A double-blind, placebo controlled trial of solidified benzyl benzoate applied in dwellings of asthmatic patients sensitive to mites: Clinical efficacy and effect on mite allergens

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Background: *The aim of this double-blind, randomized study was to investigate the effectiveness of an acaricidal cleaning product in modifying both clinical symptoms and mite allergen levels over a period of at least 1 year.*
Methods: *Twenty-six asthmatic patients with proven* Dermatophagoides pteronyssinus (Dp) *asthma were selected; three were withdrawn from the trial. The patients' homes were divided into two groups; 11 homes were treated with solidified benzyl benzoate and tenside agents (A), and 12 were treated with a placebo (P). Two applications were performed at the beginning of the trial and at least 6 months later. Patients were examined 1 month before the trial, at the beginning of the trial, and every 3 months over a period of 1 year. Indoor mite exposure was evaluated by three methods: semiquantitative guanine determinations, quantitative guanine determinations, and the measurement of* Der p *I* + Der f *I (antigen P, of* Dp + *antigen* F_1 *of* D. farinae*) levels.*
Results: *The symptom scores established at the beginning of the trial and 12 months later showed a statistically significant improvement only in the A group ($p < 0.01$). The visual analog scale also showed a statistically significant difference both in the A ($p < 0.05$) and P groups ($p < 0.01$). No statistical differences were found between medication scores in the A or P groups. A statistically significant increase was also observed for forced expiratory volume in 1 second and maximal expiratory flow rate 25/75 in the two groups ($p < 0.05$ for P group; $p < 0.01$ for A group). The mean decreases in* Der p *I* + Der f *I in patient mattresses between the beginning of the trial and after 12 months were 20% for the acaricide group and 17% for the placebo group, respectively (NS). For house dust samples with origins other than the patients' mattresses we found significant decreases in* Der p *I* + Der f *I in the A group ($p < 0.01$ for carpets and $p < 0.05$ for upholstery elements). (J ALLERGY CLIN IMMUNOL 1993;91:738-46.)*

Key words: *Mites, mite allergens, asthma prevention, acaricides*

House dust mite allergy has been widely implicated in the etiology of asthma, and mite allergy is now considered to be a worldwide problem.[1] Studies of subjects living in naturally or artificially mite-free environments have shown improvement of asthma and bronchial hyperresponsiveness.[2, 3] During the last 20 years many attempts have been made to exterminate breeding mites, remove mite allergens, and prevent mite infestation. However, conventional dust mite

Abbreviations used

Dp:	*Dermatophagoides pteronyssinus*
HDS:	House dust samples
RAST:	Radioallergosorbent test
MAb:	Monoclonal antibody
FEV_1:	Forced expiratory volume in 1 second
FVC:	Forced vital capacity
FEF:	Forced expiratory flow
PEFR:	Peak expiratory flow rate
Der p I:	Antigen P_1 of *Dp*
Der f I:	Antigen F_1 of *D. farinae*

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avoidance is difficult to carry out, and the efficacy of hygienic measures has been equivocal.[4-9] The role of acaricides in the overall strategy for managing treatment of patients allergic to mites needs to be assessed. The aim of this study was to investigate the effectiveness of an acaricidal cleaning product (Acarosan; Werner & Mertz GmbH, Mainz, Germany) in modifying both clinical symptoms and mite allergen levels over a period of at least 1 year.

MATERIAL AND METHODS

Patients

Twenty-six preselected patients attending our outpatient chest clinic, and ranging in age from 13 to 58 years (mean, 35 ± 14.9 years), were chosen on the basis of the following criteria:

(1) A definite history of perennial asthma with or without rhinitis and compatible with house dust mite allergy.
(2) A positive intradermal test after injection of 0.04 ml of a 10^{-5} mg/ml *Dermatophagoides pteronyssinus (Dp)* extract (Institut Pasteur, Paris, France); details of the preparation have been previously described.[10]
(3) A Phadebas radioallergosorbent test (RAST; Pharmacia AB, Uppsala, Sweden) for *Dp* 3 or greater in the Pharmacia screening system.
(4) An Acarex (Werner & Mertz) value in their mattresses equal to or greater than + +.

Informed consent was obtained from all patients before the study.

Study protocol

The patients were divided into two groups by a randomization procedure. In a double-blind fashion eleven homes were treated with Acarosan product and 12 with the placebo.

All patients were examined by the same physician 1 month before beginning the trial, at the beginning of the trial, and every 3 months over the course of 1 year. At each visit the patients gave their subjective impression of the severity of their asthma by placing a cross on a 10 cm linear analog scale from 0 (severe dyspnea) to 10 cm (no dyspnea). The following spirometric parameters were measured by a dry wedge spirometer Autospiro 500 (Minato, Osaka, Japan): FEV_1, FVC, and FEF 25-75. For each visit the patients had kept a diary during the previous month, recording PEFR measurements every morning and evening (Mini-Wright peak flow meter; Clement Clark Int. Ltd., London, England) to determine a functional score.

