

Mouse and cockroach allergens in the dust and air in northeastern United States inner-city public high schools

Abstract Considering that high school students spend a large proportion of their waking hours in the school environment, this could be an important location for exposure to indoor allergens. We have investigated the levels of mouse and cockroach allergens in the settled dust and air from 11 schools in a major northeastern US city. Settled dust samples were vacuumed from 87 classrooms, three times throughout the school year. Two separate air samples (flow = 2.5 lpm) were collected by 53 students over a 5-day period from both their school and their home. Mouse allergen (MUP) in the dust varied greatly between schools with geometric means ranging from 0.21 to 133 $\mu\text{g/g}$. Mouse allergen was detectable in 81% of the samples collected. Cockroach allergen (Bla g 2) ranged from below limit of detection ($<0.003 \mu\text{g/g}$) to 1.1 $\mu\text{g/g}$. Cockroach allergen was detected ($>0.003 \mu\text{g/g}$) in 71% of the dust samples. Bla g 2 was detected in 22% of airborne samples from the schools. By comparison, mouse allergen was only detected in 5%. These results indicate that the school may be an important location for exposure to allergens from mice and cockroaches and is an indoor environment that should be considered in an overall allergen intervention strategy.

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Practical Implications

To date, cockroach and mouse allergen intervention strategies have been mainly focused on the home environment. Considering that children spend a significant amount of time in schools, some studies have assessed cockroach allergen levels in schools. This study provides a clearer picture of the distribution and variability of not only cockroach allergen, but also mouse allergen in the school environment. In addition, this study describes limitations of personal air sampling in a student population. Our results suggest that although cockroach and mouse allergens are commonly recovered in classroom dust samples of inner city schools, cockroach allergens are recovered in the personal air samples with a greater frequency relative to mouse allergens.

Introduction

The prevalence of asthma in school age children in the USA has increased in the past several decades with a disproportionate frequency observed in the inner-city populations (Grant et al., 1999; Rosenstreich et al., 1997). The association between asthma exacerbations and exposure to allergens in the home environment has been well documented and the home has been a target for allergen avoidance interventions (Platts-Mills et al., 1997). Another location where children spend a great deal of time, the school, has also been investigated as a potentially important location for allergen exposure (Custovic et al., 1996; Dungy et al., 1986; Neuberger et al., 1991; Tortolero et al., 2002; Wickens et al.,

1997). The importance of the school in exposure to cat and dog allergen has been shown, with studies demonstrating that for many allergic children, the school represents a location of greater exposure than the home (Dotterud et al., 1997; Dybendal and Elsayed, 1992; Munir et al., 1993; Perzanowski et al., 1999). One study of Swedish school children found that cat allergic children returning after the summer break to classrooms with higher cat allergen experienced a decrease in peak expiratory flow, an increase in symptoms, and an increase in medication use (Almqvist et al., 2001).

In the urban northeastern USA, cockroach is one of the most important allergens for allergic asthma and exposure to these allergens in the homes is common

(Rosenstreich et al., 1997). Mouse allergen has also recently been reported as important and is also prevalent in inner-city homes (Chew et al., 2003; Phipatanakul et al., 2000a,b). Two previous studies have reported measurable cockroach allergen in schools in inner-city Baltimore (Amr et al., 2003; Sarpong et al., 1997). One of those studies also examined mouse allergen, but little was found (Amr et al., 2003). Both mouse and cockroach allergen exposure have been the target of asthma intervention studies conducted in the inner-city (Carter et al., 2001; Eggleston et al., 1999; Kinney et al., 2002; Phipatanakul et al., 2004). Therefore, it seems relevant to have a more comprehensive knowledge regarding mouse and cockroach allergen exposure in the school.

The purpose of the present study was to characterize mouse and cockroach allergen levels in dust and air in a group of high schools located in a major city in the northeastern USA. Within the context of the overall goal, we assessed seasonality of cockroach and mouse allergens in classroom dust and compared the airborne levels of these allergens in students' home and school environments. Because environmental sampling and allergen analyses require much time, effort, and cost, our final aim was to assess the predictive value of visual inspection for infestation relative to allergen concentrations measured in the dust.

