Distribution, aerodynamic characteristics, and removal of the major cat allergen Fel d 1 in British homes

Adnan Custovic, Angela Simpson, Harpreet Pahdi, Rosalind M Green, Martin D Chapman, Ashley Woodcock

Abstract

***Background* – Sensitisation to cat allergen (Fel d 1) is an important risk factor for asthma in the UK. A study was undertaken to investigate the distribution of cat allergen in British homes, the aerodynamic characteristics and particle size distribution of airborne Fel d 1, and the method of removing it.**

***Methods* – Dust was collected from 50 homes with a cat and from 50 homes without a cat, and airborne levels of Fel d 1 were measured in 50 homes with a cat and 75 homes without a cat. Particle size distribution was determined using an Andersen sampler (8 hours/day) in 10 homes with cats. This was repeated on five separate days in a house with four cats, and then one, two, four, seven, and 14 days after the cats were removed from the living room area. The effect of high efficiency particulate air (HEPA) cleaner on airborne levels of Fel d 1 was investigated in seven homes with cats. Samples were collected on two separate days from two rooms of each house concurrently, one of which contained the cat, one day with the HEPA cleaner on and the other day as a control. Three one hourly samples were collected over a nine hour period (baseline, 4–5 hours, 8–9 hours) using a high volume dust sampler (air flow rate 60 l/min) and the air sample was collected onto a microglass fibre filter (pore size 0.3 μm).**

***Results* – Fel d 1 concentrations were much lower in houses without a cat than in those with a cat (260-fold difference (95% CI 167 to 590) in living room carpets: geometric mean (GM) 0.9 μg/g (range 0.06–33.93) versus 237 μg/g (range 2.8–3000); 314-fold difference (95% CI 167 to 590) in upholstered furniture: 1.21 μg/g (range 0.06–61.9) versus 380 μg/g (range 7.1–6000); 228-fold difference (95% CI 109 to 478) in bedroom carpets: 0.24 μg/g (range 0.06–2.24) versus 55 μg/g (range 0.06–2304); and 215-fold difference (95% CI 101 to 456) in mattresses: 0.2 μg/g (range 0.06–2.3) versus 55 μg/g (range 0.06–3400)). Airborne levels of Fel d 1 were detected in all houses with cats, and the levels varied greatly between the homes (range 0.7–38 ng/m^3). Low concentrations of airborne Fel d 1 (range 0.24–1.78 ng/m^3) were found in 22 of 75 homes without a cat. Although airborne Fel d 1 was mostly associated with large particles (>9 μm, approximately 49% of the allergen recovered), small particles (<4.7 μm) comprised approximately 23% of the total airborne allergen. Total airborne Fel d 1 was reduced by 61.7% two days after removal of the cat but this was due predominantly to the decrease in larger particles (>4.8 μm) which fell to 13% of their baseline level. Fel d 1 levels associated with small particles (<4.8 μm) remained largely unchanged on days 1, 2 and 4 and then slowly decreased to 33% of the baseline levels at day 14. With HEPA cleaner a significant reduction in airborne Fel d 1 was observed compared with the control sampling (GM 5.04–0.88 ng/m^3 versus 3.79–1.56 ng/m^3 at baseline and 8 hours, active versus control group; p = 0.008).**

***Conclusions* – Airborne Fel d 1 was detectable in undisturbed conditions in all homes with cats and in almost a third of homes without cats. In houses with cats a significant proportion (23%) of airborne Fel d 1 was associated with small particles (<4.7 μm diameter). Removal of the cat from the living room and bedroom areas of the home and the use of HEPA air cleaner reduced airborne levels of cat allergen in homes with cats, but the reduction following cat removal was not evenly spread across the particle size range.**

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Keywords: asthma, cat allergen, airborne, reservoir, avoidance.

