

Does benzyl benzoate prevent colonization of new mattresses by mites?

A prospective study*

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In 12 house-dust-mite-infested double beds, one of the mattresses was replaced by a new one, the other being regarded as a mite source. All new mattresses were treated in a double-blind fashion, with either benzyl benzoate or placebo before being placed on the bed as well as 1 year later. They were examined for mites and allergen concentrations over a period of 18 months. This period of time covered two mite seasons. Dust samples were taken bimonthly and analyzed by guanine test strip, microscopic mite counting, and determination of the mite allergens Der p 1 (*Dermatophagoides pteronyssinus*) and Der f 1 (*D. farinae*) by ELISA. Although, at the end of the observation period, the new mattresses still had significantly lower mite and allergen levels than the old mattresses, there were no significant differences between the placebo and the benzyl benzoate groups. In our setting, benzyl benzoate plus frequent cleansing was not significantly more effective in controlling mites than frequent cleansing alone.

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House-dust mites produce one of the most important groups of indoor allergens. Domestic furnishings, according to the Western lifestyle, favor the multiplication of mites.

Many measures proposed to reduce the domestic mite allergen burden have been ineffective so far. (1, 2). Benzyl benzoate (BB) has been proven to be acaricidal *in vitro* (3). According to all published data, its toxicity in low concentrations is tolerable by man (4–8). Therefore, it seemed to be a highly promising agent for the prevention of mite-allergen exposure. Preliminary results with a formulation of BB combined with cleansing tensides have strengthened this expectation (9, 10). Besides minimizing a given allergen burden, it may also protect new domestic goods against mite infestation.

We therefore performed a double-blind, placebo-controlled study in order to determine whether this BB formulation (9, 10) could prevent mite

infestation of new mattresses in homes favorable for mites.

Material and methods

Conception of the study

The basic idea of the study was to look for homes with matrimonial double beds infested by mites and to replace one mattress with a new one prophylactically treated with an acaricide. The other, old mattress was left in place as a source of mites.

Selection of homes

Homes and beds were selected by the guanine strip test. A bed had to test positive, preferably "medium" or "strong" in degree. Altogether, 12 homes were selected in spring 1989, six in Berlin, northeastern Germany, and six in Tübingen, southwestern Germany.

In each subregion, the mattresses were allocated at random to the BB and placebo groups. The code

*This study includes data from the dissertations of I. Focke and A. K. Weber.

was not revealed until all measurements of dust samples were concluded.

Acaricidal treatment of mattresses

The study started in early summer 1989 and lasted until late autumn 1990, covering two mite seasons. One new foam-rubber mattress was placed in each matrimonial bed, unwrapped, and covered only by a sheet. These *new* mattresses were treated four times with BB: before being placed, 1 month later, and twice again with a monthly interval in summer 1990. The foamy acaricidal product (Acarosan) was supplied by the manufacturer (Werner & Mertz GmbH, Mainz, Germany), the active substance and placebo being packed in identical spray canisters. The active substance consisted of the acaricidal part (7.1% BB combined with acrylates), the cleansing and foam-producing part (1.5% anionic tensides), perfume, and 91.4% water. The placebo contained only water, tensides, and perfume and was visually and aromatically identical to the active foam. According to the manufacturer's recommendations, the whole content of one spray can (300 ml) was used for each mattress treatment, the mattresses amounting to a surface of 4–5 m² each.

Dust samples

Dust samples were taken from the old, untreated mattresses before and at the end of the study. The new mattresses were vacuum-cleaned for samples before placement and every 2 months until the end of the study. All samples were taken by one person with one vacuum cleaner each in each location. Every time both large surfaces of the mattresses were vacuum-cleaned as follows: the covering cloth for 1 min, and the uncovered surfaces for 2 min. Each mattress sample was collected in a separate, new vacuum-cleaner bag.

Analysis of dust samples

The dust samples were analyzed by three methods: mite counting was performed according to the flotation method described by van Bronswijk (11). All organisms clearly recognizable as mites were counted. We did not distinguish between different species, and viability was not taken into account. Mites were counted by one person. The guanine test strip system (Acarex), an indirect semiquantitative indicator of mite allergens, has been described elsewhere (12). It was applied according to the manufacturer's recommendations (Werner & Mertz GmbH, Mainz, Germany). Briefly, the results were expressed in four test classes: 0=*negative*,

meaning a guanine concentration of less than 0.6 mg/g dust, 1=*weak* for $>0.6 < 2.5$ mg/g, 2=*medium* for $>2.5 < 10$ mg/g; and 3=*strong* for >10 mg/g. If a color tone could not be assigned to a test class exactly enough, it was located intermediately as an 0.5 value.

