Allergy 1994: 49: 210–216
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ALLERGY
ISSN 0105-4538

Dust from carpeted and smooth floors

VI. Allergens in homes compared with those in schools in Norway

Dybendal T, Elsayed S. Dust from carpeted and smooth floors. VI. Allergens in homes compared with those in schools in Norway. Allergy 1994: 49: 210–216. © Munksgaard 1994.

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The amounts of dust, protein, and allergens in vacuumed floor covering samples taken from both schools and homes in Norway have been investigated. Classrooms contained significantly more crude dust per unit area than homes, while the mean protein content per unit area was somewhat higher in homes. The main allergen exposure problem in classrooms was that of pet allergens; the contents of dog allergens and the major cat (*Felis domesticus*) allergen *Fel d* I per unit area were significantly higher in school classrooms than in homes of families who do not keep pets. The mite and food allergens, on the contrary, were more prevalent in homes. Carpeted floors in schools and homes contained significantly more dust, proteins, and allergens than smooth floors ($P < 0.05$). *Fel d* I was detected in the vacuum cleaners' filter system in concentrations from < 1 ng to 1080 ng, confirming that this allergen can pass through the dust bag during vacuuming.

Key words: allergens; child; dust; hypersensitivity; schools.

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Accepted for publication 27 May 1993

Exposure to indoor allergenic proteins is a risk factor for sensitization (increased IgE antibody response) in genetically susceptible persons and for the exacerbation of symptoms in those with already established allergy and asthma. We have previously investigated the occurrence of pollen, cat, dog, mite, mold, and food allergens in dust samples from schools in Norway (3, 4). The prevalence of cat and dog allergens was surprisingly high in the classrooms, and the major cat allergen *Felis domesticus* I (*Fel d* I) was detected in quantities varying from 31 to 16840 ng per m^2 floor area after a 10-day sampling period (2). The occurrence of pet allergens in floor dust in schools suggested an unobserved exposure which may cause sensitization and symptoms among pupils. By contrast, the mite allergens *Dermatophagoides pteronyssinus* allergen I (*Der p* I) and *D. farinae* allergen I (*Der f* I) were detected in very low quantities in the school dust, ranging from undetectable (< 1 ng) to 104 ng per m^2 floor area (2).

In this investigation the dust, protein, and allergen contents in schools and homes were compared in order to determine whether allergen exposure levels in schools were equal to or greater than in homes where no allergen avoidance methods were employed. The allergen exposure levels in homes of allergic children can be controlled by elimination measures initiated by the parents, while in schools the children and their parents may not be aware of any allergen exposure. The other aim of the study was to quantify the allergens escaping the vacuum cleaner bags by analyzing the *Fel d* I, *Der p* I, and *Der f* I levels in the main filters and microfilters after the dust sampling.

Material and methods

Sampling procedure and preparation of dust extracts

Dust samples were collected from one carpeted and one smooth floor room in each of 10 schools and 16 homes. In homes, 7/16 smooth floor rooms had one or two scattered rugs, which covered from 12–43% of the floor area (mean value 20%). Homes with three or more inhabitants including one child or two or more children were favored. Six of the schools and 10 of the homes were situated in western Norway, while the rest were in eastern Norway. The schools were sampled during February, whereas most homes were sampled in April. The outdoor relative humidity and average temperature during sampling of homes in western Norway were 76.6% and 10.7°C and in eastern Norway 74.3% and

3.2°C, respectively (Norwegian Meteorological Institute, Oslo, Norway). The whole floor area was vacuumed during a 10-day sampling period with a Nilfisk GS 90 vacuum cleaner (Fisker and Nielsen A/S, Denmark) supplied with a double disposable filter bag, a main filter, and a microfilter, as previously described (5). According to the manufacturer, the main filter retains 99.5% of particles >2 μm, whereas the microfilter retains 99% of particles >0.7 μm and 90% of particles >0.2 μm. While fresh main filters and microfilters were used for each floor sampled in the schools, the same filters were used in each of the five vacuum cleaners during sampling in different homes. The dust and the filters were extracted in 50 mM NH_4HCO_3, and the extracts were dialyzed and lyophilized as previously described (5). The lyophilized extracts were stored at −80°C until analyzed for allergen contents.

