

Risk levels for mite allergens. Are they meaningful?

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Allergens found in house dust are among the most common environmental antigens to which man is naturally exposed. Standardized methods for measuring allergen exposure are essential for assessing the relationship between exposure, sensitization, and the severity of asthma. Monoclonal antibody-based assays are the most widely used method for assessing allergen exposure. In the effort to define the best "index of exposure" to mite allergens, several factors need to be investigated, including:

- 1) whether allergen should be measured in reservoir dust or airborne
- 2) whether the results of reservoir measurement should be expressed as recovered allergen per unit weight or per unit area.

As yet, airborne sampling is insufficiently sensitive to produce reliable and repeatable results. Therefore, measurement of house-dust-mite allergen concentration in reservoir dust should be regarded as the best-validated index of exposure. The results should be expressed and reported both per unit weight (concentration) and per unit area. The strongest predictor of chronic symptoms and acute exacerbation of asthma is sensitization to indoor allergens. A simple dose-response relationship between IgE-mediated hypersensitivity and allergen exposure has been established. For example, exposure to more than 2 µg Group 1 mite allergen/g dust should be regarded as a risk factor for the development of IgE antibody and asthma in susceptible children. The quantitative relationship between exposure and symptoms in patients already sensitized is complex due to a number of possible confounding factors (e.g., other allergens, viruses, asthma medication). A simple threshold level for provocation of asthmatic symptoms has not been clearly defined.

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Allergens present in house dust are among the most common environmental antigens to which man is naturally exposed. They can comprise up to 20% of the protein in house-dust extracts (1). Standardized methods for measuring indoor allergen exposure are essential for assessing the relationship between exposure and sensitization and the degree of exposure associated with provocation of asthmatic symptoms.

Measurement of exposure to mite allergens

There are three generally accepted methods of estimating exposure to dust-mite allergens: mite

counts, assays of specific mite allergens, and measurement of guanine (the major nitrogenous excretory product of arachnids) (2). Mite counts have been shown to be a good index of exposure, but the technique is time-consuming and requires considerable acarologic expertise. Furthermore, mite counts do not necessarily reflect the level of mite allergens, as allergen levels in house dust can remain high for several months after the fall in mite numbers (1). A semiquantitative assay for guanine (Acarex test) may provide a useful screening test in clinical practice for assessment of the presence of mites, but it is not specific, as it measures guanine from other sources as well (e.g., spiders) (3). A new semiquantitative monoclonal antibody (mAb)-based immunodot strip technique for the detection

Table 1. Indoor allergen measurement by mAb ELISA

Allergen	Capture ¹ mAb	Second ² antibody	Reference standards
Der p 1	5H8	4C1	NIBSC 82/518, UVA 93/03
Der f 1	6A8	4C1	CBER E1-Df, UVA 93/02
Group 2	1D8	7A1	CBER E1-Df or Dp, UVA 92/01, 92/02
Fel d 1	6F9	3E4	CBER Cat E5
Can f 1	Cf-1b	Rabbit IgG Ab	NIBSC 84/685
Bla g 1	10A6	Rabbit IgG Ab	UVA 93/04
Bla g 2	7C11	8F4	UVA 93/04
Asp f 1	4A6	Rabbit IgG Ab	Affinity purified Asp f 1

¹ Used as 50% ammonium sulphate fractions of ascites and coated on microtitre plates at 1 µg/well.

² Biotinylated mAb used at ~20 ng/well. Rabbit secondary antibodies are detected by peroxidase-labelled polyclonal goat antirabbit IgG.

of house-dust-mite, cat, and cockroach allergen has been developed (4). In research, mAb-based assays are the most widely used method for assessing allergen exposure.

The assays

Immunoassays have had a major impact on many areas of biomedical research. They have been developed for a wide range of antigenic substances; are sensitive, specific, and reproducible; and can give quantitative measurement of a given antigen in absolute units (e.g., ng or µg of protein). Over the past decade, immunoassays for measuring dust-mite, cat, dog, cockroach, rodent, and some fungal allergens have been developed. This technology has been used to compare allergen levels in reservoir dust samples from the homes of patients with asthma and other atopic disorders; to analyse the effect of different avoidance procedures on the allergen levels in homes; and to investigate the particle size distribution and concentration of airborne allergens. As a result of these studies, it has been possible to propose thresholds for allergen levels that are clinically important and to achieve a better understanding of the dose-response relationship between asthma and indoor allergen exposure.

