

Short-term effect of solidified benzyl benzoate on mite-allergen concentrations in house dust

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*The effect of solidified benzyl benzoate (bb) foam and powder on concentrations of the major allergens of *Dermatophagoides pteronyssinus* (Der p I) and *D. farinae* (Der f I) in dust samples from mattresses and carpets in 22 households was investigated in a randomized, double-blind, placebo-controlled trial. All houses had been demonstrated to have significant (≥ 2000 ng/gm of dust) mite-allergen concentrations on mattresses. Dust samples were collected from mattresses and carpets before treatment and on days 10, 30, and 60 after the first treatment. There was a second application on day 10. Der p I and Der f I were determined by a sandwich-type ELISA with monoclonal noncross-reacting antibodies to Der p I and polyclonal monospecific antibodies to Der f I. There was a significant reduction of mite allergens in mattresses and carpets within both bb-treated and control groups, but only the results on carpets of the bb-treated group were significantly different from results of the control group on days 30 and 60 ($p < 0.05$). (J ALLERGY CLIN IMMUNOL 1991;87:41-7.)*

For more than 20 years, house dust mites of the genus *Dermatophagoides* have been known to be associated with allergic airway disease.¹ It could be demonstrated that mite-allergic patients improve clinically if they move to an area with low or no mite-allergen exposure²⁻⁴ or if mite-avoidance procedures were advocated.^{5, 6}

Mite-avoidance measures, such as vigorous vacuum cleaning, the use of washable synthetic bedding encasings, or the removal of carpets and soft furnishings may have some effect^{4, 5, 7} but mostly do not achieve sufficient mite and mite-allergen reduction.⁸⁻¹⁰

There have been attempts to change the microclimate by killing molds that favor mite growth^{11, 12} or by controlling humidity, which is expensive and technically difficult.^{5, 9}

Several chemicals have been examined for their acaricidal effect. There have been studies on the effectiveness of acaricides, such as paragerm,¹³ lin-

Abbreviations used

Der p I: Antigen P₁ of *Dermatophagoides pteronyssinus*

Der f I: Antigen F₁ of *Dermatophagoides farinae*

LD₅₀: Lethal dose that kills 50% of laboratory animals

bb: Benzyl benzoate

dane^{14, 15} (the use was discouraged because of potential toxicity), pirimiphos methyl,^{15, 16} tannic acid,¹⁷ and even liquid nitrogen.^{6, 18} Recently the mite-reducing effect of bb-impregnated sheets¹⁹ and sprays containing benzyl derivatives²⁰ has been reported.

In Europe, sprays and powders containing bb have been produced, but at this time, no randomized, placebo-controlled study has been made. However, some preliminary data indicate mite-allergen controlling effects.^{21, 22}

We investigated the short-term efficacy of solidified bb-containing foam and powder when these are applied to mite-infested mattresses and carpets in a randomized, double-blind, placebo-controlled study.

MATERIAL AND METHODS

Dust collection and application of solidified bb

Twenty-two homes of atopic children from our outpatient clinic with significant mite-allergen concentrations in mattress-dust samples (≥ 2000 ng of Der p I plus Der f I per gram of dust) were included in our study. (These dust

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TABLE I. Statistical evaluation within bb-treated and control group at four different days

		<i>Der p 1 + Der f 1</i> (ng/gm dust)		
		Median	SD	Range
bb				
Mattress				
Day				
0		31,480	40,347	3,975-135,350
10		17,000*	33,653	2,075-120,142
30		12,800†	60,324	1,395-210,150
60		9,050†	42,724	1,880-150,145
Carpet				
Day				
0		16,900	22,267	415-73,500
10		7,387	28,724	200-90,000
30		3,550†	7,578	90-21,125
60		5,950†	8,355	135-25,500
Control group				
Mattress				
Day				
0		24,700	40,116	1,450-120,750
10		28,187	34,211	1,460-107,250
30		17,875†	22,035	1,420-65,000
60		14,700	20,869	1,819-56,300
Carpet				
Day				
0		2,221	6,460	164-18,532
10		2,209	6,898	180-20,776
30		575†	3,434	145-8,500
60		962†	5,786	63-17,030

SD, Standard deviation.

