

# Airborne Concentrations and Particle Size Distribution of Allergen Derived from Domestic Cats (*Felis domesticus*)

## Measurements Using Cascade Impactor, Liquid Impinger, and a Two-site Monoclonal Antibody Assay for *Fel d 1*<sup>1-4</sup>

CHRISTINA M. LUCZYNSKA, YIN LI, MARTIN D. CHAPMAN,  
and THOMAS A. E. PLATTS-MILLS

### Introduction

A large proportion of patients presenting with asthma or rhinitis will give a history of rapid onset of symptoms on entering a house with a cat (*Felis domesticus*) (1, 2). When tested, almost all of these patients will show a positive immediate hypersensitivity skin test to extracts of cat dander and will have serum IgE antibodies against cat allergens (3, 4). Further, it has recently been shown that the presence of serum IgE antibodies to cat allergen is a significant risk factor for acute attacks of asthma presenting to an emergency room (5). Because 28% of homes in the United States have at least one cat (which equals at least 50 million cats), it is difficult for anyone to completely avoid exposure to cats (6). A recent survey suggested that approximately 6 million Americans are allergic to cats, and although many persons allergic to cats do not have cats in their own houses, almost one third do (1, 7). It is now well established that the most important cat-derived protein stimulating IgE antibodies in allergic persons is a salivary glycoprotein with a molecular weight of 37,000 (*Fel d 1*), which was first purified by Ohman and colleagues (8-11). We have recently reported the production of monoclonal antibodies (mAb) to *Fel d 1*, which can be used both to purify the protein and for a sensitive two-site immunometric assay (12, 13). The fact that patients allergic to cats develop symptoms rapidly on entering a house with a cat suggests that the allergen must be continuously airborne (1, 2). This is in striking contrast to patients allergic to house mites who rarely react rapidly in a house unless the dust is disturbed. Several investigators have previously reported on measurements of airborne cat allergen using a variety of techniques for detec-

tion and Anderson samplers for separating particles (14-16). Those studies showed the presence of airborne cat allergen and suggested that it was carried on a range of particle sizes (14-16). The development of a more sensitive assay has made it possible to measure cat allergen using lower sampling rates, i.e., 1.2 m<sup>3</sup>/h (20 L/min). In the present studies, we have used two types of air sampling devices designed to collect particles by inertial impact. The cascade impactor is an instrument consisting of a series of stages on which particles can be separated on the basis of their aerodynamic diameter. It was originally developed for sampling microbiologic aerosols, but it has since been adapted for measuring airborne allergens (17-19). Air is drawn through progressively smaller rectangular jets and particles impact onto agarose-coated slides, which are then eluted and assayed for allergen content. In addition, we used a multistage liquid impinger that directs air through three progressively smaller round jets against a glass sinter surface under wa-

ter. Airborne particles of sufficient inertia deposit on the surfaces, and the final jet of the liquid impinger is operated as a critical orifice so that particles < 5 µm impinge at sonic velocity (20, 21). In the present experiments, the chambers of the impinger were filled with a buffer solution, which was assayed directly for allergen.

The experiments reported here show that a significant percentage (i.e., 10 to 60%) of airborne *Fel d 1* in domestic

**SUMMARY** The recent development of a sensitive two-site monoclonal antibody immunoassay for the major cat allergen (*Fel d 1*) has made it possible to make accurate measurements of airborne cat allergen using low volume samplers that do not disturb the room. Houses with cats had from 2 to 20 ng *Fel d 1*/m<sup>3</sup> air compared with < 0.2 ng/m<sup>3</sup> in houses without cats. Using a cascade impactor and a multistage liquid impinger, the particle size distribution of airborne *Fel d 1* in nine houses was 75% on particles ≥ 5 µm in diameter and 25% (range, 10 to 62%) on particles < 2.5 µm. In a cat vivarium with 12 cats, the air contained 40 ng *Fel d 1*/m<sup>3</sup>, but < 2% was detected on particles < 2.5 µm. The air exchange rate in the vivarium (~15 changes/h) appears to be the major difference from domestic houses (< 0.5 changes/h). Repeated studies in one house confirmed a very high proportion (~60%) of *Fel d 1* on small particles. During domestic cleaning, the levels of small particle allergen in this house approached those produced by a nebulizer for bronchial provocation, i.e., 40 ng/m<sup>3</sup>. These results show unequivocally that significant airborne *Fel d 1* is associated with small particles, which remain airborne for long periods. These findings are strikingly different from previous results obtained with airborne dust mite allergen. The results provide an explanation for the distinctive rapid onset of asthma or rhinitis in patients allergic to cats and a basis for designing a policy to reduce airborne allergen in houses with cats. AM REV RESPIR DIS 1990; 141:361-367

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<sup>4</sup> Requests for reprints should be addressed to Dr. Thomas A. E. Platts-Mills, Division of Allergy and Clinical Immunology, Box 225, University of Virginia Medical Center, Charlottesville, VA 22908.

houses is associated with particles  $\leq 2.5$   $\mu\text{m}$  in diameter. In keeping with their predicted properties, these particles remain airborne for prolonged periods, i.e., many hours after disturbance. We have also investigated some of the factors that influence both the quantity of airborne *Fel d 1* and the particle size carrying this allergen. The results suggest that the presence of small airborne particles may be very important both in determining the symptoms experienced by patients and in designing strategies to reduce airborne cat allergen.