Moreover, clinical scores were determined by the physician at each visit, taking into account the number of asthmatic episodes. Scores of 0 to 4 were assigned to the periods of no asthmatic episode, at least one asthmatic episode per month, per week, per day, respectively.

In the same fashion, a medication score was determined:
- score 0 corresponds to no medication,
- score 1 corresponds to no more than 4 inhalations per day of β_2-agonists or disodium cromoglycate intake or both,
- score 2 corresponds to continuous bronchodilator treatment with β_2-agonists, with or without long-acting theophylline (twice a day), and beclomethasone dipropionate at a dose equal to or less than 1000 μg/day,
- score 3 corresponds to β_2-agonist inhalation with or without long-acting theophylline and beclomethasone dipropionate at a daily dose equal to or greater than 1000 μg.

TABLE I. Initial clinical and functional data of the active and the placebo group

		Active	Placebo
Clinical score	m	2.54	2
	M	3	2
	CI	1.5	0.4
Drug score	m	2.27	1.92
	M	3	1.5
	CI	0.66	0.5
Visual score (cm)	m	5.7	5
	M	5	5
	CI	0.84	0.78
FEV_1	m	63.45	72.73
	M	63	72
	CI	14.32	16.4
FEF 25-75	m	48	56.34
	M	46.2	52
	CI	16	15.5
Mean morning PEFR	m	67.85	75.38
	M	71	81
	CI	13.6	11.6
Mean evening PEFR	m	67.14	79.25
	M	70	75
	CI	13.3	11.6

m, Mean value; *M*, median; *CI*, 95% confidence interval. Functional data expressed as percentages of predicted values.

Determination of indoor mite exposure by environmental analyses

Indoor mite exposure was evaluated by three methods:

(1) Semiquantitative guanine determinations (Acarex test),[11] with results expressed in four classes from 0 with a guanine content less than 600 μg/gm of dust; +, between 600 and 2500 μg/gm of dust; + +, between 2500 and 10000 μg/gm of dust; and + + +, at least 10000 μg/gm of dust, respectively.
(2) Quantitative guanine determinations expressed in micrograms per gram of house dust. These two methods are based on results of a chemical color paper test[12] and a spectrophotometric method,[13] respectively.
(3) *Der p* I and *Der f* I (antigen P_1 of *Dp* and antigen F_1 of *D. farinae*) determination, expressed in micrograms per gram of house dust, with use of a two-site monoclonal antibody (MAb) ELISA (MAb 5H8 specific for *Der p* I and 6A8 specific for *Der f* I).[14]

Samples were collected according to a standardized method by the same technician with the same vacuum cleaner (PA5-800 W Philips, Gröningen, The Netherlands) and a constant vacuum time of 2 minutes per square meter. Dust samples were stored at 4° C in sealed plastic bags.

Twenty-three house dust samples (HDS) were collected from all patient mattresses and were analyzed by the three

TABLE II. Initial data of the active and placebo groups

		Active	Placebo
No.		11	13
Sex (M/F)		5/6	7/6
Age (yr)	m	36.8	35.4
	M	31	36
	CI	11	6.7
Duration of asthma (yr)	m	17.4	13
	M	10	11
	CI	10.6	6.4
Dp-specific IgE (RAST classes)	m	3.45	3.72
	M	3	4
	CI	0.3	0.25
Dp-intradermal tests ([D + d]/2) mm	m	11.8	14
	M	10	13
	CI	2.7	1.6

TABLE III. Initial data of the allergenic content of homes in the active and placebo group (μg/gm)

		Active	Placebo
Quantitative guanine levels in mattresses	m	8241	13692
	M	6890	9187
	CI	3314	4436
Der p I + *Der f* I in mattresses	m	43.53	84.66
	M	27.5	76.75
	CI	24.6	52.5
Der p I + *Der f* I in various upholstery elements	m	30.4	40
	M	14.7	33.8
	CI	31.5	18.4
Der p I + *Der f* I in carpets	m	4.95	13
	M	2.18	13.7
	CI	3.23	6.6

methods described: initially and every 3 months after acaricidal or placebo application.

Twenty-three HDS were collected from carpets (11 in actively treated homes, 12 in placebo-treated homes).

Fifteen HDS were collected from various upholstery elements (i.e., sofas, armchairs) (nine in actively treated homes and six in placebo treated homes).

The latter two groups of HDS, including potential house dust allergen sources besides the patient mattresses, were collected initially and after 12 months. They were analyzed by semiquantitative guanine determination and major allergen measurements.

