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WITH WHEEZING IN INFANTS

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ABSTRACT

Fungi are one of the indoor biocontaminants that can cause allergic symptoms and diseases. They can grow on almost any building material if there is enough moisture available. Therefore, water damage in buildings is often associated with mold problems. Epidemiological studies have shown that children living in mold/water-damaged houses exhibit more respiratory symptoms and diseases than children in non-problem houses. Indoor mold exposure has been assessed through parental questionnaire, visual inspection by trained personnel, short-term air sampling, or dust sampling. However, the relationship between the exposure level assessed by these methods and their association with health outcomes are not clearly understood. Moreover, fungal fragments are emerging as a potential allergenic airborne contaminant, but their health-related characteristics and methods for exposure assessment are poorly explored.

On-site home visits, which included a questionnaire, visual observation and dust sampling, was conducted in 777 homes enrolled in the CCAAPS (Cincinnati Childhood Allergy and Air Pollution Study). The relationship between home characteristics and level of dustborne Alternaria allergen was investigated by using both concentration (µg/g) and loading (µg/m²). Home characteristics that were most strongly associated with the Alternaria allergen levels were those affecting the transport and penetration of particles from outdoors to indoors: the presence of dogs, the season, and the type of the infants’ primary activity room. This indicates that the major amount of indoor Alternaria originated from outdoor air. Another group of home characteristics was related with the indoor microclimate, which may affect mold growth and spore release: use of dehumidifier, cloth dryer venting into living quarters, high temperature, and low relative
humidity. Visible mold was not associated with *Alternaria* allergen level. Allergen levels measured in concentration and loading units were associated with different home characteristics, and therefore, both of these units deserve to be included in the future studies. As multiple home characteristics, not including visible mold growth, affected allergen level, both visual observation and allergen sampling are needed for a more comprehensive exposure assessment.

Airborne and dustborne allergen levels of *Alternaria* were examined in selected homes. We used 48-hour air sampling, which is much longer than conventional bioaerosol sampling of 15-30 minutes. Quantities of the allergen in air and dust samples varied widely and only a poor correlation was found between airborne concentration and dustborne concentration/loading [$R = 0.47$ (Air$_{\text{concentration}}$ vs. Dust$_{\text{concentration}}$) and 0.28 (Air$_{\text{concentration}}$ vs. Dust$_{\text{loading}}$)]. The allergen mass in floor dust samples is considerably affected by the presence of large allergen particles, which may exist in substantial amounts mainly in floor dust. Therefore, dust sampling may not be a good surrogate for inhalation exposure of *Alternaria* allergen. The 48-hour long-term air sampling, however, have not been evaluated in the relationship with non-acute health outcomes. Therefore, both air and dust sampling are recommended at this time for investigation of the relationship between cumulative exposure to fungal allergen and respiratory illnesses. Future analysis on the association between airborne and dustborne allergen levels and the clinical outcomes of the children in CCAAPS study will shed more light on this issue.

Fungal fragments were investigated through a laboratory study including characterization of their size distribution and respiratory deposition. Fungal fragments of *Stachybotrys chartarum* (in the range of aerodynamic diameters of 0.03-0.79 µm) were
released in 514 times larger quantities than spores (3.12-5.11 µm). The model calculation indicated that 60% of fragments that were retained in the respiratory tract were deposited in the alveolar region in infants. The number of deposited fragments in the bronchiolar and alveolar region was 4500-6200 times greater than that of spores in infants. Considering their biological origin as well as small size and large quantity, fungal fragments could cause allergic lower respiratory symptoms and illnesses in infants, including wheezing.

Finally, a preliminary analysis of an association between mold exposure and health outcomes in infants was performed. Our semi-quantitative observation of visible mold/water damage was able to identify an environmental risk group showing significant increase of wheezing in homes with visible mold $\geq 0.2 \text{ m}^2$. Visible mold was shown to be a significant risk factor for lower respiratory symptoms increasing the risk of persistent wheezing [RR (95%CI): 2.8 (1.2-6.5)], atopic persistent wheezing [RR (95%CI): 6.8 (2.1-21.6)], and allergic persistent wheezing [RR (95%CI): 8.5 (2.4-30.8)] in infants whose parent is atopic. Future data analysis which will include both semi-quantitative observations and quantitative measurements of airborne and dustborne mold is expected to give even higher RR-values.