The allergen ELISA was performed in a sandwich technique with monoclonal monospecific Der p 1 (*Dermatophagoides pteronyssinus*) antibodies and polyclonal immunoadsorbed, non-cross-reacting Der f 1 (*D. farinae*) antibodies in the first capture layer, and polyclonal anti-Der p 1 and anti-Der f 1 in the second layer, as described elsewhere in detail (1). For statistical analysis, the allergens Der p 1 and Der f 1 were summed together. The guanine test was applied in the homes immediately after dust collection. The rest of the dust samples were divided into two samples each. For mite counting and ELISA alike, both parts were stored at 4°C in plastic bags for further assessments. Mites were counted some days later, whereas the dust sample for the ELISA was extracted in 0.125 mol/l ammonium hydrogen carbonate over 3 h, allowing the big dust particles to separate by a serum stamp. The supernatant was filtered and kept frozen at -20°C until the end of the study in order to assess allergen concentration with the same charge of test antibodies.

Statistical methods

The results of the guanine test strips were looked upon descriptively. Mite numbers and allergen data underwent analysis of variance after log transformation in order to stabilize variances. The model for the analysis of variance takes into account, first, that there are repeated observations per object (in fact, there were nine); second, that there is a treatment effect; third, that mattresses within one group and between the groups differ widely in the levels of mite numbers and allergens as well as in the variations of these levels during the study period (mattress effect); and fourth, that there may be a time factor (period effect or time trend). The difference between old and new mattresses was examined by the Wilcoxon matched pairs signed rank test within and by the Mann-Whitney-U-test between the groups. Statistical significance was assumed at $P < 0.05$. Finally, a power analysis of the mite numbers ($\ln \text{mites} + 1$) and the allergens ($\ln \text{allergen}$) was calculated.

Results

Table 1 shows the situation in the *old*, untreated mattresses left in place, grouped with their "new" partners as actively treated and placebo groups:

Table 1. Old, untreated mattresses left in place. Results of guanine test strips, mite counting, and allergen ELISA

	Guanine test		Mites/g dust		Allergen (Der p 1 + Der f 1) ng/g dust	
	Active	Placebo	Active	Placebo	Active	Placebo
Beginning						
Median	2.25	2	60	95	8502	13 521
Mean value	2.25	2.25	80	151	20 559	40 099
Minimum/maximum	1–3	1.5–3	40–185	15–405	675–55 350	6000–125 150
End						
Median	2	2	102	82	17 962	12 283
Mean value	2.16	1.75	129	151	33 512	40 817
Minimum/maximum	1.5–3	0.5–3	60–275	25–520	1600–100 015	4632–140 020

These old mattresses were *not* treated. In Table 1, they are grouped according to their new “partners”.

there was no significant difference in mite numbers and mite-allergen concentration between the start and end of the study within and between the two groups. In the old mattresses of the actively treated group, the values increased. Therefore, it may be concluded that conditions for mites remained favorable in the homes. This was reflected consistently by all three tests. The graphic presentation of the old mattresses is included in Figs. 1–3.

Vacuum-cleaning of the *new* mattresses coming straight from the factory did not yield enough dust material for measurement; therefore, we have no results of the time *before* placing.

Fig. 1 and Table 2 show the guanine test strip values of the *new* mattresses. In some cases, there were no results, due to insufficient dust volumes for all three tests. The actively treated group showed many more negative test results (33 versus 19), lower median values, and a lower cumulative sum of all results (13.5 versus 30) than the placebo group.

Fig. 2 and Table 3 show the numbers of mites. Again, in the actively treated group, lower median values and lower cumulative sums were found. In total, the placebo group samples contained 2.05 times more mites expressed as concentration sums. The highest numbers of mites were found in mattresses 8, 11, and, in the beginning, 7. This is in good accordance with the other applied tests (guanine strip and ELISA). For the treatment effect in the analysis of variance for the mites, no significant difference between active treatment and placebo was found ($P=0.44$).

Fig. 3 and Table 4 display the mite-allergen results. In each sample series, the actively treated group had lower median values; in total, the placebo samples contained 4.09 times higher allergen concentration sums. Again, the statistical analysis of variance showed no significant effect of treatment ($P=0.1$).

