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## The role of water temperature and laundry procedures in reducing house dust mite populations and allergen content of bedding

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*The effects of various laundry procedures on house dust mites and their allergens have been established. All mites were killed by water temperatures 55° C or greater. Killing at lower temperatures was not enhanced by any of the pure detergents or laundry products tested. A cold cycle of laundry washing with or without laundry powder did not remove most live mites from bedding, however, the allergen concentration (Der p I/gm fine dust) was reduced by more than 90%. Dry cleaning did not reduce the allergen concentration of the dust, although most, if not all, mites were killed. (J ALLERGY CLIN IMMUNOL 1992;90:599-608.)*

**Key words:** House dust mites, mortality, water temperature, detergents, laundry procedures

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There is increasing evidence that allergens, and particularly house dust mite (HDM) allergens, are important in the etiology and perhaps the pathogenesis of asthma.<sup>1</sup> Several trials of allergen avoidance have demonstrated significant clinical benefits when methods that are likely to produce substantial reductions of allergen exposure in houses have been applied.<sup>2,3</sup> However, the concentration of allergen in many houses exceeds the proposed minimum threshold level

associated with sensitization (2  $\mu\text{g}/\text{gm}$  fine dust<sup>4</sup>) by up to 100-fold.<sup>5</sup> Methods to achieve reductions in allergen to below this level require further development and refinement.

Bedding, carpets, furnishings, and clothing may all contain large reservoirs of mites and allergen. Beds are an important source of mite allergen and for this reason have been the principal focus of many trials of the effects of allergen avoidance. The large proportion of our lives spent in beds and the possibility that exposure at night causes attacks of asthma has also focused attention on reducing HDM allergen in beds. Exposure to mites and allergens in mattresses and pillows can be controlled by use of occlusive covers,<sup>6</sup> but these are impractical for upper bedding, and methods such as washing to reduce the allergen concentration of the upper bedding need to be studied. Such methods could also be applied to clothing, which may be an important vector for mite redistribution and also an important source of allergen exposure.

Simple laundry procedures may have an important role in mite and mite allergen reduction but have not been systematically investigated. The water temperature needed to kill mites has not been adequately established. Temperatures of up to 70° C have been recommended,<sup>7</sup> although temperatures of 55° C have also been advised on the basis of experiments with dry heat.<sup>8</sup> Few domestic hot water systems reach 70° C, although most reach 55° C. However, many people prefer to wash in warm or even cold water. The effectiveness of water temperatures below 55° C in killing mites is not known.

The effect of different detergents or laundry products on mite survival during water immersion has not been explored. Some experiments have demonstrated up to 70% kill of mite populations with disinfectant and soap solutions at room temperatures.<sup>9-11</sup> This suggests that if a suitable reduction of surface tension can be provided, and if the hydrophobic barrier surrounding the mites is broken, most mites will drown. The efficacy of laundry procedures and laundry cleaning products in reducing mite populations and allergen levels has also not been assessed.

Dry cleaning with organic solvents might be expected to both kill mites and denature or extract allergens. One anecdotal report exists of complete extermination of mites and large reductions in allergen in a jacket after dry cleaning with perchloroethylene.<sup>12</sup>

In this article we report the results of four studies undertaken to determine the role of simple laundry procedures in reducing mite populations and allergens. Specifically we have investigated the water temperature required to kill mites, the effect of a range of detergents (anionic, cationic, nonionic, and zwitter-

#### Abbreviations used

|          |                                                              |
|----------|--------------------------------------------------------------|
| HDM:     | House dust mite                                              |
| % RH:    | Percent relative humidity                                    |
| SDS:     | Sodium dodecyl sulfate                                       |
| CTAB:    | Cetrimonium bromide                                          |
| CHAPS:   | (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) |
| ELISA:   | Enzyme-linked immunosorbent assay                            |
| Der p I: | Major allergen from <i>Dermatophagoides pteronyssinus</i>    |

ionic) and laundry products on mite survival, the effect of washing on mites and allergens in bedding, and the effects of dry cleaning.

## MATERIAL AND METHODS

### Determination of the thermal death point of mites in water

With use of a technique developed in this laboratory, live *Dermatophagoides pteronyssinus* were obtained from a laboratory culture grown on liver powder at 25° C, 75% relative humidity (RH). An aqueous suspension of mite culture (0.5 gm/5 ml) was layered on the surface of a 0% to 100% saturated NaCl gradient. The gradient was centrifuged at 1000 g for 5 minutes, and live mites were extracted from a band by use of a syringe and tubing and recovered on a filter. More than 95% of mites recovered were full-bodied and active.

Sets of three microcapsules containing 50 to 100 live mites were immersed for 10 minutes in water at 17°, 25°, 35°, 45°, 50°, or 55° C. The microcapsules were contained in a sample tube, and the temperature was maintained by a water bath. A total of 12 microcapsules were immersed for each temperature tested.

