

## Short communication

# Variability of house-dust-mite allergen levels within carpets

Simpson A, Hassall R, Custovic A, Woodcock A. Variability of house-dust-mite allergen levels within carpets.  
Allergy 1998; 53: 602–607. © Munksgaard 1998.

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Sensitization and exposure to house-dust-mite allergens is an important cause of asthma. Standardized, reliable, and reproducible methods for measuring exposure are essential for the assessment of the relationship between exposure, sensitization, and asthma. This study investigated the variability of the house-dust-mite allergen Der p 1 concentration in reservoir dust collected within whole carpets in living rooms and bedrooms. The carpets of nine bedrooms and 11 living rooms were sampled. Each room was divided into 1 m<sup>2</sup> areas measured from wall to wall where the carpet was accessible. Reservoir dust samples were collected by vacuuming each 1 m<sup>2</sup> area for 2 min. Der p 1 was assayed by a two-site monoclonal-antibody-based immunometric ELISA. Der p 1 was detectable in the carpets of all nine bedrooms and six of the 11 living rooms. Within these 15 rooms, there was a wide range of Der p 1 levels. The smallest range of allergen within a single room was 0.9 µg Der p 1/g dust (0.2 and 1.1 µg/g; 5.5-fold difference), and the largest was 149.2 µg Der p 1/g dust (0.8 and 150 µg/g; 192-fold difference). The mean range of allergen levels in the living rooms was 11.5 µg Der p 1/g of dust, and the mean coefficient of variation of these rooms was 80.2%, illustrating the huge variation of mite allergen levels within each room. The variation within bedrooms was also large, with a mean coefficient of variation value of 88.7%. The coefficient of variation was significantly lower around soft furnishings or beds (57%) than in the rest of the room (89.3%), with the mean difference being 32% (95% CI 2–63%; *P*=0.04). In conclusion, this study has shown that there is a great variation of Der p 1 levels between areas within a room. No consistent pattern of distribution of mite allergen within a room was found. Der p 1 levels in areas around soft furnishings and beds varied less than the levels in the rest of the room.

Key words: allergen; Der p 1; exposure; mites; reservoir; sampling.

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Accepted for publication 3 February 1998

Sensitization and exposure to house-dust-mite allergens is firmly established as an important risk factor for asthma (1). Standardized, reliable, and reproducible methods for measuring exposure are thus essential for the further investigation of the relationship between exposure, sensitization, and asthma. There are three generally accepted methods of estimating exposure to dust-mite allergens: mite counts, assays of specific mite allergens, and measurement of guanine (2). In addition, a new semi-quantitative monoclonal antibody-based immunodot strip technique for the detection of house-dust mite, cat, and cockroach allergen has recently been developed (3). In research studies, monoclonal-

antibody (mAb)-based assays are the most widely used method for assessing allergen exposure. Immunoassays are sensitive, specific, and reproducible and can give quantitative measurement of a given allergen in absolute units (e.g., ng or µg of protein). Allergens are usually measured in dust samples collected by vacuuming reservoirs of settled dust within the home (e.g., carpet, mattress, bedding, and upholstered furniture) for a standardized period of time (usually 2–5 min/m<sup>2</sup>); in epidemiologic studies, the results are usually expressed as allergen per unit weight (e.g., µg Der p 1/g of fine dust) (2, 4). This method for measuring exposure has remained virtually unchanged for the last two

decades (5). However, the area sampled may be only a small fraction of the total area in the room available for sampling (e.g., 5–20% of the available floor area). Samples are usually taken from standard areas within a particular room; an area in front of a sofa in living rooms and an area to the left side of the head of the bed in bedrooms. However, the variability of the allergen concentration between sites within a room has been poorly examined. The aim of this study was to look at the variability of house-dust-mite allergen Der p 1 concentration in reservoir dust collected by vacuuming the whole carpet in 1 m<sup>2</sup> areas in living rooms and bedrooms in domestic dwellings in South Manchester, UK.

