

Comparison of four allergen-sampling methods in conventional and allergy prevention classrooms

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Summary

Background Several allergen-sampling methods are used to assess level of personal or indirect exposure to cat in homes, schools and other public buildings and working environments.

Objective To compare four different allergen-sampling methods (dust collectors, Petri dishes, person-carried pumps and intranasal samplers) by simultaneous sampling in classrooms and to compare the cat allergen levels between conventional classrooms and allergy prevention classrooms. Another aim was to relate the results to self-reported frequency of allergy and asthma symptoms among the children, to their perception of the school environment.

Methods Among all compulsory schools ($n = 257$) in the Stockholm suburban area, 35 classrooms (five with implemented allergy prevention measures, seven with additional cleaning and 23 with normal cleaning routines) were chosen for allergen-sampling. Dust collectors (two models), Petri dishes, person-carried pumps and intranasal samplers were used simultaneously. All children ($n = 829$) received a self-administered questionnaire which included questions about home and school environment, allergic disease, asthma symptoms and pet contact.

Results The correlation between sampling methods was generally poor. Furthermore, there was no significant difference in allergen levels between allergy prevention and allergen avoidance classes compared to conventional classes. Median levels were generally, but not significantly, lower in classes with few cat owners, compared to classes with many cat owners. Children in allergy prevention classes were more satisfied with the indoor air quality and cleaning than children attending classes with fewer or no allergy prevention measures ($P < 0.0001$). Nine per cent of all children reported allergic symptoms while at school.

Conclusion The lack of correlation between sampling methods used simultaneously demonstrates the difficulty in assessing allergen levels in schools and similar environments. The implemented intervention measures (allergy prevention/allergen avoidance) did not influence cat allergen levels at school.

Keywords air sampling, allergen, dust collector, ELISA, Fel d 1, intranasal sampling, Petri dish, prevention, sampling

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Introduction

A number of different sampling methods are used to assess levels of personal or indirect allergen exposure in homes, schools and other public buildings and working environments. To date, analysing allergen levels in reservoir dust from upholstery, mattresses and floors have been used most commonly and a number of sampling devices are available on the market for this purpose [1–3]. Previous comparative studies have suggested that reservoir allergen concentrations are poor predictors of airborne concentrations [4–6]. For allergens such as cat (Fel d 1) and dog (Can f 1), it has been suggested that airborne

sampling may be a better index of personal exposure due to the aerodynamic properties of the allergens [7]. Aeroallergen levels can be assessed by different methods. We have evaluated the Petri dish sampling method for collection of airborne settling dust, first introduced by Tovey et al. [8, 9]. This method can be used as an estimation of aeroallergen exposure. For assessment of personal aeroallergen exposure, person-carried pumps may be considered as the gold standard, and it is used especially in occupational exposure assessments for shift and task measurements. Another method to assess personal aeroallergen exposure using intranasal samplers was introduced a few years ago [10].

Pet allergens are often found in classrooms where no pets are allowed, as well as in pet-free homes. The allergens are indirectly transported to and deposited in the pet-free environments mainly via clothing [11, 12]. In a recent study it was shown that cat-sensitized asthmatic children had lower PEF and increased medication upon returning to school after the summer holidays.

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Worsening of asthma was found among children attending classes with many cat owners but not among those attending classes with few cat owners [13]. To fulfil the needs of children with asthmatic and allergic symptoms, some schools and day-care centres in Sweden have established allergen avoidance classrooms or, in some cases, allergy prevention sections in separate school buildings. A study by Wickman et al. showed that cat allergen levels were significantly lower in both airborne and reservoir dust collected in allergen avoidance day-care centres with pet ownership restrictions and other measures in order to reduce allergen levels, compared to conventional day-care centres [14].

Few studies have investigated the relationship between measured allergen levels at school and self-reported symptoms and perceived school environment among school children. Symptoms occurring every week and dissatisfaction with the air quality and cleaning were more common among allergic than non-allergic students [15]. The authors concluded that the awareness of these risk factors may lead to anxiety, which may even worsen the symptoms. In another study, Wålinder et al. showed that the amount of settled dust was positively related to self-reported nasal obstruction and reduced nasal patency among school personnel [16].

Our first aim of the study was to compare four different allergen-sampling methods (dust collectors, Petri dishes, person-carried pumps and intranasal samplers) by simultaneous sampling in classrooms with different rates of cat ownership. The second aim was to compare the collected allergen levels between conventional classrooms with normal cleaning routines and allergy reduction classrooms with more frequent cleaning. In addition, we were interested in relating the results to self-reported allergy and asthma symptoms among the children and their perception of the school environment.