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One in four homes in the UK contains a cat (RSPCA, personal communication). Our own data would suggest that as many as one third of cat sensitised individuals live in a home with a cat. Up to 40% of children with asthma are sensitised to cat allergen, and this is a significant risk factor for acute asthma in patients seeking treatment in emergency rooms.[1] Even brief exposure to cats can precipitate severe asthma symptoms in susceptible individuals. The only major cat allergen (Fel d 1) is responsible for a large proportion of the cat specific IgE in patients allergic to cats.[2] Fel d 1 is produced primarily in the sebaceous glands and in the basal squamous epithelial cells of the skin, and is stored mainly on the surface of the epidermis and the fur.[3] Fel d 1 production is under hormonal control[4] and a single cat can produce between 3 and 7 μg per day.[5] Castration of

1.5–2 year old male cats results in a 3–5-fold reduction in the level of Fel d 1 in skin washing, and testosterone treatment of the castrated cats restores the Fel d 1 concentration to pre-castration values.[6]

The primary method of reducing exposure to cat allergen is to remove the cat from the house. If cat sensitised asthmatics insist on keeping a cat, efforts should be made to ensure that it is kept out of the bedroom, and preferably that it remains outdoors.[7]

We have undertaken a study to investigate the distribution and aerodynamic characteristics of cat allergen in British homes, and to examine possible measures for controlling airborne Fel d 1 whilst keeping the cat by investigating the effect of excluding the cat from the living room and bedroom areas of the home on particle size distribution of airborne Fel d 1 and the use of high efficiency particulate air (HEPA) filtration to reduce airborne levels of the allergen.

Methods

DISTRIBUTION AND AIRBORNE LEVELS OF CAT ALLERGEN IN BRITISH HOMES

Dust samples were collected by vacuuming a 1 m^2 area of mattress, living room carpet, bedroom carpet, and upholstered furniture for two minutes in 50 homes with a cat and 50 homes without a cat using a Medical Dust Sampler (Medivac plc, Wilmslow, UK) with an air flow rate of 45 l/s, through a 355 μm diameter mesh screen onto a 5 μm vinyl filter (Plastok Associates Ltd, Wirral, UK) which enabled collection of fine dust samples. Each sample was transferred into a preweighed petri dish, weighed, coded, and stored at 4°C until extraction. One hundred mg of fine dust was extracted with 2 ml borate-buffered saline with 0.1% Tween 20 (BBS-T), pH 8.0. The dust was resuspended using a vortex mixer and samples were rotated for two hours at room temperature before being centrifuged for 20 minutes at 2500 rpm at 4°C. Supernatants were stored at −20°C prior to allergen analysis. The Fel d 1 content was determined using a two-site monoclonal antibody based ELISA[8] with a limit of detection of 0.06 μg Fel d 1/g fine dust.

Airborne Fel d 1 concentrations were measured in 50 homes with a cat and 75 homes without a cat. Air samples were collected in the absence of artificial disturbance using a fixed location sampler sampling volumes of 3–4.3 m^3 of air. The sampling head was positioned in the middle of the living room at a height of 1.2 m. The limit of detection of the assay was 0.1 ng Fel d 1/m^3 of air.

Further personal air sampling was performed in 20 homes without cats and seven homes with a cat. The samples were collected overnight (eight hours) in the bedroom using a Casella personal sampler with a sampling head attached to the subject's pillows onto a 25 mm Whatman GFA microglass fibre filter. The pumps were precharged for a minimum of eight hours the day before sampling. The flow rate was adjusted before sampling to 2 l/min, then rechecked immediately on cessation of sampling on site, and the volume of air sampled was calculated.

PARTICLE SIZE DISTRIBUTION OF AIRBORNE FEL D 1 AND EFFECT OF CAT REMOVAL FROM THE LIVING ROOM AND BEDROOM

The study was designed to quantitate the airborne level of Fel d 1 over a period of eight hours per day. Air sampling for particle size distribution was performed using an Andersen 1 non-viable ambient particle sizing sampler Mark II (Graseby Andersen, Spirotech Division, Atlanta, Georgia, USA). A low volume pump (6 l/min; Medic-Aid, West Sussex, UK) sampled the air parallel to the Andersen sampler to collect total airborne particles.

The Andersen sampler is a multi-stage, multi-orifice cascade impactor which is comprised of eight aluminium stages. The particle fractionation at different stages is as follows: pre-separator and stage 0: >9 μm; stage 1: 5.8–9 μm; stage 2: 4.7–5.8 μm; stage 3: 3.3–4.7 μm; stage 4: 2.1–3.3 μm; stage 5: 1.1–2.1 μm; stage 6: 0.65–1.1 μm; stage 7: 0.43–0.65 μm. A continuous duty, carbon vane vacuum pump attached to the sampler drew room air through the sampler at a constant rate of 28.3 l/min. This flow minimises particle bounce and fragmentation.