## Methods

### Air Sampling

Air sampling was carried out using a Cassella Mark II cascade impactor (Cassella London, London, UK) and a multistage liquid impinger (Hampshire Research Glassware, Southampton, UK). The four stages of the cascade impactor (figure 1) were loaded with glass discs 2.5 cm in diameter (T13206, Cassella) coated with 1 mm of 5% agarose-sorbitol gel [5 g agarose (AX0417-3; Matheson, Coleman, and Bell, Norwood, OH), 50 g D-sorbitol (s-1876; Sigma Chemical Co., St. Louis, MO) in 100 ml borate-buffered saline at pH 8.0]. Sorbitol was added to decrease water evaporation during sampling and to increase the adhesive properties of the agarose. A glass fiber filter was run in parallel at the same flow rate to collect total airborne particles. The cascade impactor and parallel filter were connected via a flow meter (British Oxygen Co., Boreham Wood, UK) to a vacuum pump. Air was sampled for periods as long as 1 h at flow rates between 1 and 1.3  $\text{m}^3/\text{h}$  ( $\sim 18$  to 22 L/min). The agarose-sorbitol gel was removed from the discs and eluted in 0.5 ml 1% bovine serum albumin, phosphate-buffered saline, and 0.05% Tween<sup>®</sup> 20 (assay buffer) (BSA-PBS-Tween) overnight at 4°C. In all experiments, the results for the fourth stage of the cascade impactor were combined with the results for the final filter and expressed as particles  $\leq 2.5$   $\mu\text{m}$  in diameter. This

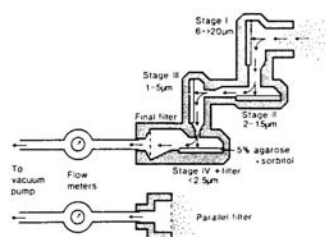


Fig. 1. Diagrammatic representation of Cassella Mark II cascade impactor showing size distribution of particles collected on the stages and final filter, according to the manufacturer's specifications. A glass fiber filter is run in parallel to collect total airborne particles.

was done for two reasons: first, because we had no way of confirming the difference between particles 2  $\mu\text{m}$  in diameter and those of less than 1  $\mu\text{m}$ ; second, because examination of the agarose on the stages of the impactor with a hand lens after 1 h of sampling revealed indentation at the site of impactation on the fourth stage. Indentation would increase the gap and therefore could allow particles through. The eluate from the glass fiber filters was collected in 1 ml buffer by compressing the filters in a 3-ml plastic syringe.

The chambers of the liquid impinger (figure 2) were filled with sufficient assay buffer to wet the glass sinter collecting surfaces (4 to 6 ml) and a filter holder (Millipore Corp., Bedford, MA) containing a glass fiber filter was connected on line to the vacuum pump. A parallel glass fiber filter to collect total airborne particles was also included. The sampling period was 1 h at flow rates of 1 to 1.3  $\text{m}^3/\text{h}$  ( $\sim 18$  to 22 L/min). After sampling, the assay buffer was removed from the chambers and assayed directly. Results for the final stage of the impinger were combined with results from the final filter because of variable frothing in some experiments.

### *Fel d 1* mAb Radioimmunoassay

The assay used here for cat allergen has been described in full elsewhere and is described in outline only here (12, 22). Immulon 2 Removawell Strips (011-010-6302; Dynatech, Chantilly, VA) were coated with 10  $\mu\text{g}/\text{well}$  6F9 anti-Fd1B mAb in 0.05 M carbonate-bicarbonate buffer (pH 9.6) overnight at 4°C. The plate was washed twice with PBS-Tween and blocked for 30 min with 1% BSA-PBS-Tween (assay buffer). After a further two washes with PBS-Tween, either 100  $\mu\text{l}$  of several dilutions of cat allergen standard (E3, containing 10.5 U *Fel d 1*/ml; Office of Biologicals and Research Reagents of the Food and Drug Administration) or eluate from the agarose-sorbitol discs or assay buffer from the liquid impinger (dilutions: neat, one-half and one-fifth) were then added to the wells and incubated for 2 h at room temperature. One unit of E3 cat allergen standard has been shown to be equal to 4  $\mu\text{g}$  of *Fel d 1* by assaying weighed samples of purified *Fel d 1* (12). After five washes with PBS-Tween, 100  $\mu\text{l}$  (approximately 5 ng)  $^{125}\text{I}$ -labeled 3E4 anti-Fd1A mAb (12) (100,000 cpm/well; specific activity, 30  $\mu\text{Ci}/\mu\text{g}$ ) was added and incubated for a further 2 h, then washed again and counted in a gamma counter (4/200; Micromedex Systems, Horsham, PA). *Fel d 1* values were obtained from the calibration curve (range, 0.04 to 84 ng/ml) and expressed as ng *Fel d 1*/m<sup>3</sup> air sampled, correcting for the actual time and flow rate in each sampling period. The sensitivity of the assay was 0.5 ng/ml or 0.2 ng/m<sup>3</sup> for 1-h sampling at 1.2  $\text{m}^3/\text{h}$ .