Methods

Selection of classrooms

All compulsory schools (grade 1–6) situated in the Stockholm suburban area ($n = 257$ according to the phone directory) received a questionnaire in the spring of 1999 including questions about allergy prevention measures. Of the 202 answers (79% response rate), 38 (19%) schools had introduced some allergy prevention measures, mainly with more frequent cleaning in one or a few classrooms ('allergen avoidance'). Only two (1%) schools had a whole section with allergy prevention classrooms where a number of measures had been implemented for several years, for instance cleaning of classrooms twice a day, absence of open shelves, upholstery, curtains and plants and all school material stored in cupboards with doors. These school-building sections were originally established for children with severe asthma and allergic symptoms with the aim to create an acceptable working environment.

In order to assess the extent of the stated allergy prevention measures, these schools were contacted by telephone. All classrooms with implemented allergy prevention measures ($n = 5$) and a random selection of classrooms with additional cleaning ($n = 7$) were chosen for allergen-sampling. Conventional (control) classrooms ($n = 23$) were, for practical reasons, mainly selected among the schools above provided that the cleaning

procedures (2–3 times a week) and other features were comparable among these classes. There was no indication of a generally better cleaning standard in schools where allergy prevention/allergen avoidance classrooms had been established. In summary, 35 classrooms (children 7–12 years of age) situated in 12 different schools were included in the study for allergen-sampling. Median rate of cat ownership among all 35 classes was 19% (range 0–43%) and each class was visited on one occasion in April or May 1999 when all sampling was made.

Permission for this study was obtained from the Ethics Committee of the Karolinska Institute.

Allergen-sampling with person-carried pumps with filters

Air sampling took place during whole-class morning lessons. Three pumps (Airchek 52 with IOM sampling head, SKC Inc., Eighty Four, PA, USA) with 25-mm diameter polytetrafluoroethylene (PTFE) filters, 1 μ m pore size (Millipore AB, Sundbyberg, Sweden) were carried by three children (non-cat owners) in each class. The sampling head was attached in the breathing zone. Airflow rate was adjusted to 2 L/min and the total sampling time was approximately 180 min. In total, 105 samples were collected of which 18 were discarded due to pump flow rate failures ($> 10\%$ drop in flow rate). In addition, one or two adults (non-cat owners) in 29 classrooms carried a pump and an intranasal sampler (see intranasal sampling below) simultaneously for about 40 min ($n = 43$). This parallel sampling was, however, not possible in all cases due to lack of equipment. Air filters were stored at -20°C until extraction.

Filters were thawed and extracted in 2 mL phosphate-buffered saline (PBS) with 0.1% Tween-20 and 0.15% Kathon (Rohmand Haas, Hydrosupa Kemiservice, Helsingborg, Sweden; PBST_K) rotating overnight at room temperature. After removal of the filters, samples were stored at -20°C until analysis.

Allergen-sampling with intranasal samplers with adhesive tapes

The intranasal sampler was worn for 40 min by one or two adults in 29 classrooms ($n = 48$). As intranasal samplers suitable for children were not available at the time of the present study, intranasal sampling was performed in adults only. The intranasal sampler collects inhaled particles by impaction on an adhesive tape (Inhalix, Camperdown, NSW, Australia). The samplers were stored at $+4^{\circ}\text{C}$ until allergen extraction. Within a few days of sampling, the adhesive tape from the left nostril ($n = 47$, one sample was discarded) was eluted in 0.5 mL PBS with 0.5% Tween-20 and 0.15% Kathon during gentle rotation for 2 h at room temperature. After discarding the adhesive tape, the elute was transferred to another tube containing 5 mg bovine serum albumin (final concentration 1%). After rotation for 5 min, samples were stored at -20°C until analysis.

Allergen-sampling with Petri dishes

Two pre-treated [8, 9] Petri dishes were placed with base and lid facing upward 120 cm above the floor level on aluminium holders (in-house workshop, National Institute for Working Life, Stockholm, Sweden) attached to the wall in 34 classrooms. Sampling of airborne settling dust was made for 180 min during the morning classes. In total, 68 Petri dishes were used of which two were discarded due to tampering with the dish during

sampling. All Petri dishes were stored a few days up to a week at room temperature until extraction.