Acaricide and placebo treatments

The acaricidal products were used as a foam or wet powder. Acarosan foam consists of a copolymer of benzyl benzoate (2.6%) and methacrylate, anionic tensides, fatty acids, perfume, propane-butane (13% as propellent), and water. The control foam consisted of tensids, perfume, propane-butane and water. The polymers are supposed to agglomerate particles. Acarosan powder consists of benzyl benzoate (5%), cellulose fibers, isoparaffin, sodium aluminum silicate, silicium dioxide, sodium chloride, an antimicrobial preserving agent, and water. The placebo powder consisted of cellulose, water, and an antimicrobial preserving agent. Acarosan foam as well as placebo foam were applied on all mattresses and upholsteries (60 gm/m^2) in each home. The foam was rubbed in, and the treated material was neither cleaned nor vacuumed for 1 week. The powder (acaricide or placebo) was sprinkled on all carpets and rugs in each home and distributed evenly with a scrubbing brush. After a drying period of 4 hours, the carpets were vacuumed. An initial acaricidal or control treatment was performed at the beginning of the trial; a second application was performed in the 23 homes after an interval of at least 6 months.

Statistics

A Mann-Whitney U test was used to assess the initial comparability of the acaricide and placebo groups in terms of sex, age, duration of asthma, clinical and drug scores, visual analog scale, functional scores (mean initial morning and evening PEFR, FEV_1, FEF 25-75), *Dp* RAST classes, and *Dp* skin tests (half sum of the cross diameters of the wheal for the intradermal reaction with an extract of 10^{-5} mg/ml).

To evaluate the differences in clinical and medication scores between the two groups at the beginning and after a period of 12 months we also used the nonparametric Mann-Whitney U test. For each group, initial scores and scores after 12 months were compared by a Wilcoxon test for matched pairs.

Both quantitative guanine determinations and the sums of *Der p* I and *Der f* I values were compared between the active and placebo groups (i.e., initial and final values after 12 months) by a Mann-Whitney U test. For each group, active or placebo, initial and final values were compared by a Wilcoxon test for matched pairs.

RESULTS

Three patients, initially allocated to Acarosan, were withdrawn from the trial: one patient changed his mattress after 3 months, and two did not stay continuously in the treated homes. These patients were not initially different from the other patients of the active group. The placebo-treated group (n = 12) and acaricide-treated group (n = 11) were similar in respect to age, sex, duration of asthma, clinical, medication, spirometric, and visual scores determined during the 1-month period before the beginning of the trial. The mean value of clinical, medication scores, visual an-

alog scale, and functional data are shown on Table I. Both groups were equally sensitive to *Dp* when they were compared by serial dilution intradermal skin testing and specific IgE measurements (Table II). The two groups were also compared as to their allergen exposure: despite patient selection based on the Acarex test, we found higher guanine and *Der p* I + *Der f* I contents among the placebo group, but the difference was only significant for the measured allergen level *Der p* I + *Der f* I in mattresses and carpets ($p < 0.05$) (Table III).

Within-group comparison

Effectiveness of the treatment on the patients' clinical and functional evolution (Table IV). The clinical scores established at the beginning of treatment and 12 months later showed a statistically significant improvement only for the actively treated group ($p < 0.01$). The visual analog scales showed a statistically significant difference between the beginning of the trial and after 12 months for the actively treated group ($p < 0.05$) and the placebo-treated group ($p < 0.01$). No difference could be found for the drug score. No statistical difference occurred between values at the beginning and 12 months after treatment, either in the actively treated group or in the placebo-treated group. A statistically significant increase was observed between the beginning of the trial and 12 months later in both groups for FEV_1 ($p < 0.05$ for the placebo group; $p < 0.01$ for the acaricide group), for FEF 25-75 ($p < 0.05$ for the placebo group and $p < 0.01$ for the acaricide-treated group). For the morning and evening PEFR values no statistically significant increase was observed.

Effectiveness of the treatment on the mite allergenic load (Table V, Fig. 1). The level of *Der p* I + *Der f* I in patient mattresses between the beginning of the trial (day 0) and after 12 months decreased 20% for the acaricide group and 17% for the placebo group, respectively. However, these decreases were not statistically significant. The decrease in guanine levels was significant only in the placebo-treated group (Fig. 2).

For HDS with origins other than the patient mattresses, we found significant decreases in *Der p* I + *Der f* I in the Acarosan-treated group ($p < 0.01$ for carpets; $p < 0.05$ for upholstery elements). The mean decrease in *Der p* I + *Der f* I levels was 74% of the initial level for carpets and 67% for upholstery elements in the active group. In placebo-treated houses the *Der p* I + *Der f* I content also decreased in the two different samples, but the decrease was not significant (i.e., only 27% for carpets).

TABLE IV. Results: percentage changes from baseline

	Within-group comparison		Between-group comparison
	Active	Placebo	
Clinical score	−45%**	−41% NS	NS
Drug score	−12% NS	−19.8% NS	NS
Visual score	+36%*	+46.6%**	NS
FEV_1	+14%**	+0.08%*	NS
FEF 25-75	+24.6%**	+12%*	NS
Mean morning PEFR	+0.05% NS	−0.014% NS	NS
Mean evening PEFR	+0.03% NS	−0.02% NS	NS

NS, No statistical significance.
*$p < 0.05$.
**$p < 0.01$.