Environmental allergen measurement

Immunoassays currently in use for the measurement of indoor allergens are summarized in Table 1 (5–17). ELISA assays are technically straightforward and can be used for testing of large numbers of samples, thus making them ideal for large-scale epidemiologic studies and clinical trials. However, there is still considerable debate as to whether exposure should be assessed by measuring settled dust from allergen reservoirs or by sampling ambient air; and whether results for settled dust

should be expressed as recoverable allergen per unit weight or per unit area (18–22).

Monitoring exposure to house-dust-mite allergens

It is critical to define what constitutes clinically relevant mite allergen exposure, both in determining the quantitative relationship between exposure and allergic disease and in designing a domestic environment which can provide lower allergen exposure. It is obvious that measurement of inhaled allergen would be desirable for assessing exposure. Unfortunately, it is technically impossible to measure the amount of allergen inhaled by an individual. The aim of measuring the concentration of house-dust-mite allergen within the individual's environment is to determine the measure that will represent an "index of exposure". However, there is still no universally accepted agreement on the best way to estimate the exposure.

Airborne house-dust-mite allergen

Airborne sampling is widely used in the assessment of occupational exposure and, as this is probably the primary route of exposure, it might be considered more representative as a measure of inhaled allergen than allergen levels in the reservoir dust. However, apart from the results of one study (21), there have been few data demonstrating a relationship between airborne levels of mite allergens and sensitization or asthma symptoms (19). As determined in most laboratories, airborne mite allergens are below the detection limit in undisturbed conditions. The major obstacle to the successful measurement of airborne mite allergen is its very low concentration in the absence of vigorous disturbance. De Blay et al. measured airborne concentrations and particle size distribution of both Group 1 and Group 2 mite allergens in seven houses with high levels of mite allergens in the carpet and furniture dust before, during, and after disturbance (23). In none of the houses were Group 1 or Group 2 mite allergens measurable in the air. However, during artificial disturbance (vacuum cleaning without a filter), mite allergens were measurable in all of the houses. Both Group 1 and Group 2 allergens were predominantly associated with large particles (>20 µm), and fell rapidly after disturbance (no detectable airborne mite allergens were found 20 min after the disturbance). Other investigators tried various methods to overcome the difficulties of airborne sampling. Sakaguchi et al. used a more sensitive radioimmunoassay for allergen measurement (24). The airborne levels of Der p 1 were very low (around 30 pg/m³), and no correlation between

airborne and reservoir levels was found. Aeroallergen level increased about 1000-fold in disturbed conditions (bed making). Swanson et al. used a mAb-based ELISA for allergen determination, but increased the volume of air sampled to 288 m³ by using high-volume samplers (200 l/min) operating for 24 h (25). Again, the concentration of airborne mite allergens was very low in the absence of disturbance (20–30 pg/m³), but increased greatly in the bedroom during bed making.

It is likely that most exposure to mite allergens occurs overnight in bed. Sakaguchi et al. reported that airborne levels of Group 1 and Group 2 mite allergens in bed were approximately 10 times as high as those during everyday life in the living room (26).

Measurements in the reservoir dust: µg/g or µg/m²?

The vast majority of mite allergens in domestic dwellings is contained in the dust reservoirs in beds, furnishing, carpets, and clothing. Allergens are usually measured in dust samples collected by vacuuming dust reservoirs within the home (carpet, mattress, bedding, and upholstered furniture) for a standardized period of time (usually 2–5 min/m²) (2, 19). This method of measuring exposure has remained virtually unchanged for the last two decades (1). The basis of our understanding of the dose-response relationship between exposure and allergic sensitization was formed by the measurement of allergen concentration in settled dust. Results of reservoir samples are usually expressed as allergen per unit weight (e.g., µg Group 1 mite allergen/g of dust), and a standardized procedure was given in the report of the First International Workshop on Mite Allergens and Asthma (2).

