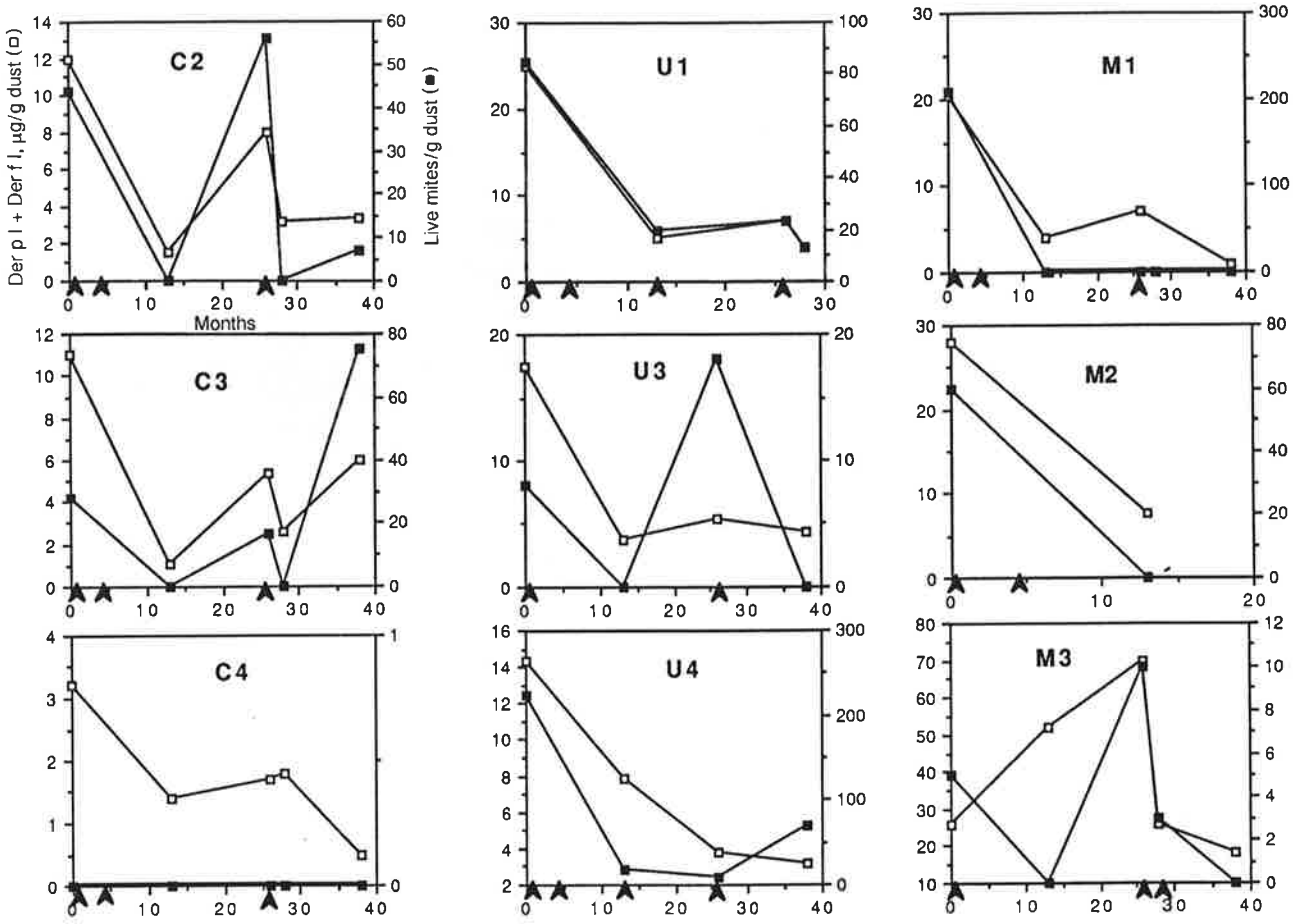

Fig. 3. Long-term effects of acaricidal treatments (arrowheads) on carpets (C), upholstery (U) and mattresses (M). Evolution of live mite population (■) and mite allergen concentration (Der p I + Der f I) (□) from June 1985 to August 1988 (0–38 months).

method, i.e. counting after flotation, agree with those of previous studies performed on experimental carpet biotopes using the 'heat escape method' [13]. After 2 acaricidal applications, mite control remained effective up to 13 months, as shown during the 1st year of our study. This long-term acaricidal effect appears to be consequential to the use of the solidified BB form, which reduces the vapor pressure of the reagent and allows its long-lasting presence in dust. Long periods without treatment, as in the 2nd year of study, led to the occurrence of new mite populations. Repeated treatments seem necessary to prevent reinfestation of the sites and, thus, a renewed production of allergens.