The lid and base of the Petri dish were rinsed seven times, respectively, with 2 mL PBST_K. The extract was then rotated overnight at room temperature and finally centrifuged at 1000 g for 10 min. Samples were stored at -20 °C until allergen analysis.

Allergen-sampling with dust collectors

Two different models of pre-weighed dust collectors were used: ALK Dustcollector with a 6-µm pore size filter (ALK Sverige AB, Kungsbacka, Sweden) and Dustscreen™ with a 15-µm pore size nylon mesh 'sleeve' (Medeca AB, Uppsala, Sweden). One square metre on the classroom floor was vacuumed using a 1000-W Volta Lite vacuum cleaner for 1 min with the ALK dust collector attached ($n = 35$). After changing to the Dustscreen dust collector, a square metre adjacent to the first was vacuumed for 1 min ($n = 19$). Large items like pieces of paper, stones and other objects were removed before vacuuming. This procedure was repeated on a square metre elsewhere in the classroom, with each previously used dust collector, respectively. The discrepancy in numbers between the two collectors was due to a limited number of Dustscreen dust collectors available from the supplier. Each dust collector was thus used for sampling of 2 m², vacuumed at total 2 min. The dust collectors were stored at -20 °C until allergen extraction.

The ALK dust collector was thawed and weighed and the filter was then transferred to a tube and 10 mL PBST_K was added. The tube was rotated for 2 h at room temperature and then centrifuged at 1000 g for 20 min. The supernatant was transferred to a new tube and centrifuged at 1000 g for 10 min. Samples were stored at -20 °C until analysis.

The Dustscreen dust collector was thawed and weighed and 5 mL PBST_K was added into the collector. It was then sealed and rotated for 2 h at room temperature. The extract was transferred to a tube and centrifuged at 1000 g for 10 min. Samples were stored at -20 °C until analysis.

In cases where more than one sample was taken per sampling method and class, the mean value between the samples was used in the calculations.

Analysis of cat allergen content with monoclonal antibody 2-site ELISA assays

All Petri dish and dust collector samples were analysed in a monoclonal sandwich enzyme-linked immunosorbent assay (ELISA) as presented previously [9]. Samples near or below the detection limit (0.156 ng/mL) and samples from intranasal adhesive tapes and pump filters were analysed in an amplified ELISA (using AMPAK signal amplification) according to the manufacturer's recommendations. The standard curve range in the amplified assay was 2.5–160 pg Fel d 1/mL. For statistical calculations, samples below this detection limit were assigned a value of half of the detection limit.

Rare samples with intra-assay coefficient of variation (CV) > 10% or interassay CV > 20% were re-assayed. The Fel d 1 mAb-kit including standard was purchased from Indoor Biotechnologies Ltd (Cardiff, UK).

Analysis of allergen-carrying particles on adhesive tapes

Detection of particles containing Fel d 1 was performed using the HALOgen assay according to the manufacturer's

recommendations (Inhalix, Camperdown, NSW, Australia). Briefly, the adhesive tape from the right nostril ($n = 48$) was laminated with a polyvinylidene difluoride (PVDF) protein-binding membrane (kindly provided by Dr Euan Tovey), and after a brief wetting in 80% methanol, the allergen was eluted onto the membrane overnight in borate buffer (0.2 M, pH 8.2). After elution, the borate buffer was removed and 5% skim milk (in PBS) was added and incubated for 60 min. After rinsing with PBS with 0.05% Tween (PBST), monoclonal antibody (mouse-anti Fel d 1) diluted in 2% skim milk/PBST was added and incubated for 2 h. After washing in PBST, alkaline phosphatase-conjugated anti-mouse IgG was added and finally membranes were stained with BCIP/NBT substrate kit according to the instructions (Bio-Rad Laboratories AB, Sundbyberg, Sweden).

Questionnaires

A questionnaire, which is frequently used to assess the school environment, was modified for this study [17]. All children in 35 classes ($n = 829$) received the self-administered (child together with parent) questionnaire which included questions about home environment, perception of the school environment, allergic disease, asthma symptoms, medication and pet contact. Most rating scales were ordinal.

Statistical methods

All data were analysed using Statview 5.0 software (SAS Institute Inc., Cary, NC, USA). Comparisons were made using the unpaired Mann-Whitney *U*-test and Spearman rank correlation test. The questionnaire answers from the three different groups of classes were compared and analysed with χ^2 -test. Sample distribution is presented as median and 25–75 percentiles. A *P*-value < 0.01 was regarded as significant when multiple correlations were calculated. Otherwise *P* < 0.05 was considered significant.

