

Mite allergens. Collection, determination, expression of results, and risk levels for sensitization and symptom induction

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The presence of mite allergens in dust can be determined by counting mites at different stages of development in dust and by determination of the major allergen content (Der p 1) in dust and air, which is crucially important to allergic patients. For comparison of results, similar methods for collection of dust and air must be used. Due to their size, mite bodies and fecal particles are airborne only directly after disturbance. Special filters should be used for dust collection, and upholstered surfaces should be vacuumed for 2 min/m² (minimum 4 m²) and hard surfaces for 1 min/m² (minimum 8 m²). Heavy contaminations should be removed. Preferably, the method given in the ISAAC study should be followed. Samples should be deep-frozen for at least some days to kill the mites. ELISA techniques, preferably using polyclonal antibodies and antigen with all isoforms present, should be used for determination of allergens. The allergen load has been given in ng/g of dust, but allergen/m² or per sample area should be preferred. Allergen in the air should be given in pg/m³. A tentative limit of 2000 ng/g was proposed for sensitization and asthma. This limit is still valid on a population basis, but recent data indicate that highly susceptible young children become sensitized at concentrations 10–100 times lower and that ng levels of cat allergen/m³, as found in schools, induce chronic asthma.

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Mites and mite allergens

Exposure to mites and mite allergens has been estimated by counting mites in dust and by determination of the amount of major allergens of mites in dust. There are about 15 different allergenic mite proteins in mite extracts. The most important are the fecal group 1 allergens, of about 25 kDa (Der p 1, Der f 1, Der m 1, Der s 1, etc.), and group 2 allergens, of about 14 kDa (Der p 2, Der f 2, etc.), originating from the mite bodies. Groups 1 and 2 allergens are present in all house-dust-mite species studied. They are called major allergens, because they sensitize most patients exposed to house-dust mites and are therefore considered clinically important. Today, the most convenient and rapid way to estimate mite-allergen exposure is by determination of mite allergens in dust by immunoassay (1–3).

Collection of mites/mite allergens in dust

To make it possible to compare results from different studies, similar methods must be used.

Most commonly, dust is sampled by vacuuming surfaces; however, in recent years, attempts have been made to collect mite allergens in the air (3). Very little mite allergen has been found in the air because after disturbances mites, as well as mite fecal particles, fall too quickly to the floor. Mite-allergen levels have been determined in dust from homes and other sites. In some cases, it is important to investigate the number of living mites and their different stages of development; however, for patients with allergies, the amount of mite allergen available for sensitization and induction of allergic reactions is far more important.

Since mites feed on human dander and need high humidity in order to survive and reproduce, the principal habitat of mites is probably the bed. Other surfaces and locations have also been investigated. The major sources of mite allergens are beds, carpets, and upholstery (4). They have been collected by scraping or vacuuming. Since it is very difficult to collect minute amounts of dust from paper bags (5), special filters should be used (6). The best way of determining the amount of

dust collected on a filter and of removing the dust from the filter, i.e., whether to weigh the filter before and after dust collection and then extract the total filter, or to scrape the dust from the surface of the filter (7), has not been properly studied. If the filter is extracted, it should be pretreated with serum albumin to avoid adsorption of proteins (allergens) on the surface (Munir & Dreborg, unpublished results).

The time of collection from the various types of surfaces must be standardized, e.g., 1 min/m² from hard surfaces and 2 min/m² from upholstery (6), to ensure that comparable results are obtained. The type of vacuum cleaner must also be standardized.

Collection of mites/mite allergens in the air

Few studies have found mite allergen in the air (3). Moreover, the data (ng/m³) obtained in these studies can be questioned because the amounts found were higher than the amounts of animal-dander allergens in the air, although animal-dander particles are airborne for much longer periods of time than are mite bodies and fecal particles. Therefore, the most important exposure to mites is probably in bed and through contact with textiles and upholstery in general.

Handling and extraction of dust

If the collected dust is not investigated immediately, it should be kept frozen at -20°C for a long enough period to ensure that all mites are killed. Stones, sticks, and similar debris should be removed. Whether the dust should be sieved or not is still under dispute (7). The impact on the results of the various ways of treating the dust samples has not been investigated. Usually, extraction is performed at 4°C overnight at 1/10 to 1/30 w/v, depending on the amount of material available, and preferably with ammonium carbonate as extraction solution, especially if the sample will be freeze-dried (in order to make it more concentrated when allergen levels are low).

One way to avoid confusion is to use the methods of dust collection (6) of the large ISAAC study (8) in which many centers all over the world are participating. The ISAAC study will probably serve as the standard, and important information about mite-allergen exposure and its relation to sensitization and reactivity will emerge from it over the next few years.

Determination of mite allergens

Either monoclonal or polyclonal antibodies have been used to measure mite allergens in the dust

extract. More or less modified ELISA assays have been developed, using purified allergen or mite-allergen extracts with known, quantified amounts of allergen as standards. Monoclonal antibodies recognize just one epitope, which can be present on all isoforms of the allergen to be measured, or only on some of them; whereas polyclonal antibodies recognize all epitopes/isoforms. We have observed that the content of the major allergen of the mite *Dermatophagoides siboney*, Der s 1, of different batches of *D. siboney* extracts assayed with monoclonals directed against Der s 1 differed unpredictably between batches from values obtained with a kit for determination of the highly cross-reactive mite allergen Der f 1 (Chapman) for estimation of the content of Der s 1. Sometimes the Der f 1 kit gave higher values than the Der s 1 kit, probably because of varying isoallergen recognition. Thus, one must consider that different monoclonals do not give the same result. The data obtained are not absolute, nor universally valid. Furthermore, the method of purification of the major allergen used as standard is important. If affinity chromatography with monoclonal antibodies is used to purify the major allergen used as standard (8), only a few isoallergens may be purified, and the standard curve will depend not on the total amount of the allergen, but on the presence of those isoforms. Therefore, the use of polyclonal antibodies in these assays is an advantage (9).