Disappearance of mite allergens in treated furnishings was more progressive than the mite eradication. Mite

Table 2. Guanine content (Acarex-test®) of dust samples from mite-infested sites before and after acaricidal treatment during a 3-year period

Acarex class	Number of Acarex class related sites			
	before treatment June 1985	after acaricidal treatments		
		July 1986	Aug. 1987	Aug. 1988
0	0	3	0	5
1	2	7	5	4
2	2	0	4	0
3	7	1	1	0
Total number of sites tested	11	11	10	9

allergen levels were monitored by: (1) determination of guanine, a marker of total fecal mite allergens, and (2) measurement of specific major allergens, such as Der p I and Der f I. Similar results were obtained, confirming changes of mite allergen levels obtained during the study. Nevertheless, there is a theoretical risk of underestimating the allergen concentration (w/w) in carpet dust if acaricidal powder has not been completely removed by vacuum cleaning. In fact, most of the powder is eliminated after complete drying (a few hours after application) and afterwards by regular household vacuuming. For this reason, the mite allergen concentrations of dust samples with and without a small amount (20%) of dry powder were compared. No statistical difference was found, lessening the likelihood of possible interference with the results by the remaining powder.

A short-term allergen-reducing effect was obtained, agreeing with other authors [15]. The long-term effect obtained depended on the number of applications per year. If allergen removal was achieved by means of tensioactive and adsorbant components combined with the chemical BB, which afterwards was collected by vacuuming, subsequent cleaning would result in the more thorough elimination of allergens. Indeed, an effective mean reduction in mite allergens was obtained at the end of the 1st and 3rd years of the study. During the 2nd year, when the number of acaricidal applications was reduced, we observed an increase in mite allergen levels due to allergen accumulations produced by new mite populations. The influence that the type of furnishings has on mite allergen elimination, a factor not taken into account in this study because of the small number of objects investigated, deserves further study. However, it was noticed that the allergen reduction in carpets seemed more efficient than in mattresses, as previously observed after a short-term treatment [15].

The measurement of group I allergens, markers of airborne mite allergens [25], enables evaluation of the efficiency of acaricidal products in reducing exposure to house dust mites. At the beginning of the study, all sites except 1 presented mite allergen levels superior to 10 μg/g dust; these levels are considered a risk factor for acute asthma sufferers [4]. Although there were no continuous acaricidal treatments during this study, at the end of the 3rd year, 7 out of 9 sites contained 6 μg/g of mite allergen or less, showing that effective mite allergen avoidance was achieved. Further investigations are necessary to determine the optimal frequency of acaricidal treatment that would reduce mite allergen concentration to a minimum.

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References

1 Voorhorst R, Spieksma FTHM, Spieksma-Boezeman MIA: Is a mite (*Dermatophagoides* sp.) the producer of the house dust allergen? Allergy Asthma 1964;10:329–334.

2 Miyamoto T, Oshima S, Ishizaki T, Sato S: Allergenic identity between the common floor mite (*D. farinae* Hugues 1961) and house dust as a causative antigen in bronchial asthma. J Allergy 1968;42:14–28.

3 Pauli G, Bessot JC, Guisard G, Roegel E, Oudet P: Importance clinique des acariens chez les asthmatiques allergiques à la poussière domestique. Rev Fr Allerg 1972;12:141–153.

4 Platts-Mills TAE, De Weck AL: Dust mite allergens and asthma: A worldwide problem. J Allergy Clin Immunol 1989;83:416–427.

5 Dowse GK, Turner KJ, Stewart GA, Alpers MP, Woolcock AJ: The association between *Dermatophagoides* mites and the increasing prevalence of asthma in village communities within the Papua New Guinea Highlands. J Allergy Clin Immunol 1985;75:75–80.