Protein determinations

The protein content in solutions of lyophilized dust extracts was determined by the method of Lowry et al. (11), with bovine serum albumin as standard.

Allergen disks

Allergen disks of alder, birch, codfish, and hen egg white were prepared as described previously (4, 20). Other paper disks (mold, cat and dog dander, timothy, and mite) were purchased from Pharmacia AB (Uppsala, Sweden).

Human sera

The sera from patients with Type I allergy used in the specific IgE inhibition tests have been described previously (4).

Specific IgE inhibition test

The radioallergosorbent test (RAST) inhibition assays of dust samples were performed as previously described (5). In brief, 50 μl patient serum pool was added to 100 μl dust extract solution (corresponding to lyophilized material from 1 m^2 floor area) and incubated for 1 h at room temperature. An allergen disk coated with the appropriate allergen was added to the mixture and incubated for 3 h. The disk was further incubated overnight with 50 μl ^{125}I-labeled-anti-IgE (Phadebas RAST isotope, Pharmacia AB, Uppsala, Sweden). The tests were performed in duplicate. The value of percent inhibition was calculated by the formula

$$\frac{(\text{cpm of noninhibited serum} - \text{cpm of inhibited serum})}{\text{cpm of noninhibited serum}} \times 100$$

Enzyme-linked immunosorbent assay (ELISA) procedure

Reagents for the *Der p* I, *Der f* I, and *Fel d* I sandwich ELISAs were obtained from Dr Martin D. Chapman, University of Virginia, Charlottesville, VA, USA. Reference cat dander preparation OBRR Cat E3 was obtained from Dr Christine Anderson, Office of Biologics and Research Resources, Department of Health and Human Services, Bethesda, MD, USA. The assays were performed as previously described (1, 2, 12).

Dog dander extract

One gram of dog dander (batch no. 228688904, Allergon AB, Engelholm, Sweden) was extracted overnight in 100 ml 0.125 M NH_4HCO_3, pH 8.6, containing 0.02% (w/v) NaN_3. The extract was filtered (Schleicher and Schüll folded filters, Dassel, Germany) and centrifuged at 6000 rpm. in a Beckman model J-21B centrifuge (45 min at 4°C). The supernatant was dialyzed (mol. wt. cutoff 3500) against 5 mM NH_4HCO_3 for 2 × 24 h, followed by distilled water for 24 h, and was finally lyophilized.

Sodium dodecyl sulfate (SDS) – polyacrylamide gel electrophoresis (PAGE)

The SDS–PAGE gels were molded in Protean® II slab cell equipment (Bio-Rad Laboratories, Richmond, CA, USA), according to the instruction manual. Reagents and gel preparation were according to the Laemmli buffer system (9), with a polyacrylamide stacking gel (4%) on top of a separation gel (12%). Aliquots of 50 μl of solution of lyophilized dust extracts (33 mg/ml) in sample buffer were applied in the wells, and electrophoresis was performed at 30 mA for about 3 h. The SDS low mol. wt. markers for mol. wt. 10000–70000 (Sigma Chemical Company, St. Louis, MO, USA) were included for mol. wt. determination. The gels were stained for protein with Coomassie brilliant blue or used for immunoblotting as described below.