Regarding the number of mites, the power analysis showed that with 68 instead of 12 mattresses the given difference would have been significant. Regarding the mite allergens, 16 instead of 12 mattresses would have been needed.

The dynamics of the values shows that the mites invaded the mattresses rather quickly: regarding the numbers of mites, in eight cases the highest values were found in the first test series after placing the new mattresses. In two additional mattresses, the first value was the second highest in the course of measurements of this object. Regarding the allergens, in seven cases, the highest values were also found in the first series. The same was true of the median values of mites and allergens. The statistical analysis confirmed that there were no differences between the actively and placebo-treated groups in these aspects (time trend). On the other hand, there was a good concordance of numbers of mites and allergens, especially in mattresses 1, 4, 6, 7, 9, 10, and 11, but also in nos. 3 and 8.

In the comparison of the new mattresses to the old at the end of the study, the new mattresses of the actively treated group had a significantly lower amount of allergens ($P=0.028$) and lower numbers of mites ($P=0.028$). The new placebo-treated mattresses had significantly lower allergens ($P=0.043$), too, but did not significantly differ in the numbers of mites.

Discussion

As expected, the numbers of mites and the allergen values as medians of the groups peaked in summer and autumn. In the course of the allergen concentrations of every mattress, there was no uniform tendency: there were mattresses with consistently low values; some declined constantly, others showed a variability consistent with the expected

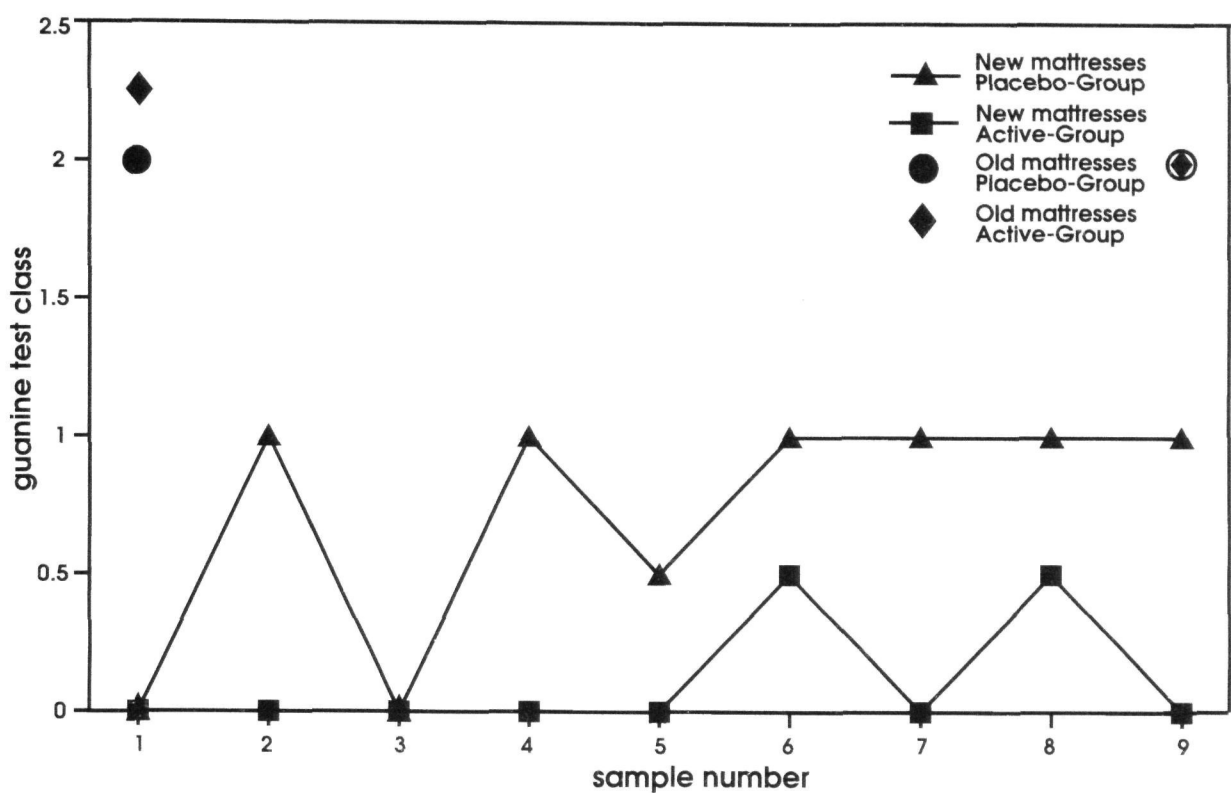


Fig. 1. Guanine test strip values: medians of groups.