### Vacuum Cleaners, Room Air Cleaners, and Air Exchange Measurements

The effect of vacuum cleaning and use of room air cleaners on airborne *Fel d 1* concen-

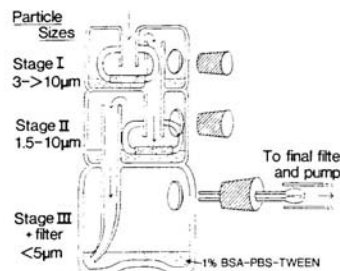


Fig. 2. Diagrammatic representation of an all-glass multistage liquid impinger showing 50% cutoff values of particle sizes collected in each chamber, according to May (21).

trations was assessed in a house with two cats. The overall air exchange rate in the house was measured throughout the experiment period by a multizone tracer gas technique (Air infiltration measurement service, National Association of Home Builders, National Research Center, Upper Marlboro, MD). The cat vivarium was designed to specifications in the guide to the care of laboratory animals [no. (NIH) 78-23; Department of Health Education and Welfare, Bethesda, MD], which recommends an air exchange rate of 10 to 15 changes/h. The rates in the vivarium were confirmed with an anemometer. We monitored airborne *Fel d 1* during 15 min of vacuuming using two domestic vacuum cleaners: a Rainbow vacuum cleaner (Model no. D3C; Rexair, Inc., Cadillac, MI), which uses "water impingement" as a means of filtering small particles, and a Nilfisk vacuum cleaner (Model no. GS90; Nilfisk Inc., Malvern, PA) with a high efficiency particulate air (HEPA) filter. HEPA filter room air cleaners (Enviroaire Corp., Hagerstown, MD) with flow rates of 300 or 600  $\text{m}^3/\text{h}$  were tested to assess their effectiveness in reducing total airborne *Fel d 1*. An aqueous carpet extractor vacuum (Allergy Control Products, Ridgefield, CT) was used for aggressive cleaning of carpets before some air cleaning experiments.

## Results

### Particle Sizes and Total Quantities in a Cat Vivarium Compared with Nebulizer Output and One Domestic House

In preliminary experiments it was found that the cat vivarium in the university had high levels of airborne cat allergen, i.e., 22 to 57 ng/m<sup>3</sup> (table 1). Using either the cascade impactor or the liquid impinger, this allergen was predominantly, i.e., 96 to 99%, on the first two stages, suggesting a mean particle size  $\geq 4$   $\mu\text{m}$  in diameter. Although there was considerable variation in the distribution of particle sizes from day to day in the vivarium,

TABLE 1  
AIRBORNE *Fel d 1* IN CAT VIVARIUM HOUSING 12 CATS\*

Particle Size ( $\mu\text{m}$ )	<i>Fel d 1</i> ( $\text{ng}/\text{m}^3$ )		Mean on Stage (%)
	Mean	Range	
Cascade impactor, n = 6			
6-20 or more	18.3	2.6-37.5	50
2-15	16.8	6.8-23.3	46
1-5	1.1	< 0.2-2.8	3
< 2.5	< 0.2	< 0.2-0.5	< 1
Total	36.2		
Parallel filter	40.8	22.2-57.5	
Liquid impinger, n = 5			
3-10 or more	34.4	11.3-63.0	84
1.5-10	6.4	< 0.2-14.0	16
< 5	0.2	< 0.2-0.8	< 1
Total	41.0		
Parallel filter	40.1	14.6-55.8	

\* Air sampling carried out at  $\sim 1.2 \text{ m}^3/\text{h}$ ; extracts of the agarose on the discs of impactor and assay buffer from the chambers of the impinger were assayed by two-site mAb RIA. Values in  $\text{ng}/\text{m}^3$  air were calculated from the flow rate and sampling time. Values < 2.5 and < 5 represent combined values for the final stage and final filter of the cascade impactor and liquid impinger. Air exchange in the cat vivarium was 10 air changes/h measured by anemometer.

