

Exposure to mite and cat allergens on a range of clothing items at home and the transfer of cat allergen in the workplace

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Background: Clothing has been proposed as an additional source of exposure to mite and cat allergens. Dispersal of allergen into public places has also been attributed to clothing.

Objectives: We sought to study the contribution of various types of clothing on mite and cat exposure in a domestic environment. Also, we studied the ability of clothing to transfer allergen in a workplace.

Methods: Personal exposure to mite and cat allergen from a range of clothing was measured by using intranasal air samplers in 11 homes. Five categories of clothing were tested. Wearing no upper clothing was the sixth category tested to distinguish the contribution of clothing over ambient background exposure. An adhesive tape was used to sample allergen from the surface of clothing, and reservoir dust samples were also collected. The above techniques were also used in the workplace to examine the amount of cat allergen transferred from cat owners to non-cat owners.

Results: The amount of mite and cat allergen inhaled differed among the clothing types worn and whether they had been washed recently. Wearing a woolen sweater increased personal allergen exposure to cat and mite allergen by a mean of 11 and 10 times, respectively. Clothing items that were less frequently washed carried more allergen whether assessed by vacuuming or sampled with adhesive tape. This corresponded to the amount of allergen inhaled. We also found that cat levels on non-cat owners' clothing increased significantly at the end of a working day, which lead to the increase in their personal allergen exposure to cat.

Conclusions: These studies strongly support the emerging model that personal clothing is an important source of both mite and cat allergen exposure. This article also demonstrates the importance of clothing as a means of distributing cat allergen into cat-free environments. (*J Allergy Clin Immunol* 2000;106:874-9.)

Key words: Clothing, aeroallergens, *Der p 1*, *Fel d 1*, immunostaining

Exposure and sensitization to a specific allergen continues to be one of the major risk factors for the development of allergic diseases.^{1,2} In groups with asthma or atopy, the prevalence of such sensitization can be as high

Abbreviations used

IOM: Institute of Occupational Medicine

PVDF: Polyvinylidene difluoride

as 60% for cat^{3,4} and up to 85% for house dust mites.⁵

High levels (>10 µg/g) of cat allergen (*Fel d 1*) can be found in reservoir dust from homes with cats.^{3,6} Cat allergen has also become ubiquitously distributed in public places, such as schools and day care centers, as well as in houses where there is no cat present.⁷⁻⁹ The presence of *Fel d 1* in cat-free environments has been attributed to allergen dispersed from cat owners to non-cat owners by means of clothing.^{8,10}

For house dust mite allergens, the most commonly cited reservoirs are beds, carpets, and soft furnishings. In temperate climates mean mite (*Der p 1*) allergen concentrations of 20 to 40 µg/g are not uncommon.^{11,12} Clothing has also been shown to be an additional source of mite allergen. In a study conducted by Tovey et al,¹³ a mean level of 15.9 µg/g of *Der p 1* was found in the upper body clothing of 35 adults. These results suggest that clothing has the potential to be a potent source of both inhaled cat and mite allergens^{8,13,14} because of its ability to accumulate such allergens, its close proximity to the wearer, and the disturbance of dust that occurs while wearing the item of clothing. Expressing personal allergen exposure on the basis of reservoir concentrations remains of limited value for assessing an individual's exposure because allergen concentrations in reservoir dust have been found to be a poor index of airborne allergen exposure.¹⁵⁻¹⁷

In a study from our group in which personal exposure was measured by using intranasal air samplers, inhaled particles eluting cat allergens were collected from clothing in time frames as short as 10 minutes.¹⁸ We found that personal exposure at work was massively increased if the person sampling wore an item of clothing belonging to a cat owner.

We have further applied these technologies to study a group of subjects at home with a range of clothing items and distinguished the contribution of clothing over ambient background allergen exposure. In addition, we were interested in measuring the transfer of cat allergen from cat owners to non-cat owners by means of clothing during the working day. We used the results obtained from the intranasal samplers to explore the relationship of airborne allergen exposure to the quantity of allergen in dust samples collected from the clothes with a vacuum cleaner and

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also by another technique that involves sampling the allergen available on a clothing surface with adhesive tape. This technique was applied to examine the changes in the amount of allergen on the surface of garments.