* $p < 0.01$, significance in mite-allergen decrease compared to day 0 ("within-group comparison").† $p < 0.05$, significance in mite allergen decrease compared to day 0 ("within-group comparison").

samples were collected and investigated in spring 1988.) When we investigated the dust samples collected on day 0 at the beginning of our study, 21 of 22 mattress-dust samples still revealed a mite-allergen concentration ≥ 2000 ng/gm of dust; in one sample we could only find 1450 ng of *Der f 1* per gram of dust (No. 5 of the control group).

Parents of the patients were informed about the aim of our study and gave consent. Study period was August to September 1988. Each home was visited four times (days 0, 10, 30, and 60) by the same investigator. Dust samples were collected from the mattress and carpet of the child's room with the same vacuum cleaner (800 W) and new disposable paper dust bags. Vacuuming time was 5 minutes. Mattress surfaces (2 m²) and 4 m² of floor were vacuumed. For repeat sampling, a note was made of the location of floor area sampled, and a sample was obtained approximately in the same floor area at the next visit. Carpets and mattresses were vacuumed by the families between the experimental vacuuming times. Dust samples were kept at 4° C until analysis. Humidity and temperature of the child's room were recorded every week by parents.

The 22 homes were randomly allocated into two groups

of 11 children. Carpets were only treated in eight homes of each group. (Four families, two in each group, did not have any carpet in the child's room; two families, one in each group, removed the carpets during the study period.) Mattresses were treated with a foam (60 gm/m²) and carpets with a powder (60 gm/m²) in a double-blind fashion on days 0 and 10. After 4 hours, residues were removed by vacuuming. Foam and powder were provided by Werner & Mertz GmbH, Mainz, West Germany (Acarosan). The bb foam (2.6% concentration of solidified bb) also contained methacrylate-copolymerisate, tensides (sodium lauryl sulfate and olefine sulphonate), fatty acids, sodium hydroxide, antimicrobial preserving agent (1,2 benzisothiazoline-3), propane/butane, and water. The polymers are supposed to agglomerate allergenic particles. The bb powder (5% concentration of solidified bb) also contained cellulose, isoparaffin, sodium aluminum silicate, silicon dioxide, sodium chloride, antimicrobial preserving agent (1,2 benzisothiazoline-3) and water. The placebo foam contained sodium hydroxide, water, fatty acids, and perfume. The placebo powder contained cellulose, water, and antimicrobial preserving agent.

BB and Acarosan toxicity

BB is considered to be nontoxic (single oral dose LD_{50} : 2.8 gm/kg in rats,²³ and dermal LD_{50} : 4 ml/kg in rats).²⁴ The chemical is well-known for the treatment of scabies, and few side effects have been reported.²⁵ In animal models, no sensitization to bb could be demonstrated.¹⁵ The other components of spray and powder were investigated for sensitizing and irritating properties. Sodium lauryl sulfate, isothiazoline, and monomeric methacrylate copolymerisate can cause sensitization.²⁶ These data can be found in a study of the product, Acarosan, published by Werner & Mertz.

Indoor humidity and temperature

Mean indoor relative humidity and indoor temperature were comparable in both groups. (Mean relative humidity was 64% in the bb-treated group, and mean temperature was 21° C; in the placebo-treated group, the values were 63% and 22° C, respectively.)

Dust extraction

Dust extracts were obtained by agitation for 3 hours at room temperature, 1:5 (wt/vol) in 0.125 mol/L of ammonium hydrogen carbonate containing 0.1% sodium azide. After centrifugation, the supernatants were filtered (220 nm pore diameter, Millex GV, Millipore Ltd. Harrow, Middlesex, U.K.) and stored at -20° C.