the results showed a consistent pattern with a very high percentage on the first two stages (table 1). These results were then compared with both a domestic house with two cats and the output from a commercial nebulizer used clinically in the hospital (table 2). In the house, using either the cascade impactor or the impinger, a significant percentage of cat allergen was collected on the final stages. Analyzing the output of a nebulizer, the particles were predominantly on the final stages of both samplers as would be predicted (table 2). The overall correla-

tion between results with the cascade impactor and the glass impinger was very close ( $r = 0.97$ ,  $p < 0.005$ ). The results for the domestic house showed tenfold less allergen on the first stages, but tenfold more on the final stage of the impactor. Although there are many differences between the vivarium and the domestic house, the most striking are the furnishings and the air exchange rate. The vivarium has an air exchange rate of 10 air changes/h, whereas modern energy efficient houses often have exchange rates lower than 1/h. The house shown in ta-

TABLE 2  
PARTICLE SIZES CARRYING AIRBORNE *Fel d 1*: COMPARISON BETWEEN A DOMESTIC HOUSE, A CAT VIVARIUM, AND OUTPUT FROM A NEBULIZER

Particle Size ( $\mu\text{m}$ )	<i>Fel d 1</i> ( $\text{ng}/\text{m}^3$ )					
	Domestic House*		Vivarium†		Nebulizer‡	
Cascade impactor						
6-20 or more	3.5	39%	35.0	59%	< 0.2	0%
2-15	2.4	27%	23.3	39%	< 0.2	0%
1-5	0.7	8%	0.8	1%	3.0	16%
< 2.5	2.3	26%	< 0.2	0%	16.0	84%
Total	8.9		69.1		19.0	
Parallel filter	11.1		55.8		—	
Liquid impinger						
3-10 or more	3.0	42%	63.0	82%	< 0.2	0%
1.5-10	2.5	35%	14.0	18%	< 0.2	0%
< 5	1.7	24%	< 0.2	0%	17.0	100%
Total	7.2		77.0		17.0	
Parallel filter	10.8		55.8		—	

\* Two cats present in house; air exchange, 0.3 air changes/h measured by tracer gas technique (NAHB National Research Center).

† Twelve cats present in cages in a vivarium room; air exchange, 10 air changes/h.

‡ Nebulizer contained 1 ml of saline containing 1 U OBRR/FDA E3 cat standard equal to 4  $\mu\text{g}$  *Fel d 1*; using a nebulizer and a model 5610D pump (DeVilbiss Corp., Somerset, PA).

ble 2 was measured using a tracer gas technique and was found to have a mean value of 0.33 air changes/h.

#### Airborne Cat Allergen in Domestic Houses

In a series of nine houses that had from one to six cats, the quantity of airborne *Fel d 1* without disturbance was 2.9 to 19.7  $\text{ng}/\text{m}^3$  (mean, 8.9  $\text{ng}/\text{m}^3$ ) (figure 3). In houses without cats, airborne cat allergen was not detectable, i.e., < 0.2  $\text{ng}/\text{m}^3$ . The total *Fel d 1* did not correlate with the number of cats present, but it was higher when the cats were house-bound (see figure 3), and also was higher when there were soft furnishings in the room. The particle size distribution was very variable from house to house. In most houses, more than 50% of the allergen impacted on the first two stages; however, small particles carrying *Fel d 1* were present in all nine houses, and a mean level of 26% landed on the last stage or the final filter. One house was identified that appeared to have a very high proportion of small particles. This house was studied repeatedly to confirm the results, to see how variable the values were, and to study the effects of disturbance (table 3). The results for eight visits show that this house, which was kept fully closed up, had consistently greater than 50% of the airborne *Fel d 1* associated with particles  $\leq 2.5 \mu\text{m}$  in equivalent diameter. During and after vigorous disturbance with a vacuum cleaner with no filter, the levels in this house rose from  $\sim 6$  to  $\sim 110 \text{ ng}/\text{m}^3$ . The particle size of cat allergen during disturbance increased so that more than 50% was on large particles,

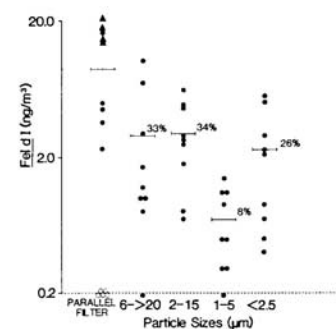


Fig. 3. Total airborne cat allergen ( $\text{ng}/\text{m}^3$ ) collected on the parallel filter and distribution of airborne particles associated with *Fel d 1* on the stages of the cascade impactor in nine houses containing one to six cats (total of 16 visits). The triangles indicate values for houses with house-bound cats.