METHODS

Sampling conducted at home

This study was performed in 11 homes; 4 of the homes shared residence with a cat. Informed consent was obtained from each subject, and experiments were approved by the Human Ethics Committee of the University of Sydney. Subjects were instructed to select a representative item from the following 5 categories of clothing: (1) a freshly washed unstored T-shirt; (2) a T-shirt that had been worn in the previous week and had not been washed; (3) a woolen sweater; (4) a jacket (sleeved outer garment type, nonwater proof type); and (5) a type of long-sleeved cotton top (sweat shirt). The sixth test category was to wear no upper body clothing. Each item of clothing was worn in the above order. After putting the clothing item on, subjects were instructed to wait for 10 minutes before commencing nasal sampling to allow disturbed dust to settle. Intranasal samplers were then worn for 15 minutes while seated on a nonfabric chair (wood or vinyl chair) in the kitchen (noncarpeted floor). No vigorous movements were made during this time to reduce the active disturbance of allergen from clothing. This procedure was repeated for all 6 categories. After the completion of all the nasal sampling, surface tape and reservoir dust samples were collected, as described below. Information on the fabric type, along with the last time the item was washed and its usual washing frequency, was recorded.

Allergen sampling at work

All sampling was conducted among the staff at one of the Institute of Respiratory Medicine's laboratories in Sydney. Informed consent was obtained from each subject, and experiments were approved by the Human Ethics Committee of the University of Sydney. Of the 15 staff present, 5 had cats at home. Non-cat owners were instructed to wear clothing that had been freshly washed. On arrival to work, all participants were given intranasal samples to wear while sitting down for 15 minutes. After nasal sampling, the upper clothing was sampled for surface allergen by rolling upper clothing with a tape adhesive. During the day, 3 air samplers operating at 2 L/min were spaced in the room at head height. Each sampler had an Institute of Occupational Medicine (IOM) sampling head containing a polyvinylidene difluoride (PVDF) membrane (1- μ m pore size). These were operated for 8 hours. Participants carried out their normal duties, and at the end of the working day, intranasal samplers were worn again for 15 minutes in the same area as worn at the beginning of the day. The clothing were resampled again with tape adhesive, and this time reservoir dust samples were also collected from the clothing.

Intranasal sampling

Inhaled personal exposure was sampled by using intranasal samplers (Inhalix, Sydney, Australia). These collect most particles larger than 10 μ m and greater than 50% of particles larger than 5 μ m in aerodynamic diameter that are inhaled during normal respiration.¹⁹ Inhaled particles were impacted onto a strip of transparent tape adhesive (Inhalix) inside the sampler. For each pair of nasal samples in the domestic study, one was immunostained to detect Der p 1, and the other was immunostained to detect particles eluting Fel d 1. Nasal samples from the work study were all immunostained to detect Fel d 1.

Rolled samples

After nasal sampling, a hand-held roller, which was covered with a 4 cm $\times$ 9 cm section of the same adhesive tape used in the nasal sam-

pler, was used on the surface of the clothing. The front of the garment was rolled once from the top of the garment to the bottom (total area covered, 144 cm²). The rolled tape samples from the domestic study were cut into 12 squares, 6 of which were immunostained for Der p 1 and 6 for Fel d 1. No rolled tape sample was collected for category 6 (no upper clothes). The rolled tape samples for the work experiment were cut into 6 (1 cm²) pieces and immunostained to detect Fel d 1.

Reservoir dust

After the completion of all the nasal and rolling sampling, separate reservoir dust samples were collected for 2 minutes from each item of clothing by using a hand-held vacuum cleaner (Makita Electric Works Ltd 4071D, Anjo, Japan). Dust was collected from the outside of the clothing while it rested on a table. A dust sample was also collected from the kitchen floor (total area of 1 m²) during the domestic study. Samples were analyzed by using a standard ELISA assay for Der p 1 and Fel d 1. The same protocol was used to collect reservoir dust from the clothing items at the end of the work day.

Immunostaining. The Halogen assay (Inhalix) was used to immunostain nasal and rolled samples. The adhesive tapes were removed from the nasal samplers and overlaid with PVDF membrane (pore size, 1 μ m; Inhalix). Similarly, the adhesive films used for rolling were laminated with a section of PVDF membrane. Conversely, the PVDF membranes used for IOM air sampling were laminated with the adhesive tape. The resultant membrane-adhesive sandwich was wetted briefly with 80% methanol and then incubated in borate buffer overnight to allow allergens to elute from the particles and bind to the membrane. After blocking the membranes, the bound allergens were detected by immunostaining with an allergen-specific mAb (5H8 for Der p 1 or 6F9 for Fel d 1; Indoor Biotechnologies, Charlottesville, Va) followed by an anti-mouse alkaline phosphatase conjugate (Sigma, St Louis, Mo) and developed by using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate (Pierce, Rockford, Ill). The number of particles containing allergen (as determined by the presence of a stained halo around them) were counted under 100 $\times$ magnification.