The microcapsules consisted of a 1 cm plastic tube closed at one end with 30  $\mu\text{m}$  wire mesh and at the other end by nylon cloth (25  $\mu\text{m}$  pore size). These allowed for complete immersion of the mites in the test solution.

After 10 minutes the mites were rinsed onto the nylon cloth by passing water of the test temperature through the wire mesh of the capsule. The nylon cloth was removed to a dish. Fifty mites were counted with a stereomicroscope, and the area in which they were located was delineated. Each dish was sealed with a glass slide and left for 12 to 15 hours. This gave mites that were stunned and appeared dead time to recover. After this period the number of dead mites in the previously delineated area was recorded and expressed as the percent of mites killed of the 50 originally counted. Only mites that showed no sign of activity, even on prodding with a blunted needle, were classified as dead.

### Effects of detergents on mite survival

The effect on mites of a variety of pure detergents and commercially available laundry products was measured with use of the same procedure described above. Live mites

**TABLE I.** Percentage composition of typical Australian, Canadian, or American laundry powder and liquid products<sup>13</sup>

| Ingredient                                                                        | Powders    | Liquids    |
|-----------------------------------------------------------------------------------|------------|------------|
| Detergents                                                                        | 8%-20%     | 18%-30%    |
| Foam boosters                                                                     | 0%-2%      | —          |
| Builders-sodium tripolyphosphate                                                  | 25%-35%    | —          |
| mixed or non-phosphate                                                            | 15%-30%    | 25%-45%    |
| sodium carbonate                                                                  | 0%-50%     | 15%-30%    |
| Antiredeposition agents-sodium carboxymethylcellulose or other cellulose polymers | 0.1%-0.9%  | 0.2%-0.3%  |
| Anticorrosion agents-sodium silicates                                             | 5%-10%     | 3%-8%      |
| Optical brighteners                                                               | 0.1%-0.75% | 0.05%-0.3% |
| Moisture                                                                          | 6%-20%     | —          |
| Fillers-predominantly sodium sulfate                                              | 20%-45%    | —          |
| Others-predominantly sodium bicarbonate                                           | —          | 15%-20%    |

**TABLE II.** Detergents and commercial laundry products screened for miticidal properties

| Detergent/laundry product                 | Supplier                                            | Ionic properties/additional known components |
|-------------------------------------------|-----------------------------------------------------|----------------------------------------------|
| Tween-20                                  | Sigma, St. Louis, Mo.                               | Nonionic (polar)                             |
| Lubrol PX                                 | Sigma, St. Louis, Mo.                               | Nonionic (polar)                             |
| SDS                                       | Sigma, St. Louis, Mo.                               | Anionic                                      |
| CTAB                                      | Sigma, St. Louis, Mo.                               | Cationic                                     |
| CHAPS                                     | Sigma, St. Louis, Mo.                               | Zwitterionic (amphoteric)                    |
| OMO                                       | Lever & Kitchen: Rexona Pty Ltd., Sydney, Australia | Nonionic/anionic detergents, fluorescer      |
| Spree                                     | Colgate-Palmolive, Sydney, Australia                | Anionic detergents                           |
| Sards laundry liquid                      | Sards Corporation, Sydney, Australia                | Unknown                                      |
| Sunlight soap                             | Lever & Kitchen: Rexona Pty Ltd., Sydney, Australia | Pure soap                                    |
| Eucalyptus wool wash (Double "D" Premium) | Sheldon Drug Co., Sydney, Australia                 | Eucalyptus oil                               |
| Fabric softener (Add Soft)                | Colgate-Palmolive, Sydney, Australia                | Quaternary ammonium compounds                |

contained in microcapsules were exposed for 10 minutes at 50° C to three concentrations of detergent or to water alone. The concentrations of 0.1%, 0.25%, and 1.0% were representative of the range of dilutions recommended by manufacturers for use in 50 to 70 L top-loading washing machines.

The detergents included representatives of the different ionic properties available—anionic, nonionic, cationic, and zwitterionic. The commercial products were representative of most of the groups of laundry products available. The composition of a typical laundry product is outlined in Table I. Laundry soaps from Australia, Canada, and the United States are very similar in composition to the European soaps, with added foam boosters and depressants, enzymes, and bleach.<sup>13</sup> Any additional information known about the specific products used is outlined in Table II.

Fabric softener is a conditioning solution that is added to washing cycles in addition to laundry soaps, and eucalyptus wool washes are widely recommended and used in Australia for washing woolen items as an alternative to using general laundry soaps.