Airborne particles were collected on 0.3 μm glass fibre filters (Whatman International Ltd, Maidstone, UK) placed into the inverted stainless steel collection plates. At the end of a sampling period the sampler was disassembled and the glass fibre filters were placed in petri dishes and kept at 4°C until extraction. The filters were cut into eight pieces and placed into a 10 ml syringe. Three ml of 1% bovine serum albumin in phosphate buffered saline with 0.1% Tween 20 (1% BSA PBS-T) were added and the samples were extracted at 4°C overnight. The extraction fluid was aspirated backwards and forwards several times through a three-way stop lock into a second syringe, transferred into a test tube and centrifuged at 3000 rpm for 30 minutes at 4°C.

Air sampling for particle size distribution was performed in 10 homes with a cat. A house with four cats and a high level of Fel d 1 in the reservoir dust was then selected to investigate the effect of cat removal on particle size distribution of airborne Fel d 1. Airborne measurements were initially performed for five consecutive nights in the absence of disturbance, with windows closed and the cats in the living room. Following this period all four cats were removed from the house and kept outdoors for most of the day. Changes were made to the cat flaps so that, when the cats were allowed inside, they only had access to the kitchen. Further collections of airborne particles using the Andersen sampler were carried out in the living room under similar conditions one, two, four, seven, and 14 days after the cats were excluded from this area. The allergen content in ng/m^3 was calculated, taking into account the total amount of allergen recovered, the length of the collection period, and the flow rate of the sampler.

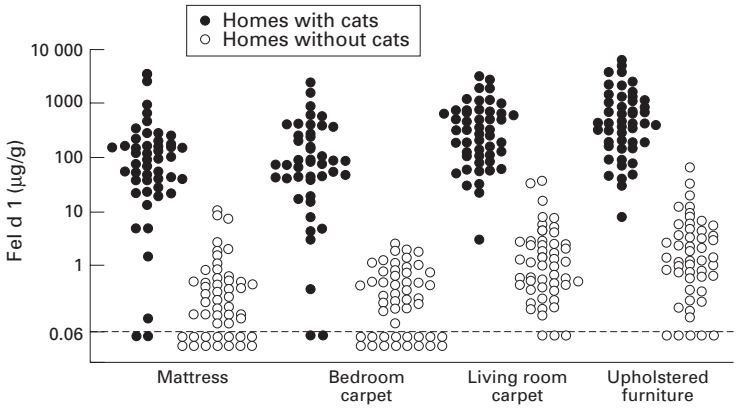

Figure 1 Distribution of cat allergen Fel d 1 in the settled dust from four sampling sites in the homes with and without cats. The lower detection limit is 0.06 µg/g.

EFFECT OF HEPA FILTRATION ON AIRBORNE FEL D 1

The effect of HEPA air cleaner (Philips Clean Air System HR4320) on airborne levels of Fel d 1 was investigated in seven houses with a cat. Samples were collected from two rooms of each house concurrently, one of which contained the cat, on two separate days. All doors and windows were closed and the sampling was performed in the absence of any artificial disturbance. To quantify the airborne Fel d 1 during the course of each day, three one hour air samples were collected at four hourly intervals from each room. The sampling pumps (60 l/min large volume dust samplers, Rotheroe-Mitchell, Greenford, UK) were placed in the centre of the room 0.75–1 m above ground and the air sample was collected onto a 37 mm Whatman GFA microglass fibre filter. Flow rates were measured at the start of sampling and then at 10 minutes, 30 minutes, and one hour. The early flow rate checks were performed to ensure that the considerable early fall in flow rates that occurred due to the machine warming to a full running temperature was measured. The volume of the air sampled was calculated by multiplying the geometric mean flow rate by the sampling time. On day 1 ("active") an HEPA air cleaner was placed on the floor in the corner of each room and left running for eight hours, starting after the first one hour air sample had been collected. On day 2 ("control" day) the sampling procedure was repeated at least 24 hours later in the same rooms and under the same conditions, but without the HEPA air cleaner.

All filters were cut into four pieces and placed into a syringe and 1 ml of 1% BSA PBS-T was added. After overnight extraction at 4°C the extraction liquid was aspirated backwards and forwards several times through a three-way stop lock into a second syringe, then transferred into a test tube and centrifuged at 3000 rpm for 30 minutes at 4°C. The supernatants were removed, coded, and stored at −20°C for future analysis of allergen content.

