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Use of a robotic sampling platform to assess young children's exposure to indoor bioaerosols

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Abstract

Indoor exposures to allergens, mold spores and endotoxin have been suggested as etiological agents of asthma; therefore, accurate determination of those exposures, especially in young children (6–36 months), is important for understanding the development of asthma. Since use of personal sampling equipment in this population is difficult, and in children < 1 year of age impossible, we developed a personal sampling surrogate: the Pretoddler Inhalable Particulate Environmental Robotic sampler (PIPER) to better estimate their exposures. During sampling, PIPER simulates the activity patterns, speed of motion and the height of the breathing zones of young children, and mechanically resuspends the deposited dust just as a young child does during running and crawling. The concentrations of allergens, mold spores and endotoxin measured by PIPER were compared to those measured using traditional stationary air sampling in 75 homes in central New Jersey, US.

Endotoxin was detected in all homes with median concentrations of 1.0 and 0.55 EU/m³ for PIPER and stationary sampler, respectively. The difference in median concentrations obtained using the two methods was statistically significant for homes with carpeted floors ($p = 0.0001$) in the heating season. For such homes, the average ratio of endotoxin concentration measured by PIPER and the stationary sampler was 2.96 (95% CI 2.29–3.63).

Fungal spores were detected in all homes, with median fungal concentrations of 316 and 380 spores/m³ for PIPER and stationary sampler, respectively. For fungi, the difference between the two sampling methods was not statistically significant. For both sampling methods, the total airborne mold levels were statistically significantly higher in the non-heating season than in the heating season. Allergens were detected in ~15% of investigated homes.

The data indicate that the traditional stationary air sampling methods may substantially underestimate personal exposures to endotoxin, especially due to resuspension of dust from carpeted floor surfaces. A personal sampling surrogate, such as PIPER, is a feasible approach to

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Practical implications. This study investigated potential indoor bioaerosol exposure of young children using a Pretoddler Inhalable Particulate Environmental Robotic sampler (PIPER) platform. The results show that the traditional stationary air sampling methods can substantially underestimate personal exposures to resuspended material, and that a personal sampling surrogate, such as PIPER, offers a feasible surrogate for measuring personal inhalation exposures of young children.

estimate personal exposures in young children. PIPER should be seriously considered as the sampling platform for future exposure studies in young children.

Keywords

Children's exposures; Robotic sampling platform; PIPER; Bioaerosol; Resuspension; Floor type

Introduction

The prevalence of asthma in the U.S. and the associated morbidity, mortality, and economic burden has increased sharply over the past 40 years, particularly in children (Braman, 2006). The most recent estimate of lifetime asthma prevalence in adults and children in the U.S. indicated it to be between 13% and 14%, respectively (USEPA, 2011).

Over the last 10 years evidence has been increasing that asthma is not just triggered by allergens and pollutants, but that they may also play a significant role in the development of asthma in early childhood (Brussee et al., Pearce et al., 2000; 2005; Selgrade et al., 2006; Yeatts et al., 2006; Zeldin et al., 2006). The suspected agents in the etiology of asthma include house dust, mold, allergens, endotoxins, second hand smoke, plasticizers and pesticides (Delfino et al., 2004; Douwes and Pearce, 2003; Pino et al., 2004; Larsson et al., 2010). Indoor exposures to these agents are of particular concern in Westernized countries where people spend as much as 90% of their time indoors (Klepeis, et al., 2001). The indoor exposure levels of contaminants are thought to have increased with the modernization of housing design, including higher indoor temperatures, extensive use of furnishings, improved insulation and low ventilation rates (Platts-Mills, 1994; Platts-Mills et al., 1997).

One important factor necessary for better understanding the role of allergens and pollutants in the etiology of asthma is the accurate characterization of airborne exposures to those pollutants, especially in children in their first years of life (Selgrade et al., 2006). Toddlers and young children spend much of their time playing on the floor where their actions can resuspend dust, allergens, mold etc, to which they can then be exposed via inhalation (Cohen Hubal et al., 2000; Liroy et al., 2002; Ferro et al., 2004; Gomes et al., 2007; Qian and Ferro, 2008; Raja et al., 2010; Zhang et al., 2008).