Table 4. Mite allergens Der p 1 plus Der f 1 in nanogram per gram dust in new mattresses

	Sample no. ↓↓ 1	2	3	4	5	6↓	↓7	8	9	Sum/mattress	Sum/group
Active group mattress no.											
1	1610	1365	828	550	500	904	670	844	1287	8558	78 197
2	225	485	460	265	89	155	138	215	345	2377	
3	505	565	740	702	300	380	250	300	303	4045	
4	10 500	210	250	150	70	385	40	42	105	11 752	
5	2040	683	790	1530	1100	4427	4200	5500	4000	24 270	
6	7075	740	1040	1340	4010	4540	1750	3000	3700	27 195	
Medians	1825	624	765	626	400	644	460	572	816		
Placebo group											
7	7700	3350	3100	2300	1390	1180	1700	1170	1170	23 060	319 657
8	5860	10 400	5500	500	5050	2220	6850	4200	3350	48 430	
9	1278	570	804	405	250	159	295	286	286	4333	
10	7515	1700	1900	400	270	294	700	570	470	13 819	
11	27 520	15 517	17 528	7000	4500	13 515	5215	6400	97 015	194 210	
12	2190	18 100	550	415	185	640	8500	4575	650	35 805	
Medians	6780	8010	2500	1357	830	910	3457	2680	910		

variations of mite growth in different seasons, and some showed unexpected changes. Two values (those of mattresses 4 and 11) appear inexplicable. This is probably due to unknown and uncontrollable influences and was taken into account as “mattress effect” in the statistical analysis. If one compares the allergen values in the old and in the adjacent new mattresses, there is a general but not a consistent correlation, and it does not appear probable that the allergens were merely transported passively by contact or by air from the old to the new objects (data not shown). As all mattresses consisted of the same material and were

treated in the same way, the differences were probably due to differing climate and feed conditions in the individual homes.

This study was planned under the hypothesis that BB in the given formulation could prevent colonization of new mattresses by house-dust mites even under difficult conditions (the remaining old mattress as a mite source). Because a distinct effect was expected, we chose a small number of mattresses for the study. This hypothesis was not confirmed by the results of the study. The actively treated mattresses showed better results with each of the three applied dust test methods, but