Electrophoretic transfer and immunologic detection

Proteins were transferred from the separating gel to a nitrocellulose membrane (0.45 μm, Schleicher and Schüll, Dassel, Germany) in a Trans-blot electrophoretic transfer cell (Bio-Rad Laboratories) according to the instruction manual. Gels and transfer membranes were preequilibrated in transfer buffer before the electrophoretic transfer. The blotting was performed at 50 V for 1 h (plate electrodes). The nitrocellulose membrane was incubated overnight with blocking solution (phosphate-buffered saline

(PBS) with 3% gelatin). After washing, the membrane was incubated with patient sera in PBS with 0.05% Tween overnight and finally with ^{125}I-labeled-anti-IgE (Phadebas RAST isotope, Pharmacia, Sweden) at >80000 cpm/ml. The membrane was washed, dried, and subjected to autoradiography at −80°C with Hyperfilm-MP (Amersham, UK). The autoradiographs were developed in a Kodak X-Omat RP automatic processor.

Statistics

The Wilcoxon matched-pairs test was used to compare the different variables in dust from carpeted and smooth floors within the homes and schools. The Wilcoxon two-sample test was used to compare similar floor types in schools and homes. The limit of significance chosen was 0.05.

Results

The mean weights of total vacuumed material, lyophilized crude and dialyzed extracts and protein per m^2 floor area in schools and homes are shown in Table 1. The mean weights of total vacuumed material were two to four times higher in schools than in homes when similar floor types were compared, while the mean protein content per unit area was somewhat higher in homes. There were significant differences in weight of vacuumed material and proteins per m^2 between carpeted and smooth floors in both schools and homes ($P<0.05$). Furthermore, a positive correlation (r) was found between age of carpet and weight of vacuumed material ($r=0.434$) and protein ($r=0.641$), respectively, per unit area in homes (results not shown). No such correlation was found in schools.

Table 1. Weights of vacuumed material, crude and dialyzed extracts, and protein (arithmetic mean±SD, range) per m^2 floor area after sampling of 10 schools and 16 homes. In each school and home, samples were collected from one carpeted floor (CF) and one smooth floor (SF), occasionally a smooth floor with scatter rug(s) (SFR)

	Schools		Homes	
	CF (n=10)	SF (n=10)	CF (n=16)	SF/SFR (n=16)
Vacuumed material (g)	*†6.4±4.4 (1.1–14.8)	‡3.5±2.3 (0.6–6.5)	§2.7±2.3 (0.4–7.9)	0.8±0.6 (0.2–2.0)
Crude extract (mg)	†146±95 (49–342)	32±21 (9–71)	§212±302 (25–387)	99±118 (6–463)
Dialyzed extract (mg)	†35±22 (11–74)	‡7±5 (1–18)	§38±40 (4–89)	21±23 (1–86)
Protein (mg)	†11±7 (2–26)	‡2±1 (1–4)	§22±25 (3–55)	7±7 (1–22)

* $P<0.05$; comparison between CF in schools and homes.
† $P<0.05$; comparison between CF and SF in schools.
‡ $P<0.05$; comparison between SF in schools and homes.
§ $P<0.05$; comparison between CF and SF/SFR in homes.

The percent specific IgE-binding inhibitions in solutions corresponding to dust from 1 m^2 floor area are shown in Table 2. Data of specific IgE inhibition percentages for alder and birch were excluded in the comparison of allergen contents in homes and schools because sampling in schools ended at the beginning of March, while the dust sampling in most homes was performed during the alder pollen season (April). Classrooms contained significantly more dog allergens per unit area carpeted and smooth floors than homes of families without dogs ($P<0.05$). For cat allergens, the mean percent inhibition values were higher in schools than in homes when similar floor types were compared, but the differences were not statistically significant ($P=0.08$). On the other hand, significantly higher amounts of mite and fish allergens were detected in the homes. No statistically significant differences were found in timothy and mold allergen contents between homes and schools. With the exception of timothy allergens in schools and food allergens in homes, there were highly significant differences in allergen contents between carpeted and smooth floors in homes and schools, respectively ($P<0.05$).