This study provides comprehensive information on current measurement strategies of indoor fungal exposure. None of the currently used methods is superior, and a combination of different methods is needed to obtain a more inclusive exposure profile for fungi and their allergens. A field-compatible method needs to be developed for the measurement and analysis of fungal fragments so that this overlooked exposure type can be included in future population-based studies.
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While preparing this dissertation, I looked back upon my five-year PhD study and remembered people who supported me in every step of my professional and personal life. It is my pleasure to express my gratitude to them.

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LIST OF PEER REVIEWED PUBLICATIONS


The full texts of peer-reviewed publications are attached in Appendices A1 through A3.

Note: Specific Aim 3 was reprinted from:
1. Aerodynamic characteristics and respiratory deposition of fungal fragments, Atmospheric Environment. (In press), Copyright (2005) with permission from Elsevier.
In addition, this thesis contains data from the following papers that are in preparation:

IV. Seung-Hyun Cho, Tiina Reponen, Linda Levin, Xiaolei Liu, David I. Bernstein, Rolanda Olds, Anne Storrs, Kimberly Wilson, Grace LeMasters. The effect of home characteristics on dust allergen levels in homes. [Specific Aim 1]

V. Seung-Hyun Cho, Tiina Reponen, David I. Bernstein, Rolanda Olds, Linda Levin, Supriya Satwah, Sergey A. Grinshpun, Grace LeMasters. The relationship between airborne and dustborne allergen levels in homes. [Specific Aim 2]
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BACKGROUND

Atopic disorders such as asthma, rhinitis, and eczema have long been recognized, but have become steadily more prominent in recent decades. There is evidence for an increase in the frequency of asthma, wheeze, and other atopic syndromes in developed countries. A study of Swedish army recruits showed a doubling of the prevalence of seasonal allergic rhinitis in one decade from 4.4% in 1971 to 8.4% in 1981 (Åberg, 1989). Similarly, studies on hayfever symptoms in school children aged 8-13 years showed increased prevalence from around 3% in 1964 to 12% in 1989 in Aberdeen, Scotland (Ninan et al., 1992). In Melbourne, Australia, an increase of 26.9% in the prevalence of asthma in children was measured between 1964 and 1990. Cross-sectional surveys performed in the USA in 1982 and 1992 showed that prevalence of wheezing in primary school children increased by 6.6% and asthma diagnosis increased by 17.6% (Peat et al., 1994). Currently, allergic rhinitis is the most common disease in humans and allergens are the major reason for hospital emergency room trips (IOM, 1993).

While such an increase in atopic disease over a relatively short period of time cannot be related to a genetic change, this predisposition to atopy appears to be determined early in life, and there is growing evidence that allergen exposure occurring in early age including in utero can influence the future development of atopic conditions (Howarth, 1998; Salam, 2004). These allergic conditions involve excessive generation of IgE (specific serum immunoglobulin E) against environmental antigens (Howarth, 1998; Peat et al, 1995).
Exposure to aeroallergens is of particular concern because about 75% of asthma cases are triggered by aeroallergens and thus have considerable medical and economic impact on the population (Platts-Mills et al., 1997; Ward et al., 1998). Various studies have evaluated the levels of common aeroallergens in indoor environments in order to investigate the development of atopic diseases in children. Cockroaches and house dust mites have been reported as important allergen sources causing adverse health effects in humans (Antony et al., 2002; Salam et al., 2004; Squillace et al., 1997; Wickmann et al., 1993; Leung et al., 1997; Sporik et al., 1990; Custovic et al., 1996). Contact with domestic animals such as cat, dog, birds, and rodents are also known to cause allergic asthma and rhinoconjunctivitis (Eggleston et al., 2001; Carrer et al., 2001).