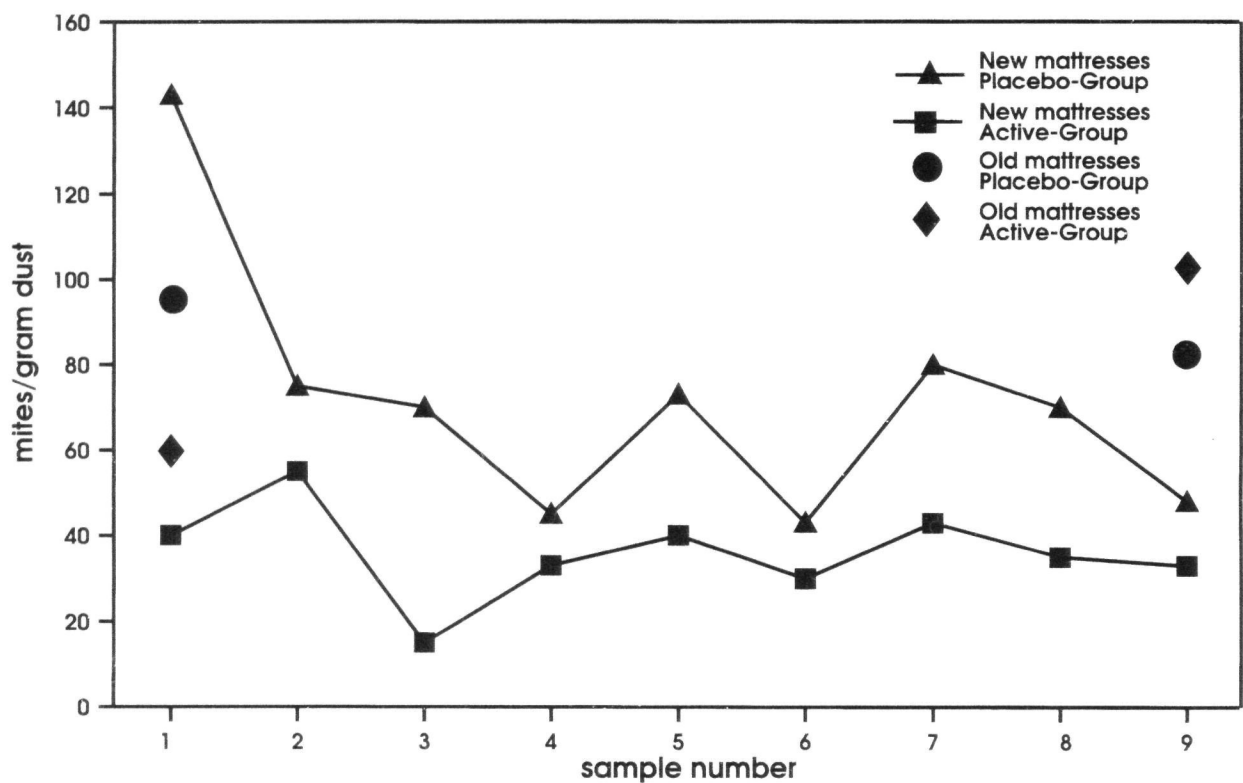


Fig. 2. Mite numbers: medians of groups.

Table 3. Mite numbers per gram dust in new mattresses

	Sample no. ↓↓ 1	2	3	4	5	6↓	↓7	8	9	Sum/mattress	Sum/group
Active group mattress no.											
1	105	90	20	35	15	0	30	15	40	350	2325
2	15	35	5	35	60	35	55	55	75	370	
3	35	40	10	0	20	15	10	5	10	145	
4	45	10	15	5	0	25	0	0	0	100	
5	35	75	15	30	100	90	80	115	25	565	
6	120	70	35	105	115	70	130	100	50	795	
Medians	40	55	15	33	40	30	43	35	33		
Placebo group											
7	195	140	120	110	125	65	105	65	50	975	4800
8	305	190	135	180	130	160	410	225	110	1845	
9	80	40	40	25	15	10	30	30	30	300	
10	90	0	40	35	20	20	55	75	45	380	
11	275	110	100	55	170	170	110	170	90	1250	
12	15	5	0	10	0	0	5	0	15	50	
Medians	143	75	70	45	73	43	80	70	48		

↓ = acaricidal treatment.

even in the actively treated group, no mattress remained completely free of mites and allergen, and the differences from placebo did not reach statistical significance. As the power analysis shows, concerning the allergens, with two more mattresses in each group, the difference might have been significant.

The applied “placebo” was more than conventional cleaning: twice a year, a cleansing foam was applied and the mattresses were vacuum-cleaned every 2 months. This was probably enough to reduce mite replication by removing nutrition and disturbing the biotope. We believe that this interpretation

is strengthened by the observation that in more than half of the cases in the actively treated as well as in the placebo group, the highest allergen values were found in the dust samples after placing the new mattresses and – with one exception – the allergen level was decreasing toward the end of the study. On the other hand, in the actively treated group, frequent vacuum-cleaning reduced the concentration of BB in the mattresses, thereby weakening the treatment effect. In this sense, the placebo would have been “too strong”.

Taking dust samples only at 9 and 18 months probably would have demonstrated the relations

