

Quantitative measurement of airborne allergens from dust mites, dogs, and cats using an ion-charging device

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Summary

Background Increasing evidence suggests that children raised with an animal(s) in the house have a decreased risk of becoming sensitized. However, it is not clear whether this phenomenon is related to airborne exposure.

Objective To estimate airborne exposure to animal dander and dust mite allergens using a device that can sample large volumes of air silently.

Methods The device, which uses an ion-charging technique to move air and to collect particles, was run at 1.7 m³/min for 24 h in 44 homes with and without animals. The allergen collected was measured by ELISA for Fel d 1, Can f 1, Der p 1, and Der f 1.

Results Airborne Fel d 1 was present in all homes with a cat ($n = 27$). The quantities measured, i.e. 0.5–20 µg in 24 h, represent 0.01–0.3 µg Fel d 1 inhaled/day at normal breathing rates (20 L/h). Values for houses without a cat were 0.01–0.05 µg inhaled/day. Airborne Fel d 1 correlated significantly with floor Fel d 1 ($r = 0.58$, $P < 0.001$). Results for Can f 1 were similar in houses with a dog, but this allergen was only detected airborne in two houses without a dog. Neither Der p 1 nor Der f 1 (i.e. < 0.01 µg) was detected, which represents ≤ 1 ng inhaled/day during normal domestic activity. During disturbance airborne mite was detected with both the ion-charging device and a filter run in parallel. For cat and mite allergens there was a close correlation between the two techniques ($r = 0.84$, $P < 0.001$).

Conclusion Exposure to cat or dog allergen airborne in homes with an animal can be up to 100 times higher than exposure to mite allergen. The results are in keeping with a model where immunological tolerance to animal dander allergens results from high exposure.

Keywords airborne, allergen, cat, dog, dust mite, ion charging

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Introduction

Sensitization to allergens found in homes is strongly associated with the prevalence, incidence, and severity of asthma [1, 2]. However, the dose–response relationship between cat allergen exposure and sensitization to cat allergens appears to be different from that for dust mite or cockroach [3]. In particular, high-dose exposure to cat allergen (i.e. the presence of an animal in the home) has been associated with a *decreased* prevalence of sensitization [4–8]. Furthermore, a large proportion, in some studies as high as 75%, of cat-allergic children have never lived in a house with a cat [9]. Although many possible explanations for differences between animal dander and other allergens have been considered, the most obvious is that exposure to animal allergen is higher than for other allergens [10, 11]. Most of the available evidence about exposure is based on measurements

of the concentration of allergen in floor dust or the presence of animals in the home.

Theoretically, measurements of airborne allergen would be a better approach for evaluating exposure to an inhaled allergen. However, there are major problems with using airborne measurements to study exposure during normal domestic life. Nasal samplers can be used to estimate the number of allergen particles that are inhaled, but samplers can only be worn for a limited time and the results are difficult to quantitate [12, 13]. Most other techniques for measuring airborne allergens have required a method for moving air through a filter, over impactor plates, or through an impinger [10, 14–17]. These methods all require an electric pump and airflow through a narrow orifice, each of which creates noise and therefore restricts their use in homes. Ion charging has been widely used as a technique for removing particles from the air, and one previous report adapted this technique to collect and quantify airborne allergen [18]. The technique of positively charging ions and then accelerating them past negatively charged plates was developed by NASA as a possible method of propulsion in space. The realization that negatively charged plates could be designed to collect

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particles suggested that the principle could be adapted to create an air-cleaning device. We report here the use of an ion-charging device (ICD) to measure airborne allergen. For this purpose, this ICD has three major advantages: it is silent; it collects particles from a relatively large quantity of air, $\sim 1.7 \text{ m}^3/\text{min}$; and the plates that collect the particles are designed to be removed and cleaned. The charging technique is not selective and should charge all allergen-containing particles equally [19]. We have taken advantage of these features to develop a sensitive technique for measuring airborne allergen during normal domestic activity.