However, the accurate exposure characterization of toddlers and young children is especially challenging. Currently, it is suggested that indoor PM levels be measured using stationary air samplers at a height of 110 cm (U.S. EPA, 2003). This height is supposed to represent the breathing zone of an average adult, but is much higher than that of children playing on the floor. In addition, the use of area sampling is not an accurate representation of personal exposures (Rodes and Wiener, 2001). Also, human activities can resuspend house dust, and the resuspended dust has been identified as a cause for the “personal dust cloud” effect, i.e., an observation that personal exposures are often higher than indoor concentrations determined by area sampling (Özkaynak et al., 1996), which could also be visually described as the “pig-pen effect” in the Shultz comic strip Peanuts (Liroy, 2006). Resuspension of floor-deposited dust would especially elevate exposures of children due to the proximity of their breathing zone to the floor. Ferro et al. (2004) examined a wide variety of indoor human activities, such as walking, folding of clothes and blankets, vacuuming, and making of bed and the relationship between these activities and dust resuspension. They observed that as indoor activities increased, human exposure to PM increased as well and contributed to the “personal dust cloud.” According to their research, vigorous dancing on a rug disturbed deposited dust and produced the highest exposure to PM₁₀. Qian and Ferro (2008) investigated particle resuspension from human activities in a

full-scale experimental chamber. They found that “heavy and fast” walking was associated with higher resuspension rates than less active walking. They also found that given the same size and mass distribution of test particles per unit floor area, resuspension rates of larger particles (1.0–10 μm) from the new level-loop carpet were significantly higher than those from the vinyl tile flooring.

The traditional method of exposure assessment with personal samplers and pumps cannot be used with children in most cases, especially of young age, due to the size and weight of the equipment. In addition, compliance of a young child with the sampling protocol in this particular population could be a substantial issue. Thus, in order to better understand personal exposures of young children, especially when it comes to their exposures to airborne etiological agents of asthma, different exposure characterization tools are required. One possibility is the use of robotic samplers as personal air sampling surrogates that can simulate the behavior and breathing zone height of young children during their floor activities.

The development of a personal sampling surrogate, the Pretoddler Inhalable Particulate Environmental Robotic (PIPER) sampler has been ongoing since 2006 (Shalat et al., 2007) and the latest version of PIPER, the Mk IV, represents a significant development of this concept and is described in detail by Shalat et al. (2011). Briefly, PIPER Mk IV is completely autonomous and is capable of maneuvering in a typical home setting without an operator's intervention. It can carry up to two personal air sampling devices and can mimic the speed and pattern of motion as well as breathing height of boys and girls in three age groups: 6 months–1 year, 1–2 years and 2–3 years.

PIPER has been used to examine the role of near floor activity on the resuspension of particulate matter (PM) and potential exposure of young children (Shalat et al., 2011). To compare PIPER sampling to traditional stationary monitoring, the concentration ratios (PIPER PM concentration/stationary PM concentration), or relative differences (RD), were calculated for both bare surfaces and carpeted floors for each home. Using this method, inhalable PM concentrations measured by PIPER were 2–3 times higher on carpeted floors than those measured by stationary sampling. In this paper, we extend our investigation to exposures to airborne allergens, mold and endotoxin in indoor environments in central New Jersey, US, employing the PIPER robotic sampling system..

Materials and Methods

Characteristics of the robotic sampler

The main technical characteristics of PIPER have been described elsewhere (Shalat et al., 2011). Briefly, PIPER is a four-wheeled robot with a variable height air sampling tower (Figure 1). The sampling system consists of PIPER and a laptop computer that the operator uses to program and monitor the robot. Room air can be sampled at any height from 20 to 100 cm by using various sampling heads mounted on PIPER's sampling tower. A variety of aerosol and bioaerosol samplers, such as Button Aerosol Sampler (SKC Inc., Eighty Four, PA), PEM sampler (SKC Inc.), Air-O-Cell cassette (Zefon International, Inc., Ocala, FL) and others, can be installed on the sampling tower by using snap-on attachments that allow quickly changing samplers for evaluating particles of different types and size fractions. The control software program that allows selecting one of the robot's six activity profiles which mimic the speed and pattern of motion of boys and girls of different age groups.