Methods

Eleven public high schools were selected from a major metropolitan area in the northeastern USA (Table 1). The school buildings ranged in age, and many were

multi-story buildings located near major commercial zones (restaurants, grocery stores, subway stations). None of our schools had carpeting in the classrooms. Since the geometry and architecture of each building was different, in general, at least one classroom was picked from every floor (that had classrooms) and wing of the buildings. Dust samples from six to 10 classrooms were vacuumed from each school during the winter of 2002, and these same classrooms were vacuumed again in spring and fall of 2003. In each classroom, dust was collected onto 70 mm cellulose filters with a particle retention size $> 11 \mu\text{m}$ (Whatman International, Maidstone, UK) with a canister vacuum cleaner (Eureka Mighty Mite, Bloomington, IN, UK) and a modified collection nozzle (ALK, Horsholm, Denmark) for 3 min. Since the classrooms had chairs and desks arranged in an assortment of patterns, we chose to standardize our dust sampling as best possible by collecting dust from the edges of floor while avoiding vacuuming chalk dust. Samples were returned to the laboratory for post-weighing and then stored at -20°C . Dust samples were not sieved. Depending on the amount of dust, phosphate-buffered saline with 0.05% Tween 20 (PBS-T) was added in quantities sufficient to create a slurry. The exact extraction concentration was recorded. Next, the mixture was shaken at 200 rpm for 1 h at 30°C . Samples were centrifuged at 11 000 rpm (9875 g) for 1 min, and the supernatant was removed and frozen at -20°C until assayed. After extraction dust samples were assayed as previously described for Mouse Urinary Protein (MUP, Greer Laboratories Inc., Lenoir, NC) (Chew et al., 2003) and Bla g 2, a major allergen from the

Table 1 Description of schools visited and dust and air samples collected

School ID	Date of building	Building area (m ²)	School size (number of students) ^a	Total number of classrooms	Dust sampling			Air sampling (number of students that conducted sampling)	
					Number of classrooms sampled ^b	Floors/stories of classrooms sampled	Seasons of sampling	School	Home
1	1958	24 341	2000	104	8	1–5	Spring, fall	5	5
2	1967	26 384	3800	60	8	0–3	Winter, spring, fall	5	5
3	1939	24 341	3200	80	9	1–4	Winter, spring, fall	5	5
4	1941	22 854	4500	110	8	1–3	Winter, spring, fall	5	5
5	1931	23 412	175	14	7	1, 2	Winter, spring, fall	5	5
6	1972	38 369	3800	200	8	2–8	Winter, spring, fall	2	3 ^c
7	1937	24 155	525	40	6	1, 2	Winter, spring, fall	5	5
8	1904	13 564	1200	44	9	1–4	Spring, fall	4	5
9	1972	34 653	3700	200	9	0–3	Winter, spring, fall	5	5
10	1904	5203	300	60	7	2, 4, 5	Winter, spring, fall	5	5
11	1913	29 636	2800	100	8	2–9	Winter, spring, fall	5	5

^aApproximate numbers of students per school.

^bSome schools share space within a building and therefore the number of classrooms seems small compared with the reported area of the building. Also, not all schools utilize all classrooms.

^cFor three of the schools, fewer than five students successfully completed air sampling. Among the 104 filters from completed air sampling, four did not have a sample volume properly recorded (see Methods).

German cockroach which is the most commonly recovered cockroach in homes in our area (Indoor Biotechnologies, Charlottesville, VA, USA) (Pollart et al., 1991). For samples that were below the assay's limit of detection (0.4 ng/ml), Bla g 2 was assayed using an amplified ELISA in order to increase the sensitivity to 0.04 ng/ml (AmpliQ, Dako Cytomation, Ltd, Cambridgeshire, UK).