However, the Position paper of the British Society of Allergy and Clinical Immunology Working Party on the control of allergens of dust mites and domestic pets suggested that recoverable allergen per unit area may be a better “index” of exposure than allergen per unit weight of dust (20). This was particularly advocated for the trials of mite control which involve killing mites followed by repeated vacuum cleaning in order to remove dead mites and the allergen pool, or when mite control involves addition of certain chemicals (e.g., acaricides) (20). However, the recovery is influenced by a number of factors (flow rate of the vacuum cleaner used for dust sample collection, the technique of the collector, the cleaning regimen in the house where sampling is taking place, etc.) (27). We have observed that using a different collection head for a vacuum cleaner can significantly affect the weight of dust sample. There was up to an eight-fold difference in weight of dust

collected from adjacent areas of carpet by the same collector and the same vacuum cleaner when different sampling heads and different filters were used (e.g., cotton sheet or glass microfiber filter). This was probably due to the effect of sampling head, filter, and the dust sample itself on the actual airflow rate of the vacuum cleaner. There is no point in trying to explain how different research groups define cleaners, filters, sampling sites, and times used during collection; they vary widely, the effect of this being largely unknown. Even if an agreement could be reached on the type of equipment used, a number of variables (e.g., enthusiasm of the collector) would still contravene any attempt at standardization. It is therefore not possible to standardize recovery, making comparison between the different studies very difficult.

Expressing results per unit weight is easier to standardize, and sieving dust makes it easier to compare samples collected at different sites (e.g., mattress and living-room carpet). The majority of studies have used this method, and it is therefore easy to compare data between different trials. We found a very good quantitative correlation between the results expressed as µg/g and µg/m² (28). Measurements per gram of dust are therefore appropriate for most epidemiologic studies. However, assessment of exposure by this method may underestimate the effect of allergen-avoidance technique performed at home. This was illustrated in a study by Neary et al., in which 12 sequential samples were collected from a fixed area of carpet by vacuuming for 12 min (29). When the results were expressed as µg/g, there was a marginal reduction in Der p 1 over the first three samples, and the concentrations remained the same in the remaining nine samples. However, when the results were expressed in µg/m², the levels fell linearly by up to 10-fold. On the other hand, the application of certain chemicals on carpets in an attempt to control dust mites (e.g., acaricides) might “dilute” the dust sample, giving a false impression of reduction in allergen level when the measurement is only made in µg/g. This can be important in trials in which effectiveness of mite-avoidance measures is being investigated. Therefore, expressing the results per unit area is useful, particularly in trials in which the size of the dust reservoir is changed by intervention.

The choice of whether to express the results of reservoir sampling as Group 1 mite allergen per unit weight of dust or per area sampled should depend on the question the study addresses. In epidemiologic studies, expressing the data as µg Group 1 allergen/g dust is appropriate. For mite-avoidance studies, quantification of allergen per unit area is a more logical way to express the

results. Practically, the easiest way to get around this problem is to report allergen levels both per unit weight and per unit area.

Whichever method is used for monitoring exposure to mite allergens, it has to be clinically valid, robust, reproducible, and reliable. Reservoir measurements have proved to have all of these characteristics. A spot measurement of very low airborne concentration of mite allergens cannot be considered a better "index of exposure" than reservoir measurements. As yet, airborne sampling is insufficiently sensitive to produce reliable and repeatable results and should be avoided as a method for the routine assessment of exposure to mite allergen until more sensitive assays or better sampling methods are developed.

Concept of threshold levels for exposure to indoor allergens

The relationship between exposure to indoor allergens and asthma can be investigated both in relation to sensitization and to symptoms and severity of asthma. With respect to sensitization, the evidence strongly suggests that there is a simple dose-response relationship between IgE-mediated hypersensitivity and allergen exposure; i.e., that the prevalence of sensitization is directly related to the concentration of allergen in the individual's indoor environment.

The First International Workshop on Mite Allergens and Asthma proposed two threshold levels for mite allergens that represent risk for sensitization and asthma: $\geq 2 \mu\text{g}$ Group 1 mite allergen/g was regarded as a risk for the development of IgE antibody and asthma, and a higher level of $10 \mu\text{g}$ Group 1 mite allergen/g was regarded as a risk for acute attack of asthma (2).