Methods

The Ionic Breeze Quadra is a commercially available ICD that utilizes a technique in which particles are charged and adhere to one of three negatively charged plates. The result is that air is accelerated through the plates and the charged particles adhere to the plates (see Fig. 1). The apertures for airflow are 54 lateral slits $0.5 \text{ cm} \times 12 \text{ cm}$ that go from 17 to 57 cm above the floor. Using the hot-wire technique, the airflow through the ICD at high setting was $1.7 \pm 0.15 \text{ m}^3/\text{min}$ [20]. The method of ion charging generates ozone; the quantity produced is controlled by the voltage pattern and the model consistently complies with Underwriters Laboratory Standard #867. Studies were carried out in the homes of 44 adults involved in a study of immune responses to cat allergens. Of these, 27 had a cat and 17 had a dog; of these 12 had both a cat and a dog, and 12 had neither. The ICDs were placed at least 2 ft away from any surface. In the living rooms ($n = 44$), the two ICDs were placed at least 6 ft apart. Only 29 bedrooms were studied and only one ICD was used in the bedrooms because many of the rooms were too small. Values for living rooms are the mean of results from two separate ICDs; the individual values were closely reproducible, κ value = 0.93. We are grateful to Dr. Chuck Taylor of The Sharper Image, San Francisco, for kindly providing the ICDs used in these studies.

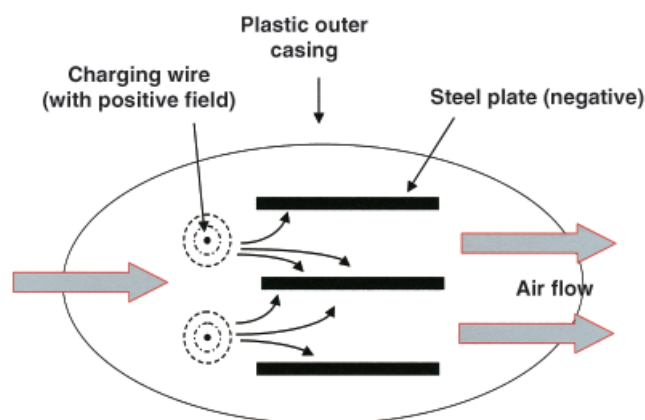


Fig 1. Cross-sectional view through the device to show wires that give a positive charge to airborne particles and the negatively charged plates that attract and bind the particles. The plates can be removed and cleaned. The ICD stands approximately 66 cm high and has cross-sectional diameters of $17 \text{ cm} \times 11 \text{ cm}$. The air entering and leaving the device flows through 54 horizontal slits $0.5 \text{ cm} \times 12 \text{ cm}$ which are from 17 to 57 cm above the ground.

Cleaning the device

After running, the stainless-steel plates were lifted out of the device and cleaned with a series of three filters (Millipore prefilters, AP20, 35 mm) that had been dampened with sterile water. All three plates were thoroughly wiped with the first filter and then with the second and third filters; each filter was folded and inserted into a 3 cm^3 syringe. Using a second 3 cm^3 syringe, 2 mL of sterile phosphate-buffered saline (PBS) was injected into each of the syringes, and they were then rotated overnight at 4°C . Extracts were recovered by dispensing the contents of the syringes into 2 mL microcentrifuge tubes and were assayed by using two-site monoclonal antibody ELISA techniques. Preliminary experiments established that wiping the plates 3 times removed $\sim 98\%$ of the allergen on the plates. Typically, the three sequential samples yielded 85%, 13% and 2% of the total. All data are presented as the sum of the results from the three filters. Overloading of the plates can result in decreased efficiency of binding, and in some cases particles on the plates can be dislodged by impact. However, this was never observed with up to 6 days running even in houses with very high levels of airborne allergen. In each case, the visible dust was adherent to the plates and required physical contact (i.e. a damp filter) to remove it.

Air sampling using a pump

Two 35 mm Millipore filters were placed in filter holders (Cassela Lovidon Ltd., Bedford, UK) and assembled 6 in apart with each face at 90° to each other at a height of 60 cm from the floor. The filter holders were connected to a stationary vacuum pump (Alitea AB, Stockholm, Sweden). Air was sampled for 45 min (disturbed) or 2 h (undisturbed) at a flow rate of 18 L/min through each filter and was monitored using a flow meter (Mark 8 Wright Respirometer, Ferraris, Edmonton, UK) [10]. After sampling, filters were removed and treated as previously described.