PIPER is also equipped with on-board sensors and programmed to effectively avoid obstacles. Areas to be sampled can be delimited by infrared emitters that create virtual walls

which PIPER can detect. This feature allows PIPER to move autonomously in the room selected for sampling.

Recruitment of Participants

Participation in the study was voluntary and the participating families were recruited through two pediatric clinics in central New Jersey and through a local health fair. The study was approved by the UMDNJ-IRB (IRB Protocol 0220070004) and consents were obtained from all participants. Eligible participants had at least one child between 3 and 59 months of age. If participants had more than one child in this age range, the youngest child was selected for the study. Participants were asked to do no special cleaning prior to sampling. On the day of sampling, parents were asked to complete a short questionnaire about their child's health and about their household, including indicating which room was the child's main play area.

Indoor Bioaerosol Sampling Procedure

PIPER and the stationary monitor were equipped with identical samplers, sampling lines and pumps. The airborne PM was collected using Button Aerosol Samplers (SKC Inc.), which collect inhalable particles ($< 100 \mu\text{m}$ in aerodynamic diameter). The Button Aerosol Sampler is a lightweight sampling device featuring porous curved inlet and a 25-mm filter placed directly behind the inlet to avoid transmission losses in the sampler (Aizenberg et al., 2000; Hauck et al., 1997; Yao and Mainelis, 2007). Teflon (PTFE) filters (Pall Corporation, Ann Arbor, MI) with $3.0 \mu\text{m}$ pore size were used. In order to collect higher PM mass within the limited time, the Button Aerosol Samplers on both PIPER and stationary sampler were operated at a flow rate of 10 L/min provided by a Leland Legacy Pump (SKC Inc.). This sampling flow rate was also used in the study of the prototype Button Sampler (Hauck et al., 1997) and the authors concluded that the higher sampling flow rate does not negatively affect sampler's performance.

The filters were pre-weighed and post-weighed with a calibrated microbalance (MX-5, Mettler-Toledo, Inc., Columbus, OH) and their mass difference was recorded. Before weighing, filters equilibrated for 72 hours at 21°C and 30–40% relative humidity. The filters were then sent to an external accredited laboratory (EMLab P&K, Cherry Hill, NJ) for analysis of collected endotoxin and the following allergens: Cat Allergens (Fel d 1), Dog Allergens (Can f 1), Cockroach Allergens (Bla g 1), Dust Mite Allergens (Der p 1, Der f 1). The detection limits of the allergen assay were 3.3 ng/m^3 for Der p1, Der f1 and Can f1, 0.026 ng/m^3 for Bla g1 and 5.2 ng/m^3 for Fel d1. The results were reported as ng/m^3 . The airborne mold spores were collected with Air-O-Cell sampling cassettes (Zefon International, Inc.) and Leland Legacy pumps (SKC Inc.) operated at 15 L/min. This type of cassette is a widely used spore trap (Trunov et al., 2001) that collects airborne particles onto an adhesive-covered glass slide. They are simple to use and cost effective samplers for fungi. Based on our earlier investigations, we chose 20 min sampling because we found that longer collection times would overload the samples. The flow rates of both sampling pumps were set using a mass flow meter (model 4000, TSI Inc., Shoreview, MN) and were measured before and after sampling. An average of the two measurements was used to determine the sampled air volume. After the sampling, all Air-O-Cell cassettes were sealed in accordance to the manufacturer's instructions, placed in Ziploc storage bags and shipped to an accredited laboratory (EMSL Analytical Inc., Westmont, NJ) for analysis. EMSL Method 05-TP-003 with analytical sensitivity of 11 spores/m^3 under $600\times$ microscope magnification was used for sample analysis.