DATA ANALYSIS

The data followed a log-normal distribution. A mixed design ANOVA, including a mix of between group factors (active and control) and repeated measures factors (baseline, hour 4 and hour 8), was used to evaluate changes over the study periods. Statistical significance was set at the 5% level.

Results

DISTRIBUTION AND AIRBORNE LEVELS OF CAT ALLERGEN

In the homes with cats the highest levels of Fel d 1 were found in upholstered furniture (geometric mean (GM) 380 µg/g, range 7.1–6000) followed by the living room carpets (237 µg/g, range 2.8–3000). Bedrooms contained lower levels with carpet levels 4.3-fold (95% CI 2 to 9.4) lower (55 µg/g, range 0.06–2304; mattress: 55 µg/g, range 0.06–3400; fig 1). Fel d 1 was readily detectable in homes without cats, but the levels were much lower than in houses with cats (260-fold difference (95% CI 167 to 590) in living room carpets, 314-fold difference (95% CI 167 to 590) in upholstered furniture, 228-fold difference (95% CI 109 to 478) in bedroom carpets, and 215-fold difference (95% CI 101 to 456) in mattresses, fig 1). The highest levels of Fel d 1 in homes without a cat were found in the upholstered furniture from the living room (1.21 µg/g, range 0.06–61.9), followed by the living room carpet (0.9 µg/g, range 0.06–33.9). Bedrooms contained significantly lower levels than living rooms (carpet levels 3.8-fold (95% CI 2.2 to 6.6) lower; 0.24 µg/g, range 0.06–2.24; mattress: 0.25 µg/g, range 0.06–2.3). The Fel d 1 concentration in upholstered furniture was significantly higher than in any other sampling site within these homes – for example, 4.7-fold (95% CI 2.5 to 8.8) higher than mattress levels.

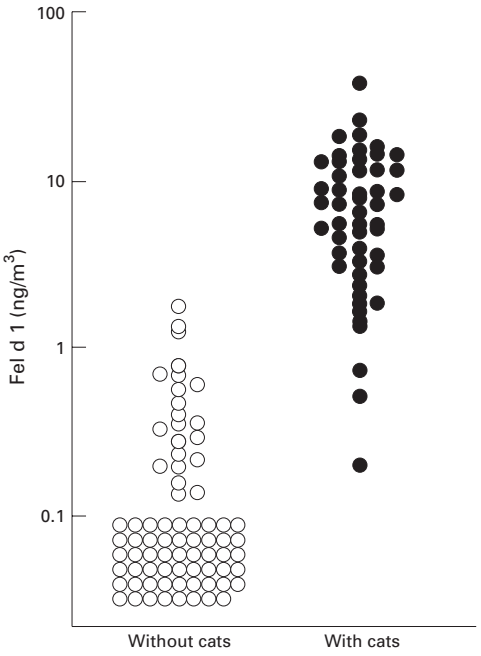

Figure 2 Airborne Fel d 1in 50 homes with cats and 75 homes without a cat. The lower detection limit is 0.1 ng/m^3.

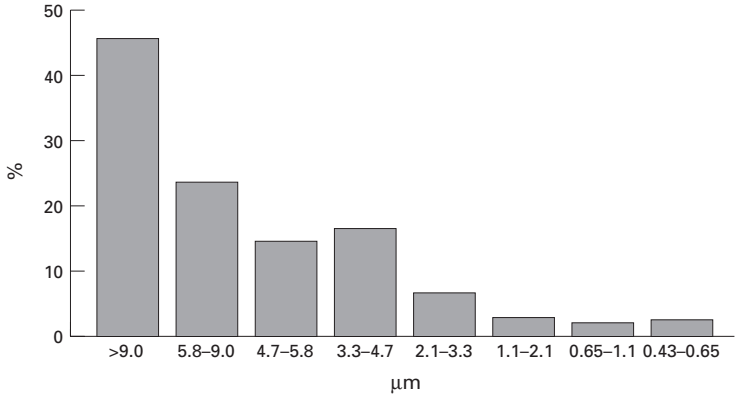

Figure 3 Particle size distribution of airborne Fel d 1 (mean daily percentages) collected from 10 homes with cat(s).