TABLE 3  
AIRBORNE *Fel d 1* IN A DOMESTIC HOUSE\* IN QUIET AND  
IN DISTURBED CONDITIONS

Particle Size ( $\mu\text{m}$ )	Quiet Air ( $\text{ng}/\text{m}^3$ )			Disturbed Air† ( $\text{ng}/\text{m}^3$ )		
	mean	range	%	mean	range	%
6–20 or more	0.7	< 0.2–1.7	10	25.3	< 0.2–100.0	23
2–15	0.8	< 0.2–1.7	12	31.9	6.0–91.7	29
1–5	1.1	< 0.2–4.0	16	8.8	1.3–6.7	8
< 2.5	4.2	2.1–8.0	62	45.9	5.8–85.3	41
Total	6.8			111.9		
Parallel filter	5.7	2.4–8.0		117.0	66.7–183.0	

\* Non-air-conditioned house with two cats, with windows closed and curtain drawn during period of experiments ( $n = 8$ ).

† Vacuuming without filter in hand-held vacuum cleaner for 15 min of air sampling period ( $n = 4$ ).

but there was still a significant percentage of *Fel d 1* on small particles. Indeed, the level of small particles present during disturbance in this house was equal to or greater than the output of a nebulizer (compare tables 2 and 3).

*Falling Properties of Airborne Cat Allergen Studied by Artificial Disturbance of House Dust in a Laboratory Room*

Using vacuum cleaner dust that contained  $500 \mu\text{g}$  *Fel d 1*/g of dust, we measured the airborne allergen over a 3-h period after vigorous disturbance of the dust in an airtight laboratory room (figure 4A). The total quantities airborne were very high with a large proportion of large particles. After 20 min, less than 1% of the large particles remained airborne, and after 3 h, the only *Fel d 1* detected airborne was that associated with particles of apparent diameter less than  $2.5 \mu\text{m}$ . This result is consistent with the expected settling velocity of these particles; particles  $10 \mu\text{m}$  in diameter are expected to fall at  $\sim 0.33 \text{ cm/s}$ , i.e., within 15 min; particles  $2 \mu\text{m}$  in diameter are expected to fall at  $\sim 0.013 \text{ cm/s}$ , i.e., in about 6 h in still air (23). The experiment was then repeated using a 12-inch diameter domestic fan (Windermere model no. NR-12/412) to create an air flow to disturb the dust in the same airtight room. The results show that although the maximal levels were lower, the falling pattern after stopping the fan was very similar (figure 4B). In addition, this experiment shows that modest levels of disturbance such as that created by an electric fan can dramatically increase airborne cat allergen.

*Airborne Cat Allergen during the Use of Two Different Vacuum Cleaners or a Room Air Cleaning Device*

Two domestic vacuum cleaners were test-

ed in two houses with two cats each. In this experiment, the amount of *Fel d 1* collected by the vacuum cleaner was measured, and airborne *Fel d 1* concentrations were monitored during and immediately after vacuuming (table 4). The water impingement vacuum collected  $30 \text{ mg}$  *Fel d 1* in the 2-L reservoir, and the airborne *Fel d 1* was approximately doubled during and immediately after use, with a predominant increase in the proportion of small particles. The HEPA filter vacuum collected  $61.4 \text{ mg}$  *Fel d 1* and had no effect on the total level or particle size distribution of airborne cat allergen during vacuuming. Very similar results were obtained in a second house, and to further analyze the apparent rise in small particle allergen, the two vacuum cleaners were tested in a  $14\text{-m}^3$  airtight laboratory room (table 5). After measuring background levels of *Fel d 1*, the water impingement and HEPA filter vacuum cleaners were filled with 10 to  $20 \text{ mg}$  *Fel d 1* and run for 15 min, during which time air sampling was carried out using the cascade impactor. Under these circumstances, the water impingement vacuum cleaner produced approximately  $100 \text{ ng}/\text{m}^3$  *Fel d 1*, of which  $> 98\%$  was associated with small particles. In contrast, the HEPA filter vacuum cleaner effectively contained the allergen. In a subsequent experiment, the water filter vacuum cleaner was filled with dust outside the room and transferred to the room after running for 15 min. After running a further 15 min in the room with no further dust intake, this cleaner had generated a similar high level of small-particle-associated allergen (table 5).

The results of experiments carried out to test the effectiveness of a HEPA filter room air cleaner in removing airborne *Fel d 1* are shown in table 6. Preliminary experiments showed that 3 h of filtration were sufficient to reach equilibrium