Table 2. Percent specific IgE-binding inhibition (arithmetic mean±SD, range) in dust extracts corresponding to dust from 1 m^2 floor area in 10 schools and 16 homes. In each school and home, samples were collected from one room with carpeted floor (CF) and one room with smooth floor (SF), occasionally a smooth floor with scatter rug(s) (SFR)

	Schools		Homes	
	CF (n=10)	SF (n=10)	CF (n=16)	SF/SFR (n=16)
Alder*	†20±15 (0–48)	5±7 (0–16)	‡64±26 (20–96)	44±21 (10–81)
Birch*	†16±12 (0–40)	3±4 (0–12)	‡50±28 (11–93)	29±16 (8–64)
Timothy	13±11 (0–27)	7±6 (0–20)	‡26±20 (0–63)	16±14 (0–52)
Cat§	†73±9 (62–85)	44±13 (26–68)	‡59±21 (24–78)	34±21 (13–70)
Dog‖	†§§84±11 (62–95)	**54±18 (24–76)	‡65±19 (28–90)	45±17 (15–74)
Mite	†§§31±17 (11–59)	**9±12 (0–40)	‡66±19 (33–95)	40±23 (10–81)
Egg white	†33±8 (17–44)	**16±10 (2–34)	37±17 (11–68)	38±23 (7–74)
Codfish	†§§37±11 (19–53)	**9±11 (0–25)	75±12 (42–93)	71±19 (32–93)
Mold	†85±8 (69–95)	59±20 (29–84)	‡69±23 (26–95)	52±24 (12–91)

* Data of specific IgE inhibition percentages for alder and birch were excluded in comparison of allergen contents in schools and homes because of different sampling periods (February/March and April).
† $P<0.05$; comparison between CF and SF in schools.
‡ $P<0.05$; comparison between CF and SF/SFR in homes.
§ Samples from homes without cats were analyzed: CF (n=10); SF/SFR (n=10).
‖ Samples from homes without dogs were analyzed: CF (n=14); SF/SFR (n=14).
§§ $P<0.05$; comparison between CF in schools and homes.
** $P<0.05$; comparison between SF in schools and homes.

The *Fel d* I contents in ng per m^2 carpeted and smooth floors in homes and schools are shown in Fig. 1. The *Fel d* I quantities varied from 4 ng/m^2 to 16840 ng/m^2, a difference of more than 4000 times. The mean *Fel d* I contents per m^2 carpeted floors in schools and homes were 3275 and 336 ng, respectively; that is, about 10 times more in schools ($P = 0.002$). When comparing smooth floors, the mean value in homes was 64 ng/m^2 and in schools 283 ng/m^2 ($P = 0.003$). The *Fel d* I concentrations per g of unsieved dust are shown in Fig. 2. The difference in mean contents per g of dust between similar floor types in homes and schools was not significant.

The *Der p* I contents in ng per m^2 floor area in homes and schools are shown in Fig. 3. Carpeted floors in homes contained significantly more *Der p* I than carpeted floors in schools ($P < 0.05$). The *Der p* I concentrations expressed in ng per g of unsieved dust were very low, as shown in Fig. 4. Mean values were < 48 ng for homes and < 3 ng for schools.