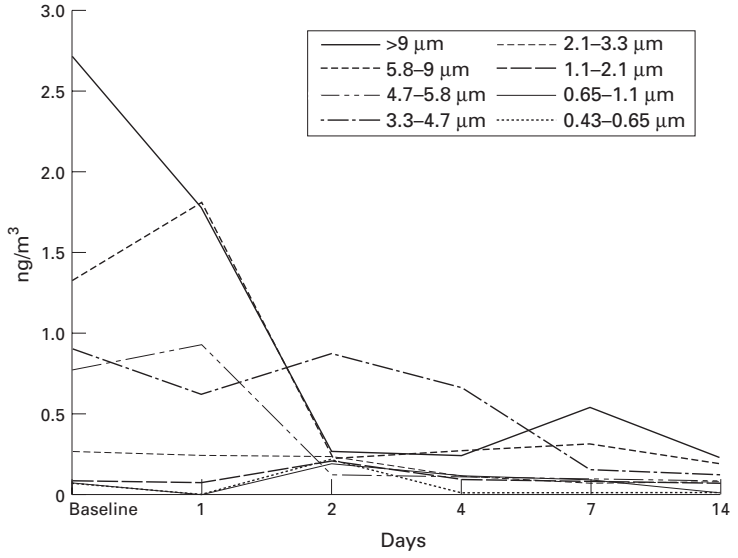

Figure 4 Airborne Fel d 1 (total allergen recovered; ng/m^3) measured on different stages of an Andersen sampler before and one, two, four, seven, and 14 days after removal of cats.

Airborne Fel d 1 was detected in all houses with cats, but the levels varied greatly between homes (range 0.7–38 ng/m^3). Low concentrations of airborne Fel d 1 (range 0.24–1.78 ng/m^3) were found in 22 of 75 living rooms in homes without a cat (fig 2). However, airborne Fel d 1 was undetectable in all 20 samples collected overnight from beds in homes without cats (mean sample volume 1 m^3, detection limit 0.4 ng Fel d 1/m^3). Fel d 1 was present in all seven samples collected overnight from beds in homes with cats, the levels ranging from 0.4 to 28 ng/m^3).

Table 1 Effect of HEPA air filtration on airborne levels of Fel d 1 in houses with cats (geometric means and ranges for each day and time; ng/m^3). Also presented are percentage reductions with 95% confidence intervals for each time point compared with the baseline levels for active and control days

	Active	*Control*
Baseline	5.04 (0.5–63.7)	3.79 (0.5–169.2)
4 hours	0.95 (0.1–27.4)	2.45 (0.5–75.4)
	81.2% (53.2% to 92.5%)	35.3% (20.4% to 47.4%)
8 hours	0.88 (0.1–27.1)	1.56 (0.17–40.8)
	82.5% (55.4% to 93.1%)	58.8% (36.8% to 73.1%)

PARTICLE SIZE DISTRIBUTION OF AIRBORNE FEL D 1

The particle size distribution of airborne Fel d 1 on the stages of the Andersen sampler is shown in fig 3. Fel d 1 was predominantly associated with large particles collected on the first stage of the Andersen sampler (>9 μm) which averaged approximately 49% of the total allergen recovered. Almost 23% of the airborne Fel d 1 was carried on small particles (<4.7 μm diameter). There was a good concordance in the total airborne Fel d 1 recovered from the Andersen sampler (an aggregate of all stages) and a parallel filter (5.5 ng/m^3 and 4.7 ng/m^3, respectively).

EFFECT OF CAT REMOVAL FROM LIVING ROOM AND BEDROOM AREAS ON PARTICLE SIZE DISTRIBUTION OF AIRBORNE FEL D 1

A house with four cats and a high level of Fel d 1 in the dust (3700 μg/g in sofa, 2420 μg/g in living room carpet, 1200 μg/g in bedroom carpet, and 520 μg/g in mattress) was selected. Figure 4 shows the airborne Fel d 1 recovered on the different stages of the Andersen sampler before (mean of the measurements obtained on five consecutive nights) and one, two, four, seven, and 14 days following the removal of cats from the living room area. Total airborne Fel d 1 was reduced by 61.7% two days after removal of the cat but this was due predominantly to the decrease in larger particles (>4.8 μm) which fell to 13% of their baseline level. Fel d 1 associated with small particles (<4.8 μm) remained largely unchanged on days 1, 2 and 4, and then slowly decreased at days 7 and 14. The proportion of Fel d 1 carried on small particles (<4.8 μm) increased to 73% of the total airborne level two days following cat removal. At day 14 the total airborne Fel d 1 fell by 87% compared with the mean of five nights of baseline sampling, with larger particles being reduced by 89% and small particles by 67%.