### Effects of laundry procedures on mite and allergen content of bedding

To determine if washing can reduce live mite numbers and remove allergen from bedding, items including doonas (duvets), blankets, and sleeping bags were subjected to four different treatments. Each treatment group contained one of each type of bedding ( $N = 3$ ). One group was machine washed (General Electric, Mobile 12, Sydney, Australia) in cold water with 250 gm (approximately 4 gm/L) of a non-

ionic/anionic laundry powder (Cold Power, Colgate-Palmolive, Sydney, Australia). The machine program included a 9-minute wash, 3-minute rinse, and a 2-minute spray and spin cycle. This was followed by a 4- to 5-hour period of drying in full sunlight. The second group was machine washed in cold water without any soap added and also dried in the sun. A third group was subjected to a 4- to 5-hour period in the sun to determine if the drying period alone had any influence on mites and allergen levels. The final group was not treated and was included as a control.

**Measurement of mites/item.** The number of mites per item of bedding was estimated before and within 48 hours after each treatment. Items were not in contact with other allergen sources until the posttreatment samples had been collected. Mites were classified as live if they were full-bodied, whitish in color, and active; that is, if they were mobile or if movement was noted in response to prodding. Ex-live mites were full- to half-bodied, remained white in color, but were not active even if prodded. These mites are most likely to have died recently, but may simply be inactive live mites. For this reason they are categorized separately. Dead mites were dehydrated and generally appeared brown in color and were most likely to have been dead before the washing procedure. No distinctions were made between different life stages or between male and female mites.

Two common methods of estimating numbers of mites were used. The first method was based on the number of live, ex-live, and dead mites in dust samples vacuumed from 600 cm<sup>2</sup> of an item for 2 minutes with a Volta (model 4229; Electrolux, Melbourne, Australia) or a Hoover (model 2614; The Hoover Co., Great Britain) vacuum cleaner. Mites were separated from a known weight of dust by use of the flotation method in a 30% sucrose/saturated sodium chloride (NaCl) solution.<sup>14</sup> The data were converted to the number of mites per item on the basis of the proportion of dust examined and the area of the item.

Mites were also estimated by use of the heat-escapement method.<sup>15</sup> The number of mites adhered to a 55 cm<sup>2</sup> strip of adhesive tape was counted after heat had been applied to the opposite side of the item for 10 minutes. Four strips of tape were applied to each item, and the sum of the number of mites on each strip was used to estimate the total number of live mites per item. Four strips were used to reduce the influence of clustering. A 150 W spotlight positioned 30 cm from the item was used as the heat source in these experiments. These data were also converted to a mites/item value on the basis of the area of the item. All full-bodied mites were considered live because the method is based on the principle that mites captured on the tape were mobile.

**Estimates of mites removed during the washing procedure.** Estimates of mites removed during the washing procedure were obtained to confirm that live mites were removed from the bedding during washing. Water discharged from the washing machine during the wash cycle was passed through a 25  $\mu$ m pore size nylon cloth bag (450 cm<sup>2</sup>) to collect mites and lint. A weighed portion of the collected material was examined for mites by the flotation method, and the total number of mites washed from the item was estimated on the basis of the total weight of collected material.

**Measurement of Der p 1 allergen.** Extracts of fine dust samples were prepared,<sup>14</sup> and the mite allergen *Der p 1* concentration was measured by the ELISA monoclonal antibody assay.<sup>16</sup> Allergen concentration was expressed as micrograms of mite allergen per gram of fine dust.

## Effects of dry cleaning on mite and allergen content of bedding

To determine if dry cleaning could reduce mite populations and remove allergen, groups of items were commercially dry cleaned; the first group with perchloroethylene and the second with a white spirit-based cleaning system that contained the soap Lelite (Manville Sales Corp., Denver, Colo.). Items are placed in a front-loading washing machine into which the cleaning solution is introduced. The solution infiltrates the items dissolving oils and dirt. At no stage are the items in contact with any water, hence the term dry cleaning is used.

To test the direct effect of dry cleaning on mites, two nylon cloth satchels (25  $\mu$ m pore size) containing 1 gm of mite culture were pinned to each of the items that underwent the second commercial dry cleaning process. After dry cleaning the culture was observed under a stereomicroscope, and the presence or absence of any live or ex-live mites was observed.

## Statistical analysis

A chi-square test established homogeneity of the proportions of mites killed by each temperature (12 replicates of 50 mites each). Hence data for each temperature were pooled, and differences between the proportion of mites killed at each temperature were assessed by use of a 2  $\times$  4 contingency table. Confidence intervals for each temperature were calculated on the basis of binomial distribution.<sup>17</sup> Comparisons between the temperatures were tested with use of the Tukey test for multiple comparisons, which uses the arcsine transformed proportions to calculate the parameter  $q$ .<sup>17</sup>

This analysis was repeated for each of the detergents and commercial laundry products tested. With use of an arcsine transformation of the proportion of mites killed by each dilution, Sheffe's test for multiple comparisons tested each dilution against the control. Pooling of these data was considered appropriate because all dilutions of a product were tested in the same conditions at the same time.

Differences in the changes in levels of allergen between experimental and control groups were tested by a one-way analysis of variance (ANOVA) in which the natural logarithm of the ratio obtained by dividing after by before levels of *Der p 1*/gm fine dust was used.