In each participating home, sampling was performed in the room in which the child spent most of his/her playtime. Once the room was selected, both the stationary monitor and

PIPER were set up. In order to maximize the floor surface for sampling, small objects and furniture (toys, ottomans, and children's chairs) were removed from the area. The stationary monitor was located at least 0.5 m from the nearest wall and the inlet was placed at a sampling height of 110 cm (U.S. EPA, 2003). The stationary monitor consisted of one mold spore sampler and two PM samplers (one for endotoxin and one for allergen). PIPER was initially equipped with one spore sampler and then manually placed in the middle of the room. The flooring type, temperature and humidity of the room were noted and recorded. Field blanks comprised ~15% of all samples.

Air sampling took approximately 2 hours in each home. During the first 30 minutes after set-up, PIPER remained motionless and no active samples were collected by either PIPER or the stationary sampler. This delay allowed larger dust particles that were potentially disturbed during the setup of the measurement to settle down. After 30 minutes, the sampling pumps were turned on; PIPER was programmed by one of the activity profiles and set in motion. Spore samples were collected by both PIPER and the stationary monitor for 20 minutes. Once the sampling was completed, PIPER was manually piloted to the exit of the room and its batteries were replaced. In addition, 2 Button samplers were placed on PIPER and its program was restarted. The overall time to sample the airborne PM was 90 min for the stationary monitor and 60 minutes for PIPER (the stationary monitor started collection of PM when sampling for fungi). We elected to sample PM longer with the stationary sampler because it collected lower PM concentrations compared to PIPER (Shalat et al., 2007) and we wanted to ensure that a sufficient PM mass was collected for sample analysis. Study personnel monitored the sampling progress from an adjacent room to minimize the interference to the sampling in the investigated room.

The sampling was completed in 75 homes. Paired (from stationary and PIPER samplers) endotoxin samples (as PM on filters) were collected from all 75 homes. To conserve resources, paired fungi samples were collected and analyzed only for the first 50 homes. Although the initial protocol called for paired allergen analyses, we initially analyzed allergens only from the PIPER samples in the first 26 homes. Based on the minimal allergen prevalence in these dwellings, as described below, and to conserve resources the rest of the samples were not analyzed for allergens. Sampling dates were divided into a heating season (from October 1st through April 30th) and a non-heating season. The floor material in the sampled room was divided into two categories: bare surface floor (vinyl, tile or wood) and carpeted floor (wall-to-wall or area rug).

Statistical Analysis

Statistical analyses were performed at $\alpha=0.05$ level using STATISTICA version 8.0 software (StatSoft, Inc., Tulsa, OK) to examine the bioaerosol concentration differences between PIPER and the stationary measurements, and as sub analyses the effect of seasons and flooring type on the measured airborne bioaerosol concentrations. Nonparametric analysis methods were used because the mold and endotoxin concentration data did not fit either normal or lognormal distribution. Sign test was used to assess the absolute difference between PIPER and stationary measurements. The mean and 95% confidence intervals of paired ratios (RD) between PIPER and stationary samples were computed. Mann-Whitney U test was performed to assess the effect of heating season and flooring type on both PIPER and the stationary measurements. The Pearson Chi-square test was used to evaluate the association between season and floor type.

Results and discussion

Endotoxin

Differences between PIPER and stationary sampler—In all 75 homes, paired airborne endotoxin samples were collected (Table 1). Concentrations detected in individual homes and summary statistics are presented in Figure 2 and Table 2. As could be seen, there was a substantial variation in endotoxin concentration from home to home. The level ranged from 0.09 to 16 EU/m³ (median = 1.0 EU/m³) for PIPER samples and from 0.03 to 8.55 EU/m³ (median = 0.55 EU/m³) for stationary samples. Our results are in general agreement with other studies. Park et al. (2001) studied the level of airborne endotoxin in 111 Boston area homes and found that the median value was 0.72 EU/m³ and the range was 0.01–30.23 EU/m³.