## Material and methods

### Sampling

The study was conducted in nine homes in South Manchester. The whole carpets of nine bedrooms and 11 living rooms were sampled. Each room was divided into 1 m<sup>2</sup> areas measured from wall to wall where carpet was accessible; areas under heavy, static furniture, such as cupboards and bookcases, were not sampled. Half-metre squares were measured where a full 1 m<sup>2</sup> could not be obtained to complete the sampling of the room. A plan was drawn of each room illustrating each numbered 1 m<sup>2</sup> sample, and the position of any furniture within the room and the site of windows and doors were noted. Reservoir dust samples were collected from the carpet by vacuuming each 1 m<sup>2</sup> area for 2 min; any half-metre squares were vacuumed for 1 min.

Dust samples were collected with a Medical Dust Sampler (Medivac plc, Wilmslow, UK) with air-flow rate of 45 l/s, through a 355-µm-diameter mesh screen onto a 5-µm vinyl filter (Plastic Associates Ltd, Wirral, UK). This method enabled collection of fine dust samples. Each sample was transferred into a preweighed Petri dish, weighed, coded, and stored at 4°C until extraction.

An amount of 100 mg of fine dust was extracted with 2 ml borate-buffered saline with 0.1% Tween 20 (BBS-T), pH 8.0. For samples less than 50 mg, 1 ml BBS-T was added. Samples between 50 and 100 mg were extracted in the proportional amount of BBS-T (1:20). The dust was resuspended with a vortex mixer (Vortex-Genie, Fisher Scientific, USA). Samples were then mixed end over end on an orbital rotator (Rotator SB-1, Stuart Scientific, UK) for 2 h at room temperature before being centrifuged for 20 min at 2500 revolutions per min at 4°C (BR 401 Refrigerated Centrifuge, Denley, UK). Supernatants were removed with a Pasteur

pipette and stored at –20°C for future analysis of allergen content.

### Allergen assay

Der p 1 was assayed by a two-site immunometric ELISA, as previously described (6, 7). The standard used to establish the control curve for Der p 1 assay (UVA 93/02) was considered to contain 2500 ng Der p 1/ml (relative to WHO/IUIS *Dermatophagoides pteronyssinus* standard NIBSC 82/518, which has been estimated to contain 12.5 µg Der p 1 per ampoule). Extracts were initially assayed in duplicate at 5-, 25-, and 125-fold dilution. For concentrations lying off the sloping portion of the standard curve, the assays were repeated at an appropriate dilution. Paired samples were rejected if they differed by more than 5% (restricting intra-assay variation to this level).

### Statistical analysis

The allergen data were found to follow a log-normal distribution; coefficients of variation were calculated, and the results reported as geometric means (GM). Values were compared by Student's *t*-test. Statistical significance was set at the <5% level.

## Results

Of the 11 living rooms, five had mite allergen levels below the limit of detection of the assay (0.2 µg Der p 1/g of fine dust) in every 1 m<sup>2</sup> area sample. Der p 1 was detectable in the reservoir dust of the carpets of nine bedrooms and the remaining six living rooms. Within each of these 15 rooms, there was a wide range of Der p 1 levels. The smallest

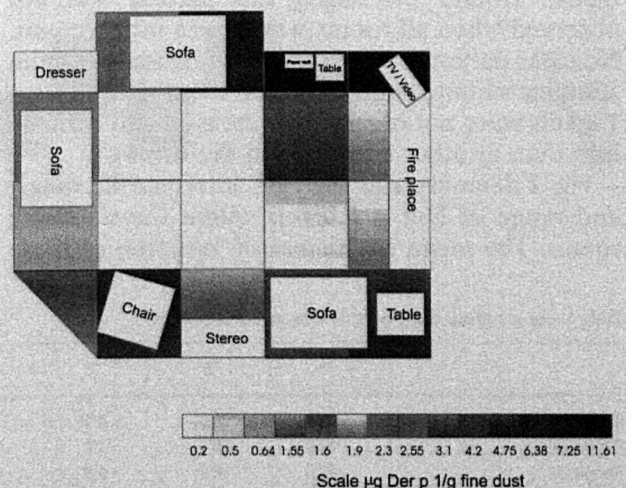


Fig. 1. Distribution of house-dust-mite allergen Der p 1 in living room.