EFFECT OF HEPA FILTRATION ON AIRBORNE FEL D 1

In the homes with cats the baseline airborne Fel d 1 levels were 5.5-fold (95% CI 2.1 to 14.4) greater when the sampling was performed with a cat in the room than when the cat was elsewhere in the house (GM 11.7 ng/m^3 (range 0.83–169.2) versus 2.1 ng/m^3 (range 0.5–22.3)). No significant difference was found in the baseline levels of Fel d 1 between the active and control sampling days. The effect of HEPA air filtration on airborne Fel d 1 levels is presented in table 1. Total airborne Fel d 1 on the active days was reduced by 5.3-fold (95% CI 2.13 to 13.2) at four hours and by 5.7-fold (95% CI 2.24 to 14.4) at eight hours. Some reduction in Fel d 1 was observed on the control days as well (1.54-fold (95% CI 1.25 to 1.9) at four hours and 2.4-fold (95% CI 1.6 to 3.7) at eight hours), but the fall was significantly greater on the active day when the HEPA air cleaner was on than on the control days ($p = 0.008$).

Discussion

The Fel d 1 levels found in reservoir dust samples in Manchester, UK are similar to the data reported recently by Ingram *et al* from Los Alamos, USA.[9] Although all homes without cats contain quantifiable levels of cat allergen in at least one dust reservoir and, in one third of cases, in the air, the levels in the homes of cat owners are about 250-fold higher. These results are similar to the findings of Bollinger *et al*.[10] Our data confirm the previous findings in other countries that cat allergen is ubiquitous in areas with a high proportion of cat ownership.[11 12] In homes with cats the highest levels of Fel d 1 are found in the upholstered furniture. This may reflect the tendency of cats to spend time on the soft furniture rather than the floor. In contrast, we have previously reported that the levels of dog allergen in homes with dogs are highest in the living room carpet (possibly reflecting the tendency of these animals to spend more time on the floor).[13] In homes without cats the levels of cat allergen are also highest in the upholstered furniture in the living room. This finding supports the view that passive transfer of allergen occurs from the clothing of cat owners. Significantly lower levels of Fel d 1 were found in the bedrooms than in the living rooms, both in the homes with and without cats. Unlike house dust mite allergen, where the highest levels are found in mattresses, these results suggest that the most significant exposure to cat allergen may not occur in the bedroom. This view is further supported by the fact that we failed to detect airborne Fel d 1 during personal sampling overnight in bed in any of the 20 homes without a cat (seven of which had measurable airborne levels of Fel d 1 in the living rooms). Furthermore, we have shown in previous studies that cat allergen is detectable in most dust reservoirs collected from public places in the UK (hospitals, cinemas, public houses, etc) and also in the air in about 25% of the areas sampled.[14 15] This again is unlike the situation with mite allergen where very little allergen is found outside domestic dwellings. Although the levels of pet allergens are lower, the passive exposure in homes without pets and in public places may be important and perhaps one ought to think in terms of community exposure for pet allergens, rather than just domestic exposure. Such exposure may be sufficient to cause sensitisation in those susceptible individuals who have never been pet owners. Subsequent pet ownership may then put them at risk of developing symptomatic disease. Furthermore, it has been suggested that even the low levels of pet allergens found in the community may be sufficient to induce symptoms of asthma in highly sensitive individuals.[16]

The cat is clearly the major source of allergen in homes with cats and therefore removal of the cat from the home must be the recommendation of physicians treating asthmatic patients who are allergic to their cats. It is, however, important to note that, even after cat removal, it can be many months before the levels of cat allergen in the dust reservoirs of the home have fallen significantly.[17] Unfortunately, many cat sensitised asthmatics refuse to part with their pets. In some cases this may be because the cat sensitised asthmatic cat owner is unable to recognise the cause and effect relationship between the presence of the cat and the asthmatic symptoms. In some homes with cats we found very low levels of airborne Fel d 1 in the absence of disturbance; such levels may be high enough to cause and maintain the chronic airway inflammation but not enough to cause acute asthma symptoms. Investigators have therefore endeavoured to devise a set of measures which effectively reduce exposure to cat allergen whilst keeping the cat. Knowledge of the aerodynamics of allergen-carrying particles is essential for the development of such strategies.