Collection and extraction of floor dust samples

Dust samples were collected using previously described techniques [14, 21], with a Hoover Sprint 100. Samples were sieved using a $300 \mu\text{m}$ mesh wire sieve (Newark Wire Cloth Company, Newark, NJ, USA), and were extracted with 2 mL of borate-buffered saline (BBS).

ELISA for *Fel d 1*, *Can f 1*, and mite allergens

Two site monoclonal antibody (mAb)-based ELISAs were used to measure *Fel d 1*, *Can f 1*, and group 1 (*Der f 1* and *Der p 1*) mite allergens as described previously [10, 11, 22]. Airborne samples were assayed undiluted and at a 1:5 dilution. Samples collected from the three plates of the device were assayed at 1:5, 1:25, 1:125, and 1:625 dilutions. Values were obtained from curves using University of Virginia standards for *Fel d 1*, *Can f 1*, and group I mite allergens.

Results

Optimal sampling time for the ICD

Our objective was to develop a sensitive technique for measuring airborne allergen. Because the ICD is silent, it was possible to run it for prolonged periods in living rooms or bedrooms. Preliminary studies in houses with a cat established that collection rates were linear over hours (data not shown). Sampling was carried out in duplicate over 8 days at seven sites. The results showed progressive increases in allergen up to 4 (or 6) days, but a decreased total at 8 days. Three sites remained fully negative for 4 days, i.e. $<0.01 \mu\text{g}$ collected. In one house with cats, values up to $30 \mu\text{g}$ Fel d 1 at 4 days were observed, but all samples were negative for Der p 1 and Der f 1. Typical sequential data showed $2.8 \mu\text{g}$ at 2 days, $4.9 \mu\text{g}$ at 4 days, $9.0 \mu\text{g}$ at 6 days, but only $7.6 \mu\text{g}$ at 8 days. The sampling time of 24 h was chosen because there was no chance of overloading the ICD at this time, it provided a full cycle of domestic activity, and it allowed for rapid turn around of ICDs in a way that was acceptable to the subjects.

Comparison of air filters and the ICD as techniques for measuring airborne allergen: airborne mite allergen during vigorous disturbance

Comparison of the ICD with traditional filter-based techniques had two objectives: (i) to assess the efficiency of collection and (ii) to answer whether the ICD would collect airborne particles carrying dust mite allergens. Experiments were carried out with two filters run at 18 L/min in parallel with two ICDs with or without vigorous disturbance in homes with known levels of floor dust allergens (Fig. 2). Since cat allergen is continuously airborne, sampling for 2 h without disturbance was used, and only four disturbance experiments were carried out: 15 min with a domestic vacuum cleaner, and sampling was continued for a total of 45 min. For airborne mite allergen, disturbance was carried out in 10 houses for 25 min using a cleaner from which the filters had been removed, and sampling was continued for a total of 45 min. The results show a close correlation between the two techniques ($r = 0.84$; $P < 0.001$). With both techniques, dust mite was only detected during disturbance.

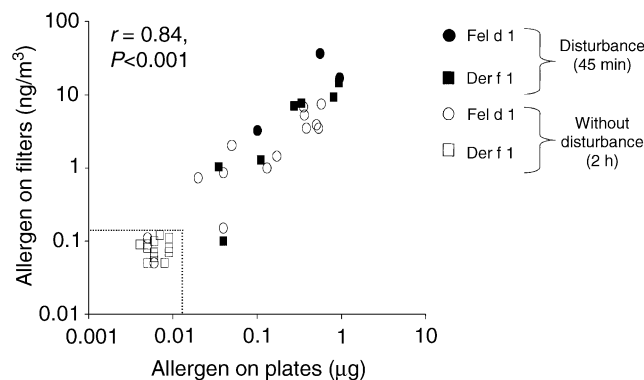


Fig 2. Comparison of allergen collected with two ICDs with the allergen collected on filters sampling at 18 L/min . Values shown are the mean value for duplicates of each collection system run for the same time. Results are shown for Fel d 1 in undisturbed (2 h) ($\circ$) and disturbed (45 min) ($\bullet$) conditions. In addition, the results for Der f 1 undisturbed (2 h) ($\square$) and disturbed (45 min) ($\blacksquare$) are shown. The disturbance for cat allergen used a domestic vacuum cleaner with filters. In contrast, disturbance to measure airborne mite allergen used a vacuum cleaner without filters. The correlation between the results using the two methods was highly significant ($r = 0.84$; $P < 0.001$).