House dust-mite ELISA

Precoated polystyrene microtiter trays and reagents for sandwich ELISA were obtained from ALK, Copenhagen, Denmark. For assay of *Der p* I, plates coated with monoclonal, noncross-reacting antibodies²⁷ and horseradish peroxidase-conjugated, species-specific, rabbit antibodies to *Der p* I were used. The *Der f* I assay was a combined determination of the two species, *D. farinae* and *D. microceras* of the *D. farinae* group. The rabbit antibodies used (coating as well as horseradish peroxidase-conjugated antibody) were anti-*Der f* I, immunoabsorbed on a *D. pteronyssinus*-Sephadex column and further affinity purified by acid elution from a *Der m* I-Sephadex column. The assay was performed as described by Lind.²⁸

In brief, 100 µl of dust extract and dilutions were added to the precoated wells for overnight incubation, followed by repeated washings. After a 1-hour incubation with enzyme-conjugated antibody and repeated washings, substrate (hydrogen peroxide/orthophenylene amine) was added to each well, and wells were left for 15 minutes in darkness. Absorption at 492 nm was measured 5 minutes after addition of 1.0 mol/L of sulphuric acid (Titertek Multiskan MCC 340, Flow Laboratories, Rickmansworth, Hertfordshire, England). Standard response curves were plotted from reference antigen solutions of each species with concentrations of major allergens in the range 1.6 to 400 ng/ml (internal standards of ALK; the *Der p* I standard related to National Institute of Biological Standards and Control code 82/518.²⁹ Results were expressed as nanograms per gram of dust. Assay sensitivity was 5 ng/ml or 15 ng/gm of dust. Previous experiments demonstrated an

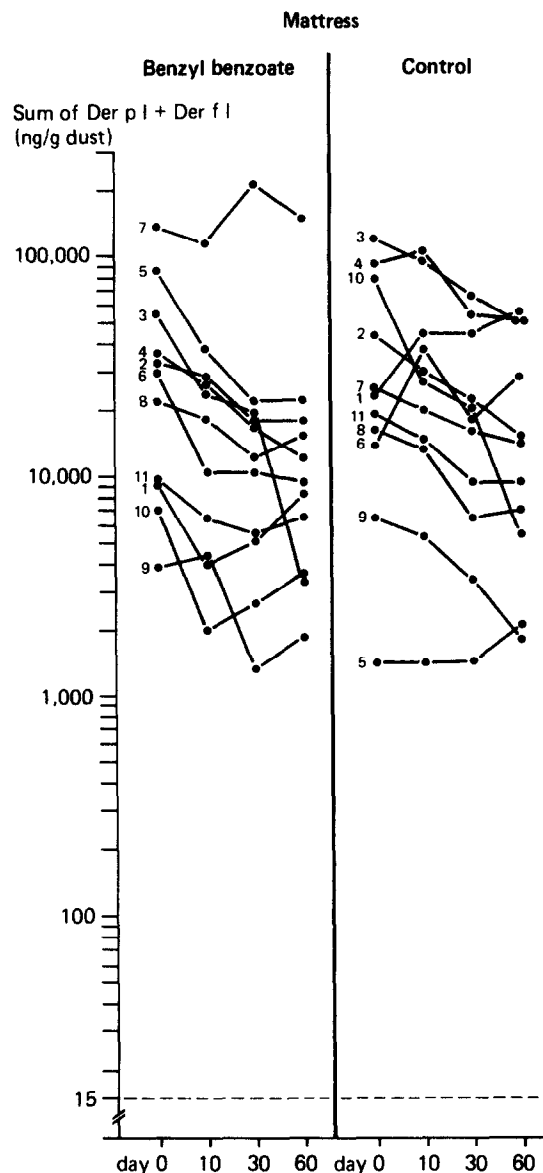


FIG. 1. Mite-allergen concentrations (sum of *Der p* I plus *Der f* I) on mattresses (day 0, 10, 30, and 60) in the bb-treated group and control group.

interassay coefficient of variation of 17% to 25% and an intra-assay coefficient of variation of 5% to 10%. Every analysis was carried out in duplicate and was repeated on 3 separate days. Means of the results were taken for statistical analysis.