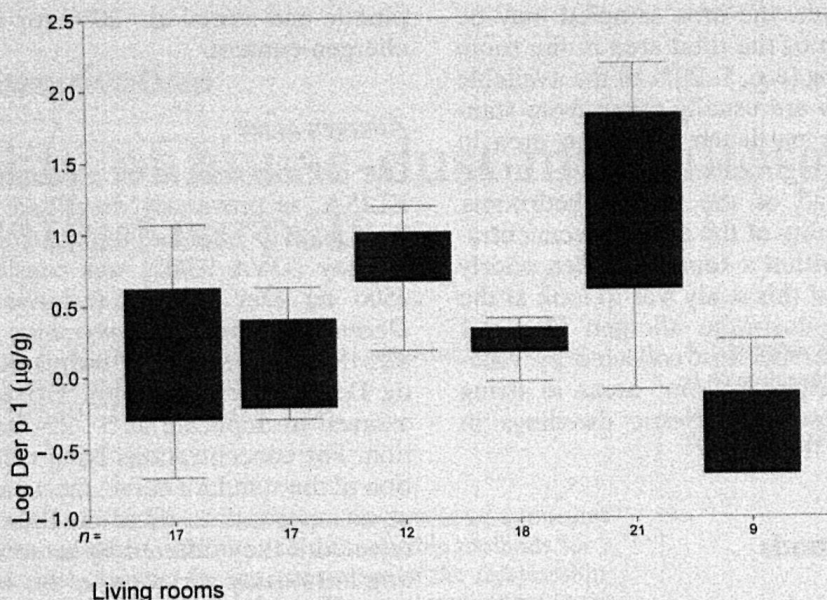


Fig. 2. Variability of house-dust-mite allergen Der p 1 levels in reservoir dust collected from carpets in six living rooms. Box shows geometric mean (—), 25th percentile (bottom of box), and 75th percentile (top of box). Whiskers show minimum and maximum values. Number of 1 m<sup>2</sup> samples collected in each of rooms is indicated on x-axis.

range of allergen within a single room was 0.9 µg Der p 1/g of dust (0.2 and 1.1 µg/g; 5.5-fold difference), and the largest was 149.2 µg (0.8 and 150 µg/g; 192-fold difference) Der p 1/g of dust. Three rooms in the study had mite allergen levels below the limit of detection in a small number of areas, while the rest of the room had quantifiable levels. The mean range of allergen levels in these rooms was 11.5 µg Der p 1/g of dust.

As an illustration of the distribution of Der p 1 in different areas of carpet, allergen levels from one of the living rooms analysed are presented in Fig. 1. Although from the figure it may appear that the concentrations in this particular room are higher around the edges, this pattern was not observed when all rooms were taken into account. No clear pattern of distribution of house-dust-mite allergen within rooms could be found (e.g., Der p 1 levels were not higher near areas of soft furnishings than at other areas within the room).

Fig. 2 demonstrates the GM, interquartile range, and range of Der p 1 levels within the six living rooms. The mean coefficient of variation of these

rooms was 80.2%, illustrating the huge variation of mite allergen levels within each room.

Similarly, GM, interquartile range, and range of Der p 1 levels within the nine bedrooms are presented in Fig. 3. The variation within these rooms was also large, with a mean coefficient of variation value of 88.7%.

GM, ranges, and coefficients of variation of Der p 1 levels in each of the bedrooms and living rooms, respectively, are presented in Tables 1 and 2. Taking only the rooms in which the levels of Der p 1 were above the detection limit in at least one sample, we compared the mean level of Der p 1 for the whole room (GM 4.95 µg/g) to the value obtained with the standard sampling technique (sampling only a single 1 m<sup>2</sup> area in front of the sofa or to the left side of the head of the bed; GM 5.36 µg/g) and found no significant difference between these values (mean difference 8.3%, 95% CI -43.4 to 107.5, *P*=0.8).