Reservoir allergen concentrations. Samples of fine dust were extracted (50 mg/mL buffer) and assayed for Der p 1 and Fel d 1 by using the established monoclonal-based ELISA assays.^{20,21} Results were calculated in micrograms of allergen per gram of fine dust. The detection limit of the mite and cat ELISA assays were 0.5 and 0.2 ng/mL, respectively.

Data analysis among inhaled allergen, surface tape sampling, and reservoir dust

Because the distributions of reservoir dust allergen concentrations and particle counts for rolled samples were positively skewed, values for these measures were log₁₀ transformed before estimation of geometric means and for testing differences. Differences between clothing types in allergen concentrations and particle counts were tested with repeated-measures ANOVA. The distribution of nasal air sampler particle counts was not normal and included many zero values. It was not suitable for log transformation, and hence these data were analyzed by using nonparametric statistics: medians, interquartile ranges, and Friedman nonparametric 2-way ANOVA.

Pairwise correlations among reservoir dust concentrations, nasal particle counts, and rolled sample particle counts were estimated by using the Spearman rank correlation (ρ) separately for mite and cat allergen.

RESULTS

Domestic study

Reservoir dust allergen in clothes. Cat and mite allergen was detected in all reservoir dust samples taken from

TABLE I. Allergen concentration of mite and cat allergen in reservoir dust from clothing items

Item sampled	Concentration of Der p 1 ($\mu\text{g/g}$)	Concentration of Fel d 1 ($\mu\text{g/g}$)
T-shirt (clean)	3.6 (1.4-15.8)	3.5 (1.7-8.3)
T-shirt (worn and unwashed)	5.4 (1.7-12.4)	5.9 (2.6-36.6)
Woolen sweater	25.5 (13.3-82.4)	13.1 (6.1-56.1)
Jacket	22.8 (8.9-79.2)	8.5 (4.4-53.4)
Cotton sweat shirt	20.1 (5.2-181.3)	5.4 (1.9-32.9)
Kitchen floor samples	2.3 (0.3-5.1)	1.9 (0.4-10.9)

Values are given as geometric mean (range).

TABLE II. Mite and cat particles detected on the surface of clothing by using the rolled tape samples

Item sampled	No. of Der p 1 particles	No. of Fel d 1 particles
T-shirt (clean)	2 (1-21)	5 (2-14)
T-shirt (worn and unwashed)	16 (5-50)	21 (7-212)
Woolen sweater	128 (21-416)	82 (19-158)
Jacket	35 (25-336)	26 (4-309)
Cotton sweat shirt	29 (8-437)	16 (2-92)

Values are given as median (range).

the clothing items and the kitchen floors (Table I). The 5 categories of clothing items were found to contain higher concentrations of both mite and cat allergen compared with the dust sample taken from the kitchen floor ($P < .05$). The effect of clothing type on allergen concentration differed between mite and cat allergen ($P = .03$). For mite allergen, woolen sweaters and jackets contained higher levels than clean and worn T-shirts ($P < .05$). The cotton sweat shirt also contained a higher concentration of mite allergen than clean or worn T-shirts ($P < .05$). All other comparisons of mite allergen in clothing items were not significant ($P > .05$).

Levels of cat allergen were significantly less in clean T-shirts compared with worn and unwashed T-shirts, sweat shirts, jackets, and woolen sweaters ($P < .05$). Cat levels were higher in woolen sweaters than jackets, sweat shirts, and worn and unwashed T-shirts ($P < 0.05$). All other comparisons of cat allergen in clothing items were found to be not significant ($P > .05$).

Nasal sampling. The number of inhaled mite and cat allergen particles detected during 15 minutes of intranasal sampling differed among the clothing items worn (Figs 1 and 2). The amount of mite and cat allergen inhaled increased significantly when worn and unwashed T-shirts, woolen sweaters, jackets, and sweat shirts were worn ($P < .05$). Subjects inhaled less allergen while wearing a clean T-shirt compared with the other items of clothing ($P < .05$). The highest levels of inhaled allergen occurred when jumpers and jackets were worn ($P < .05$). All other comparisons were not significant ($P > .05$).