Statistics

The allergen levels on days 0, 10, 30, and 60 were compared within each group with Wilcoxon matched-pairs signed-ranks test (within-group comparison). To assess statistically significant differences between the placebo-treated and bb-treated group, we subtracted the allergen levels on days 10, 30, and day 60, respectively, from day 0 in each

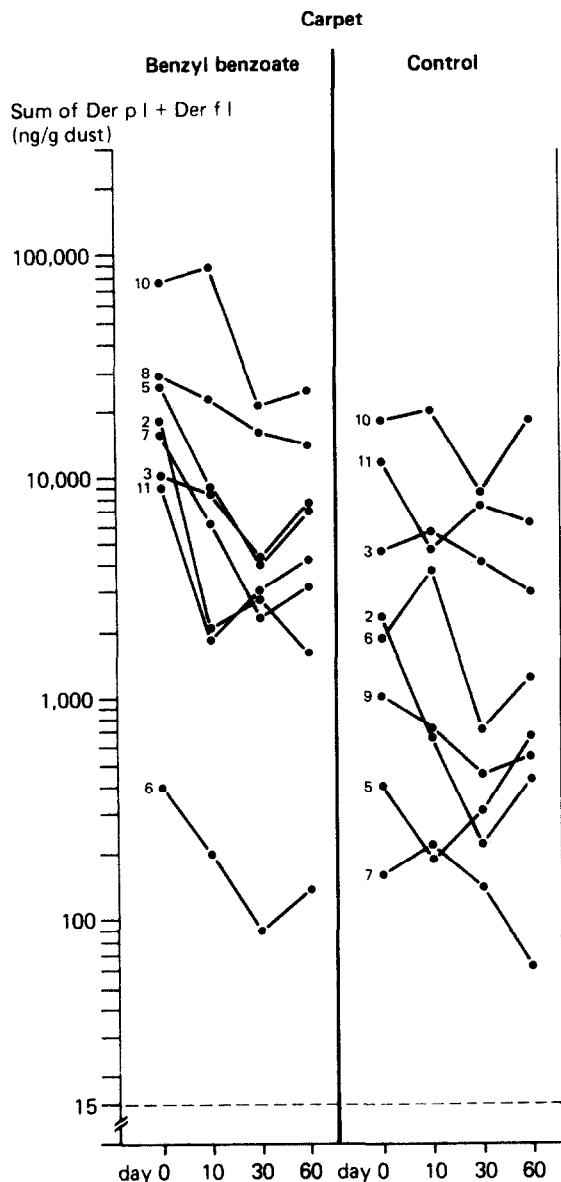


FIG. 2. Mite-allergen concentrations (sum of *Der p I* plus *Der f I*) on carpets (days 0, 10, 30, and 60) in the bb-treated group and control group.

group and analyzed the results with Mann-Whitney U-Wilcoxon test (between-group comparison). Furthermore, we calculated the area under median curves in the two groups. For evaluation, *Der p I*, *Der f I*, and the sum of both allergens were considered. Statistical significance was assumed at $p < 0.05$.

RESULTS

The mite-allergen levels in the two groups on the various days are presented in Table I. The median values for the sum of *Der p I* plus *Der f I* were comparable for mattress dust in both groups (31,480

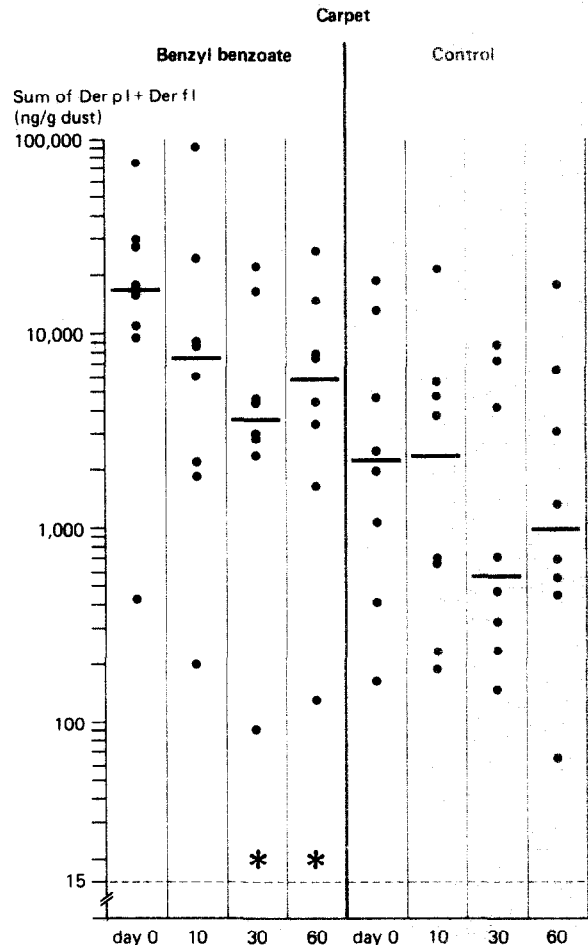


FIG. 3. Comparison between mite-allergen concentrations (sum of *Der p I* plus *Der f I*) and medians for carpets on day 0 and days 10, 30, and 60 in bb-treated and control groups. Asterisks indicate the significance in mite-allergen decrease compared to day 0; * $p < 0.05$ (between-group comparison: bb-treated group versus control group).