## RESULTS

### Thermal death point of *Dermatophagoides pteronyssinus* in water

The effect of water temperature on the percentages of mites killed are shown in Fig. 1. Water temperatures below 45° C did not kill significant numbers of mites. At 50° C, 47.9% of the mites were killed, and at 55° C all of the mites were killed (Fig. 1).

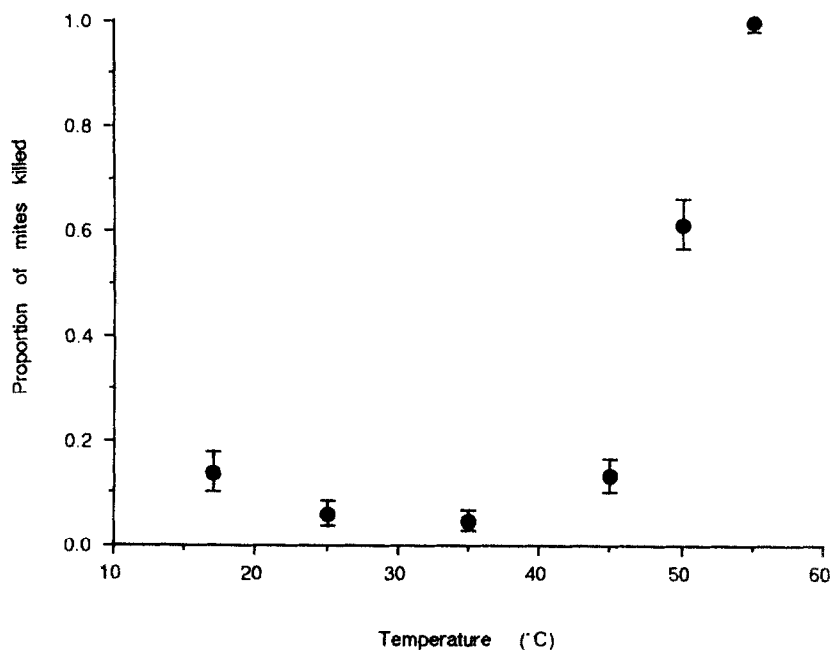


FIG. 1. Mean percentage ( $\pm 95\%$  CI) of mites killed at water temperatures ranging from 17° to 55° C. Twelve replicates of 50 mites each were tested at each temperature.

### Effects of detergents on the thermal death point of mites

With the exception of eucalyptus wool wash, no pure detergent or commercial laundry product increased the proportion of mites killed at 50° C compared with water only ( $S = 11.536$ ;  $p = 0.0001$ ). Only a 10% solution of eucalyptus wool wash at 50° C killed a significantly greater number of mites compared with water alone (Table III). Some products reduced the number of mites killed. (Tween-20, sodium dodecyl sulfate, Spree, OMO, Sard's, and Sun-light soap). Four of these products (SDS, Spree, OMO, and Sard's) have high sodium ion concentrations. To determine if the sodium ion was playing a protective role, an NaCl solution was also tested. The salt solution also killed significantly fewer mites than water alone.

In the Lubrol PX (Sigma), CHAPS, and CTAB trials, the proportion of mites killed in the control groups did not fall within the 95% confidence intervals (CI) calculated for 50° C in the initial water temperature experiments. This greater survival may be due to a slight drop in the water bath temperature below 50° C.

### Effects of laundry procedures on mite and allergen content of bedding

Having determined that none of the available commercial washing powders could kill mites more effectively than water alone, washing trials were per-

formed according to the manufacturers' instructions to determine if the washing powder in its capacity to remove particles during the washing procedure was effective in removing the mites from the bedding.

No treatment resulted in large reductions in mites (Table IV), although estimations of the changes were confounded by large variations in the estimates of mites before and after treatment. This variation in estimating mite numbers occurred with both methods of assessing the mite populations.

The change in the number of mites in three items of bedding after treatment by cold washing with soap or water alone or placing in the sun are shown for both sampling methods in Table IV.

Although no significant reductions in the mite populations were observed after washing, mites were collected in the water and lint flushed out of the washing machine (Fig. 2). The total number of mites flushed from items washed in soapy water (geometric mean,  $285.63 \pm 2.84$ ) did not differ significantly from the total number flushed from the water only group (geometric mean,  $247.85 \pm 3.13$ ).

*Der p 1* were significantly reduced after cold washing in both soapy water and water only, with the mean amounts of *Der p 1*/gm of fine dust falling from  $41.21 \pm 2.34 \mu\text{g}$  to  $2.03 \pm 4.28 \mu\text{g}$  and  $52.6 \pm 2.28 \mu\text{g}$  to  $3.9 \pm 2.43 \mu\text{g}$ , respectively (Fig. 3, A). The inclusion of laundry powder in the wash cycle did not produce a greater reduction in allergen levels compared with reductions observed with use of water only.