In each school, teachers were asked to select five responsible students who volunteered to collect personal samples of air at school and at home using a personal sampling pump (SKC Inc., Eighty Four, PA, USA) equipped with 25 mm PVC filters (Omega Specialty Instrument Company, Chelmsford, MA, USA) (Table 1). The pumps were set at a flow rate of 2.5 lpm and placed inside a foam-insulated pack that attached to the waist (for school sampling) or foam-insulated lunch bag (for home sampling) in order to dampen the noise. For the school sampling, students were trained to operate the pump and wear it during school hours for a period of 5 days. For home sampling, students were instructed to place the pump at their headboard, turn it on before they went to sleep and turn it off in the morning for a period of 5 days. Air samples were collected once during the heating season (i.e. winter or spring). Samples were extracted as described above and assayed for Mus m 1 (Indoor Biotechnologies, Charlottesville, VA, USA) and Bla g 2. Bla g 2 was assayed using an amplified ELISA to increase the sensitivity. Because of the anticipated low levels of mouse allergen in the air, the samples were analyzed for Mus m1 which can be measured with greater sensitivity than that of MUP. Mus m 1 is a component of MUP and the relationship between the assays has been described in previous literature (Chew et al., 2003). In addition, we re-analyzed 238 dust samples for Mus m 1 and the correlation coefficient between Mus m 1 and MUP was 0.94 ($P < 0.01$). The median ratio of Mus m 1 to MUP was 0.35, and this was consistent through a range of MUP values (0.04–1100 $\mu\text{g/g}$).

For four of the air samples (two from home and two from school), the volume of air sampled could not be determined because the subject wearing the pump had re-programmed the pump. Of these four, one was positive for cockroach (>0.06 ng/filter) and none were positive for mouse (>0.16 ng/filter). Results from the remaining 100 samples are described in Table 2. For five of the air samples, the volume of air sampled was determined using a diary of how long the pump was reported in use and a pre- and post-calibration of the flow rate.

Statistics

Allergen concentrations were logarithmically transformed for all analyses. Values below the limit of

Table 2 Airborne mouse and cockroach allergen in schools and homes of school children

	School samples		Home samples	
	% Positive ^a	Range of positives (ng/m ³)	% Positive	Range of positives (ng/m ³)
Mouse (Mus m 1)	5 (2/43) ^b	0.09–0.25	6 (3/51)	0.10–0.15
Cockroach (Bla g 2)	22 (11/49)	0.058–3.8	6 (3/51)	0.11–0.13

^aThe lower limit of detection was 0.089 ng/m³ for Mus m 1 and 0.052 ng/m³ for Bla g 2.

^bSix of the mouse allergen school samples are not represented in this table because of a high limit of detection (driven by a lower air sampling volume). The limits of detection for these samples were 0.098, 0.10, 0.11, 0.12, 0.19 and 0.34 ng/m³.

detection were assigned the limit of detection (Gilbert, 1987). Geometric means (GM) are reported with 95% confidence intervals (CI). Paired *t*-tests were used to compare mean allergen levels in different seasons.

Results

Mouse allergen (MUP) in the dust from the school samples ranged from below limit of detection (<0.2 $\mu\text{g/g}$) to 1125 $\mu\text{g/g}$ (Figure 1). Mouse allergen varied greatly between schools with geometric means ranging from 0.21 to 133 $\mu\text{g/g}$. Mouse allergen was detectable in 81% of the samples collected. Over the three sampling times, mouse allergen was detected at least once in 89% of the classrooms. High mouse allergen (≥ 2 $\mu\text{g/g}$) was measured in 78% of the classrooms on at least one occasion (one of the three seasons). Cockroach allergen was also common and ranged from below limit of detection (<0.003 $\mu\text{g/g}$) to 1.1 $\mu\text{g/g}$ (Figure 2). Cockroach allergen was detected (≥ 0.003 $\mu\text{g/g}$) in 71% of the samples and 95% of the classrooms on at least one occasion.

The season in which dust sampling occurred seemed to be a factor for the level of mouse and cockroach allergen. Mouse allergen was significantly higher in the samples collected in the spring [GM = 5.3 $\mu\text{g/g}$, 95% CI (3.3–9.3)] as compared with the fall [2.7 $\mu\text{g/g}$ (1.8–4.1), $P = 0.008$, paired *t*-test]. The samples from the winter [4.0 $\mu\text{g/g}$ (2.2–7.3)] were also higher than those from the fall, but this difference was not significant ($P = 0.064$). Conversely, cockroach allergen was significantly lower in spring compared with fall ($P = 0.001$) or winter ($P = 0.022$). The mean cockroach allergen levels for the fall, winter, and spring were GM = 0.014 $\mu\text{g/g}$, 95% CI (0.011–0.018), 0.011 $\mu\text{g/g}$ (0.009–0.0114), and 0.008 $\mu\text{g/g}$ (0.006–0.010), respectively.