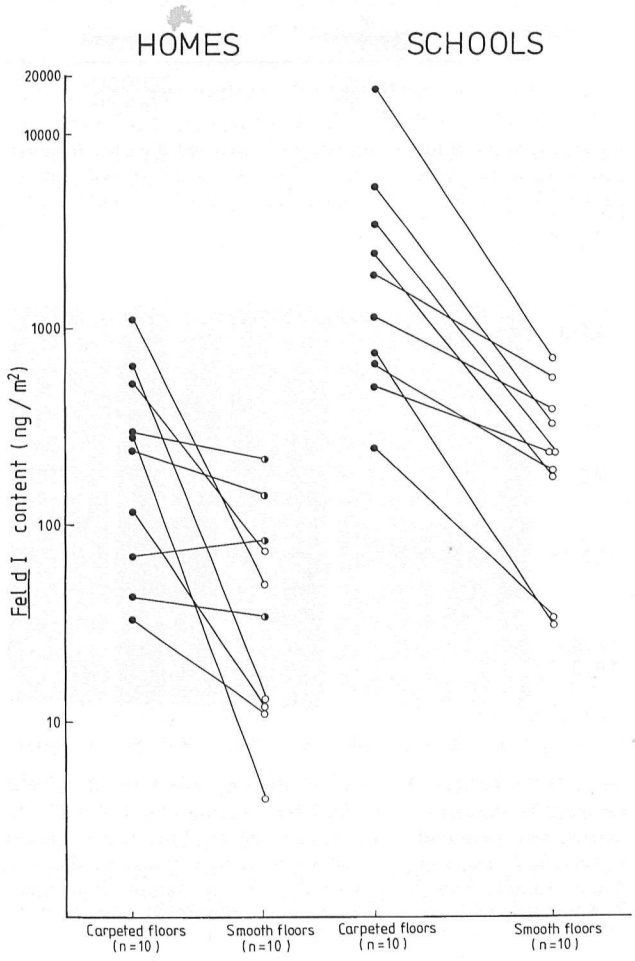

Fig. 1. Fel d I content expressed in ng per m^2 floor area in 10 homes without cats and 10 schools. Lines designate results from paired sampling of carpeted and smooth floor rooms in same home or school. When similar floor types in homes and schools were compared, *Fel d* I content per m^2 was significantly higher in schools ($P = 0.002$; comparison between carpeted floors in schools and homes; $P = 0.003$; comparison between smooth floors in schools and homes). ● = carpeted room; ◑ = smooth floor room with scatter rug(s); ○ = smooth floor room.

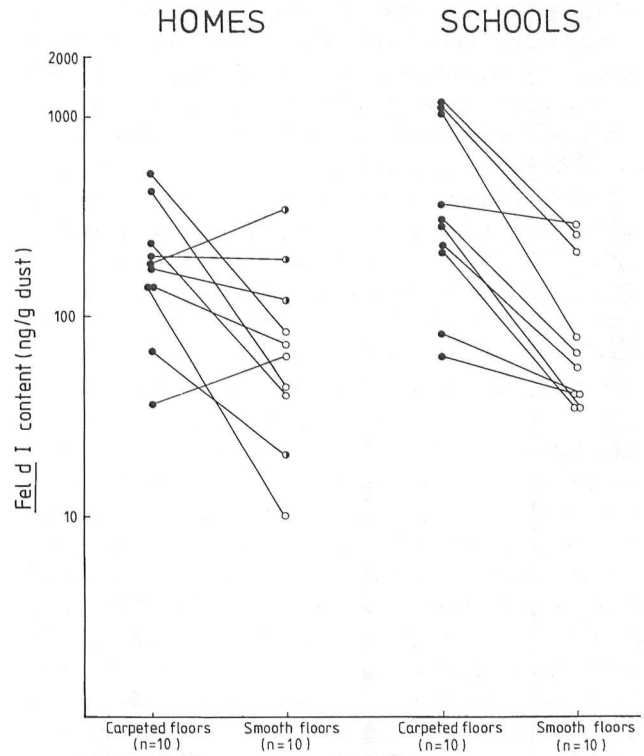

Fig. 2. Fel d I content expressed in ng per g of dust in 10 homes without cats and 10 schools. Lines designate results from paired sampling of carpeted and smooth floor rooms in same home or school. No significant difference was detected in allergen content per g of dust when similar floor types in homes and schools were compared. Symbols are described in Fig. 1.

The *Der f* I contents were not detectable in most dust samples, and the mean value for homes and schools was < 10 ng/m^2 (range < 1–72 ng/m^2). Likewise, the *Der f* I content per g of unsieved dust was almost unmeasurable, ranging from < 1 to 19 ng in homes and from < 1 to < 5 ng in schools.