Fungi are also known to cause allergic symptoms and diseases. Numerous fungi produce highly allergenic proteins or glycoproteins that can cause hypersensitivity diseases in susceptible hosts (Flannigan et al., 1991; Nevalainen et al., 1991). Between 10% and 60% of genetically susceptible (atopic) persons develop immediate hypersensitivity (allergy) to fungi, as demonstrated by skin tests (IOM, 1993). Furthermore, several other mechanisms such as infection, irritation, and toxigenic effects have been implicated with adverse health effects of fungi and fungal products as summarized in several review papers (Burge et al., 2001; Husman et al., 1996; Chapman et al., 2003; Terr, 2004).

Fungi can grow on almost any building material if there is enough moisture available. Therefore, water damage in buildings is often associated with mold problems. The National Institute for Occupational Safety and Health (NIOSH) conducted indoor
environmental health hazard evaluations for 105 office buildings based on employees’ requests (Crandall et al., 1996). Eighty trained NIOSH field investigators collected building and indoor environmental quality information including the presence of water damage and visible mold. Water damage was identified in 42% of the evaluated buildings. A survey of 24 communities in the United States and Canada found that 36% of homes were contaminated with mold/mildew. When combining a positive response to any of three moisture/mold-related questions, 50% of the homes reported dampness. Furthermore, the dampness condition was reported for more than 55% of the homes in seven communities (Spengler et al., 1993). In England and Wales, the Building Research Establishment (BRE) estimated that 2.8 million dwellings had mold growth or damage caused by dampness (Hunter et al., 1994). In the Netherlands, about 15% of homes were estimated to have some degree of dampness (Verhoeff et al., 1990). Among various studies in Finland, surveys conducted on the condition of the home showed that 15% - 23% of residents reported at least one of the following: water/moisture damage, moisture stains, visible mold, and moldy odor (Pirhonen et al., 1996; Kilpeläinen et al., 2001). In another study, some signs of current or previous moisture problems were found in 80% of randomly selected houses inspected by civil engineers who were trained to identify signs of water leaks and condensation. It was also reported that most houses had more than one sign of moisture problems (Nevalainen et al., 1998). In the USA, the Midwest has high humidity throughout the summer months and frequent flooding in spring. Thus, moisture and mold problems are believed to be common. There are, however, no statistics available on the prevalence of mold in Midwestern homes.
As people spend more time in indoor environments, the prevalence of water-damage and mold growth in buildings has gained more attention and has been linked to potential adverse health effects (Husman, 1996; Dearborn et al., 1999; Etzel et al., 1998; Meklin et al., 2002). Since infants spend most of their time at home, exposure to indoor biocontaminants can be a critical factor for developing illnesses in early life. Epidemiological studies have shown that children living in mold/water damaged-houses exhibit more respiratory symptoms and diseases than children in non-problem buildings (Dearborn et al., 1999; Etzel et al., 1998; Meklin et al., 2002; Belanger et al., 2003; Zock et al., 2002; Waegemaekers et al., 1989; Andrae et al., 1988; Dales et al., 1991; Dekker et al., 1991). In most studies listed above, the mold/water damage was determined by parental questionnaire or by visual inspection performed by trained teams. Among these studies, only Belanger et al. (2003) and Waegemaekers et al. (1989) showed an association between air sampling results and the health outcome in children. Furthermore, several studies failed to find a relationship between visible mold/water damage and respiratory symptoms or asthma (Burr et al., 1993 and 1997; Gent et al., 2002; Jaakkola et al., 2005). Thus, the literature shows confusing information on the cause-and-effect relationship of mold exposure in children.

It is important to define what kind of sampling method quantifies personal exposure best. In principle, the technique of measuring exposure must be simple, reliable, reproducible, and not time-consuming (Custovic et al., 1999). For respiratory exposure, air sampling is believed to be the most representative method (Burge, 1995). Traditionally, fungal exposures have been assessed by short-term air sampling combined
with microscopic or culture-based spore counting, which have their limitations. Due to the short sampling period of commercially available instruments, temporal variation of spore concentration (Hyvärinen et al., 2001; Pasanen et al., 1991; Górny et al., 2001) might result in under/overestimation of fungal exposure. Several field studies have shown that the concentration of airborne fungal spores in mold problem buildings is not necessarily higher than in non-problem buildings. In a Finnish study the mean fungal spore level in the samples collected in moldy homes was even lower than that in the reference homes (Nevalainen et al., 1991). A British study could not find a significant difference between median counts of colony forming units obtained in moldy and non-moldy homes, respectively (Strachan et al., 1990). Similarly, studies performed in Australian and the Boston area homes did not show a significant association between evidence of dampness or visible mold and the spore concentration (Garrett et al., 1998; Chew et al., 2003).