For the 75 homes, the association between season and floor type was statistically significant (Pearson Chi-square, $p=0.03$) and the data were divided into four subsets: non-heating season with bare surface floor, non-heating season with carpeted floor, heating season with bare surface floor and heating season with carpeted floor (Figure 3). Based on the Sign test, the difference in endotoxin levels between the stationary and PIPER samples was statistically significant for carpeted floor ($p=0.0001$ and 0.012) but not for bare surface floor ($p=0.45$ and 0.81), in both heating and non-heating seasons.

The relative differences (PIPER/stationary concentration) of paired endotoxin samples are shown in Figure 4. The RD values were statistically significantly above 1 for carpeted floor in both the non-heating season (mean RD = 2.67, 95% CI 2.71–3.63, median RD = 2.0) and the heating season (mean RD = 2.98, 95% CI 1.08–2.23, median RD = 1.96). For bare surface floors, the average RDs were 1.68 for non-heating and 1.82 for heating seasons, but they were not statistically significant for both seasons. Since the RD value is >1.0 for carpeted flooring during both seasons, but not for hard surface flooring, endotoxin RD ratios for different seasons are pooled together in Figure 4. Thus, on average, PIPER measured endotoxin concentrations by approximately a factor of 2 higher compared to those measured by a stationary sampler. The difference was especially pronounced when considering carpeted flooring separately. This mean relative difference of 2.9 in endotoxin concentration is similar to that observed for inhalable particles measured in the same homes with carpeted flooring – 2.3 (Shalat et al., 2011). In fact, the relative difference in endotoxin concentration was significantly correlated with relative difference of inhalable particle concentration taken in the same homes: $p<0.0005$, $r=0.4$. Chen and Hildemann (2009) found that coarse fraction of airborne particles ($>2.5 \mu\text{m}$) contained most of the airborne endotoxin. Particles with endotoxin attached to them might have been resuspended due to PIPER's motion. If the resuspension occurred away from the stationary sampler or if the resuspended particles remained close to the floor, the stationary sampler did not capture these particles thus resulting in the underestimation of the endotoxin concentration to which a child would have been exposed. This resuspension caused the “pig-pen effect” which was more pronounced in homes with carpet floors because carpets are more effective in capturing and retaining particles for resuspension compared to bare floors. This difference in particle resuspension, from bare floors and carpeted floors, has been previously observed by other researchers as well (Ferro et al., 2004).

We examined an association between reported visible mold and the difference between PIPER and stationary measurements of endotoxin. Of the 75 homes, one did not respond to the question whether visible mold was noticed in any area during the past year. The difference between the two sampling methods was statistically significant for homes where no visible mold was reported ($N=42$) as well as where visible mold was reported ($N=32$):

$p < 0.01$ for both cases, according to the Sign test. In both cases PIPER reported higher median endotoxin concentrations compared to the stationary sampler.

Effect of environmental determinants on airborne endotoxin levels—We also examined the effect of season and floor type on airborne endotoxin concentration measured by each sampling method (Figure 3). According to the Mann Whitney U test, the heating season was not a significant factor for airborne endotoxin levels measured on bare surface floors by either PIPER ($p=0.75$) or stationary sampler ($p=0.53$). For carpeted floors, the heating season also was not a significant factor for airborne endotoxin level measured by either PIPER ($p=0.31$) or stationary measurements ($p=0.09$). During non-heating season, the floor type was not a significant factor for airborne endotoxin level measured by either PIPER ($p=0.77$) or stationary measurements ($p=0.15$). However, the floor type was a significant factor for both PIPER ($p=0.031$) and stationary measurements ($p=0.004$) during the heating season, based upon the Mann Whitney U test.

The significance of seasonality on endotoxin concentration is not yet clear in the literature. Park et al. (2001) did not find a significant seasonal effect on airborne endotoxin levels. Several other studies in Brazil, Germany, the US and Taiwan measured the endotoxin levels in floor dust samples (Thorne et al., 2009; Abraham et al., 2005; Rizzo et al., 1997; Park et al., 2000; Su et al., 2001; Heinrich et al., 2003) and showed that the seasonal pattern of endotoxin levels differed by study location and year.