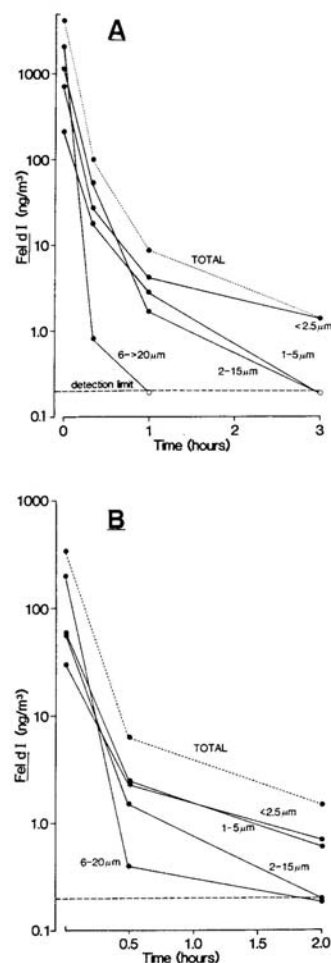


Fig. 4. Time course of falling of particles of different sizes carrying airborne *Fel d 1* in an airtight room after (A) physical disturbance; (B) disturbance using a 12-inch domestic fan. The room contained  $545 \text{ g}$  of dust containing  $500 \mu\text{g}$  *Fel d 1*/g. Airborne samples were collected with a cascade impactor.

whether or not the cats were present (data not shown). When one filter unit was used, this achieved a 70% fall in airborne *Fel d 1*. Using two units at a low flow rate achieved only marginally better reduction, whereas using high flow rate even off the floor was less effective, i.e., only 60% reduction. A charcoal "pre-filter" alone was partly effective, and when the air cleaner was run with the filter unit removed, there was no detectable reduc-

TABLE 4  
COMPARISON OF AIRBORNE CAT ALLERGEN DURING USE OF TWO  
VACUUM CLEANERS IN A DOMESTIC HOUSE\*

Particle Size ( $\mu\text{m}$ )	<i>Fel d 1</i> ( $\text{ng}/\text{m}^3$ )							
	Water-impingement				HEPA-Filter			
	Baseline		Cleaning		Baseline		Cleaning	
6-20 or more	11.3	46%	5.3	13%	10.0	73%	4.9	36%
2-15	10.8	44%	3.3	8%	2.3	17%	5.6	41%
1-5	1.7	7%	0.2	1%	0.4	3%	1.8	13%
< 2.5	1.0	4%	31.3	78%	1.0	7%	1.5	11%
Total	24.5		40.1		13.7		13.8	
Parallel filter	21.7		41.7		18.3		13.3	
<i>Fel d 1</i> collected, $\text{mg}^\dagger$			30.0				61.4	

\* Vacuuming separate areas (approximately  $6 \text{ m}^2$ ) of carpet for 15 min at beginning of a 30-min air-sampling period, each experiment conducted 24 h apart. Each vacuuming was preceded by 30 min of air sampling in undisturbed conditions to obtain baseline measurement.

$^\dagger$  Quantity of *Fel d 1* collected in the water reservoir or vacuum cleaner bag.

TABLE 5  
COMPARISON OF AIRBORNE CAT ALLERGEN DURING USE OF TWO  
VACUUM CLEANERS IN AN AIRTIGHT LABORATORY ROOM

Particle Size ( $\mu\text{m}$ )	<i>Fel d 1</i> ( $\text{ng}/\text{m}^3$ )			
	Water-impingement			HEPA-Filter
	Baseline	Collect/Run*	Run Only $^\dagger$	Collect/Run*
6-20 or more	< 0.2	0.4	0.4	< 0.2
2-15	< 0.2	0.4	< 0.2	0.4
1-5	< 0.2	0.4	< 0.2	< 0.2
< 2.5	< 0.2	93.7	118.0	< 0.2
Total	< 0.2	94.9	118.4	0.4
Parallel filter	< 0.2	129.9	134.9	< 0.2
<i>Fel d 1</i> collected, $\text{mg}^\ddagger$		17.6	15.2	17.0

\* Vacuum cleaner filled with 10 g house dust containing 10 to 20  $\text{mg}$  *Fel d 1* in laboratory room and run for first 15 min of a 30-min sampling period.

$^\dagger$  Vacuum cleaner prefilled with 10 g dust containing 10 to 20  $\text{mg}$  *Fel d 1* outside laboratory room and run for 15 min. The vacuum was then brought into the room and run for first 15 min of a 30-min air-sampling period.

$^\ddagger$  Quantity of *Fel d 1* collected in the water reservoir or vacuum cleaner bag.

TABLE 6  
EFFECT OF HEPA FILTER AIR CLEANER ON AIRBORNE  
*Fel d 1* IN A HOUSE WITH TWO CATS

Number and Type of Filter	Height off Floor	Air Flow ( $\text{m}^3/\text{h}$ )	Cats Present during Experiment	Decrease in 3 h (%)
Living room*				
1 HEPA	On floor	300	Yes	70
2 HEPA	On floor	600	Yes	80 $^\dagger$
1 HEPA	30 cm	600	No	80
2 HEPA	30 cm	1,200	Yes	60
Charcoal	30 cm	1,200	Yes	35
None	On floor	600	Yes	0
Cats' room $^\ddagger$				
1 HEPA	On floor	300	Yes	30
2 HEPA	On floor	600	No	30
1 HEPA	On floor $^\S$	600	No	90

\* Living room: mean background airborne *Fel d 1* before experiments 21.8  $\text{ng}/\text{m}^3$ ; mean floor dust 4  $\text{mg}/\text{g}$  *Fel d 1*; volume of room 130  $\text{m}^3$ .