ng/gm of dust in the bb-treated group versus 24,700 ng/gm of dust in the control group), whereas carpets in the control group demonstrated lower concentrations of mite allergen than carpets of the bb-treated group (medians 16,900 ng/gm versus 2221 ng/gm). This finding was due to the fact that homes were selected only according to their allergen exposure in mattress dust samples. In the control group, there was an increase in mite allergen on mattresses in homes Nos. 1 and 6 and on carpets in homes Nos. 5 and 6 (No. 6 only on day 10).

Within-group comparison

When data within each group were evaluated individually, there was a statistically significant decrease of mite-allergen concentrations within each group comparing day 0 with day 30 and day 60

TABLE II. *p* Values for the significance of mite-allergen decrease (*Der p* I, *Der f* I, and both allergens) in the bb-treated group

	Day		
	10	30	60
Mattress			
<i>Der p</i> I	ns	ns	ns
<i>Der f</i> I	ns	ns	ns
<i>Der p</i> I + <i>Der f</i> I	ns (<0.08)	ns	ns
Carpet			
<i>Der p</i> I	ns	ns (<0.06)	ns
<i>Der f</i> I	ns	ns (<0.07)	<0.05
<i>Der p</i> I + <i>Der f</i> I	ns	<0.05	<0.05

ns, Not significant ($p \geq 0.05$).

Mite-allergen decrease was calculated compared to day 0 (between-group comparison: bb carpet-treated group versus control group).

($p < 0.05$). This was not the case for *Der p* I on carpets on day 60, *Der f* I on mattresses (days 30 and 60) and on carpets (day 60), and for the sum of both allergens on mattresses (day 60) in the control group. The *p* values of significances are presented in Table I.

On day 10, there was a significant decrease in mite-allergen concentration in mattress-dust samples of the bb-treated group ($p < 0.01$). Median mite-allergen concentrations (sum of *Der p* I plus *Der f* I) on mattresses were lower on day 60 compared to day 30. On carpets, mite-allergen concentrations were slightly higher on day 60 compared to day 30. These differences were not significant and could be observed in both groups. Mite-allergen concentrations of the two groups for mattress and carpet dust, respectively, are illustrated in Figs. 1 and 2. Both groups demonstrate similar curves. In the bb-treated group, there was a reduction ranging from 0% to 74% for mattresses and from 46% to 86% for carpets. In only one case was there no reduction of mite allergen in mattress dust. In the control group, percentage of mite-allergen reduction (sum of *Der p* I plus *Der f* I) ranged from 0% to 75% on mattresses and from 23% to 91% on carpets. We observed no reduction on three mattresses.

Between-group comparison

We compared the values of mite-allergen decrease between the two groups. Significantly lower allergen contents (sum of *Der p* I plus *Der f* I) were only found for carpets in the bb-treated group (Fig. 3) on days 30 and on day 60 ($p < 0.05$) (Table II) compared to day 0. Evaluating *Der p* I and *Der f* I alone, only *Der f* I in carpet dust was significantly lower in the bb-treated group compared to the control group on day 60 ($p < 0.05$).

On day 10, the sum of both allergens in mattress dust and *Der p* I and *Der f* I in carpet dust on day 30 tended to be lower in the bb-treated group, but the difference did not reach statistical significance (Table II).

DISCUSSION

In our study solidified bb was more effective than placebo for both allergens only on carpets on days 30 and 60.

It is obvious that placebo treatment itself achieved a certain amount of mite-allergen reduction on days 30 and 60, which might be explained by the fact that participating in a study has an effect itself. Furthermore, the placebo products contained substances such as tensides that could have bound allergens and had some cleaning effect. The placebo spray, but not the placebo powder, contained a tenside that was indispensable for the production of foam. This could explain the poor results obtained by the spray containing bb compared to the placebo spray.