We then divided the rooms into two areas – one consisting of samples taken adjacent to the soft furnishings (living rooms) or beds (bedrooms), and

Table 1. Der p 1 levels (µg/g dust) in nine whole bedrooms

|                              | 1     | 2     | 3    | 4     | 5     | 6     | 7    | 8     | 9    |
|------------------------------|-------|-------|------|-------|-------|-------|------|-------|------|
| Geometric mean               | 12.8  | 24.61 | 0.58 | 19.4  | 8.55  | 19.9  | 1.52 | 3.11  | 7.14 |
| Single value                 | 72.5  | 25    | 1.1  | 37.25 | 2.78  | 8.43  | 6.96 | 13.23 | 15   |
| Range                        | 71.5  | 16.3  | 0.9  | 37.05 | 13.57 | 57.02 | 6.76 | 12.52 | 12.9 |
| Coefficient of variation (%) | 138.1 | 73    | 57   | 64.5  | 49.2  | 79.9  | 181  | 98.59 | 57.2 |

Single value denotes Der p 1 level in 1 m<sup>2</sup> area that would have been sampled by normal procedures if only 1 m<sup>2</sup> were sampled per room.

Table 2. Der p 1 levels ( $\mu\text{g/g}$  dust) in six whole living rooms

|                              | 1     | 2      | 3    | 4     | 5     | 6    |
|------------------------------|-------|--------|------|-------|-------|------|
| Geometric mean               | 2.89  | 38.5   | 1.51 | 7.24  | 1.79  | 0.61 |
| Single value                 | 0.2   | 24.25  | 0.55 | 9     | 1.9   | 0.8  |
| Range                        | 11.41 | 149.22 | 3.7  | 13.75 | 1.6   | 1.55 |
| Coefficient of variation (%) | 108.4 | 129.4  | 75.7 | 54.14 | 27.06 | 85.5 |

Single value denotes Der p 1 level in  $1 \text{ m}^2$  area that would have been sampled by normal procedures if only  $1 \text{ m}^2$  were sampled per room.

the other consisting of samples from the remaining area of the room – and calculated the coefficient of variation of each part. When the values of the two parts of the room were compared, the coefficient of variation was found to be significantly lower in the part of the room with samples taken around soft furnishings or beds (57%) than in the rest of the room (89.3%), with the mean difference being 32% (95% CI 2–63%;  $P=0.04$ ).

## Discussion

Measuring house-dust-mite allergen content in reservoir dust samples is the accepted method of estimating exposure to mite allergen and is used in epidemiologic studies worldwide. This study suggests that sampling from a single  $1 \text{ m}^2$  area in a room may not give reliable information about the level of mite allergen in the rest of the room. Three rooms in the study had mite allergen levels below the limit of detection in a small number of areas, while the rest of the room had quantifiable levels.

These rooms were singled out to illustrate the point that taking a sample from a single  $1 \text{ m}^2$  area may lead to the conclusion that exposure to Der p 1 in that room is very low. However, the mean range of allergen levels in these rooms was  $11.5 \mu\text{g}$  Der p 1/g dust, making it clear that if another  $1 \text{ m}^2$  area had been sampled, it could have been concluded that exposure in the room was high ( $>10 \mu\text{g}$  Der p 1/g dust). We found less variation in mite allergen levels around soft furnishings. When the mean Der p 1 levels of the whole room were compared to the value we would have obtained with the standard sampling technique, there was no significant difference between these values.

In Sydney, Australia, Tovey performed a similar experiment. Allergens were measured within 10 rooms in every half-metre square (a quarter of the area sampled in this study), and no consistent pattern of distribution was found (8). From an example given in Tovey's study of the allergen distribution within a bedroom, the coefficient of variation was found to be 31.7%. This is much lower than the mean coefficient of variation of the bedrooms in our study (88%). However, the mean mite allergen levels in Sydney are  $13.7 \mu\text{g}$  Der p 1/g dust in living rooms and  $22.4 \mu\text{g}$  Der p 1/g dust in bedrooms (9, 10) compared to  $1.5 \mu\text{g}$  Der p 1/g dust in living rooms and  $1.06 \mu\text{g}$  Der p 1/g dust in bedrooms in Manchester (11).