Results

Correlation among sampling methods

The correlation among sampling methods was generally poor, regardless of type of class (Table 1). The correlation (ρ) between the two dust collectors when expressed as pg Fel d 1/m² was 0.46 ($P = 0.051$, $n = 19$; data not shown).

Allergen was detected in all samples collected with dust collectors, Petri dishes and 180-min personal air sampling. Twenty-eight per cent of the 40-min personal air samples, 51% of the intranasal samples quantified with ELISA and 39% of the intranasal samples quantified with HALOgen visualization were below the detection limit.

Relationship between allergen levels and type of class

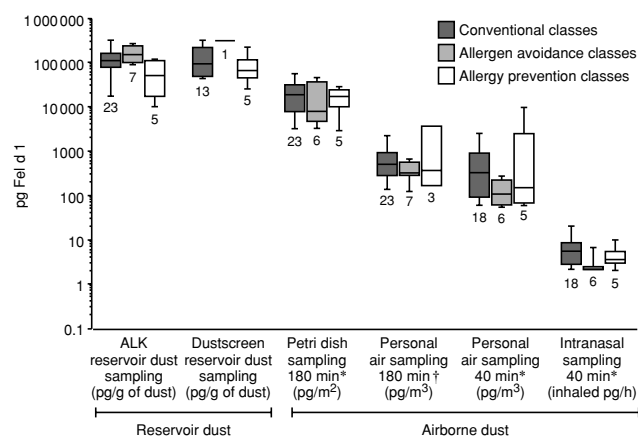
Regardless of sampling method, the levels of cat allergen and total amount of dust collected with the dust collectors did not differ significantly between the allergy prevention and allergen avoidance classes compared to the conventional classes (Fig. 1).

The median allergen levels were not significantly lower in classes with few cat owners ($\leq 19\%$), compared to classes with many cat owners ($> 19\%$), regardless of sampling method (with one exception; Fig. 2).

Table 1. Spearman rank correlation of analysed cat allergen levels sampled simultaneously in classrooms with dust collectors, person-carried pumps, Petri dishes and intranasal samplers

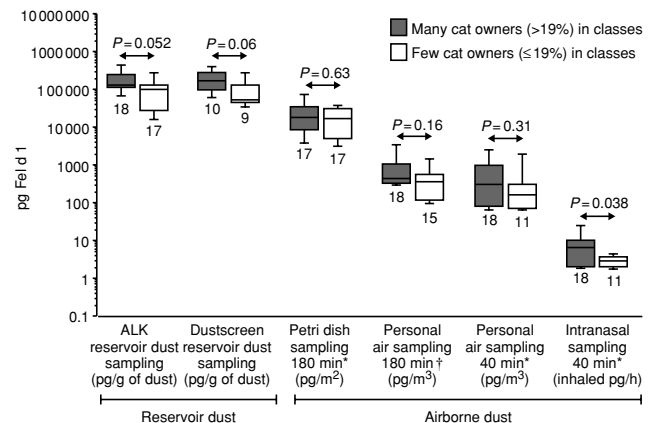
Type of sampling	Type of sampling				
	Reservoir dust	Petri dish	Personal air		Intranasal
Type of sampling	Dustscreen™	180 min*	180 min†	40 min*	40 min*
Reservoir dust	$\rho = 0.40$	$\rho = -0.049$	$\rho = 0.074$	$\rho = 0.18$	$\rho = 0.26$
ALK	$P = 0.092$	$P = 0.78$	$P = 0.68$	$P = 0.34$	$P = 0.17$
	$n = 19$	$n = 34$	$n = 33$	$n = 29$	$n = 29$
Dustscreen™		$\rho = 0.42$	$\rho = 0.047$	$\rho = 0.50$	$\rho = 0.44$
		$P = 0.075$	$P = 0.85$	$P = 0.046$	$P = 0.081$
		$n = 19$	$n = 17$	$n = 17$	$n = 17$
Petri dish 180 min*			$\rho = 0.036$	$\rho = 0.15$	$\rho = 0.28$
			$P = 0.84$	$P = 0.44$	$P = 0.14$
			$n = 32$	$n = 29$	$n = 28$
Personal air					
180 min†				$\rho = 0.30$	$\rho = 0.20$
				$P = 0.13$	$P = 0.31$
				$n = 27$	$n = 27$
40 min*					$\rho = 0.28$
					$P = 0.15$
					$n = 29$

Mean of two (*) and three (†) samples has been used in the calculations.