Vacuuming cleaning alone did not reach significant mite-allergen reduction (unpublished data), which is in agreement with other authors.¹⁰

On day 10, there was only a significant decrease in mite-allergen concentration in mattress dust of the bb-treated group comparing day 0 and day 10 within one group. Comparing both groups, this reduction did not reach statistical significance. The conclusion of these data should be made very carefully because of the small number of households investigated in this study. The results might have been more significant for bb treatment with a larger number of cases. Obviously, there are differences in results between mattresses and carpets. The products containing bb appeared to be more effective on carpets than the placebo products

after the second treatment. A significant effect on carpets lasted at least for 30 days of the study period. The reduction on day 60 was as significant as on day 30, although mite-allergen levels appeared to increase again (not statistically significant). Mite-allergen reduction was more difficult on mattresses than on carpets in this study. This finding might be due to the thickness of mattresses that impairs the rate of penetration of the spray. Our data indicate that, after the first treatment, the bb-treated mattresses tended to reveal significantly lower mite allergen on the mattress surface, but probably the spray only reaches the surface. After a short time period, mites and mite allergen from inside the mattress can be found again on the surface. Mattresses might need greater quantities of acaricides applied to reach every fiber. The percentage of mite-allergen reduction on carpets is also dependent on the length of pile and type of fiber. We did not take the different materials of carpets into consideration, which could have influenced our results. At the beginning of the study, mite-allergen concentrations on carpets were lower in the control group than in the bb-treated group. We are not sure whether this finding also may have influenced the outcome of the trial.

We did not standardize the frequency of vacuuming by the families between the experimental vacuuming times because we knew the patients usually did not obey too strict rules in previous studies. This finding also represents an unknown influencing factor. Finally, the seasonal fluctuations also might have played a role; the time that we started the treatment, the mite number could have been so high that killing of mites caused an increase of mite-allergen first, and the mite-reducing effect might be observed in the next mite generation.

Seasonal variation might also explain the increase in mite allergen in some homes of the control group.

The time for our second treatment was chosen according to the advice of the producer of the sprays and powders. Other groups suggested the best time for respraying would be 4 to 6 weeks after the initial treatments, before numbers of mites from eggs that had not been destroyed began to increase.²⁰

Other authors reported good results for bb-impregnated sheets¹⁹ or sprays containing benzyl derivate²⁰ and for the solidified bb-containing product tested in our study.^{21, 22} However, the latter studies were not controlled.

The degree of mite reduction in these trials ranged from 20%¹⁹ to approximately 90%.²⁰ In one of these studies,²⁰ a spray containing benzyl derivate and polyphenol was used, which not only kills the mites but also destroys allergenic material by chemically altering it. The reduction of up to >90% was achieved

after 4 weeks with one treatment, and the mite number was increasing again after 16 weeks, similar to our study in which the allergen concentrations tended to increase on day 60. For pirimiphos methyl, the *Der p* 1 reduction ranged from 50% to 75%, lasting from week 2 to week 6 after a single application¹⁶; for liquid nitrogen, there was a decrease in the number of living mites of about 88% after one treatment, comparing week 1 with week 5.¹⁸

In central Europe, mite-allergen concentrations of *Dermatophagoides* are frequently found to be between 20,000 and 200,000 ng/gm of dust.^{30, 31} To achieve a reduction that is relevant for the risk of sensitization, an effective acaricide should eliminate at least 90% to 99% of mite allergen to reduce mite-allergen contents lower than a concentration of 2000 ng/gm, which has been demonstrated to be the threshold above which the sensitization risk significantly increases for atopic individuals.^{30, 32} In our trial, even in houses in which a certain percentage of mite-allergen reduction was achieved, significant (≥ 2000 ng/gm of dust) or high ($\geq 10,000$ ng/gm of dust) mite-allergen concentrations still could be found.

Although bb was not found to have this degree of efficacy, long-term studies should be performed to study the effect of bb after longer treatment periods. Bronchial hyperreactivity should be monitored to assess the potentially irritating effect of bb itself and to control clinically the mite-reducing effect of the acaricide.

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