TABLE V. Results: changes in the allergenic content of house dust over 1 year (percentages of the initial data)

	Within-group comparison		Between-group comparison
	Active	Placebo	
Quantitative guanine (mattresses)	−0.03% NS	−35%*	NS
Der p I + *Der f* I (mattresses)	−19.7% NS	−17% NS	NS
Der p I + *Der f* I (various elements)	−67%*	−61% NE	*
Der p I + *Der f* I (carpets)	−74%**	−27% NS	NS

NE, Not evaluable.
*$p < 0.05$.
**$p < 0.01$.

With respect to the Acarex classes, we found a decrease in Acarex test classes at the end of the study for both groups. The percentage of HDS in low classes

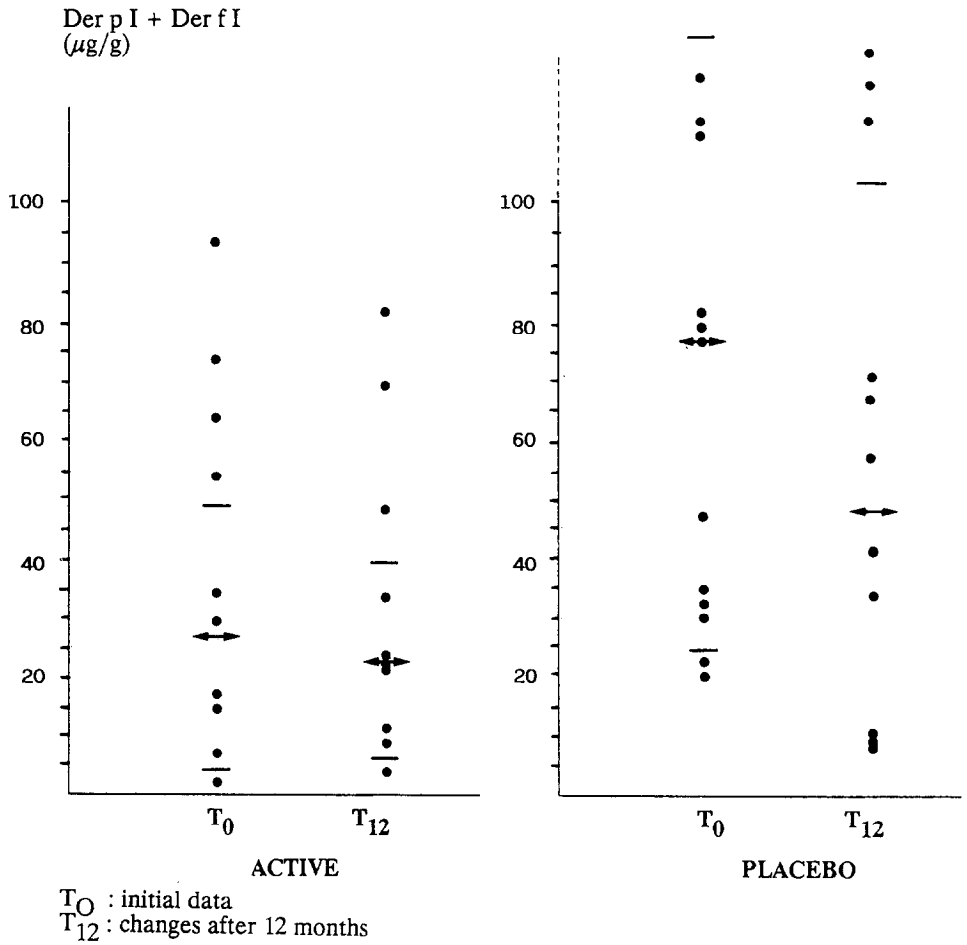


FIG. 1. Changes in the allergenic content of mattresses in the two groups.

(≤ +) was greater at the end of the study both in the placebo- and Acarosan-treated groups for the samples obtained from mattresses (i.e., from 12% initially to 32% in the placebo group and 52% in the Acarosan group), from carpets (i.e., from 60% initially to 72% in the placebo group and 82% in the Acarosan group), and from upholstery (i.e., from 24% initially to 72% in the placebo group and 90% in the Acarosan group) (Fig. 3).

Between-group comparison

The two groups did not differ significantly in the evolution of clinical scores assessed 1 month before the beginning of the trial and 12 months after treatment. Similar results were observed for the analog visual scales and the medication scores (Table IV).

The two groups showed no significant modifications in any of the functional values measured at the beginning of the trial and 12 months later (Table IV).