Park et al. (2001) suggested that indoor sources are important for airborne endotoxin. During the heating season, doors and windows are rarely open and there is less mixing between indoor and outdoor air. As a result, endotoxin stemming from indoor sources (e.g., occupants) tends to accumulate indoors. Since airborne endotoxin are associated with airborne particulate matter (Chen and Hildemann, 2009), particles with attached endotoxin are easier resuspended from carpets than from bare surface floors. During the non-heating season, the differences of airborne endotoxin between homes with different floor types are thought to be reduced as higher ventilation rates reduce their overall concentration and deposition.

Mold

Differences between PIPER and stationary sampler—For the first 50 participating residences, paired Air-O-Cell cassette samples were collected by PIPER and the stationary sampler (Table 1). For these 50 homes, no significant association was found between floor type and season (Pearson Chi-square, $p=0.49$). Total airborne mold spore concentrations from stationary and PIPER samplers in each sampled home are shown in Figure 5 and summary statistics of the measurements are presented in Table 2. The PIPER sample from Home 2 was excluded from data analysis due to equipment malfunction. The median concentrations were 316 and 380 spores/ m^3 for PIPER and the stationary, respectively. According to the Wilcoxon Sign test, the difference in mold levels between the stationary and PIPER samples was not statistically significant: $p=0.67$.

The mold concentration data for each sampling method stratified by floor type and heating season are shown in Figures 6 and 7, respectively. In each case, there was no statistically significant difference between the two sampling methods ($p > 0.7$ for each floor type and $p > 0.5$ for each season).

The RDs for paired mold concentrations stratified by floor covering are presented in Figure 3. For the RD calculation, Home 22 was also excluded due to an uncertain RD ratio (43/ <LOD). For fungi, the mean and median values of the relative difference for the bare floors ($N=17$) were 1.08 (95% CI 0.81–1.35) and 1.09, respectively. For the carpeted floors

(N=31), the mean and median RD values were 1.09 (95% CI 0.75–1.44) and 1.05, respectively. These data indicate that on average PIPER collected slightly higher spore concentrations in each home compared to the stationary sampler. However, as indicated by the 95% CI, the RD was not statistically significantly above 1. Furthermore, the relative difference in mold spore concentration was not significantly correlated with relative difference of inhalable particle concentration taken in the same homes ($p=0.92$).

We think several factors may have contributed to the lack of a statistically significant difference between mold spore concentrations measured by PIPER and stationary sampler. The endotoxin data described above suggest that carpets act as reservoirs for particles, which can be suspended by PIPER as it simulates the motion of a child. However, spores of many mold species have ridges or spikes and could have attached to carpet pile minimizing their resuspension. In such a case, both samplers would have measured airborne spores originating from other sources, such as outdoors. If the room air is relatively well mixed, the difference between the two sampling methods would be obscured. In addition, a single 300 L air sample may have been insufficient to detect the difference due to innate variation in airborne spore concentration. The issue of fungal spore resuspension, including the role of different surfaces, should be investigated in future studies.

For both mobile and stationary air samples, the most frequently detected mold species (detected in >50% of samples) were *Aspergillus/Penicillium*, *Ascospores*, *Cladosporium* and *Myxomycetes* (Table 3). *Cladosporium* and *Aspergillus/Penicillium* were also found as the indoor dominant fungal propagules by another study (Li and Kendrick, 1995). No significant difference was observed for these four fungi taxa between stationary and PIPER measurements ($p=0.88$, 0.19 , 0.65 , and 0.64 for *Aspergillus/Penicillium*, *Myxomycetes*, *Ascospores* and *Cladosporium*, respectively).

18 out of sampled first 50 homes reported visible mold appearing during some seasons. Comparison of PIPER and stationary sampler data for homes reporting/not reporting visible mold did not show statistical significance between the two samplers ($p>0.6$ for both).

Effect of environmental determinants on mold spore levels—For PIPER, the median mold concentration for carpeted floors and bare surface floors was 213 spores/m³ and for bare surface floors 454 spores/m³, respectively (Figure 6). For stationary sampler, the median mold concentration for carpeted floors and bare surface floors was 368 spores/m³ and 446 spores/m³, respectively. The effect of floor type on the spore concentration level detected by each method was not statistically significant (stationary sampler: $p=0.516$; PIPER samples: $p=0.354$).