Dust sampling has been extensively used to assess exposure to indoor allergens, such as house dust mite, cockroach, cat, and dog allergens. Some fungal spore investigations have also utilized dust sampling and combined it with modern analytical methods, such as PCR, immunochemical analysis of specific fungal allergens, or analysis of β (1→3)-D-glucan, ergosterol, or extracellular polysaccharides. Dust sampling is believed to represent long-term patterns in exposure better than short-term air sampling and is easy to perform in large population-based studies (Chew et al., 2003). However, dust sampling may not adequately reflect human inhalation exposure. Furthermore, dust samples from different places and/or different floor material at a home may have different allergen levels (Foarde et al., 2004; Price et al., 1990; Loan et al., 2003), so dust
samples might not be suitable to represent respiratory exposure in the entire home environment.

Only few studies have compared fungal spore concentrations in air and dust samples. Chew et al. (2003) indicated a weak correlation of culturable spore count between dust and air samples \( r=0.13, p<0.05 \) recommending the application of both sample types when assessing overall potential fungal exposure. More information is needed on the relationship between the levels obtained by dust and air sampling.

In a recent laboratory study, it was found that smaller-sized fungal fragments, which are pieces of fungal spore cell wall or mycelium, are released together with spores from contaminated surfaces in large quantities (Górny et al., 2002, 2003). These particles have been shown to contain fungal antigens (Górny et al., 2002) and mycotoxins (Brasel et al., 2005). This suggests that fungal fragments could contribute to the development of atopic diseases in genetically susceptible persons. However, fungal fragments haven’t been much explored so far. The aerodynamic characteristics and respiratory deposition of fungal spores has been extensively studied (Reponen 1995; Reponen et al., 1997 and 2001). However, there is a need to investigate the aerodynamic behavior of fungal fragments to better understand the potential adverse health effects caused by these particles.

This PhD study comprised a part of the Cincinnati Childhood Asthma and Air Pollution Study (CCAAPS), a prospective birth cohort study aimed to investigate the role of diesel exhaust particles and associated bioaerosols in the prevalence of atopy and
atopic respiratory disorders in early life. In addition, a separate laboratory-based study included characterization of fungal fragments.

In the CCAAPS, an on-site home visit was performed in 777 homes in the Cincinnati area and the Northern Kentucky area when the infants were on average 8 months old. During the home visit, home characteristics were investigated through a questionnaire and a visual inspection, and dust samples were collected for allergen analysis. At age of about 13 months, the infants were invited to clinics for a medical examination. Of infants who participated in the home visit, 640 visited clinics. Infants’ respiratory symptoms were asked from parents, and all infants underwent a skin prick test (SPT) for food and aeroallergens. Detailed indoor samplings were conducted in 112 selected homes through dust and air samplings. The selection criteria included SPT reactivity of infants (56 homes with SPT positive to aeroallergens, the other 56 homes with SPT negative) and the time of birth.
OBJECTIVE, HYPOTHESIS, AND SPECIFIC AIMS

Objective: The objective of this study was to investigate indoor fungal exposures and associated health outcomes of persistent wheezing, atopic persistent wheezing, and sensitization to fungal allergens in infants. The findings of this study provide important information on the methodology utilized for fungal exposure assessment in indoor environments, and the development of sampling techniques of airborne fungal particles. These data can be used when designing future field studies aimed to investigate the relationship between exposure and health outcomes.

This PhD study comprises:

1) Assessment of indoor fungal exposure through on-site home visits that included questionnaires and visual inspection about mold/water damage and floor dust sampling for fungal allergen analysis, and detailed airborne and dustborne sampling in selected homes.