Rolled samples. Particles containing mite and cat allergen were detected on all tape samples (Table II). Sig-

nificantly smaller amounts of both allergens were found on the surface of clean T-shirts compared with other items of clothing ($P < .05$). Woolen sweaters contained more allergen than jackets, worn and unwashed T-shirts, and sweat shirts ($P < .05$). Jackets also contained more cat allergen on the surface than sweat shirts ($P = .002$). All other comparisons were not significant ($P > .05$).

Correlations among inhaled allergen, surface tape sampling, and reservoir dust for the domestic study. The strongest correlation was between rolled (surface) particle counts and nasal sampler particle counts (Spearman $\rho = 0.78$ for mite allergen and 0.68 for cat allergen). The correlation between reservoir dust allergen concentrations and rolled surface particle counts was also strong (Spearman $\rho = 0.64$ for mite allergen and 0.72 for cat allergen). A weaker correlation was detected between nasal air sampler particle counts and reservoir dust allergen concentrations (Spearman $\rho = 0.60$ for both mite and cat allergen). All the above correlations were significant at a P value of less than .001 level.

Frequency of washing. The frequency of washing was recorded for all the items of clothing. Woolen sweaters and jackets were found to be washed the least number of times a year (average of 1 wash per year). T-shirts were washed more frequently (average of 40 times a year) than cotton shirts (average of 20 times a year).

Studies of allergen exposure in the laboratory

Nasal sampling. The number of inhaled particles carrying cat allergen increased significantly at the end of the day for non-cat owners ($P = .002$, Fig 3). No significant difference in inhaled cat allergen was observed between the beginning and end of the day for cat owners ($P = 1.0$).

Rolled samples. The number of particles eluting cat allergen per 6-cm^2 area collected by rolling the upper outer garments with adhesive tape on the arrival to work and again at the end of the day is shown in Fig 4. Cat particles detected from the clothing surface of non-cat owners significantly increased at the end of the day ($P = .0001$). No difference was observed in the number of cat particles from the surface of clothing from cat owners ($P = .9$).

Reservoir dust allergen in clothes. Cat allergen was detected in all of the reservoir dust samples collected from the clothing of the 15 subjects at the end of the working day. The mean concentration of cat allergen in reservoir dust collected from the clothing of cat owners was higher than the mean level in non-cat owners ($6.58 \mu\text{g/g}$ vs $1.92 \mu\text{g/g}$, $P = .03$ by 2-sample t tests assuming unequal variance).

Correlations among inhaled allergen, surface tape sampling, and reservoir dust for the work sampling. A positive relationship ($r = 0.727$) was found between samples collected with the intranasal samplers and the surface-rolled samples for cat allergen. No significant relationship could be demonstrated between reservoir dust and cat particles inhaled at the end of the day ($r = 0.240$), but a moderate relationship existed between surface tape samples taken at the end of the day and reservoir dust collected from the clothing ($r = 0.479$). All the above correlations were significant at P values of less than .001.

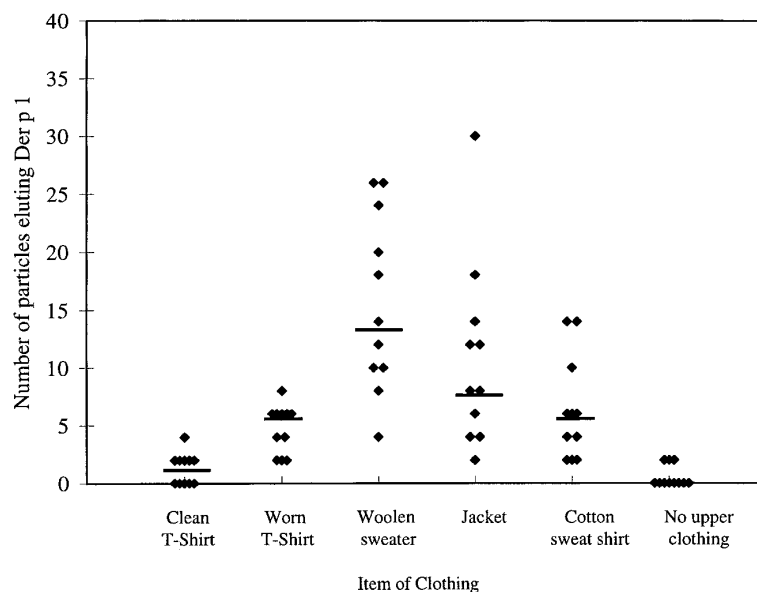


FIG 1. Number of particles inhaled eluting mite allergen (Der p 1) from various items of clothing in a domestic environment. Line indicates median score.