After 12 months, *Der p* I + *Der f* I levels in carpet dust tended to be lower in the treated group, but the difference did not reach statistical significance. No significantly lower allergen contents nor guanine levels were found in patients' mattresses for the treated and the placebo groups (Table V).

DISCUSSION

In this long-term study the application of Acarosan led to a significant decrease in major mite allergens only in carpets and upholsteries but not in patients' mattresses. The difficulty in obtaining a reduction in allergens in patient mattresses in our study could be explained by a high allergenic mite load in all the patient mattresses at the beginning of the trial, because we had selected the patients on the basis of their high Acarex test class (≥2). It can be suggested that these mattresses are true allergen reservoirs, and that repeated dust sampling removes mite feces accumulated deep into the mattress. Moreover, the acaricide may not have penetrated deep into the mattress, although studies have shown that no living mites were found after Acarosan treatment. Even if mites are killed, the

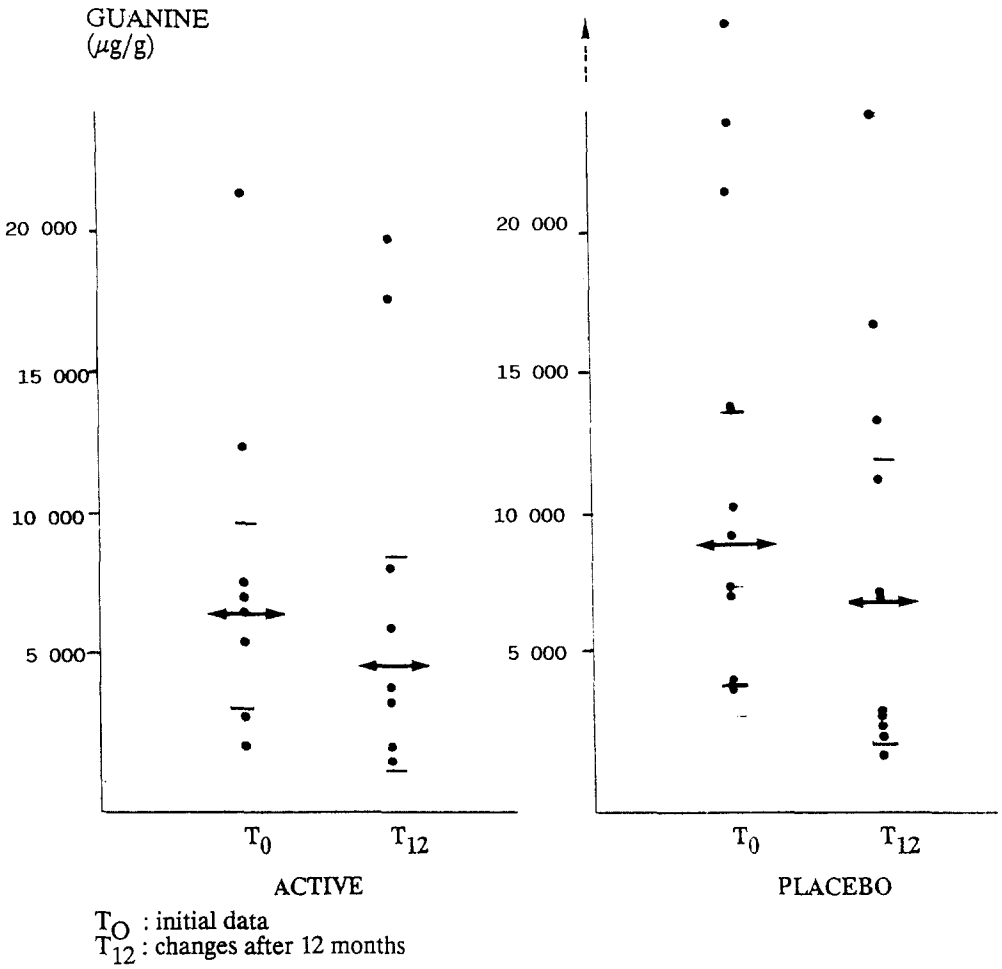


FIG. 2. Changes in the guanine levels of mattresses in the two groups.

allergenic load may not be reduced; such findings were observed earlier by Bischoff et al.[16] who demonstrated that the excrement content evaluated by Acarex test decreased in some cases but not in others. Because no effectiveness could be demonstrated on the mite allergen load of highly infested mattresses, we consider it advisable to replace or encase such mattresses. The greater percentage of mite allergen reduction in carpets and upholstery could be attributed to a lesser thickness of this material, which might favor the penetration of the acaricide foam or powder. It should also be noted that mite allergen concentrations were low in carpets at the beginning of the trial. The partial reduction obtained after a double treatment and regular vacuuming could have been improved if home cleaning and application of Acarosan had been prolonged, as has been shown by Le Mao et al.[17]