In order to address all objectives of this study it was necessary to use different samplers and sampling strategies – for example, the Andersen sampler over an eight hour period for particle size distribution, a low volume pump over the same period of time in parallel to the Andersen sampler to confirm that the total allergen recovered was equal using two samplers, a high volume fixed location sampler for "on spot" measurement of airborne Fel d 1 in living rooms of homes with and without cats in order to sample a larger volume of air and thus increase the lower detection limit; this was impossible in bedrooms, however, as the sampler is too noisy and much quieter low volume personal samplers were thus used over a longer period of time. The choice of samplers and sampling times has to be appropriate for the design of the study.

The site of deposition of particles in the lung is determined by their size and shape. Small particles of <5 μm diameter can readily penetrate into the small airways and may be responsible for the acute symptoms noted by many sensitised asthmatics on contact with the relevant animal. In line with previous results[18 19] we found in homes with cats that, although most of the allergen is carried on particles of >10 μm, about 23% of airborne Fel d 1 was associated with particles of <4.7 μm diameter. Repeated measures within the same home over five days showed consistency of particle size distribution, in keeping with the findings by Luczynska *et al*.[18] We have shown that, with the cat in the room, the level of Fel d 1 in the air is more than five times higher than when the cat is not in the room. Although exclusion of cats from the living areas of the home resulted in a dramatic fall in the total airborne level of Fel d 1, this was mostly due to a decrease in larger particles. Particles of <4.7 μm diameter remained airborne in more or less unchanged absolute levels for several days. Clearly, the relationship between removal of a cat from a room and the subsequent decrease in airborne cat allergen is more complex than it would appear when looking only at the total airborne Fel d 1, and different particle sizes carrying Fel d 1 are not reduced evenly across the range. The results of cat removal must be interpreted with caution as this was done in a single household only. However, consistency of particle size distribution, observed both in this study and

previously,[18] would suggest that the observed phenomenon is probably applicable to other houses as well.

Our results suggest that airborne Fel d 1 in homes with cats can be reduced by the use of HEPA air cleaner. It is interesting to note that over a nine hour sampling period we observed a 2.4-fold reduction in total airborne Fel d 1 even without the use of the HEPA air cleaner. This may be due to the effect of sampling – approximately 3.6 m^3 of air is filtered through a 0.3 μm glass fibre filter during one hour of sampling so the air is effectively being cleaned by the sampler. In addition, it is possible that setting up the sampling equipment may have caused some disturbance of particles carrying Fel d 1 which settled during the sampling period, although we made an effort not to create unnecessary disturbance during the sampling days to make the sampling conditions as reproducible as possible. We have observed similar phenomena when testing the effect of HEPA air cleaner on airborne dog allergen.[20] Nevertheless, a more than fivefold reduction in airborne Fel d 1 was achieved at four hours and maintained at eight hours when the HEPA air cleaner was used.

Washing the cat has been shown to reduce the amount of airborne allergen,[21] although some studies have questioned this finding.[22] The use of vacuum cleaners with HEPA filters and double thickness bags has been shown to be effective in preventing Fel d 1 from becoming airborne during vacuuming.[23] Other recommendations include polished floors and a minimum of upholstered furniture to reduce reservoirs for cat allergen.[21]

In conclusion, we found measurable levels of cat allergen in all sampled homes in the north-west of England. Furthermore, airborne Fel d 1 was detectable in the undisturbed conditions in all homes with cats and in almost a third of the homes without cats. In houses with cats a significant proportion (23%) of airborne Fel d 1 was associated with small particles (<5 μm diameter), in keeping with previous results.[18] Removing the cat from the living room and bedroom areas of the home resulted in a marked reduction in total airborne allergen, but it was not reduced evenly across the particle size range. The use of an HEPA air cleaner may reduce airborne cat allergen in homes with cats. Further studies are needed to examine the effects of stringent cat allergen avoidance on asthma severity.

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