2) Laboratory study on the aerodynamic characteristics of airborne fungal particles and computational modeling on their respiratory deposition.

3) Investigation of health outcomes in infants in association with indoor mold/water damage.

Hypothesis: Specific home characteristics are associated with the level of indoor fungal exposure and the prevalence of respiratory symptoms and sensitization in infants.
In order to achieve the objective of this study and test the hypothesis, the following four specific aims were accomplished:

**Specific aim 1:** Evaluate indoor fungal exposure by on-site home visits.

**Specific aim 2:** Evaluate indoor fungal exposure through air and dust sampling of *Alternaria* allergen in selected homes.

**Specific aim 3:** Estimate respiratory deposition of airborne fungal particles in infants.

**Specific aim 4:** Investigate the relationship between mold/water damage in homes and the prevalence of respiratory symptoms and sensitization in infants.
SPECIFIC AIM 1
EVALUATE INDOOR FUNGAL EXPOSURE BY ON-SITE HOME VISITS

1.1. INTRODUCTION

Although the best-known health outcomes of indoor mold exposure are allergic symptoms and diseases (Burge, 2001), most studies have measured the number of viable or total spores or other cellular components of fungi instead of fungal allergens. While the effect of home characteristics on other types of indoor aeroallergens such as house dust mite and cockroach has been extensively investigated, much less is known about the factors that affect fungal allergens.

In this study, the effect of home characteristics on the contamination with Alternaria allergen was investigated. Alternaria was selected because 1) Alternaria is known as the most common sensitizer among asthmatic children in the USA (Eggleston et al., 1998), 2) it is common both in indoor and outdoor environments, and 3) an immunoassay for Alternaria was available for this study.

In Specific Aim 1, the evaluation of home characteristics, which may be directly or indirectly related with fungal exposure, was accomplished through on-site home visits in 777 homes investigated in the CCAAPS. The on-site home visit consisted of two major procedures: the interview and the exposure assessment via dust sampling and visual observation of house condition. A checklist was developed to identify home characteristics including mold and water damage in homes. The dust samples were taken...
from the floor for the exposure assessment of the *Alternaria* allergen. The relationship of home characteristics with the level of *Alternaria* allergen was investigated, and specific home characteristics were identified as indicators of fungal exposure.
1.2. MATERIALS AND METHOD

1.2.1. Examination of home characteristics

1.2.1.1. Study population

Infants born in Cincinnati and Northern Kentucky between 2001 and 2003 were recruited to the CCAAPS using birth certificate data (LeMasters et al., 2005). Eligibility for the study required that at least one parent should be allergen-sensitized, which was determined by positive reaction in a skin prick test (SPT) to at least one of 15 common aeroallergens (meadow fescue, timothy, white oak, maple, American elm, red cedar, short ragweed, Alternaria spp., Aspergillus fumigatus, Penicillium spp., Cladosporium spp., cat, dog, German cockroach, and house dust mite). The study was approved by the Institutional Review Board (IRB) at the University of Cincinnati.

1.2.1.2. Design of the checklist to investigate general home/family characteristics and mold/water damage in homes

A checklist was developed to obtain information about home characteristics that might be associated with health outcomes in infants, study subjects in the CCAAPS. Existing questionnaires and home inspection protocols, which were used in previous studies (Admodt et al., 1999; Lebowitz et al., 1989; Toivola et al., 2002; Belanger, 2003) and those published by professional organizations or governmental agencies (ACGIH, 1999; US EPA, 1994 and 2001; NIOSH, 1999) were utilized in the development of the checklist. Specific home characteristics found to be associated with indoor contaminants in other studies were also included in the checklist (Kozak et al., 1979; Almqvist et al., 1999; Dornelas de Andrade et al., 1995). The checklist consists of two parts: a
questionnaire with 36 questions administered to a parent in an interview during the home visit, and an inspection list to evaluate housing conditions (Appendix B).