$^\dagger$  Mean of two experiments.

$^\ddagger$  Cats' room (containing cat food/litter/bed): mean background airborne *Fel d 1* before experiments, 16.5  $\text{ng}/\text{m}^3$ ; mean floor dust, 9  $\text{mg}/\text{g}$  *Fel d 1*; volume of room, 30  $\text{m}^3$ .

$^\S$  Floor cleaned using aqueous carpet extractor vacuum.

tion in airborne allergen. This experiment was repeated in the cats' room using the room-air cleaners on a carpet. Despite the small volume of the room (30  $\text{m}^3$ ), even two filters running at 600  $\text{m}^3/\text{h}$  produced only a 30% fall in airborne allergen. Dust from the carpet in this room contained 9  $\text{mg}$  *Fel d 1*/g and it seemed likely that the air filters were disturbing dust from the carpet. The experiment was therefore repeated after aggressive cleaning of the carpet with an aqueous carpet extractor vacuum cleaner. After the carpet was cleaned, the room air cleaner produced a 90% fall in airborne *Fel d 1*. Monitoring these same rooms with a cascade impactor showed that HEPA filtration had little or no effect on the particle size distribution of airborne *Fel d 1*.

### Discussion

The primary objective of these experiments was to obtain definitive results on the absolute quantities of airborne cat allergen associated with different particle sizes. By using two types of particle collectors and by following the rates of falling after disturbance, we have unequivocally demonstrated the presence of particles  $\leq 2.5 \mu\text{m}$  carrying *Fel d 1*. The two devices used both run at 1.2  $\text{m}^3/\text{h}$  (20 L/min), which does not change the pattern of air movement in a room more than the breathing of a single resting person. Both the cascade impactor and the liquid impinger are, in effect, measuring the terminal settling velocity of the particles, which is a function of their aerodynamic size. When used for irregularly shaped particles, the values given by these measurements are the "equivalent" diameter of a sphere. These measurements do not define what shape the particles are, and it is possible that the "small" particles are, in fact, small flakes of dried protein containing secretions coming off cat hair. In practice, the equivalent diameter is the relevant measurement both in terms of the length of time they remain airborne and their behavior on entering the lung (23-25). The small size of particles would be expected to influence their impact on the lung in several ways: first, the percentage of inhaled particles entering the lung during mouth breathing will be higher, perhaps as high as 30 to 40%; second, the number of particles necessary to carry a given quantity of allergen will be far higher; third, these small particles have the potential for penetrating further into the lung. The results obtained in a house with four cats show levels of airborne small particles

during disturbance comparable to those produced by a nebulizer. From previous reports it is possible to calculate that the dose of *Fel d 1* producing a positive bronchial provocation was in most patients between 8 and 80 ng over 2 min in the form of nebulizer droplets (2). Inhaling a "dose" of 8 ng would take only 12 min in the house shown in table 3 during disturbance, but would take greater than 4 h in some of the other houses. Thus, the results are compatible with the often reported history, that patients who have immediate hypersensitivity to cats have at some time developed acute symptoms of asthma on entering a house with a cat. On the other hand, the total levels of airborne cat allergen in some undisturbed houses are not high, e.g., 2 ng/m<sup>3</sup>, and it is perhaps not surprising that many persons allergic to cats can, despite their symptoms, continue to live in a house with a cat. Previous studies on airborne allergen derived from dust mites of the genus *Dermatophagoides* showed strikingly different results (18, 19). Measuring the dust mite antigen *Der p 1*, no allergen was found airborne in undisturbed rooms; during disturbance, levels rose to 30 ng/m<sup>3</sup>, but the allergen was almost entirely on "large" particles, i.e.,  $\geq 10 \mu\text{m}$  in diameter (18). Certainly we have never seen levels of airborne mite allergen associated with small particles comparable to those reported here for cat allergen. Using very different sampling techniques (i.e., high volume) and different assays, Swanson and colleagues (15) similarly observed that mite allergen levels fall more rapidly than do cat allergen levels. We believe that the absence of small particles carrying mite allergen is one of the reasons that patients allergic to mites are usually unaware of a direct relationship between exposure to house dust and their symptoms. Only a small proportion (i.e., 5 to 10%) of the large particles (i.e.,  $> 8 \mu\text{m}$  in diameter) carrying either mite or cat allergen would be expected to enter the lung (24, 25). However, these particles, because of their volume, can carry much larger quantities of allergen and may be more important in causing the chronic "inflammation" of the respiratory tract that is thought to contribute to bronchial reactivity in many asthmatics (26–28).