The floor type (carpeted or bare surface) had no significant effect on the airborne levels of four prevalent fungi taxa (*Aspergillus/Penicillium*, *Myxomycetes*, *Ascospores* and *Cladosporium*) for both PIPER and stationary samples ($p>0.15$ for all).

The effect of seasonality on the total airborne mold spore concentration is presented in Figure 7. For both PIPER and stationary samplers, higher median mold concentrations were observed during the non-heating season compared to the heating season. The median mold concentration detected using PIPER was 433 and 128 spores/m³ for non-heating and heating season, respectively. For the stationary air sampler, the median values were 539 and 127 spores/m³ for non-heating and heating season, respectively. According to the Mann Whitney U test, the effect of heating season was a significant factor for total airborne mold level (stationary: $p=0.006$; PIPER: $p=0.012$). Indoor humidity and temperature levels were significantly lower during heating seasons compared to non-heating season ($p<0.005$ for both).

Similar variation of indoor mold concentration by season, i.e., higher overall concentrations during warmer months, has been observed in other studies. Shelton et al. (2002) examined 12,026 fungal air samples (9,619 indoor samples and 2,407 outdoor samples) from 1,717 buildings located across the United States. The indoor fungal concentration measured as colony forming units (CFU) varied between 1 and >10,000 CFU/m³. They found that the indoor fungal levels were highest in the fall and summer and lowest in the winter and spring. Li and Kendrick (1995) examined total airborne spore concentrations (spores/m³) and found that in general the indoor concentrations were highest from April through September, a period encompassing to our non-heating season.

The effect of seasons was also examined for individual species as well. Higher concentrations of airborne *Ascospores* and *Cladosporium* spores were observed in the non-heating season for both stationary and PIPER samples compared to the heating season ($p < 0.01$ for all). During the non-heating season, the median concentrations of *Ascospores* and *Cladosporium* from stationary air samples were 127 and 84 spores/m³, respectively. For PIPER, the median concentrations were 86 and 84 spores/m³. During the heating season, the median concentrations of *Ascospores* and *Cladosporium* from stationary samples were 21 and 11 spores/m³, while for PIPER samples the median concentrations were 11 spores/m³ and <LOD from PIPER. The presence of *Aspergillus/Penicillium* and *Myxomycetes* was not affected by season. Similar to our study, Li and Kendrick (1995) also found that *Cladosporium* had highest concentration in summer time and *Aspergillus/Penicillium* spores showed no seasonal patterns.

The airborne spore levels detected in homes reporting visible mold compared to those not reporting visible mold were not statistically different for both sampling methods ($p > 0.83$ for both methods).

Allergens

As described in the Methods section, the airborne allergen concentrations were measured only in mobile samples of the first 26 homes. Thus, no comparison can be made between PIPER and stationary measurements. Among these 26 homes, allergens above detection limit were detected only in four homes (~15%) and all at low concentrations. Low prevalence of allergen-containing samples suggested that airborne allergen levels are in general below the limit of detection of our study and thus they were not analyzed for the remaining homes. The low prevalence of allergens is likely due to the relatively small volume of air (600 L) collected for the sample. A higher allergen prevalence level may have been detected with longer sampling times, however shorter sampling times placed less burden on the study participants.

Cockroach allergen was detected at 0.013 ng/m³ in two of the 26 homes. In response to questions about indoor pets, 2 of the first 26 homes reported having cats indoors and 7 reported having dogs indoors. Cat allergens was detected in both homes reporting the presence of indoor cats at concentrations of 14 ng/m³ and 9.3 ng/m³. Dog allergens were not detected in any home, including where dog presence was reported. In contrast, a study by Park et al. (2001) showed that presence of airborne endotoxin in Boston-area homes appeared to be associated with the presence of dogs, moisture sources, and increased amounts of settled dust, but not significantly associated with presence of cats and other pets. Thus additional research is necessary to further investigate factors associated with the presence of airborne allergens, including presence of pets, socio-economic levels of the residents, household cleanliness and others.