The decrease in guanine and major mite allergens in the placebo-treated homes may have several explanations: first, participation in the trial seems to have a guanine-reducing effect by itself, because the patients tend to vacuum more regularly. Second, cleaning agents present in the placebo contain tensioactive agents that could have bound dust and allergens from house dust, thus having an avoidance effect. It should also be pointed out that we evaluated the efficacy of acaricide treatment only by measuring the major allergen content of the HDS obtained by vacuuming the textile elements; however, we have no data concerning the amount of mite allergens extracted by vacuum from the mattresses, which would have been effectively rendered airborne by disturbances, nor do we have data concerning modifications of this amount by acaricide treatment. On the other hand, airborne sampling procedures recently proposed only measure the quantity of inhaled allergen at a given time[18]; the quantity measured strongly depends on disturbances, because it has been shown that mite allergens, contrary to cat allergens, are not airborne for long periods.[19] Measurement of major house dust allergens in dust samples is more representative of the total amount of mite allergens possibly accumulated over several

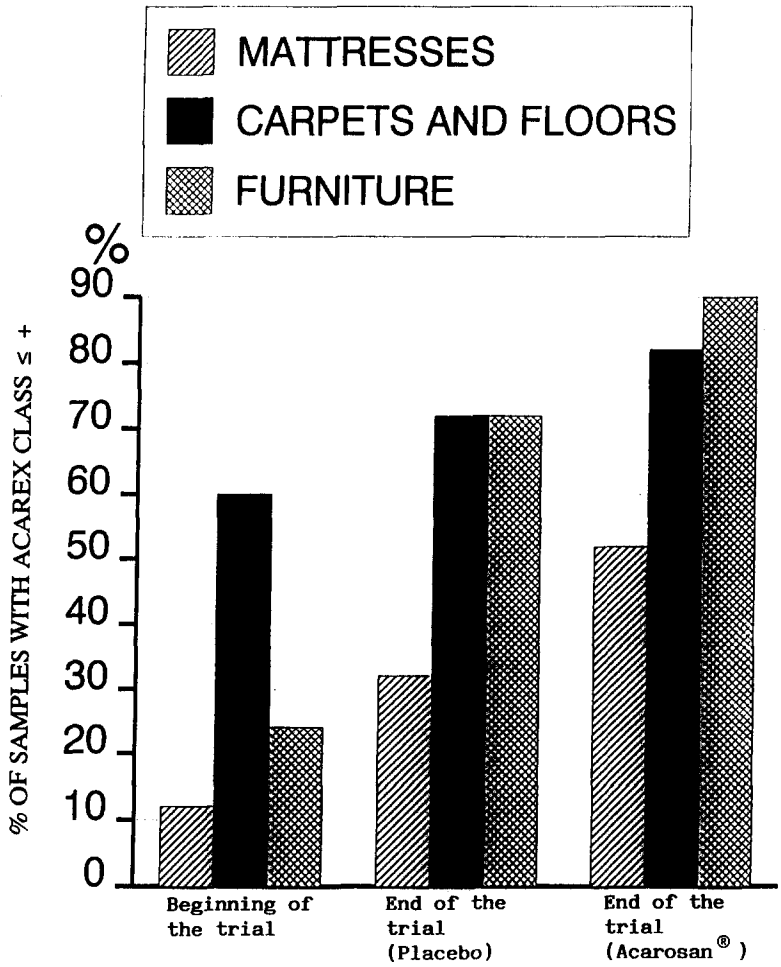


FIG. 3. Percentage of HDS with Acarex classes equal to or less than + at the beginning and at the end of the trial.

years; in our study the results were obtained by two different methods that reflect changes in allergen exposure,[20] and both showed a similar trend for active as well as placebo treatment.

The practicability of using Acarosan was not directly evaluated. Application of the acaricide products was always done by the patients' families and our technician; it seems that correct application on all textile elements in the entire home or flat represents a major task. The cost of these products may also limit their use.

The number of patients recruited for this trial was small, despite repeated efforts. There appear to be some explanations for this: it is obviously quite demanding for patients to participate in a prolonged avoidance trial. We also had to select patients having enough symptoms and medication, to appreciate an improvement, both in symptoms and medication scores. In our study asthma severity assessed by the measurement of FEV_1 and the duration of asthma was rather severe in both groups, with a more severe clinical trend for patients who had acaricide application in their environment. Such atopic asthmatic patients may not improve even after the cessation of exposure, as has been shown in occupational asthma. Even when clinical symptoms decreased and analog visual score increased, patients required medications that were not withheld because their functional values did not improve sufficiently. Moreover, nonspecific factors (i.e., irritating dusts, fumes, smoke, and cold air) may possibly be responsible for exacerbations. It is also worth noting that it is difficult to observe modifications in specific symptoms of house dust allergy. In our study, although patients were recruited over a 6-month period, all patients were followed during a period of at least 12 months. This allowed us to detect modifications of possible seasonal attacks that may occur in late summer or autumn.