Twenty-four-hour airborne monitoring in homes with and without domestic animals

Air sampling was carried out in 44 homes using two ICDs in each living room and one in each bedroom when the family agreed ($n = 29$). Results for dust mite (Der p 1 and Der f 1), cat (Fel d 1), and dog (Can f 1) allergens are shown in Table 1 and Fig. 3. Airborne dust mite allergen was not detected in any of the sites tested including the bedrooms (Fig. 3a). Thus, even in a room with $\sim 500 \mu\text{g}$ Der f 1/g dust, no allergen was detected airborne during normal domestic activity. In keeping with previous results, the cat allergen Fel d 1 was detected airborne in all homes with a cat (Fig. 3b). Quantities of Fel d 1 collected on the plates were up to $20 \mu\text{g}$ in 24 h. In homes without a cat, Fel d 1 was also detected in the air (positive results in 11 of 17 living rooms), in some homes as much as $1 - 2 \mu\text{g}$, although the quantities were in general lower. Overall, the correlation between airborne cat allergen and levels in floor dust was highly significant ($r = 0.58$, $P < 0.001$) (Fig. 3b). For dog allergen, quantities airborne in homes

Table 1. Mean allergen levels detected airborne and in floor dust in homes with or without animals

	Floor ($\mu\text{g/g}$)		Airborne ($\mu\text{g/24 h}$)	
	Living room*	Bedroom	Living room*	Bedroom
Cat: Fel d1				
Cat in house	625 (27/27)	315 (16/16)	1.52 (27/27)	1.12 (16/16)
No cat	3.68 (17/17)	4.44 (13/13)	0.049 (11/17)	0.042 (8/13)
Dog: Can f1				
Dog in house	287 (17/17)	543 (10/10)	1.48 (16/17)	2.80 (9/10)
No dog	208 † (23/27)	3.13 † (14/19)	< 0.01 ‡ (0/27)	0.116 † (2/19)
Dust mite	1.47 † (25/44)	1.69 † (18/29)	< 0.01 ‡ (0/44)	< 0.01 ‡ (0/29)

*Values reported for 44 living rooms are a mean of two ICDs run in parallel. Single samplers were used in the 29 bedrooms studied. The number of sites with a positive value are indicated in parentheses. Mean levels: bold; negative values: italics.

†Geometric mean values were calculated using only the positive values.

‡Lower limit of detection; no positives.