Conclusion

We examined the difference in airborne bioaerosol levels in residential indoor environments measured using a robotic personal sampling surrogate (PIPER Mk IV) and a stationary sampler equipped with identical sampling equipment. PIPER, which is designed to simulate resuspension of the dust from flooring by young children, measured significantly higher endotoxin concentrations compared to the stationary sampler. The mean RD was 2.9 for carpeted floors when averaged over seasons. For bare surface floor, the mean RD was above 1, however not statistically significantly. The difference in endotoxin concentration for two flooring types most likely reflected different dynamics of particle emission patterns associated with the different flooring types. This finding underscores the need to better understand the resuspension of contaminants from the surfaces and the role of that resuspension on personal exposures. The data also indicate that the traditional stationary air sampling methods may substantially underestimate exposures caused by particle resuspension; however, a large number of bioaerosols of different morphological and physical characteristics must be studied to determine the range of differences in concentrations measured by stationary air sampler versus PIPER.

In summary, a personal sampling surrogate such as PIPER can be used as a tool to investigate the effect of resuspension on personal exposures, especially those of young children. However, PIPER has not been compared to actual personal exposures of young children yet. Thus further study is needed and this might be accomplished by asking children to wear light air sampling devices for a short time in their homes and then by directly comparing their results with those obtained by PIPER carrying the same type of air sampling devices. Due to relatively small sample size of this study, additional investigations are also needed to ascertain the role of various factors, e.g., seasons and floor types, on the presence of airborne asthmatic agents.

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Figure 1.
PIPER sampler equipped with sampling pumps and sampling heads.

Figure 2. Airborne endotoxin concentrations collected measured by PIPER and stationary samplers in each home (N=75).

Figure 3. Concentrations of airborne endotoxin measured by PIPER and stationary sampler. Data was subdivided according to floor type and season.

Figure 4. Concentration Ratios (Relative Difference) of airborne mold and endotoxin concentrations measured by PIPER and stationary measurements stratified by floor surface type.

Figure 5. Total airborne mold spore concentration collected by PIPER and stationary samplers in each home (N=49).

Figure 6. Concentrations of airborne mold spores measured in rooms with carpeted and bare surface flooring.

Figure 7.
Concentrations of airborne mold spores measured during heating and non-heating seasons.

Table 1

Sample distribution by season and floor type

Floor type	Mold samples (N=50)		Endotoxin samples (N=75)	
	Non-heating season	Heating season	Non-heating season	Heating season
Bare Surface	11	6	18	7
Carpeted	18	15	23	27
Total	29	21	41	34

Table 2

Summary statistics of airborne mold and endotoxin concentration from stationary and PIPER samplers.

	Mold spores (#/m ³)		Endotoxin (EU/m ³)	
	Stationary (N=50)	PIPER (N=49) *	Stationary (N=75)	PIPER (N=75)
Minimum	<LOD	11	0.03	0.09
Maximum	11900	5770	8.55	16.00
Range	11900	5759	8.52	15.91
Median	380	316	0.55	1.00
Mean	1149	806	1.12	2.15
Standard deviation	2219	1256	1.64	3.26

* One sample excluded due to equipment malfunction.

Table 3

Frequencies of mold and myxomycetal spores as identified by microscopy. The frequency value indicates the percentage of samples where at least one spore of a given mold taxon was detected.

Taxon	Frequency (%)	
	Stationary (N=50)	PIPER (N=49)
Aspergillus/Penicillium	94	92
Ascospores	86	88
Cladosporium	74	78
Myxomycete	56	65
Basidiospores	24	18
Rust	18	18
Bipolaris	16	14
Alternaria	14	10
Epicoccum	14	16
Ganoderma	14	14
Ulocladium	14	12
Curvularia	12	16
Fusarium	8	6
Chaetomium	6	10
Pithomyces	4	10
Stachybotrys	4	8