**Fig. 1.** Cat allergen levels sampled with dust collectors, person-carried pumps, Petri dishes and intranasal samplers, respectively, in conventional (normal, without allergy prevention measures), allergen avoidance (with more frequent cleaning) and allergy prevention classes (with a number of allergy prevention measures). Box plots with medians are shown in the figure where the box corresponds to the 25–75th percentiles and vertical lines correspond to the 10–90th percentiles, respectively. Number of samples for each sampling method and type of class is stated in figure. Mean of two (*) and three (†) samples has been used in the calculations.

Questionnaire answers and allergen levels

After three reminders, 723 children (87.2%) responded to the questionnaire or actively refused to participate in the study. In total, 698 (84.2%) completed questionnaires were analysed. Most children had answered the questions about perception of indoor air quality (96.7%) and cleaning (97.3%) in the classroom. There was a significant difference in degree of satisfaction among the three groups of classes. Children in allergy prevention classes were satisfied to a larger extent and dissatisfied to a lesser extent with the indoor air quality and cleaning than

**Fig. 2.** Cat allergen levels sampled with dust collectors, person-carried pumps, Petri dishes and intranasal samplers, respectively, in classes with many (> 19%) and few (< 19%) cat owners. Box plots with medians are shown in the figure where the box corresponds to the 25–75th percentiles and vertical lines correspond to the 10–90th percentiles, respectively. Number of samples for each sampling method and group is stated in the figure. Mean of two (*) and three (†) samples has been used in the calculations. Statistical comparisons were made using Mann–Whitney *U*-test.

children attending classes with fewer or no allergy prevention measures ($P < 0.0001$ for both). Asthma and allergy symptoms in the 12 months prior to the study were more common among children in allergy prevention classes than among children in the other types of classes (Table 2). According to the questionnaires, cat exposure induced nasal and eye symptoms in 4% (for both) and pollen exposure induced nasal symptoms in 10.9% and eye symptoms in 10.5% of the children. Sixty-two (9%) children reported having experienced allergic symptoms while at school and there was no difference among the three groups of classes. Many stated pet owners and/or dusty environment as probable causes of the symptoms (data not shown).

Table 2. Asthma and allergy symptoms in the last 12 months among the children in conventional (normal, without allergy prevention measures), allergen avoidance (with more frequent cleaning) and allergy prevention classes (with a number of allergy prevention measures), respectively

Type of class	Any symptoms in the last 12 months							
	Asthma/wheezing		Nasal*		Eyes†		Eczema	
	Yes	%	Yes	%	Yes	%	Yes	%
Conventional (<i>n</i> = 439)	34	7.7	62	14.1	51	11.6	79	18.0
Allergen avoidance (<i>n</i> = 144)	19	13.2	29	20.1	13	9.0	28	19.4
Allergy prevention (<i>n</i> = 115)	16	13.9	26	22.6	25	21.7	28	24.3

*Chi-square test, d.f. = 2, $P < 0.05$. †Chi-square test, d.f. = 2, $P < 0.01$.

The rate of children reporting dissatisfaction with indoor air quality (bad/very bad) correlated positively with allergen levels sampled (ng/g of dust) with both the ALK ($\rho = 0.46$, $P = 0.049$) and the Dustscreen ($\rho = 0.36$, $P = 0.034$) dust collector. Dissatisfaction with classroom cleaning correlated positively with levels sampled with the Dustscreen dust collector ($\rho = 0.66$, $P = 0.0048$). Airborne allergen levels were not related to perception of indoor air quality (data not shown).

Discussion

In the present study, we found poor correlations among different simultaneously used methods for sampling of cat allergen in classrooms and we were not able to demonstrate a difference in allergen levels between classes with and without allergy prevention measures.

Several studies have found a weak or nonexistent correlation between airborne and reservoir dust cat allergen levels [3–5]. Despite this, reservoir dust collection is frequently used for assessment of indirect personal allergen exposure of pet allergens in environments such as homes and public buildings. Cat allergen stays airborne for a longer time than other pet allergens and mite allergens [18] and levels found in reservoir dust may not be an appropriate index of airborne cat allergen levels. Reservoir dust sampling may, however, be a suitable method when evaluating the effect of improved cleaning of floors, as shown in this study, where a positive correlation was found between dissatisfaction with cleaning/indoor air quality and allergen in floor dust. As was shown in this study, there was a poor correlation between the two dust collectors. This could be explained by the uneven distribution of dust on the floor, which may complicate comparisons between dust sampling devices. However, this was based on a relatively small number of samples (one sample per model and class).