1.2.1.3. Examination of home characteristics by an on-site home visit

On-site home visits were performed by several trained two-persons teams in 777 homes with infants of an average age of 8 months. A questionnaire on home characteristics such as surrounding environment, building characteristics, infants’ activity characteristics, and housekeeping status including history of mold/water damage was administered to a parent. The status of the building was assessed by a visual observation. Each room in the house including the basement and the attic was inspected for existence of any signs of visible mold or water damage. Location of damage, changes in the color and integrity of surface material, and the size of damaged surface were recorded. Tape samples were taken from mold-damaged surfaces to identify fungal genera. Additionally, temperature and relative humidity were measured and the existence of moldy odor was recorded in the infants’ bedroom, basement, and the room where the child spent most of his or her daytime, referred to as the child’s primary activity room (PAR). The number of plants and stuffed toys in the PAR and the infants’ bedroom were also counted.

The extent of mold and water damage in homes was categorized into three different classes (Table 1-1) based on the questionnaire and observation data. The criteria for the classification were developed by Meklin et al. (2004) based on ISIAQ guidelines for mold cleanup (ISIAQ, 1996). A ‘class 0’ home had no water damage history, visible mold/water damage, or moldy odor. A ‘class 1’ home had at least one of the signs listed
above, but the moldy area per room was smaller than 0.2 m². A ‘class 2’ home had visible mold: either the moldy area per room was greater than 0.2 m², or the combined area of mold and water damage on the same surface was greater than 0.2 m².

1.2.2. Exposure assessment of dustborne Alternaria allergen

The families were requested not to clean the floor for at least a day before the visit. At the visit, a parent was asked to identify the PAR. Dust samples were collected from flooring materials in the PAR using a vacuum cleaner (Filter Queen Majestic®; HMI Industries Inc., Seven Hills, Ohio) at a flow rate of 800 L/min. A custom-made cone-shape HEPA filter trap (Midwest Filtration, Cincinnati, OH) was attached to a nozzle of the vacuum cleaner to collect the dust sample. This equipment was chosen based on a pilot study (Appendix C). In the pilot study, flow rates, pressure changes, and collection efficiencies were tested using different types of filter traps (HEPA filter trap, dust trap with 180-thread cloth, and dust trap with 200-thread cloth). Among these filter traps, the HEPA filter trap showed a collection efficiency of over 95% for particles larger than 0.3 µm. The cone-shape HEPA filter trap (CCAAPS filter trap) increased the vacuum flow rate by 10% compared to a traditional rectangular HEPA filter trap (IBT filter trap). The selected vacuum cleaner was able to maintain a high flow rate with only a 3% pressure drop when it was used with the HEPA filter trap.

For carpeted floor, a dust sample was collected from an area of 2 m² at a vacuuming rate of 2 min/m² (1 min horizontally, 1 min vertically). Then, in order to increase an amount of fine dust, which is a fraction used for the allergen analysis, another
dust sample was collected from the same area with a new filter trap. The first sample typically had human and animal hair and other coarse particle whereas the second sample had more fine dust. If the collected dust amount was less than 1/3 of the volume of filter trap, an adjacent area in the same room was vacuumed to collect sufficient amount of dust. For non-carpeted floor (hard wood, linoleum, tile, or sheet floor), only one sample was collected from the entire room at a rate of 1 min/m². The reason for using a different sampling protocol for non-carpeted floor is that dust can be vacuumed from non-carpeted surfaces faster than from carpet (Ewers et al., 1994; Reponen et al., 2002) (we did not observe significant increase in dust amount with higher vacuuming rate in our pilot study). Furthermore, the amount of fine dust from a duplicated sample from a non-carpeted floor was not enough for analysis. Information on the size of the sampled area and floor material was recorded. The exact location of the sampled area was also recorded so that future repeat samples could be collected from the same location. The sample was taken back to the laboratory in a cooler bag with ice. The detailed protocol for the dust sampling is presented in Appendix D. The dust sample was sieved (355 µm sieve), and the fine dust was divided into sub-samples and stored at -20°C before analyses.