The difficulties in correctly assessing modifications in symptoms and medication scores have been previously described.[21] In house dust mite allergic asthma, it appears essential to take into account several

parameters over a long period. A combination of clinical evaluation, medication scores, and functional measurements are needed because of the fact that perception of breathlessness, tolerance of discomfort, and the ability to record symptoms may vary according to patients.

Although house dust mite avoidance seems to be the treatment of the future, no consensus exists about which methods should be used. Several successful avoidance studies involved removal of carpets and stuffed toys, covering mattresses with plastic, or washing bedding regularly.[5, 6, 9, 22] Some authors have reported good results with acaricides; however, very few studies exist concerning the effects of these products both on the allergen mite load of the indoor environment and on the clinical status of patients with respiratory mite-allergic symptoms. The effect of acaricides on the environment was evaluated on the basis of the percentage of mite reduction: liquid nitrogen appears to be extremely effective in reducing living mites in mattresses[8]; by treating dwellings with Acarosan, Elixman et al.[23] and J. Le Mao et al.[17] obtained a dramatic reduction in living mites both in carpets and in most furniture 1 year and more after treatment. Instead of counting mites, several studies relied on either a guanine decrease or major mite allergen decrease. For pirimiphos methyl,[24] the *Der p* I reduction ranged from 50% to 75%. In an open study with Acarosan, Kersten et al.[25] found a decrease in Acarex test values after 3 months; however, the reduction was incomplete in mattress HDS. In a personal preliminary open study, we found a decrease in guanine levels in 7 out of 12 patients.[26] In a short-term study of Acarosan and placebo on mite allergen concentrations in house dust, Lau-Schadendorf et al.[27] showed a significant reduction of mite allergens in mattresses and carpets with both Acarosan and placebo treatments; however only the results of carpets in the group treated with Acarosan differed significantly from those of the control group. Our results show a comparable trend, because we were able to show an allergen reduction both with Acarosan and placebo, and the reduction was greater with Acarosan, especially for carpets; however, it did not reach statistical significance levels. It cannot be ruled out that several repeated applications of Acarosan would remove additional mite allergens because of the mite-killing effect of Acarosan, whereas cumulated allergen loads would slowly decrease as a result of repeated vacuuming. However, it should be pointed out that even in homes in which we observed a mite allergen reduction, the final mite allergen concentration was always greater than 10 μg/gm.

Concerning efficacy as expressed by the clinical status of patients, few studies have been performed in a controlled double-blind fashion.[28, 29] Open studies appeared promising.[25, 26] In a double-blind, dust mite, Acarosan trial with patients with rhinitis who are allergic to mites, an improvement was found in 8 out of 10 patients treated with Acarosan and in 4 out of 10 treated by placebo.[29] In our study we did not find a statistically superior effect of Acarosan versus placebo. However, the possibility that more frequent vacuuming and tensides contained in the placebo are also effective in allergen avoidance cannot be ruled out; this could have concealed a positive effect of the acaricide used.

The indications for acaricide treatment should take into account both the mite allergenic load and the asthmatic status of the patients. Patients with continuous corticosteroid treatment, presenting a long duration of asthma, as well as permanently altered pulmonary function, are probably not ideal patients for such avoidance trials. For some patients, despite an objective decrease in the mite allergen content of their homes, the residual mite allergen load is sufficient to render asthma symptoms perennial. Sensitization is thought to occur mostly at an early age. Evidence from a prospective study suggests that high-level exposure within the first 2 years of life may be highly significant for both sensitization and the risk of asthma.[30] Thus it appears that the best indication for acaricide treatment is probably atopy in young children. It may also be of interest to preventively treat textile objects with acaricides, especially new mattresses, to prevent infestation by mites and new allergen production.

Finally, it is possible that acaricide treatments, conventional dust mite avoidance, hygienic measures, and mite desensitization need to be combined to increase the effectiveness of the fight against mites.

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REFERENCES

1. Platts-Mills TAE, De Weck AL. Dust mite allergens and asthma. A worldwide problem. J ALLERGY CLIN IMMUNOL 1989;83:416-27.
2. Platts-Mills TAE, Mitchell EB, Nock P, Tovey ER, Mozzoro H, Wilkins S. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982;2:675-8.
3. Vervloet D, Penaud A, Razzouk H, et al. Altitude and house dust mites. J ALLERGY CLIN IMMUNOL 1982;69:290-6.
4. Korsgaard J. Preventive measures in house dust allergy. Am Rev Respir Dis 1982;125:80-4.
5. Murray AB, Ferguson AC. Dust free bedroom in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. Pediatrics 1983;71:418-22.
6. Walshaw MJ, Evans CL. Allergen avoidance in house dust mite-sensitive adult asthma. Q J Med 1986;226:199-215.
7. Burr ML, Dean BV, Merrett TG, Neale E, St Leger AS, Verrier-Jones ER. Effects of anti-mite measures on children with mite sensitive asthma: a controlled trial. Thorax 1980;35:506-12.