Mite-allergen exposure and IgE-mediated sensitization

One of the first studies relating house-dust mites to asthma suggested that a level of 200 mites/g of dust was significant (30). Since then, a number of reports from different parts of the world have demonstrated a dose-response relationship between allergen exposure at home and IgE-mediated sensitization. However, most of the studies investigating the relationship between exposure and sensitization focused on individuals at high risk of developing atopy. In Freiburg, Germany, Kuehr et al. have recently studied 1812 schoolchildren prospectively, performing skin prick tests to inhalant allergens at 12-month intervals over a period of 2 years, and measuring Der p 1 levels in mattresses (31). The

children were divided into those with previous positive skin tests (other than to mites) and those without: in nonatopic children, only extremely high exposure to Der p 1 ($>60 \mu\text{g/g}$) represented a significant risk of sensitization. Exposure to levels of Der p 1 of $>9 \mu\text{g/g}$ conferred a definite risk of sensitization in the whole population. However, for children already sensitized to other inhalant allergens, a significant risk of sensitization was posed by exposure to levels of Der p 1 of $>2 \mu\text{g/g}$. They went on to propose this as the minimal avoidance level of primary prevention in children with sensitization to other inhalant allergens.

Lau et al. found that, although mite allergen concentrations were similar in the beds of both atopic and nonatopic children, the levels were 25-fold higher in mite-allergic children (32). In atopic children exposed to Group 1 allergen levels of $>2 \mu\text{g/g}$, the odds ratio for the risk of developing sensitization to mite allergens was 5 (32). Furthermore, population studies conducted in six different climatic regions of New South Wales, Australia, with a broad range of mean Der p 1 levels, showed that the percentage of children sensitized to house-dust mite in each region increased with increasing mean levels of Der p 1 (33). In Poole, UK, household exposure to elevated levels of Der p 1 in amounts greater than $2 \mu\text{g/g}$ of dust in carpets and bedding during infancy were associated with increased prevalence of positive skin tests and increased concentrations of IgE specific to house-dust mite by the age of 5 years in children of atopic parents (34). In addition, in the same cohort of 68 children prospectively followed from birth until the age of 11, Sporik et al. found that exposure to Der p 1 level greater than $10 \mu\text{g/g}$ of dust measured in infancy was associated with a 4.8-fold relative risk of developing atopic asthma by the age of 11 (35).

Using data from several different studies, the US Institute of Medicine analysed the dose-response relationship between exposure and sensitization and reported a significant correlation between cumulative exposure to mite allergen and the risk of sensitization (36).

Mite-allergen exposure and asthma severity

Although it was relatively straightforward to demonstrate the dose-response relationship between exposure to indoor allergen and sensitization, proving this for exposure and asthma severity has been more difficult. The relationship between exposure and asthma symptoms in already sensitized individuals is probably much more complex than in the case of exposure and sensitization. Many patients with asthma are exposed and sensitized to more than one indoor allergen, and

the relative contribution of each to the airway inflammation may be difficult to elucidate. "Trigger factors" such as viruses, air pollution, exercise, and emotion may make asthma severity worse. Some sensitized patients will react to a very low dose of allergen, while in others the level required to cause symptoms will be considerably higher than that inducing sensitization. Nevertheless, sensitized individuals are likely to have more severe asthma if exposed to high allergen levels than if their level of exposure is low (37). As the relationship between allergen exposure and symptoms of asthma among sensitized patients is very complex, simple threshold levels for provocation of asthmatic symptoms are not appropriate.

For pet and cockroach allergens, the dose-response gradient has not yet been determined; this may be because the optimum way to quantify exposure has not yet been identified. In contrast to mite allergens, exposure to dog, cat, and cockroach allergens can occur outside the home (38, 39). Both *Can f 1* and *Fel d 1* are carried on smaller particles than mite allergen and so remain airborne for longer (40, 41). Sampling reservoirs in the home (carpets, mattresses, and sofas) may not give the best index of exposure for these allergens.

Conclusions

Measurement of house-dust-mite allergen concentration in reservoir dust should be regarded as the best validated index of exposure, and quantification of allergen both per unit weight and per unit area should be reported. As yet, airborne sampling is insufficiently sensitive to produce reliable and repeatable results and should be avoided as a method for the assessment of exposure to mite allergen until more sensitive assays or better sampling methods are developed.

There is strong epidemiologic evidence that there is a quantitative dose-response relationship between IgE-mediated hypersensitivity and allergen exposure, and exposure to more than 2 µg Group 1 mite allergen/g dust should be regarded as a risk factor for the development of IgE antibody and asthma in susceptible children. This value is applicable to the population, and should not be extrapolated to the clinical situation and individual patients. A simple threshold level for provocation of asthmatic symptoms has not yet been clearly defined.

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