**TABLE III.** Percentage of mites killed by the test solution at 50° C after an initial 10 minutes of exposure and a 12- to 15-hour recovery period

| Detergent       | Dilution | Percent of mites killed in<br>test solution<br>(95% CI) | Percent of mites killed in<br>the control<br>(95% CI) |
|-----------------|----------|---------------------------------------------------------|-------------------------------------------------------|
| NaCl            | 0.9      | 13.3 (8.5, 20.0)***                                     | 42.7 (34.6, 51.8)                                     |
|                 | 2.5      | 16.7 (11.3, 23.8)***                                    |                                                       |
|                 | 0        | 16.7 (11.3, 23.8)***                                    |                                                       |
| Tween-20        | 0.1      | 24.7 (18.6, 34.7)***                                    | 46.0 (38.2, 54.4)                                     |
|                 | 0.25     | 16.0 (10.9, 25.8)***                                    |                                                       |
|                 | 1.0      | 22.0 (15.9, 32.0)***                                    |                                                       |
| Lubrol-PX       | 0.1      | 31.3 (23.9, 39.3)                                       | 27.3 (21.0, 37.1)                                     |
|                 | 0.25     | 28.7 (21.6, 38.7)                                       |                                                       |
|                 | 1.0      | 22.7 (16.6, 30.4)                                       |                                                       |
| CHAPS           | 0.1      | 23.0 (17.2, 31.2)                                       | 23.3 (17.2, 31.2)                                     |
|                 | 0.25     | 12.7 (7.8, 18.9)*                                       |                                                       |
|                 | 1.0      | 20.0 (14.3, 27.5)                                       |                                                       |
| CTAB            | 0.1      | 14.0 (8.9, 20.9)                                        | 18.7 (12.8, 25.6)                                     |
|                 | 0.4      | 25.3 (18.9, 33.1)*                                      |                                                       |
| SDS             | 0.1      | 14.9 (9.1, 19.7)***                                     | 56.1 (48.3, 64.8)                                     |
|                 | 0.25     | 9.2 (4.2, 15.7)***                                      |                                                       |
|                 | 1.0      | 16.7 (11.0, 23.1)***                                    |                                                       |
| Spree           | 0.1      | 22.0 (16.0, 29.3)***                                    | 62.7 (54.1, 70.7)                                     |
|                 | 0.25     | 19.0 (11.7, 28.0)***                                    |                                                       |
|                 | 1.0      | 26.3 (20.4, 34.6)***                                    |                                                       |
| OMO             | 0.1      | 69.3 (61.2, 76.7)                                       | 62.7 (54.1, 70.4)                                     |
|                 | 0.25     | 38.0 (30.2, 46.3)***                                    |                                                       |
|                 | 1.0      | 46.0 (38.1, 54.4)*                                      |                                                       |
| Sards           | 0.1      | 29.7 (22.7, 38.1)***                                    | 56.7 (48.3, 64.8)                                     |
|                 | 0.25     | 9.3 (5.3, 15.2)***                                      |                                                       |
|                 | 1.0      | 11.3 (6.9, 17.2)***                                     |                                                       |
| Sunlight soap   | 0.1      | 33.7 (26.4, 42.1)***                                    | 67.7 (58.9, 75.4)                                     |
|                 | 0.25     | 29.3 (22.9, 37.3)***                                    |                                                       |
|                 | 1.0      | 27.3 (21.0, 35.3)***                                    |                                                       |
| Wool wash       | 0.5      | 46.7 (38.5, 55.1)                                       | 51.7 (43.5, 60.2)                                     |
|                 | 1.0      | 44.0 (36.1, 52.4)                                       |                                                       |
|                 | 10       | 82.3 (75.4, 88.3)***                                    |                                                       |
| Fabric softener | 0.25     | 58.5 (48.2, 70.6)                                       | 51.7 (43.6, 60.4)                                     |
|                 | 1.0      | 33.0 (24.0, 44.1)*                                      |                                                       |

Percentage represents pooled results of three replicates of 50 mites each, with 95% CI given in brackets.

Differences between the control and each treatment group tested with Sheffe's multiple contrasts test.

\* $p < 0.05$ .

\*\* $p < 0.001$ .

\*\*\* $p < 0.0001$ .

### Effects of dry cleaning on mite and allergen content of bedding

Dry cleaning was the only procedure to show a reduction in the number of live or ex-live mites (Table IV). Levels of *Der p* I before and after dry cleaning did not differ significantly (Fig. 3, B).

Mite culture subjected to the second dry cleaning process returned with no live or ex-live mites. All the mites appeared completely dehydrated. In contrast, ex-live mites were recovered in dust from bedding subjected to this dry cleaning method.