8. Dorward AJ, Colloff MJ, Mac Kay NS, Thomson NC. Effect of house dust mite avoidance measures on adult atopic asthma. Thorax 1988;43:98-102.
9. Sarsfield JK, Gowland G, Toy R, Norman ALE. Mite-sensitive asthma in childhood: trial of avoidance measures. Arch Dis Child 1974;49:716-21.
10. Le Mao J, Weyer A, Pauli G, Lebel B, David B. Studies on *Dermatophagoides pteronyssinus* allergens: measurement of the relative potencies of *Dermatophagoides pteronyssinus* purified extract by in vitro and in vivo methods. J ALLERGY CLIN IMMUNOL 1980;65:381-8.
11. Bischoff E, Schirmacher W. Farbnachweis für allergenhaltigen Hausstaub. 1. Mitteilung. Allergologie 1984;11:446-9.
12. Pauli G, Hoyet C, Tenabene A, Le Mao J, Thierry R, Bessot JC. Guanine and mite allergenicity in house dust. Clin Allergy 1988;18:383-90.
13. 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;83:926-33.
14. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-side monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp allergens *Der p* I and *Der f* I. J Immunol Meth 1989;118:227-35.
15. Siegel S. Non parametric statistics for the behavioral sciences. New York: McGraw-Hill, 1956.
16. Bischoff E, Schirmacher W. Investigations of allergen containing dust samples from the interior of the house. In: Boehm G, Leuscher RM, eds. Advances in aerobiologie. Basel: Birkhauser, 1987:189-96.
17. Le Mao J, Liebenberg B, Bischoff E, David B. Changes in mite allergen levels in homes using an acaricide combined with cleaning agents: a three-year follow up study. Indoor Environ 1992;1:212-8.
18. Price JA, Pollock I, Little SA, Longbottom JL, Warner JO. Measurements of airborne mite antigen in homes of asthmatic children. Lancet 1990;336:895-7.
19. de Blay F, Heymann PW, Chapman MD, Platts-Mills TAE. Airborne dust mite allergens: comparison of group II allergens with group I mite allergen and cat-allergen *Fel d* I. J ALLERGY CLIN IMMUNOL 1991;88:919-26.
20. Hoyet C, Bessot JC, Le Mao J, Quoix E, Pauli G. Comparison between *Der p* I plus *Der f* I content determinations and guanine measurements in 239 house dust samples. J ALLERGY CLIN IMMUNOL 1991;88:678-81.
21. Pauli G, Bigot H, Pelletier A, Kopferschmitt-Kubler MC, Roegel E. A study of the criteria used for clinical evaluation of prophylactic treatment in bronchial asthma. Eur J Respir Dis 1985;67:369-77.
22. Owen S, Morganstern M, Hepworth A, Woodcock A. Control of house dust mite antigen in bedding. Lancet 1990;335: 396-7.
23. Elixman JH, Bischoff E, Jorde W, Linskens HF. Einmalige Acarosan-Applikation zur Sanierung von Wohntextilien in Haushalten von Patienten mit Milbenallergie. Allergologie 1988;7:274-9.
24. Mitchell EB, Wilkins S, Deighton J, Platts-Mills TAE. Reduction of house dust mite allergen levels in the home: use of the acaricide, pirimiphos methyl. Clin Allergy 1985;15:235-40.
25. Kersten W, Stollewerk D, Musken H. Klinische studie zur wirksamkeit der akariziden Substanz Acarosan bei Hausstaubmilbenallergiken. Allergologie 1988;11:371-90.
26. Pauli G, Dietemann A, Hoyet C, Eckert F, Bessot JC. Effets d'un nouvel acaricide sur less symptômes des asthmatiques allergiques aux acariens et influence sur l'environnement domestique. Rev Fr Allergol 1989;29:175-80.
27. Lau-Schadendorf S, Rusche AF, Weber AK, Buettner-Goetz P, Wahn U. Short-term effects of solidified benzyl benzoate on mite allergen concentration in house dust. J ALLERGY CLIN IMMUNOL 1991;87:41-7.
28. Charpin D, Birnbaum J, Haddi E, N'Guyen A, Fondarai J, Vervloet D. Evaluation de l'efficacité d'un acaricide Acardust, dans le traitement de l'allergie aux acariens. Rev Fr Allergol 1990;30:149-55.
29. Kniest FM, Young E, Van Praag MCG, et al. Clinical evaluation of a double-blind dust-mite avoidance trial with mite-allergic rhinitic patients. Clin Exp Allergy 1991;21:39-47.
30. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell J. Exposure to house dust mite allergen (*Der p* I) and the development of asthma in childhood: a prospective study. N Engl J Med 1990;323:502-7.