*Alternaria* spp. allergen (AA) was extracted from the sieved fine dust (50 mg) into 2 mL of PBS-T (0.05% Tween 20 in phosphate buffered saline, pH 7.4) by vortexing at high speed for 2 minutes with a vortex touch mixer. The extract was analyzed using a commercial polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (Indoor Biotechnologies, Inc., Charlottesville, VA). Results were given as µg allergens per ml extract, and converted to µg allergens per g of sieved dust and µg allergens per m².
of sampling area. The lower limit of detection ranged from 0.2 to 20 µg/g. For dust samples that contained non-detectable allergen, a half of the limit of detection was assigned as their allergen level. The detailed protocol for the analysis of *Alternaria* allergen is presented in Appendix E1. All dust samples were also analyzed for cat, dog, house dust mite, and cockroach allergens by ELISA using commercial immunoassay from the same company.

### 1.2.3. Investigation of the relationship between home characteristics and the level of *Alternaria* allergen

The correlation between home characteristics and dustborne AA level was investigated by Poisson ordinal regression analysis. Rate ratio and 95% confidence intervals (95%CI) were computed using the GENMOD procedure in SAS with a log link function for AA level. The data on home characteristics were used as independent variables, and the AA level was used as a dependent variable. Home characteristic data obtained through questionnaire and visual observation were divided into three groups with regard to their influence on allergen concentration: direct source or carrier of allergens (group 1); indirect source or control factors of allergens (group 2); and general housing characteristics (group 3). Continuous home characteristic variables such as temperature and relative humidity were divided into two or more subgroups to be used as an ordinal variable either by uniform proportion or by uniform unit. The response for categorical variables such as cloth dryer use and floor type of the PAR were grouped to detect specific home features that might affect the allergen level. Mold/water damage class was included in group 1 representing the source of indoor mold.
Two units were used for the AA level: allergen concentration (µg/g) and allergen loading (µg/m²). The former is the relative amount of allergen in the fine dust from floors, and the latter is the total amount of allergen collected from the unit surface area of floor materials. Both allergen levels were divided into six groups by cutoff allergen levels corresponding to frequency of 50%, 75%, 90%, 95%, and 99% specifying the Poisson distribution.

Mold/water damage was examined in an adjusted model in association with the AA level. First, three separate models were built utilizing each group of home characteristics. All independent variables of home characteristics in a group were controlled for each other in each model. Then, variables that showed a significant ($p < 0.05$) association with AA level in each adjusted model were included in the final model to investigate overall effect of home characteristics on the AA level. This data analysis was separately performed with allergen levels in concentration (µg/g) and loading (µg/m²).

1.3. RESULTS AND DISCUSSION

Table 1-2 presents the results for all three groups of home characteristics, which were used as independent variables in the model. Among 777 homes, 440 homes (56.6%) had some sign of mold/water damage (belonged to class 1 or 2). Spores of *Alternaria* were only identified on tape samples from 10% of the homes with visibly damaged surfaces. As sources/carriers of allergens, 19.7% and 31.1% of homes had cat and dog, respectively, 68.1% of homes had equal or less than 4 people as residents, and 57.7% of
homes had stuffed toys in the PAR. As control factors of allergens, 1.9% of homes had cloth dryer venting into the living quarter, which might increase relative humidity inside the home. On the other hand, 16.6% of homes were using dehumidifiers mostly in the basement to reduce excessive moisture. Most homes (91.2%) had carpets or area rugs on the floor in the PAR, which might hold fine dust along with allergens for a longer time than non-carpeted floor. Vacuum cleaners with either standard filters or HEPA filters were used in 88.2% of the homes. The cleaning frequency varied in homes, but it was not significantly associated with the type of vacuum cleaner. For general housing characteristics, more than half of the families participating in the study lived in houses older than 50 years. The infants’ PAR was mostly the room where people frequently come and go, such as the living room, family room, kitchen, or dining room, and these rooms were located mostly on the first floor. Most of the homes used either central or local air conditioning units with some type of filter. The amount of sampled fine dust from the unit surface area varied from 0.005 to 7.985 g/m². The mode, median, and mean of the dust amount were 0.17, 0.33, and 0.62 g/m², respectively.