### DISCUSSION

Controlling exposure to allergens is a primary component of any strategy to manage allergic diseases. This study demonstrates that domestic laundry procedures, a common and easily applied method of cleaning, could only effectively kill mites if a minimum water temperature of 55° C was used. Below this temperature the percentage of mites killed could not be enhanced by the addition of any pure detergents or commercial laundry products. At these lower temperatures the concentrations of allergen *Der p* I in

**TABLE IV.** Mean proportion of mites (after/before) remaining in items after washing with soap and cold water, cold water alone, drying in the sun for 4 to 5 hours, and dry cleaning

| Treatment group                 | (Mite category) | Range of mite numbers (mites/item) |            | Proportion of mites remaining | Mite category | Range of mite numbers (mites/item) |             | Proportion of mites remaining |
|---------------------------------|-----------------|------------------------------------|------------|-------------------------------|---------------|------------------------------------|-------------|-------------------------------|
|                                 |                 | Before                             | After      |                               |               | Before                             | After       |                               |
| Control                         | Ex-live         | 7 - 23                             | 8 - 27     | 1.17                          | Live          | 120 - 618                          | 0 - 756     | 0.74                          |
|                                 | Dead            | 7 - 283                            | 16 - 425   | 2.02                          |               |                                    |             |                               |
|                                 | Total           | 14 - 305                           | 25 - 449   | 1.68                          |               |                                    |             |                               |
| Soap                            | Ex-live         | 127 - 212                          | 0 - 450    | 0.95                          | Live          | 340 - 14,770                       | 10 - 10,585 | 0.27                          |
|                                 | Dead            | 191 - 916                          | 199 - 2819 | 4.46                          |               |                                    |             |                               |
|                                 | Total           | 18 - 2870                          | 199 - 2973 | 1.15                          |               |                                    |             |                               |
| Water                           | Ex-live         | 58 - 378                           | 149 - 1021 | 2.72                          | Live          | 1129 - 2754                        | 454 - 4076  | 0.78                          |
|                                 | Dead            | 265 - 1834                         | 572 - 3004 | 2.42                          |               |                                    |             |                               |
|                                 | Total           | 324 - 2010                         | 721 - 4025 | 2.43                          |               |                                    |             |                               |
| Sun                             | Ex-live         | 87 - 205                           | 0 - 42     | 0.15                          | Live          | 76 - 2034                          | 340 - 1000  | 1.05                          |
|                                 | Dead            | 131 - 1023                         | 295 - 607  | 1.19                          |               |                                    |             |                               |
|                                 | Total           | 218 - 1228                         | 321 - 649  | 0.82                          |               |                                    |             |                               |
| White spirit-based dry cleaning | Ex-live         | 69 - 1240                          | 0 - 129    | 0.08                          | Live          | 1356 - 3200                        | 0           | 0                             |
|                                 | Dead            | 69 - 2351                          | 13 - 230   | 0.11                          |               |                                    |             |                               |
|                                 | Total           | 139 - 3591                         | 13 - 359   | 0.09                          |               |                                    |             |                               |

Control bedding was untreated. Assessment of mite numbers made by two methods (a) recovery of mites by flotation from fine dust samples and (b) heat-escapement. *N* = 3 for all treatments.

dust, but not the numbers of mites in items, were significantly reduced by washing. However, some allergen did remain after a single cycle of washing. Conversely, dry cleaning probably killed all mites but did not reduce the *Der p* I concentration of dust.

**Thermal death point of *D. pteronyssinus* in water**

In these trials water temperatures of 55° C killed all mites, 50° C killed only half, whereas below 45° C there was virtually no effect at all. In the only other study of the thermal death point of mites in water, Andersen and Roesen<sup>11</sup> observed considerably higher mortality rates at 40° C and 50° C than in the current study. This may have arisen because of methodologic differences in assessing mite death. Andersen and Roesen<sup>11</sup> reported a 100% mortality rate at 58° C, but because they did not test any other temperature between 50° C and 58° C, it is not unlikely that a 100% death rate could have been achieved at 55° C, as was the case in this study. This temperature is similar to the dry thermal death point of 51° C determined by Kinnaird<sup>8</sup> at 60% RH. Knulle<sup>18</sup> suggested that above a critical temperature the mite cuticle fails to maintain the water balance between internal and external environments. Suggestions of differences in heat sensitivity between different strains of mites, particularly between laboratory and wild populations

of mites, have been made.<sup>19</sup> A possibility exists that such differences may have an impact on the results of this experiment. A laboratory culture grown in relatively constant conditions may respond to the physiologic challenges of high water temperatures differently from a wild population of mites that survive in constantly fluctuating conditions.