Immunoblotting of SDS–PAGE-separated school dust components with a pool of sera from patients with clinical dog dander allergy showed clearly a band just above the 24-kDa mol. mass marker, as shown in Fig. 5. This is probably the major allergen of dog, *Can f* I, as the mol. mass at about 25 kDa corresponds to that described by Schou et al. (14). This band was detected in all dust samples from carpeted classrooms and homes with a dog. Furthermore, mite allergens could not be detected by these methods with a pool of sera from mite-allergic patients, confirming the low levels of mite allergens in the dust samples.

Mean weight increase of the main filters and microfilters after the sampling period in homes was

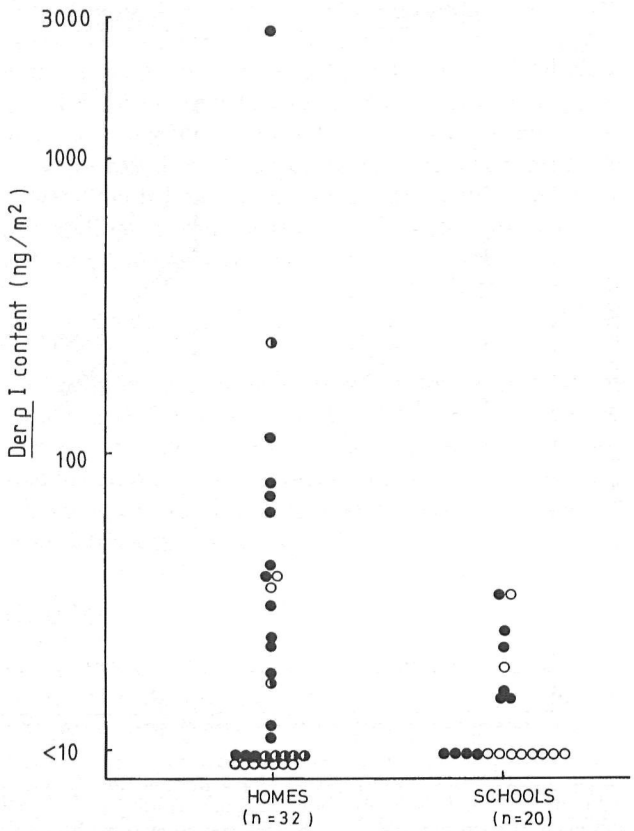

Fig. 3. *Der p* I content expressed in ng per m^2 floor area in 16 homes and 10 schools. One carpeted and one smooth floor room were sampled in each home and school. Difference in *Der p* I content between carpeted floors in homes and schools was statistically significant ($P = 0.05$). Symbols are described in Fig. 1.

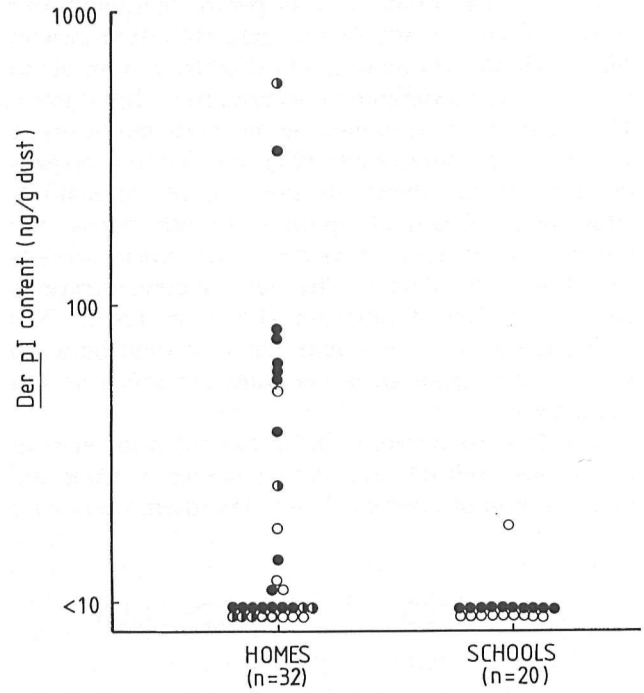

Fig. 4. *Der p* I content in ng per g of unsieved dust in 16 homes and 10 schools. In each home and school, one carpeted and one smooth floor room were sampled. The symbols are described in Fig. 1.