The mean mite allergen level measured in all 20 rooms in this study was  $2.25 \mu\text{g}$  Der p 1/g dust, which is slightly higher than the average value for Manchester (11). The selection of samples for this

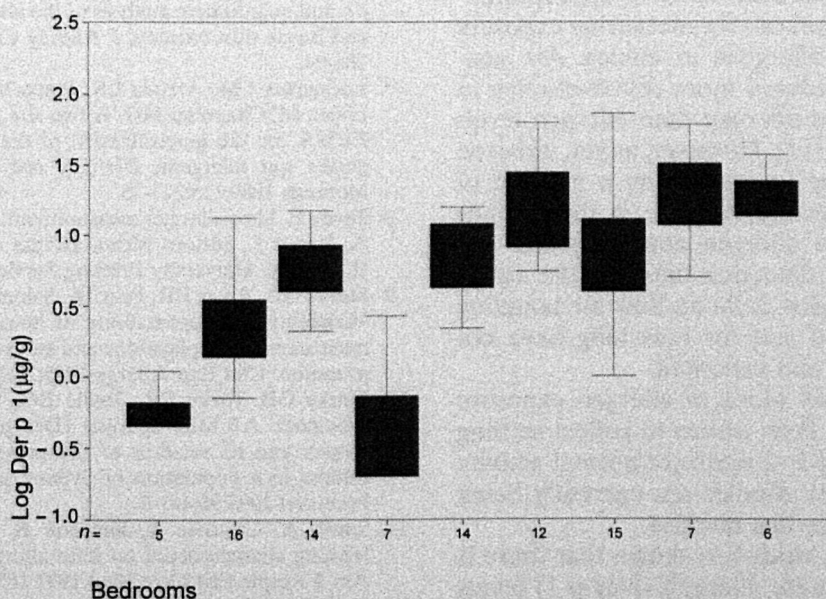


Fig. 3. Variability of house-dust-mite allergen Der p 1 levels in reservoir dust collected from carpets in nine bedrooms. Box shows geometric mean (—), 25th percentile (bottom of box), and 75th percentile (top of box). Whiskers show minimum and maximum values. Number of  $1 \text{ m}^2$  samples collected in each of rooms is indicated on x-axis.

study was not random, because we performed the study in a selection of homes previously known to have quantifiable allergen levels. If we had taken a random sample of rooms, it would be likely that the mean allergen levels would be lower and this may lead to lower variability, due to a larger number of rooms with very low allergen levels.

This study failed to demonstrate a consistent pattern of distribution of mite allergen within carpeted rooms. It would be interesting to determine whether house-dust mites are distributed similarly to allergen. It is possible that some areas of microhabitat such as carpets have a microclimate that is more suitable for house-dust-mite population growth and survival than the others, and this in turn may affect population density (and allergen levels) within each of these areas. However, the factors influencing dust-mite populations within individual homes are still poorly understood (12).

The important question arising from these results is whether a single sample collected from 1 m<sup>2</sup> can be considered an appropriate index of exposure. This would probably have little consequence in large-scale epidemiologic studies where comparisons are made between geographic areas with differing levels of mite allergens (as we have shown that in the whole sample of homes in the current study, the mean Der p 1 level of each whole room did not differ significantly from the Der p 1 level measured in the single 1 m<sup>2</sup> area which had been sampled by the usual technique). However, when one assesses the exposure of an individual patient, a single 1 m<sup>2</sup> area sample of carpet may be misleading as an index of exposure, and dust collection from the whole carpet may be more appropriate.

There are other methods for measuring exposure to house-dust-mite allergens in homes. Air sampling might be considered more representative as a measure of inhaled allergen than allergen levels in the reservoir dust (13). However, as yet, airborne sampling has serious limitations as a method of measurement of exposure, because, in the absence of disturbance, mite allergen concentrations are generally below the detection limits of the assays (2, 4, 14, 15). Strategies defining how air sampling should be performed and for how long have not yet been developed and validated.

A more satisfactory index of allergen exposure may be to use large Petri dishes to collect settling dust over a period of 1–2 weeks of normal activity within the home (16). Studies are currently being performed to validate this method.

In conclusion, this study has shown that there is a great variation of mite allergen (Der p 1) levels between areas within a room with no consistent pattern of distribution. Der p 1 levels in areas around soft furnishings varied less than Der p 1

levels in the rest of the room. Encouragingly, the mean Der p 1 level of each whole room sampled did not differ significantly from the Der p 1 level measured in the single 1 m<sup>2</sup> area which would have been sampled by the usual technique. Sampling a single 1 m<sup>2</sup> area of carpet remains the most appropriate method of assessing exposure in large-scale epidemiologic studies.

## Acknowledgments

This work was supported in part by Foundation Lancardis. Conflict of interest: none.

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