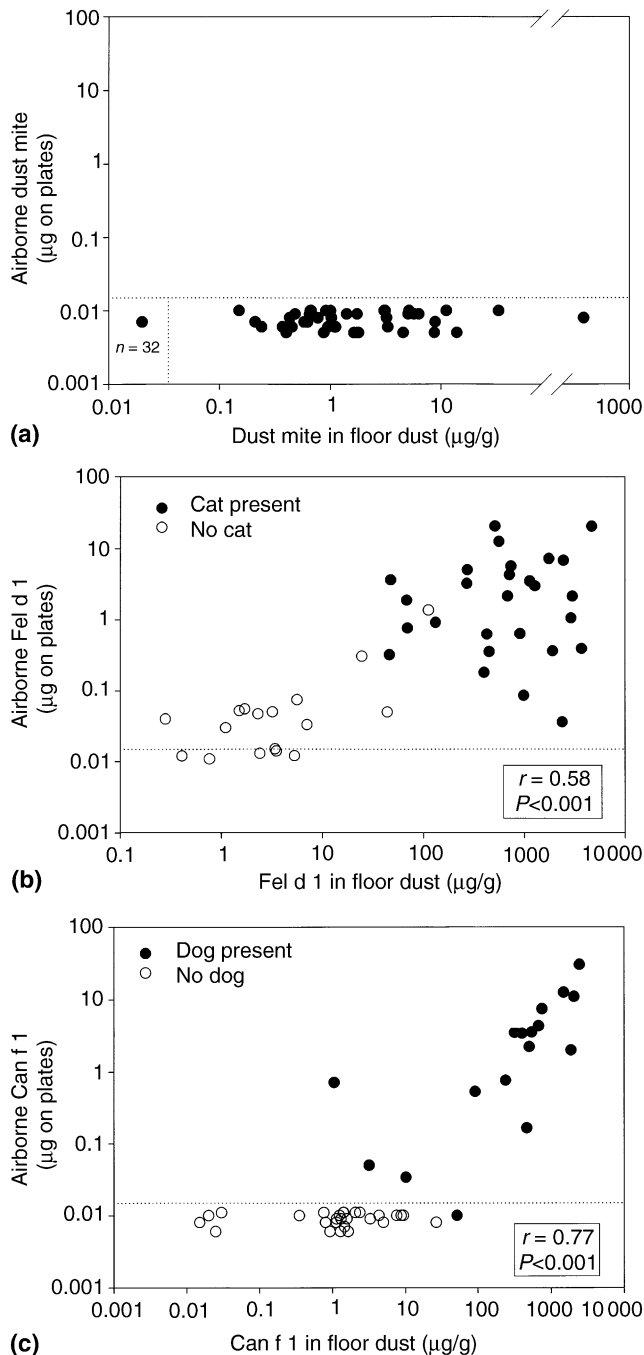


Fig 3. (a) Group I mite allergen airborne (ICD) and in floor dust in 44 living rooms and 29 bedrooms. (b) Correlation between Fel d 1 airborne (ICD) and floor dust levels in 44 living rooms of homes with (●) and without (○) cats. The correlation between the floor dust and airborne allergen was highly significant ($r = 0.58$; $P < 0.001$). (c) Correlation between Can f 1 airborne (ICD) and floor dust levels in 44 living rooms of homes with (●) and without (○) dogs. The correlation between floor and airborne allergen was highly significant ($r = 0.77$; $P < 0.001$).

with a dog were similar to those for cat allergen and correlated with the floor concentration (Fig. 3c). Airborne levels in homes without a dog were almost all negative (Fig. 3c, Table 1). No airborne dog allergen was measured in any living rooms and only two bedrooms had measurable Can f 1 in homes without an animal.

Concentrations of airborne allergen

The airflow through the ICD is $1.7 \text{ m}^3/\text{min}$, and the efficiency of collection of particles is 65% using mulberry pollen grains. This suggests that the ICD collects particles from $1.1 \text{ m}^3/\text{min}$. Using measurements of Fel d 1 collected using two filters and two ICDs in parallel in six houses, comparable values were calculated for cat allergen collection. The results were $0.95 \pm 0.06 \text{ m}^3/\text{min}$. Assuming a value of $1 \text{ m}^3/\text{min}$, the ICD would collect particles from $\sim 1400 \text{ m}^3/\text{day}$. The values in Fig. 3b can be used to calculate airborne concentrations. If $10 \mu\text{g}$ Fel d 1 was collected on the plates, then the daily average airborne allergen was 6.5 ng Fel d 1 per m^3 . Assuming a breathing rate of $20 \text{ L}/\text{min}$ for an adult, the quantity of cat allergen inhaled per day in a house with a cat could be from 0.01 to $0.3 \mu\text{g}/\text{day}$.

Discussion

The technique used here has provided quantitative measurements of airborne cat allergen both in homes with and without a cat. The values for dog allergen in homes with an animal were similar to the values for cat, but dog allergen was generally not airborne in homes without a dog. In keeping with previous results, the values for dust mite were consistently negative except during vigorous disturbance. Although it was known that cat allergen (unlike mite allergen) is airborne without artificial disturbance, the present results provide a clearer definition of the scale of the difference. Our values show that cat allergen can be 100-fold greater than mite allergens. Indeed, the quantity of Fel d 1 airborne in homes without a cat appears to be 10-fold higher than our maximum estimates of the quantities of mite allergen that are airborne without artificial disturbance. The results are in keeping with the view that mite allergen exposure occurs during disturbance or while the subject is in close contact with bedding, pillows, sofas, etc. In turn, the results suggest that there is very little mite allergen associated with small particles; if there was, it should remain airborne and be detected [23]. The results for airborne cat allergen confirm the highest previous estimates. Assuming normal breathing rates of $\sim 20 \text{ L}/\text{min}$, persons living in a house with a cat could inhale as much as $0.3 \mu\text{g}$ Fel d 1 per day. This should be compared to previous estimates for pollen or dust mite of closer to $0.5 \mu\text{g}/\text{year}$ [14, 24]. In turn, this supports the view that the reason for decreased prevalence of sensitization among individuals with high exposure to cat allergens is related to the levels of exposure. The quantities of cat allergen found airborne in homes without a cat strongly reinforce the idea that airborne cat allergen either in homes without a cat or in schools is sufficient to give rise to sensitization and to cause symptoms [25, 26].