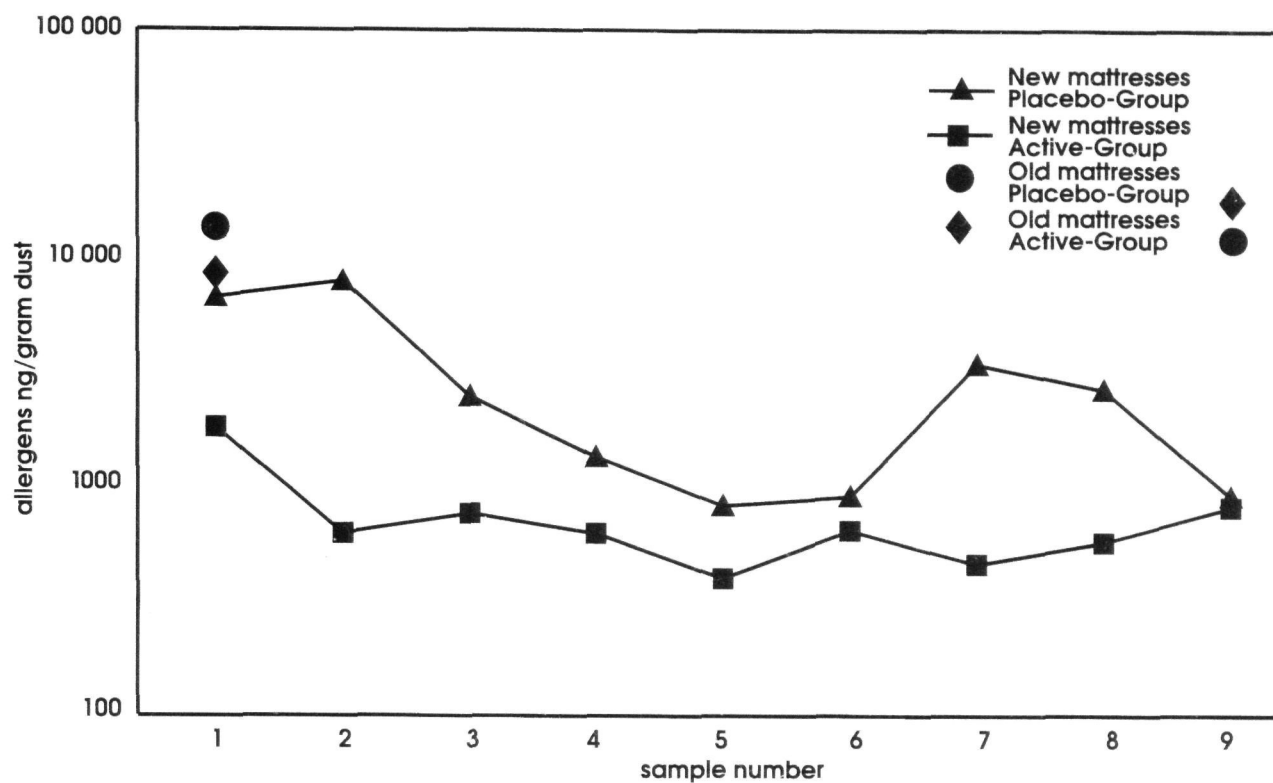


Fig. 3. Mite allergens Der p 1 plus Der f 1: medians of groups.

Table 4. Mite allergens Der p 1 plus Der f 1 in nanogram per gram dust in new mattresses

	Sample no. ↓↓ 1	2	3	4	5	6↓	↓7	8	9	Sum/mattress	Sum/group
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2	225	485	460	265	89	155	138	215	345	2377	
3	505	565	740	702	300	380	250	300	303	4045	78 197
4	10 500	210	250	150	70	385	40	42	105	11 752	
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12	2190	18 100	550	415	185	640	8500	4575	650	35 805	
Medians	6780	8010	2500	1357	830	910	3457	2680	910		

between active (BB) and placebo (cleansing foam) more clearly.

Altogether, in our experiment, the applied BB formulation compared to the applied "strong" placebo regimen was not significantly better. In no case was it possible to prevent completely the mite and allergen infestation of the new mattresses. However, the results of the placebo regimen show how potent mere cleansing methods may be in the control of house-dust mites. This is emphasized by the comparison of the old and new mattresses at the end of the study where the difference remained significant in spite of the initially rapid colonization of the new mattresses.

Several reports of experiences with BB products have been published: Burr et al. (13) used a method similar to ours: they covered mattresses with sheets impregnated with BB and collected dust after 4, 8, 12, and 24 months for mite counting. As mite numbers showed an extremely skewed distribution and the medians were low, they arbitrarily chose fewer than five mites per gram dust as the criterion of success. In this way, they found a significant difference between the actively treated and placebo groups. Comparability to our results is very limited because of different ways of BB treatment and different success criteria. A number of uncontrolled studies (14–19) found BB successful, while

controlled studies found significant (20), limited (21, 22), or no (23, 24) success.

Hayden et al. (25) confirmed the acaricidal *in vitro* effect of BB. Treating sofas with Acarosan foam had disappointing results in their study, whereas the powder was successful in reducing the allergen concentration of carpets during a 2-month observation period. As an important result, they suggest that the powder should remain long enough (>12 h) in contact with the carpet to be effective.

So far, the experiences with BB in practical use show that there is no proven specific effect, but as Hayden et al. (25) pointed out and as Burr et al.'s data (13) seem to suggest, details of the mode of application may be very important for the results, and further studies have to be done to examine these aspects. Obviously, mere cleansing itself has a controlling influence on mite numbers and mite allergens.

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