Personal sampling of airborne allergens on filters using person-carried pumps is well-established in occupational exposure assessment [19]. The sampling method is, however, time-consuming and personnel-intensive and can be disturbing during class hours. Person-carried pumps reflect the actual personal exposure because the sampling device is carried in the subject's breathing zone. However, the constant flow rate (2 L/min) is adjusted to collect the inhalable dust fraction using the IOM sampler [20] and does not reflect exposure when breathing is altered due to change of workload. It could, however, be argued that the minute ventilation does not change during the lessons when the school children are mostly sitting still.

By using intranasal samplers, personal exposure assessment is now possible without expensive equipment and great labour efforts. However, there is a limit as to the duration of intranasal sampling and hence low exposure levels may be difficult to detect. This was the case in our study in classrooms where about half of the 40-min intranasal samples were below the detection limit. Samples taken simultaneously with person-carried pumps were undetectable in only 28% (not significant) of the cases, suggesting that sampling with person-carried pumps is a more sensitive method and hence more suitable in this low-exposure environment.

There was no significant correlation among the sampling methods. This suggests that the results obtained when one sampling method is used at one occasion as an index of indirect exposure may not be representative for the 'true' allergen level. Because measured levels vary over time and within classrooms it is important to perform repeated measures within a certain time period or at least use duplicate samples, in order to obtain a good assessment of the allergen exposure. Although it would have been preferable to have more samples in the present study, duplicates were used in most cases in all 35 classrooms. In a previous study, we have found a significant correlation between Petri dishes and person-carried pumps [9]. The Petri dish sampling was, however, performed during 5 days and not, as in the present study, during 3 h. This suggests that a longer sampling time is required in this relatively low-exposure environment.

There was no significant difference in levels between allergy prevention classrooms and conventional classrooms, regardless of sampling method. However, there were few samples due to few available allergy prevention and allergen avoidance classes where samples could be collected. The rate of cat ownership among the allergy prevention classes did not differ from the rate among the conventional classes (18% compared to 21%). Children attending the allergy prevention classes were in fact allowed to keep furred pets at home but they were requested to avoid acquiring more pets while attending the class. Our results are in contrast with the results observed by Wickman et al. where levels were lower in allergen avoidance day-care centres. In that study, no furred pets were allowed among staff, children and their families [14]. Our results suggest that the studied allergen avoidance measures may not be sufficient to reduce airborne cat allergen levels as long as there are cat owners indirectly bringing allergen particles into the class. Cat allergen-associated particles stay airborne for a long time [18], which makes removal difficult. This may also explain the similar airborne allergen levels, regardless of lack of implementation

of allergen avoidance measures. We have previously found a fivefold difference in airborne cat allergen levels between classes with many and few cat owners [9, 11] and that asthmatic children in classes with many cat owners (> 18%) worsen their symptoms after returning to school after summer holidays [13]. This suggests that at least a fivefold decrease in allergen level would be required to achieve a clinically relevant effect in allergy prevention classes. No such difference was observed in the present study.

The children in allergy prevention classes were more content with indoor air quality and cleaning. However, there is an overrepresentation of allergic and asthmatic children with symptoms in the allergy prevention classes, which were established in order to fulfil the needs of symptomatic children in the first place. Reported nasal and eye symptoms following cat exposure were, apart from pollen, most common among the children. Although there was no significant difference in the present study, the number of cat owners is of importance, both regarding measured levels and as indirect exposure inducing symptoms among sensitized children, as recently shown [13]. Compared to most other non-industrial indoor environments, the person load and physical activity in classrooms are high, causing accumulation and frequent resuspension of settled dust. Smedje et al. showed that new onset of asthma and furry pet allergy was more common among children attending a school with more settled and respirable dust [21], suggesting that cleaning should at least be generally improved at school for the sake of the children. Although we did not find lower cat allergen levels in classes with more frequent cleaning, children were, however, more content with the school environment in these classes. According to an amendment in the Swedish Work Environment Act from 1990, school children are considered as employees and their working environment is as important as any other occupational environment.

In conclusion, the choice of sampling method depends on the question addressed and repeated measurements are important for a proper assessment of indirect personal allergen exposure. In this study, allergen levels were not significantly lower in classes with allergy prevention measures compared to conventional classes. We conclude that indirect transport of allergen on cat owners' clothes into the classrooms influences the cat allergen levels to such an extent that prevention measures may not be sufficient for a substantial allergen reduction.

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