Our results make it clear that different factors can alter both the total quantity and the particle size of airborne cat allergen. The most striking result was the contrast between a cat vivarium with  $< 2\%$  small particles and one of the houses

where on average 62% of airborne cat allergen was associated with particles  $\leq 2.5 \mu\text{m}$  in diameter. Although there were many differences between the house and the vivarium, we believe that the two most important factors were the absence of soft furnishings and the high level of air exchange in the vivarium. The vivarium air was exchanged at least 10 times/h with outside air; this level is required by federal regulations for the health of the animals. By contrast, "tight" houses for human habitation often have less than 0.5 changes/h, and one of the houses we studied had only 0.33 changes/h. It is important to distinguish between exchange of air with the outside air and circulation of air within a house. We believe that accumulation of small particles carrying animal allergen is yet another harmful consequence of keeping houses progressively "tighter." We assume that rapid air exchange removes small particles that otherwise remain airborne or settle on soft furnishings in a form that can easily become airborne again. Our studies on artificial disturbance not only confirm that different particles fall with their expected velocities but also show that relatively minor airflow can increase airborne cat allergen. The domestic fan used to disturb house dust in the laboratory was typical of those used widely for providing cool air. Further, the airflow provided by the fan was similar to the flow through a room air cleaner and less than most domestic vacuum cleaners. In the experiments reported here with a room air cleaner, the airflow was such that the whole volume of air would pass through the apparatus at least 10 times/h. The HEPA filters are rated to be  $> 99\%$  efficient, even for particles in the range of  $0.5 \mu\text{m}$  to  $5 \mu\text{m}$  in diameter where they are least effective. Thus, the air filter should dramatically reduce airborne allergen over 1 h. At present, it seems that the exhaust from the apparatus serves to disturb almost as much allergen as it removes. This view was strongly supported by the much improved effect of the air filter (i.e., 90% removal) when it was preceded by aggressive cleaning of the carpet. The sharp increase in airborne cat allergen associated with small particles when the water filter vacuum cleaner was used was shown to be due to the formation of fine droplets by the machine. This result leaves little doubt that this type of vacuum cleaner would not be suitable for patients allergic to cats. The results also suggest that airborne cat allergen represents an excellent model for testing these

devices. It would be more difficult to carry out comparable tests with mite allergen, which becomes airborne only during disturbance.

The conclusion both for vacuum cleaners and room-air cleaning devices is that their effectiveness depends both on the actual filtration performance and the allergen that their exhaust disturbs. In turn, the disturbance depends on the levels of allergen on the floor around the device and the route and velocity of air coming out of the cleaner. Control of environmental exposure has become normal practice in the workplace and has been successfully applied to airborne organic particulates as well as to chemicals associated with asthma (29, 30). Until recently, very little work has been carried out on controlling environmental exposure in the home (31). The present results show that the technology is available to make detailed studies of the effects of vacuum cleaner, room-air cleaners, and different forms of furniture. Currently, the only testing done relevant to this area is on the ability of room air cleaners to remove artificial fine particulates from the air of a test room (31).

In our experiments on houses, we observed a wide range of results, and initially it seemed that the variation might be either within the measurement system or might be day-to-day variation. However, repeated experiments on two houses and the vivarium confirmed that the levels were consistent. Furthermore, throughout these experiments there was an excellent correlation between the results obtained with a parallel filter and the total quantity collected on the stages of the cascade impactor. The levels were only partly related to the number of cats. The major factors that appeared to increase airborne allergen were whether the cats were kept indoors continuously and the quantity of soft furnishings. We have already discussed the possibility that the cat itself is an important source of airborne small particles. It also seems likely that soft furnishings provide an important reservoir from which small particles become airborne. Certainly, extracts of carpet and sofa dust from houses with a cat commonly contain very high levels of *Fel d 1*/ml (e.g., 0.5 to 4 mg/ml), which should be compared with levels of 0.5 to 120  $\mu\text{g}/\text{ml}$  that are present in commercially available extracts of cat dander (8, 12, 32). Recently, it has been reported that *Fel d 1* levels in a house fall slowly after removing a cat, but that aggressive cleaning can accelerate this process (33). The

present results suggest that even in a house with a cat it may be possible to reduce airborne levels of cat allergen by aggressive cleaning or removal of soft furnishings combined with air filtration.

In conclusion, the increased sensitivity of the assay for *Fel d 1* used here has made it possible to make accurate measurements of both the absolute quantities and the particle size of the airborne cat allergen. The results show that in most houses cat allergen remains airborne and that a significant proportion is associated with particles that have an aerodynamic diameter of  $\leq 2.5 \mu\text{m}$ . It is likely that these properties of airborne cat allergen are responsible for the distinctive rapid onset of symptoms experienced by patients who are allergic to cats. In addition, the results present for the first time a rational approach for reducing airborne cat allergen.

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