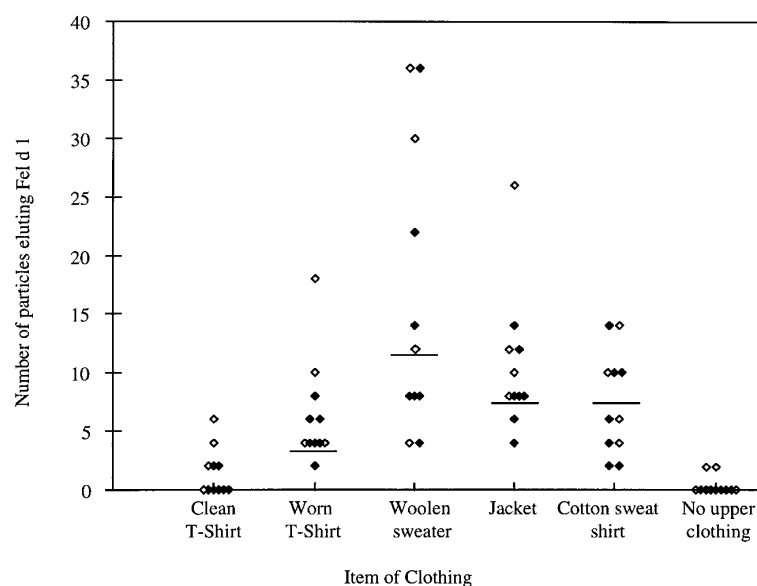


FIG 2. Number of particles inhaled eluting cat allergen (Fel d 1) from various items of clothing in a domestic environment. Line indicates median score. Open diamonds indicate subjects who owned cats.

Air sampling. The number of particles collected on the IOM samplers eluting cat allergen from the air during the work day was 12, 19, and 30 at the 3 locations in the room. This is equal to a concentration of cat allergen carrying particles in the air passing through the samplers of 12.5, 19.8, and 31.3/m³, respectively.

DISCUSSION

These studies strongly support the emerging model that clothing is an important source of exposure for both mite and cat allergen. It was found that personal exposure to

mite and cat allergen increases when items of clothing are worn that have not been freshly washed. Wearing a woolen sweater increased inhaled mite and cat levels by a mean of 11 and 10 times, respectively, above background domestic levels. It was apparent that clothing items that are infrequently washed carried more allergen, whether assessed by vacuum cleaning or sampled with an adhesive tape. This also increased personal allergen exposure because the amount of allergen inhaled also increased. The use of nasal samplers demonstrated the actual dose of allergen inhaled and enabled distinctions to be drawn between the reservoir amount and dose inhaled.

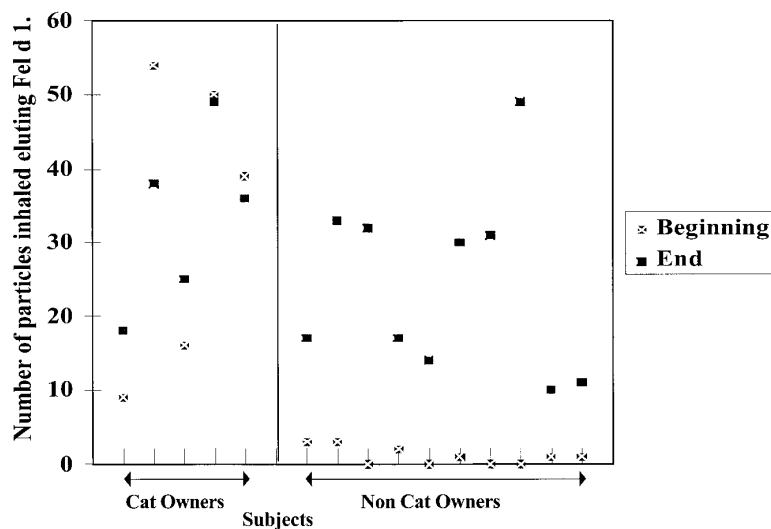


FIG 3. Each column shows the number of cat particles inhaled by a subject at the beginning and end of the day.