Of the building characteristics listed in Table 1, none were associated with cockroach allergen. However, mouse allergen levels were lower in schools with a larger number of classrooms ($r = -0.40$, $P < 0.001$), but not necessarily greater square footage. We did not sample dust in the cafeterias or kitchens; however, these were located on the ground floors in each school.

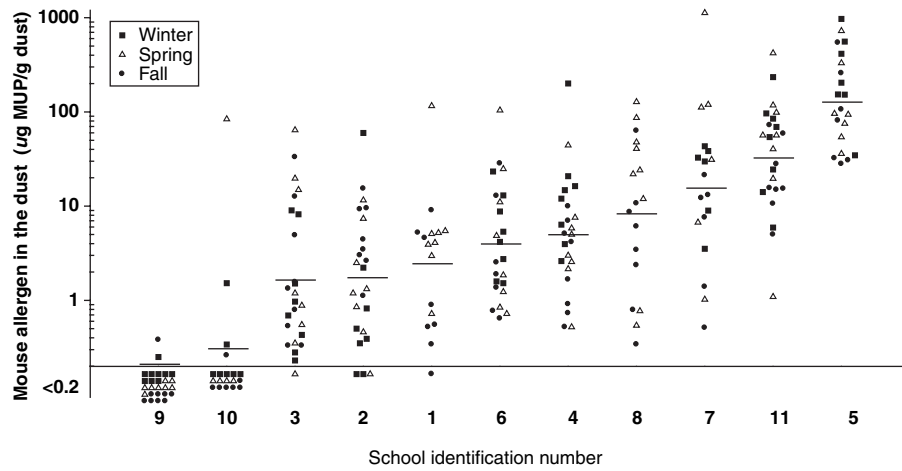


Fig. 1 Concentration of mouse allergen (MUP) in the dust from school classrooms with bars representing geometric means of allergen concentrations. Schools are ranked from left to right in order of geometric means. Each point represents one classroom visit with symbols indicating the season of the dust collection

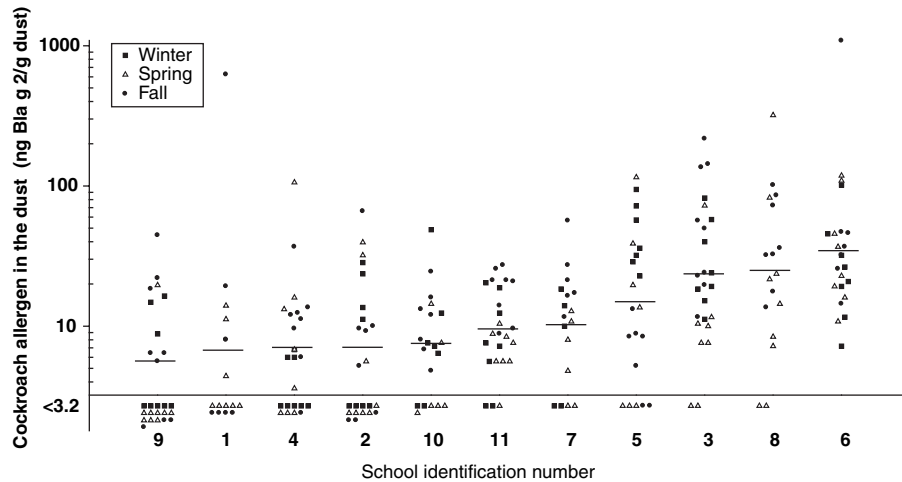


Fig. 2 Concentrations of cockroach allergen (Bla g 2) in the dust from school classrooms with bars representing geometric means of allergen concentrations

We evaluated differences in allergens between lower and upper floor/story as a proxy for distance from the cafeteria/kitchen. There also appeared to be no association between the floor/story of the classroom and either mouse or cockroach allergens. There were not significant differences in the mean levels or number of positives either when the floor was examined as the floor level above the street or when the floors were ranked relative to the other floors in the building (i.e. the lowest floor was 1, next 2, etc.).