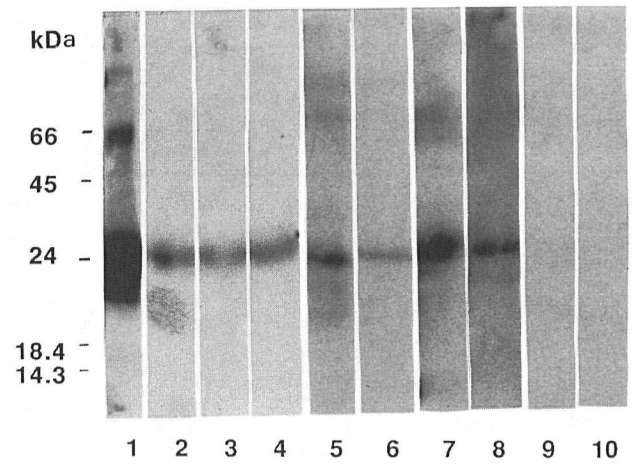

Fig. 5. SDS–PAGE and immunoblotting profile of floor dust samples for detection of major allergen from dog, *Can f* I, with patient sera containing dog-dander-specific IgE. 1: dog dander extract; 2–7: dust samples from classrooms with carpeted floors; 8: dust sample from home with dog; 9: dust sample from home without dog; 10: dust sample from classroom with smooth floor.

<10 mg and <1 mg per m^2 vacuumed floor area, respectively. The total *Fel d* I, *Der p* I, and *Der f* I concentrations in main filters and microfilters are summarized in Table 3. The highest *Fel d* I concentrations were detected in main filters and microfilters used during sampling of homes with cats and classrooms with carpeted floors.

Discussion

An important factor that correlated with the levels of dust, protein, and allergen prevalence was the presence or absence of carpeting. The samples from schools and homes were therefore divided into those from carpeted and smooth floor rooms for comparison. We have previously shown a higher allergen load per g of dust and per unit floor area from carpeted than bare floors in schools (4, 5). With the exception of food allergens, the same relation between floor type and allergen load was shown in homes in this investigation. Among the smooth floor rooms in homes were several dining rooms, and this is probably the reason that the smooth floor rooms showed approximately the same food allergen content as carpeted rooms. The vacuum cleaner model used in this investigation had a high vacuum power, and the reported allergen levels on the carpeted floors probably included allergens removed from the carpet dust reservoir in addition to those gathered during the day. The allergen levels on the smooth floors,

Table 3. Total *Fel d* I, *Der p* I, and *Der f* I concentrations in ng in main filters and microfilters after dust sampling in schools and homes. Arithmetic mean and range are given

	Main filter		Microfilter	
	Schools (n=20)	Homes* (n=5)†	Schools (n=20)	Homes* (n=5)†
Fel d I	<30 (<10–143)	<285 (<10–1080)	<4 (<1–14)	<6 (<1–20)
Der p I	<15	<15	<5	<5
Der f I	<10	<10	<5	<5

* Including homes with cats.
† Filters were not changed in the five vacuum cleaners used during dust sampling of 37 floors in homes.

on the contrary, were the amounts that were gathered during the 10-day sampling period.

The vacuumed material was coarser in schools than in homes, containing more sand and other solid particles. This was noted during the sample preparation and verified by the fact that the protein content per unit area was slightly higher in homes than in schools in spite of the high weight of vacuumed material per unit area in schools. The coarser dust in the classrooms probably resulted from the heavier traffic in schools, outdoor dirt being tracked in on children's shoes.