6 Pollart SM, Chapman MD, Platts-Mills TAE: Allergen levels inside homes: Risk factors for acute asthma attacks. J Allergy Clin Immunol 1988;81:242.

7 Vervloet D, Penaud A, Razzouk H, Senft M, Arnaud A, Boutin C, Charpin J: Altitude and house dust mites. J Allergy Clin Immunol 1982;69:290–296.

8 Platts-Mills TAE, Tovey ER, Mitchell EB, Mozarro H, Nock P, Wilkins SR: Reversal of bronchial reactivity during prolonged allergen avoidance. Lancet 1982;ii:675–678.

9 Mitchell EB, Wilkins SR, McCallum Deighton J, Platts-Mills TAE: Reduction of house dust mite allergen levels in the home: Use of the acaricide, pirimiphos-methyl. Clin Allergy 1985;15:235–240.

10 van Bronswijk JEMH, Reumer JWF, Pickard R: Effects of fungicide treatment and vacuuming on pyroglyphid mites and their allergens in mattress dust. Exp Appl Acarol 1987;3:271–278.

11 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;19:203–207.

12 Bischoff E, Liebenberg B, Wetter C: Die Bekämpfung von Hausstaubmilben in Verbindung mit Reinigungsvorgängen auf wässriger Basis. 2. Anwendung bei naturanalogen Milbenbiotopen. Allergologie 1986;9:287–292.

13 Bischoff E, Fischer A: A new method of counting mites to establish the efficacity of water-based acaricides and their application. J Allergy Clin Immunol 1987;79:194.
14 Bischoff E, Fisher A, Wetter G: Die Bekämpfung von Hausstaubmilben in Verbindung mit Reinigungsvorgängen auf wässriger Basis. 1. Grundlegende Befunde. Allergologie 1986;9:173–178.
15 Lau-Schadendorf S, Rusche AF, Weber AK, Buettner-Goetz P, Wahn U: Short-term effect of solidified benzyl benzoate on mite-allergen concentrations in house dust. J Allergy Clin Immunol 1991;87:41–47.
16 Bischoff E, Schirmacher W: Farbnachweis für allergenhaltigen Hausstaub, 1. Mitteilung. Allergologie 1984;7:446–449.
17 van Bronswijk JEHM: House Dust Biology for Allergists, Acarologists and Mycologists. Ziest, NIB, 1981.
18 Bischoff E, Shirmacher W: Farbnachweis für allergenhaltigen Hausstaub, 2. Mitteilung. Allergologie 1985;8:36–38.
19 Le Mao J, Pauli G, Tekaia F, Hoyet C, Bischoff E, David B: Guanine content and *Dermatophagoides pteronyssinus* allergens in house dust samples. J Allergy Clin Immunol 1989;83:926–933.
20 Chapman MD, Heyman PW, Platts-Mills TAE: Epitope mapping of two major inhalant allergens, Der p I and Der f I, from mites of the genus *Dermatophagoides*. J Immunol 1987;139:1479–1484.
21 Le Mao J, Weyer A, Mazie JC, Rouyre S, Marchand F, Le Gall A, David B: Identification of allergenic epitopes on Der f I, a major allergen of *Dermatophagoides farinae*, by using monoclonal antibodies. Mol Immunol 1992;29:205–211.
22 Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD: A two-site monoclonal antiboby ELISA assay for the quantification of the major *Dermatophagoides* spp. allergens, Der p I and Der f 1. J Immunol Methods 1989;118:227–235.
23 Dandeu JP, Le Mao J, Rabillon J, David B: Antigens and allergens in *Dermatophagoides farinae* mites. II. Purification of Ag 11, a major allergen in *Dermatophagoides farinae*. Immunology 1982;46:679–687.
24 Platts-Mills TAE, Hayden ML, Chapman MD, Wilkins SR: Seasonal variation in dust mite and grass pollen in dust from the houses of patients with asthma. J Allergy Clin Immunol 1987;79:781–791.
25 Platts-Mills TAE, Heymann PW, Longbottom J, Wilkins SR: Airborne allergens associated with asthma: Particle sizes carrying dust mite and rat allergens measured with a cascade impactor. J Allergy Clin Immunol 1986;77:850–857.