The field technicians' assessment of visual evidence of mice was moderately predictive of detectable mouse allergen with a positive predictive value of 0.86, but had a poor negative predictive value of 0.21. The concentration of MUP was significantly higher ($P < 0.001$, t -test of ln-transformed values) for those classrooms with visible mouse infestation [GM =

8.6 $\mu\text{g/g}$, 95% CI (5.1–14)] as compared with those without [2.3 $\mu\text{g/g}$ (1.7–3.2)]. Similarly, sightings of cockroaches were predictive of detectable cockroach allergen with a positive predictive value of 0.71, but also had a poor negative predictive value of 0.29. Visual evidence of cockroach allergen was not associated with having detectable cockroach allergen in the classroom; however, few of the classrooms had evidence of cockroaches.

Overall, 14 airborne samples were positive for Bla g 2, and 5 were positive for Mus m 1. A majority of the positive cockroach allergen samples came from the schools, while mouse positive samples were equally uncommon in both school and home environments (Table 2). There was no overlap in who had measurable cockroach allergen in the air from the home and those who had cockroach allergen in the air from the

school. Also there was no overlap in those students who had measurable mouse allergen in the air from the home and those that had mouse allergen in the air from the school. Two of the air samples were positive for cockroach allergen from each of schools 1, 2, 4 and 8, and one of the air samples was positive from each of schools 5, 6, and 7. The two positive airborne mouse allergen filters came from schools 4 and 11.

Discussion

In 2000, The National Inner City Cooperative Study (NCICAS) found that more asthmatic children were allergic to mice (assessed by skin prick test) when mouse allergen levels (Mus m 1) in house dust were greater than 1.6 $\mu\text{g/g}$ (Phipatanakul et al., 2000a,b). In a study of inner-city homes in New York City, we reported that the median mouse allergen (MUP) level in kitchens was 2 $\mu\text{g/g}$ (Chew et al., 2003). In the current study, 10 of the 11 schools had at least one classroom above this level during one of the sampling periods. Moreover, we observed that 78% of the classrooms contained MUP $\geq 2 \mu\text{g/g}$ at some point during the school year. Given that students spend 7 or more hours each day in school, this environment may represent an important location for exposure to mouse allergen for these children.

It is unlikely that exposure to mouse allergens in the schools is as high as that in an occupational setting. Although, given the levels of pest allergen in the dust, disturbance of the dust by sweeping might represent a relevant occupational exposure. A recent study by Korpi et al. (2004) found a range of Mus m 1 allergen in the air from rooms with laboratory mice (disturbed and undisturbed air) of 1–545 ng/m^3 . In a study of animal handling facilities in the Netherlands, the range of mouse allergen in the air of rooms with mice and changing rooms for the workers was 0.63–2000 ng/m^3 (Hollander et al., 1997). None of the airborne samples from the schools or homes in this study contained as much mouse allergen (Mus m 1) as was found in these occupational settings. However the evidence of the NCICAS asthma study points to important non-occupational settings for mouse allergen exposure, since it is unlikely that those mouse-sensitized children were exposed to mouse allergen in an occupational setting (Phipatanakul et al., 2000a,b).

Several investigators have seen an association between asthma and sensitization to another common urban pest, the cockroach (Call et al., 1992; Gelber et al., 1993; Rosenstreich et al., 1997). High exposure (Bla g 1 $> 8 \text{ U/g}$) among sensitized children has been reported as a risk factor for asthma medication use and number of asthma-related hospitalizations (Rosenstreich et al., 1997). Exposure to cockroach allergen in schools was previously demonstrated in a study by Sarpong et al. (1997) where a majority (69%) of dust

samples from classrooms from four schools in Baltimore had detectable Bla g 1 ($> 0.8 \text{ U/g}$). A second study from 12 Baltimore elementary schools found detectable cockroach allergen (sum of Bla g 1 and Bla g 2 $> 1.4 \text{ U/g}$) in 66% of the 120 samples collected (Amr et al., 2003). Similar to these findings, 71% of the 232 samples from the current study were detectable ($> 0.003 \mu\text{g/g}$). Some caution must be taken in comparing these studies, since the limits of detection are not directly comparable. For Bla g 2, 0.003 $\mu\text{g/g}$ corresponds to 0.08 U/g . Also, our measurement of Bla g 2 as marker of cockroach allergen rather than Bla g 1, which is cross-reactive with Per a 1 (from *Periplaneta americana*), might have failed to pick up any American cockroaches that were in the school environment.