**The effect of detergents on the thermal death point of mites**

The current study did not reveal any detergent or commercial laundry product that demonstrated any useful miticidal properties at lower temperatures. Andersen and Rosen<sup>11</sup> observed mortality rates of 96% to 100% at 50° C with soaps recommended for allergy sufferers, but as in the current study these values were no greater than the mortality rates observed in water only. Other previous studies of floor cleaning products containing SDS have demonstrated mortality rates greater than 80% but only for dilutions over 1% and only after a 3-day period.<sup>10</sup> Solutions at concentrations of less than 1% did not produce mortality rates greater than 50% even over an 8-day period. De Boer<sup>20</sup> suggested that although residual effects of carpet cleaning shampoos may slow population development, the destruction of the mite's food source would be more likely to have a significant impact on mite populations in the longer term. Residual effects of machine washed

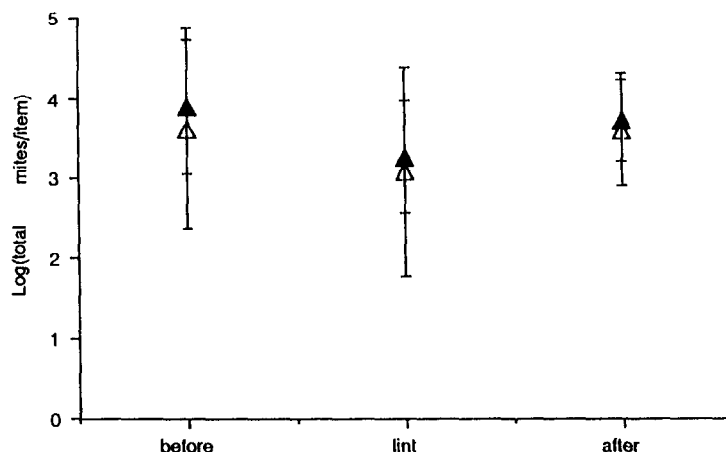


**FIG. 2.** Mean numbers of mites in items of bedding before and after washing with a commercial laundry powder (▲) or water only (Δ), and the mean number of mites collected in lint that was flushed from the washing machine during the wash cycle. Data are expressed as the  $\log_{10}$  (total mites/item)  $\pm$  SD.

items of bedding are unlikely because the rinse cycle rinses very thoroughly, however, habitat destruction may cause longer term reductions in mite numbers. On the basis of results of this study and previous studies it is unlikely that the addition of any of the detergents or soaps tested, even in greater concentrations, will improve the miticidal properties of commercially available laundry products.

Solutions of SDS and the washing products with high sodium salt content (Spree, OMO, and Sardis) would appear to provide a degree of protection for the mite near critical temperatures. A similar result was also seen when mites were immersed in saline solutions, indicating that  $\text{Na}^+$  may play a role in protecting mites against drowning at higher temperatures. This hypothesis is supported by the observation that at water temperatures greater than  $50^\circ\text{C}$  dead mites appear bloated, indicating that a breakdown of a mite's ability to control the uptake of water and subsequent drowning has been the cause of death. This breakdown in the mite's control of water uptake may be less extensive or prevented in isotonic solutions.

The only washing product showing a significant reduction in mite survival was a product containing eucalyptus oil. However, the 10% concentration, killing a mean of 82% of mites at  $50^\circ\text{C}$ , was 30-fold greater than the concentration recommended by the manufacturer. It is not known if this product is safe to use at higher concentrations or if it would be economically viable. This does suggest that the inclusion of essential plant oils may enhance mite killing, and Watanabe<sup>21</sup> has investigated the killing effects of the volatiles emitted from a number of essential oils. The

miticidal properties of essential oils including eucalyptus oil warrant further investigation and could be tested with use of current methods.

### The effects of washing on the mite and allergen content of bedding

The washing experiments demonstrated that reductions, but not complete removal, of mite allergen could be accomplished by machine washing in cold water, and that these reductions were not always to below the recommended  $2\text{ }\mu\text{g}$  of *Der p 1*/gm of fine dust. It is likely that repeated washing, perhaps fortnightly, would eventually reduce allergen levels to below the limit of detection. These reductions in allergen levels were not matched by reductions in the number of live or ex-live mites estimated to be present in items of bedding after the washing procedure. However, it is important to recognize that the cold washing procedure did remove some mites from items as is evident from the lint collected from the washing machine.

The results of the washing experiments were confounded by the large variation in the estimates of mites per item both within and between the different groups as determined by both methods of estimating populations. These variations could have occurred for several reasons. A clustered distribution of mites is more likely to introduce variation into the tape results because a smaller area was sampled compared with the area sampled during vacuuming.

When estimating the size of mite populations in bedding, the structure of the item itself may also influence the estimates of the numbers of mites per item.