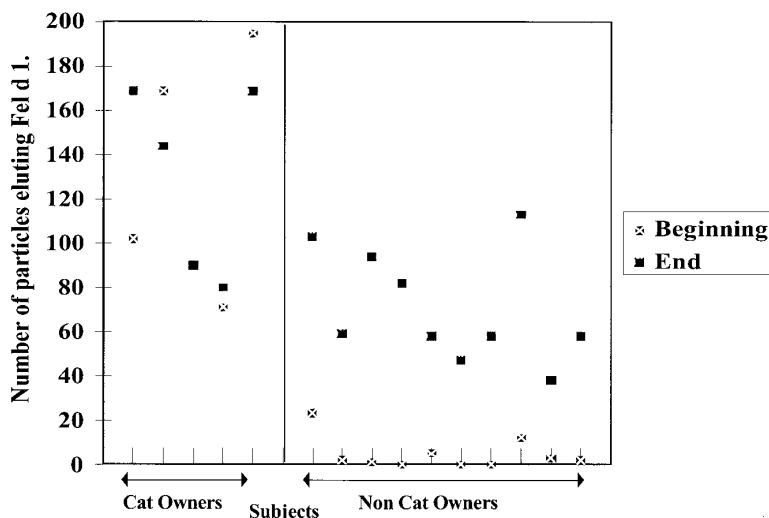


FIG 4. The number of particles eluting cat allergen collected on the clothing surface by the roller at the beginning and end of the day.

Cat allergen was detected in all reservoir dust samples collected from the kitchen floor, even in homes without cats. The clinical significance of Fel d 1 levels found in homes without cats is uncertain. Symptoms caused by cat allergen have occurred when there is no cat present and even on exposure to passively transferred allergen.^{22,23}

Levels of cat allergen on the clothing of non-cat owners increased significantly during the workday. This was positively correlated with the amount of cat allergen inhaled by these individuals. Non-cat owners' clothing throughout the day accumulated on average 48 particles of cat allergen per 6 cm², which is equivalent to approximately 24,000 particles over the entire surface of the clothing. Given that the ambient air concentration was around 21.2/m³, it is apparent that ambient allergen becomes concentrated on clothing and may therefore have the potential to be dispersed further to homes or other public places.

Almqvist et al⁹ measured cat allergen levels in homes, in schools, and on the clothing of school children. Like our study, they found that cat allergen on non-cat owners' clothing increased while at school. They also demonstrated the movement of allergen between homes and schools.

The allergen content of the clothing was assessed by 2 methods: the traditional concentration of reservoir dust and the number of particles directly sampled from the clothing surface that carried allergens. Although these 2 methods were correlated, it is perhaps not surprising that the amount of inhaled allergen, as determined by intranasal sampling, was more closely related to the quantity of allergen-carrying particles on the fabric surface than with the concentration of allergen in reservoir dust. The allergen collected from the surface of the clothing is likely to more closely represent what would be available to be released from a fabric into the ambient air.

The correlation between reservoir dust allergen concentrations and rolled surface particle counts was also strong for mite and cat allergen. The factors that determine the release of allergen from the surface of clothes have not been studied. In studies involving carpets, Price et al¹⁵ measured mite allergen in houses with woolen and synthetic carpets. They found significantly lower levels of airborne mite allergen from homes with synthetic carpets, even though the reservoir concentration of Der p 1 in the carpets was similar. It was hypothesized that a static charge develops on synthetic fibers that retain particles, thus preventing them from becoming airborne. Lewis et al²⁴ examined the recovery of dust by vacuuming new carpets with different physical structures that had been artificially loaded with dust. More allergen was found to be recovered from carpets that had a shorter pile and were treated with stain-resistant surface agents. These studies show that there may be a number of factors that influence the liberation of aeroallergen from clothing, such as surface charge, fiber structure, surface area of the clothing, the level of disturbance of the wearer, the capacity of the fiber to both accumulate and liberate allergen, and the quantity of dust involved.

This study uses 3 technologies that better enable the fine structure of the factors determining allergen exposure to be quantified. First, intranasal sampling allows the air that is inhaled by an individual to be sampled, reflecting true personal exposure. Although the samplers are highly efficient for particles with aerodynamic diameters above 10 μm ,¹⁹ we have recently become aware that many indoor natural allergen carriers are nonspherical^{25,26} and behave in a more aerodynamically complex manner, with resultant lower collection efficiency. As such, nasal sampling collects a consistent and representative portion of the entire personal exposure. Second, the sampling of surface allergen with adhesive tape is novel and provides an additional and simple index of allergen. The use of adhesive tape itself is not novel, and it has been used for forensic and contaminant sampling, but its direct use for allergen sampling is enhanced by the compatibility of this tape with the Halogen assay system. Particles eluting allergen can be examined along with the particle's morphology.

In summary, these studies strongly support the emerging model that clothing plays an important role in both mite and cat allergen exposure and in the transfer of cat allergen to cat-free environments.

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