The relative importance of cockroach allergen exposure in the school as compared with the home environment is unknown. In a study of New York inner-city homes, we found a median Bla g 2 level of 0.02 $\mu\text{g/g}$ in bedrooms (Chew et al., 2003). Only 29% of the classroom samples from our study were above this level. Among the 49 air samples collected in these schools, 22% had measurable Bla g 2, as compared with only 6% of those from homes of the same students. Interestingly, the students with measurable airborne allergens in the homes were not the same students with measurable allergen on the school filters. A possible explanation for the low level of airborne cockroach allergen in homes relative to schools could be a selection bias of students; perhaps the students who volunteered for our study knew that they did not have cockroaches at home. Collection of a floor dust sample could have confirmed this hypothesis, but we were mainly focused on comparing the breathing zone levels in homes vs. schools. A potential limitation with comparing air and dust samples is that students wore the personal samplers throughout the building including cafeterias, hallways, their lockers, and other locations. At this time, the air measurement in schools is difficult to interpret because there are no comparable studies. Cockroach allergen was detected in almost a quarter of the student's air samples from schools which warrants further investigation.

The predictive value of visual assessment of infestation was also evaluated in this study. Repeated allergen measurements for schools may not be feasible; therefore, visual assessment would be more practical. Visual inspection was most useful in discerning high mouse allergen ($\geq 2 \mu\text{g/g}$) from moderate to low levels ($< 2 \mu\text{g/g}$). Given visual evidence of mouse infestation, 70% of the samples had $\geq 2 \mu\text{g/g}$. As a cautionary note, 80% of the samples without visual evidence still had detectable levels of mouse allergen ($\geq 0.2 \mu\text{g/g}$). For cockroach allergen the positive predictive value was moderate, but only 3% of the classrooms had visual evidence of cockroach infestation.

Our study design did not permit a thorough examination of the underlying characteristics of the schools that are conducive to high levels of mouse and cockroach allergens. Although we found that the larger schools in our study tended to have lower levels of mouse allergen, we are cautious with interpretation given that the sample was limited to 11 schools. The published literature has a detailed list of home characteristics that are associated with high mouse and cockroach allergens in residential environments (Chew et al., 2003; Phipatanakul et al., 2000a,b; Rauh et al., 2002). For example, kitchens tend to have higher levels of these allergens than bedrooms, and this is most likely because of proximity of food and water sources. We used a comparison of the upper floors of the school to the lower floors as a proxy for distance to the kitchen/cafeteria, but did not detect a significant association between this proxy and allergen levels. We speculate that the ubiquitous eating of snacks by students in the classrooms led to distribution of the pests throughout the school building.

While we did observe seasonal variation in mouse and cockroach allergen levels in the schools, we cannot confirm whether or not this is a true seasonal pattern or if it reflects variation in repeated sampling. A limitation of our study was that we were not able to obtain documentation of when pesticides were applied to the schools. The school district administration has a policy that no spray pesticides are to be applied. Adequate pesticide application records were not available for confirmation. As a result, pesticide application could have contributed to the within-classroom variation that was observed in our study.

Previous studies have demonstrated the importance of the school as an environment where allergen exposure can lead to adverse respiratory effects (Almqvist et al., 2001; Dotterud et al., 1997; Dybendal and Elsayed, 1992; Munir et al., 1993; Perzanowski et al., 1999). In the dust samples from our schools, mouse and cockroach allergens were prevalent. Airborne mouse allergen was detectable, but rare, whereas, airborne cockroach allergen was more common. While our results might not be generalizable to other schools (because of different building types, furnishings, or methods of cleaning), for the buildings in this study, the school appeared to represent an important location for these allergens from pests. Recent emphasis has been placed upon allergen intervention in homes (Carter et al., 2001; Eggleston et al., 1999; Kinney et al., 2002; Krieger et al., 2002; Phipatanakul et al., 2004), but our data show that mouse and cockroach allergens are found in schools and should also be considered in interventions.

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