Presentation of data

The major allergen content is given in ng or µg of major allergen/g of fine dust. However, more clinically important may be the total amount of mite allergen vacuumed from the surface of the mattress or the total floor, since all those allergens are available for inhalation. However, there are few data supporting the use of new methods of estimation; for example, measuring the amount of allergen per m², amount per unit (mattress, floor, etc.), and amount per room or location. Until more experience has been obtained in the use of these estimates, it is advisable to state also the concentration of allergen.

Risk levels for sensitization to mite allergens

During the first international workshop on mite allergy (1), a tentative limit for the risk of sensitization was proposed; i.e., 2 µg/g of dust. A number of authors have supported this risk level. However, this risk level has been calculated on a general population basis. The amount of allergen

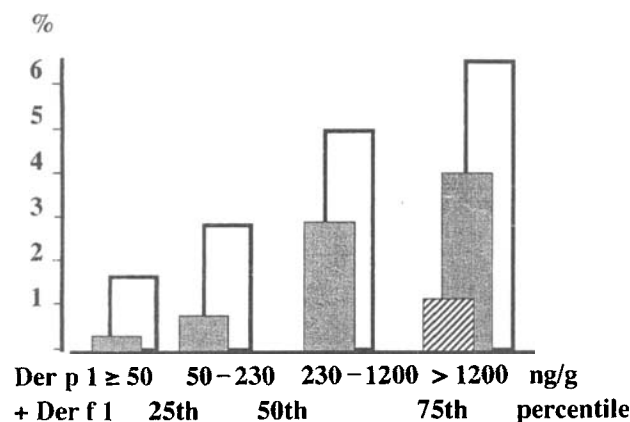


Fig. 1. Frequency of sensitization to house-dust mite at 1 ▨, 2 ■, and 3 years □ of age. Reproduced from Ref. 10.

needed for sensitization is different for individuals with low and high risk of developing allergy to mites. Most of the studies have been performed in areas with relatively high levels of mite allergens in all houses. However, children are sensitized to mites also in areas with low levels of mite allergens. Wahn et al. (10) recently reported that small children are sensitized to mites even at levels below 50 ng/g. They found that 6% of children in continental Europe were sensitized when exposed to less than 1.2 µg/g; i.e., the 75th percentile of concentration of mite allergen sensitizing 3-year-old children. Therefore, to reduce the risk of sensitization, children at risk of developing allergy should not be exposed to mite allergen at all. Furthermore, it can be concluded from Wahn et al.'s study that there is a clear dose-response relationship (Fig. 1); i.e., the higher the mite-allergen load, the more children become sensitized. Thus, according to present knowledge, sensitization may take place in a few predisposed children exposed to very low levels of allergen, and the number of children sensitized increases with time and the concentration of mite allergen in the home. It can be concluded that mite-allergen levels should be kept far below 1 µg of mite allergen/g of fine mattress dust in the homes of children at risk of developing allergies.

Risk levels for induction of symptoms in mite-sensitive patients

Risk levels for induction of allergic symptoms/asthma have recently been reviewed (3, 11). It is a complicated question. Platts-Mills et al. (referred to in Ref. 11) went to the homes of patients with acute asthma and found mite-allergen levels to be higher than 2 µg/g of dust. However, schoolchildren

with dog or cat allergy and asthmatic sensitization to cat and dog suffered worsening of their asthma when returning to school after summer vacation. School dust contains 1 µg of cat allergen and 10 µg of dog allergen per g of fine dust (12), and the air in schools contains 1–10 (100) ng/m³ of these allergens (13). Thus, the asthma of these children probably worsened due to exposure to a few nanograms of allergen per day. This corresponds to data from Ihre & Zetterström (14), who showed that inhalation of 1 ng of major allergen in the morning over a 7-day period increased bronchial hyperreactivity in allergic asthmatics. However, it should be remembered that these data cannot be easily translated to ng of mite allergen/g of mattress dust, since it is very difficult to estimate the inhaled quantity, i.e., to attach filters close to the mouths of sleeping subjects. It should also be remembered that the reactivity differs approximately a million-fold between people sensitized to the same allergen (15); e.g., mites. Thus, what induces severe symptoms in one patient does not necessarily cause symptoms in another.

According to present information, any traceable amount of mite allergen can probably worsen the asthma of asthmatic patients highly sensitive to mites. Since mite-allergic asthmatics cannot totally avoid mites, the amount should be reduced as much as possible.

Conclusions

At present, many methods are used to collect dust and air samples and determine mite-allergen content. There are many methodological problems which must be solved and the solutions then implemented. So far, methods used to estimate the amount of mite allergen inhaled are experimental.

Risk levels for sensitization and induction of symptoms can be given for populations in highly mite-infested areas; however, to avoid sensitization of the most allergy-prone individuals, as well as symptoms in those most sensitive to mites, there should be virtually no detectable mite allergen in the environment; i.e., ng/g levels.

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