Keeping domestic pets is very common in Scandinavia. An epidemiologic study in Sweden showed that out of 38000 nonasthmatic children, 52% had indoor furry pets (6). Homes with pets were omitted from the comparison of cat and dog allergens in homes and schools since we wanted to compare the children's risk of exposure to pet allergens in the school environment with that in homes without pets. The higher contents of dog dander and cat allergen *Fel d* I in schools than in homes without pets can be explained by several children's contacts with pets at or near home, bringing animal hair and dander on their clothes, shoes, and bags into the classrooms. In several of the homes without cats in this investigation, either the families were in contact with cats in their grandparents' or neighbors' homes, or their homes were regularly visited by people keeping cats. Significant levels of cat allergens were therefore detected in these homes, although no cats lived there. The major allergen of dog, *Can f* I, was detected in all dust extracts from carpeted classrooms by SDS–PAGE and immunoblotting procedures, but not from all smooth floor classrooms and homes, verifying the higher dog allergen contents in the carpeted classrooms. Dog antigens and allergens in school dust extracts have also been detected by crossed immunoelectrophoresis and crossed radioimmunoelectrophoresis, respectively, in previous investigations (3, 4). The reservoir of cat and dog allergens in schools has implications for persons attempting to live in an environment relatively free of pet allergens. The time and effort spent in reducing allergen exposure at home could be negated by exposure at school. The high prevalence of animal proteins in the classrooms emphasizes the importance of frequent cleaning to lower the exposure of pupils to these allergens. Wall-to-wall carpets act as reservoirs for various allergens, and by avoiding these the allergen contents could be further reduced. In addition, if pupils and teachers who are in contact with pets at or near home used separate clothes and shoes in school, much of the animal allergen transfer into schools could be avoided.

D. pteronyssinus was more prevalent in homes and schools than *D. farinae*, which was virtually absent. Although the dust from carpeted rooms in homes contained more mite allergens than the school dust, the levels of *Der p* I and *Der f* I in the homes investigated seemed low in contrast to more heavily mite-infested areas in Europe (10, 15). The main reason for the low mite allergen levels in the houses investigated could be the low indoor humidity during the heating period in winter. Mite development is only possible at indoor absolute humidities above 7 g/kg, corresponding to a relative humidity of 45% at indoor temperatures of 20–22°C (7). The indoor relative humidity during the heating period in winter in Norway generally varies between 20 and 40%, which is too low to permit extensive mite growth. If the mites are dried out in winter, a few months of higher humidity during the summer is too short to permit their reestablishment (7, 17). The low mite allergen levels in the homes of nonatopic persons shown in this investigation agree with other investigations from Scandinavia (16, 19). Higher levels of house-dust mite antigens have been found in many homes of house-dust-mite-sensitized children (8, 10, 19), but, so far, these have not been investigated by us. Another reason for the low mite counts might be the sampling of floor dust instead of sofa and mattress dust, which has been demonstrated to contain more mite allergens than carpets (13).

The weight increase of the main filters and microfilters after sampling in homes agreed with a previous investigation in schools (5), showing that less than 1% of the total vacuumed material weight passed through the dust bags further into the filter systems during vacuuming. The *Der p* I and *Der f* I contents in the filters were under the detection limits for the ELISA tests used, merely reflecting the low mite allergen levels in schools and homes. The main filters used during sampling of homes with cats and two classrooms with carpeted floors contained more than 100 ng *Fel d* I. Bronchial provocation of cat-allergic patients requires 3–265 ng of *Fel d* I (assuming 1 unit *Fel d* I = 4 μg protein) to induce a

20% fall in forced expiratory flow volume in 1 s. (18). This emphasizes the importance of using vacuum cleaners with filter systems during cleaning of places with a high allergen load.

Acknowledgments

We wish to thank Ms Marit Dybendal for performing the dust sampling in homes in eastern Norway and Mr Frode Abrahamsen for assistance with preparation of dust extracts and the SDS–PAGE and immunoblotting procedures. This study was supported by the Royal Norwegian Council for Scientific and Industrial Research (NTNF).

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