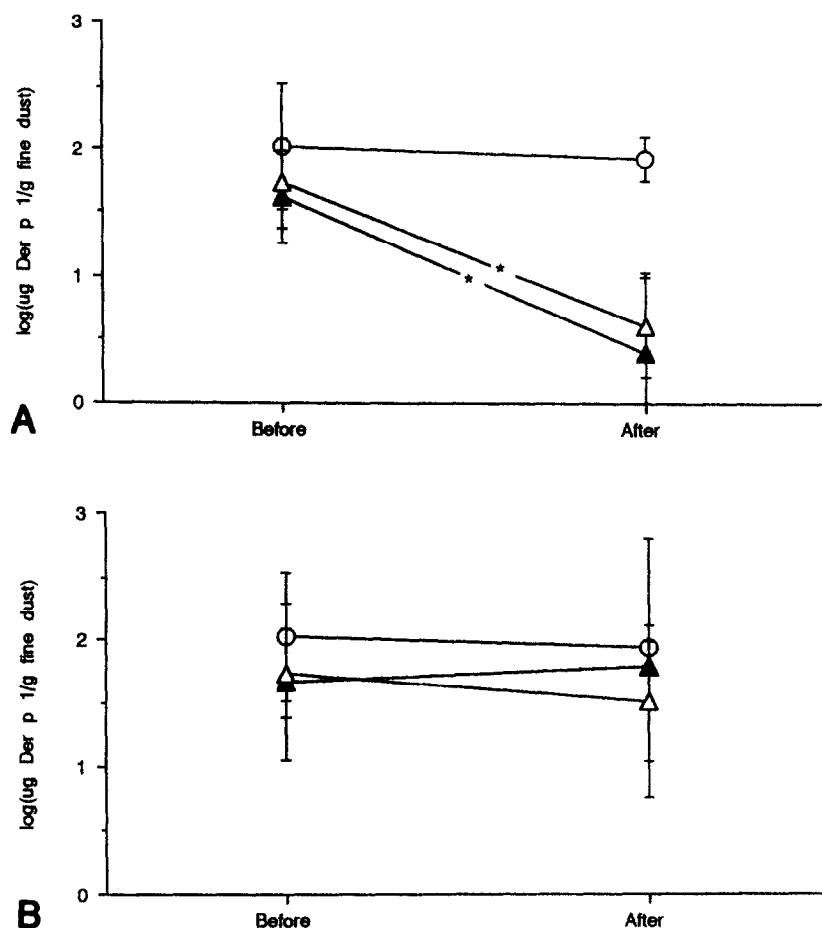


**FIG. 3.** Allergen concentration of fine dust before and after each trial. Control data (○) are the same for both groups. Data are presented as  $\log_{10}$  (*Der p* I/gm fine dust)  $\pm$  95% CI.\* These reductions were significantly greater than reductions observed for the control group ( $p < 0.0001$ ). **A**, Before and after washing with a laundry powder (▲) or water only (△). **B**, Before and after dry cleaning with perchloroethylene (△) and white spirit-based cleaning fluid (▲).

The effectiveness of the heat-escapement method will depend on the distribution of heat through the item, the ease with which mites move through the fabric, and the thickness of the item. Similarly, the proportion of dust and the number of mites collected by the vacuuming process will be influenced by the structure of the fabric. Clearly, some refinement of the methods currently used to estimate mite numbers is needed.

Although the washing process did not eliminate mites from the bedding, it was effective in significantly reducing levels of *Der p* I. The 17-fold reduction in allergen by washing was less than expected from the reports of eluting *Der p* I from mite feces and culture material where all allergen was eluted in less than 2 minutes.<sup>14</sup> Accumulated mite bodies may elute *Der p* I allergen more slowly than mite fecal material and could be a possible source of this aller-

gen. Similar quantities of dust were recovered during the sampling period before and after washing, demonstrating that washing alone may not remove significant quantities of dust from items of bedding.

### The effects of dry cleaning on the mite and allergen content of bedding

In this experiment reductions in the concentration of *Der p* I after dry cleaning with white spirit-based cleaning fluid or perchloroethylene were not observed. Anecdotal evidence has demonstrated that dry cleaning with perchloroethylene reduced levels of guanine in a jacket to below allergenically significant levels.<sup>12</sup> In the same report it was shown that with use of the heat-escapement method mites in the jacket were completely exterminated. Similarly, in this study no mites adhered to tape after dry cleaning, suggesting

that no live mites were present. However, immobile, full-bodied (ex-live) mites were present in dust in reduced numbers, and it is unclear if live mites did in fact persist in the items. One active mite was recorded after dry cleaning with white spirit, but this may have been due to contamination from the experimenter during vacuuming. Mites in nylon bags that were exposed to dry cleaning were all dehydrated and killed, but mites in fabric may be protected by a tight network of fibers, air bubbles, and moisture in the items.

The required frequency of washing to maintain low levels of allergen requires further investigation. Current recommendations suggest that bedding be washed regularly,<sup>4, 22</sup> although no data on the rate of allergen accumulation in beds have been reported. Monthly hot washing or fortnightly cold washing of all bedding seems reasonable, although this suggestion needs to be substantiated experimentally. The effect of suggested treatments on each life stage of the mite would have to be assessed to establish any model of population recovery. In addition, the rate of accumulation of allergen is likely to be dependent on the allergen levels at other untreated sites within a room, factors affecting the redistribution of this allergen (such as ventilation and dust disturbance), and the temperature and humidity in the bed. Further studies are required to determine the optimum conditions for creating "allergen-free" beds.

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