The ICD used here creates an airflow that can be felt 6 ft away, and we therefore assume that air in the room will be disturbed. However, the air velocity is not comparable to that of a fan or a HEPA air cleaner, which have been shown to disturb cat allergen from a carpet or floor [10]. We have previously shown that from 10 to 100 mg of cat allergen can be found in carpets, sofas, or the cat itself [27]. Thus, the quantity airborne is consistently less than 1% of the quantity

in the home. We assume that there is an ongoing exchange of allergen between the furniture and the air. Thus, although the air will pass through the ICD many times in 24 h, the allergen continues to accumulate.

When the plates are heavily loaded, collection of allergen becomes significantly less efficient. This leads to arcing of charge between the wires and the plates and an associated crackling sound. Overloading may occur in as little as 2 or 3 days but generally takes more than 1 week. In keeping with all ion-generating devices, the ICD produces ozone that can accumulate if it is run in a small space [28, 29]. The acceleration of the air and the generation of ozone are a function of the pattern of current in the wires of the device, which can be controlled (Fig. 1).

The studies reported here were not designed to test the ability of the ICD to clean the air. However, our experiments demonstrate that large quantities of cat or dog allergen bind to the plates. In preliminary experiments in homes with a cat, we have found that the ICD alone is not sufficient to control airborne cat allergen (data not shown). This is in keeping with results showing that controlling airborne allergen in a home with a cat would require removing carpets and sofas, controlling all reservoirs, and where possible regular washing of the cat, as well as the use of an air cleaner [30]. The results presented here together with previous results on symptoms among cat-allergic individuals and data on schools suggest that efforts to control airborne cat allergen should not be restricted to homes with a cat [25, 31, 32]. It has been known for some years that homes without a cat can accumulate cat allergen in floor dust [10, 11, 31, 32]. Previous studies on airborne cat allergen in homes without a cat required enhanced assays to detect Fel d 1 [24]. The current technique allows for longer sampling periods and consequently the quantity of allergen collected is higher. In addition, the correlation between airborne Fel d 1 and allergen in the floor dust (Fig. 3b) recorded here was closer than in previous studies [10, 17, 24, 33]. It was a surprise that dog allergen was not airborne in homes without a dog, despite the presence of measurable allergen on the floor. Published results have suggested that Can f 1 could be measured in homes without a dog, but the reported values were very low [34]. Our results cannot be explained either by lower sensitivity of the assay or by failure of binding of particles to the plates. It is possible that the results reflect different properties of the particles carrying Can f 1 or differences in the behaviour of dog owners/visitors to homes with a dog. If it is confirmed that Can f 1 becomes airborne less than Fel d 1, this could be highly significant in understanding the overall prevalence of dog sensitization.

In conclusion, the results reported here show that the ICD provides a new approach to monitoring airborne allergen. The combination of silence, moderate airflow, and ease of sampling the plates makes it suitable for monitoring airborne exposure in homes during normal domestic life. Our experience is that patients can take the device home, run it, and return it without difficulty. The device can collect the allergen particles from 1 m³ of air per minute, which with 24 h running provides great sensitivity. The results dramatically reinforce the difference between airborne cat and dust mite allergens. It is clear that further experiments are needed to (i) validate the results on airborne dog allergen in the absence of

a dog, (ii) study the relevance of cat allergen avoidance in houses and schools without a cat, and (iii) understand why or how exposure to ~0.3 µg Fel d 1 per day can give rise to immunological tolerance [35].

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