The AA level in dust samples ranged from 3.8 to 1649.4 µg/g and from 0.2 to 3350.8 µg/m², and 90% of samples contained measurable amounts of AA. The average allergen levels were 47.3 µg/g and 14.8 µg/m² (geometric mean). The distributions of AA levels were skewed toward the high level, and 10% of the homes showed AA > 121.7 µg/g (> 96.1 µg/m²).
Tables 1-3 presents the significant associations (95% confidence level) between the indoor dustborne allergen levels and a particular home characteristic adjusted for other home characteristics as potential confounding factors using a Poisson regression model. The AA levels in both concentration and loading units were associated with more home characteristics than the levels of other allergens (cat, dog, house dust mite, or cockroach), which were mainly correlated with their respective sources.

Home characteristics, which were significantly associated with the AA level in both measurement units, included the following: the existence of dogs, the existence of plants, annual season, and the type of the PAR (bedrooms or busy room with high levels of physical activity such as living room, family room, kitchen, and dining room). The AA level was significantly higher in homes that had ≥ 2 dogs. Animals can carry allergens on their feet and fur, and most dogs in the CCAAPS homes were allowed to go out. Therefore, it is suspected that the outdoor activity of dogs increases the outdoor-to-indoor transport of AA. The existence of plants was significantly associated with low level of AA. Similar associations were seen also for other allergens (cat, dog, and cockroach). Plants could have an effect of cleaning allergens by absorption similarly as they can absorb toxic vapors (NASA, 1989). The AA level showed seasonal variation being highest in fall samples and lowest in spring samples. *Alternaria* is one of the most common fungal genera found more abundantly outdoors than indoors (Shelton et al., 2002) and known to grow on rotting leaves and other biomaterial peaking in the fall (Platts-Mills, et al., 1993). Adhikari et al. (2005) reported that outdoor *Alternaria* spore concentration in Cincinnati peaks in fall. Therefore, elevated AA levels in fall dust
samples may be explained by higher rates of penetration of outdoor *Alternaria* into the homes. Busy rooms had higher levels of AA than bedrooms, which reflects the intensity of activity of humans and their pets.

Among the other home characteristics in group 1, appearance of cockroaches, the number of stuffed toys, relative humidity, and temperature in the PAR affected allergen concentration (µg/g) significantly. Homes with no cockroaches, no stuffed toys, temperature > 75°F, or relative humidity < 30% had higher levels of AA. It is difficult to explain the negative association between AA concentration and other allergen sources/carriers (cockroach and stuffed toy). Further studies are needed to explain these relationships. Homes with temperature > 75°F had favorable climate for mold growth. These homes may have more indoor mold growing either on the building surface or in the carpet. A decrease in the relative humidity is known to trigger spore release (Pasanen et al., 1991; Foarde et al., 1999). Since the measurement of relative humidity was conducted only once in the homes, however, it is not clear whether homes that had the low relative humidity (< 30%) associated with higher AA maintained the humidity level always low and it resulted in spore release from sources other than carpets. None of the mold/water classes did show any significant association with the AA level in either concentration or loading. This is not surprising as tape samples showed that only 10% of mold-damaged homes had *Alternaria* on the damaged surfaces. Moreover, only 5% of homes had mold/water damage in the PAR where the dust sample was taken. This supports the conclusion that that the main AA source was the outdoor air, rather than indoor mold/water damage.
Among groups 2 and 3, different home characteristics appeared as significant factors depending on the unit of allergen measurement. In allergen concentration (µg/g), homes with dehumidifier or cloth dryer venting into the home had significantly higher levels of AA. In allergen loading (µg/m²), homes that used any insect extermination methods, where the kitchen was within 14 ft from the PAR, or had a carpet or an area rug in the PAR had higher levels of AA. Most homes using dehumidifiers had experienced excessive moisture inside the houses. These homes might have *Alternaria* growing in the building. Humid air vented from a dryer or a kitchen close to the PAR as a moisture source may be potential causes of mold growth in the carpet in the PAR. However, measurement of relative humidity, which was performed for a short time, did not provide enough information to examine the effect of these potential moisture sources on a long-term increase in relative humidity and AA levels. Carpets or area rugs provide larger surface areas where allergens can collect and accumulate together with other dust components. Furthermore, organic particles embedded in the carpet can be nutrients for mold growth. AA loading increased with larger amounts of fine dust per unit sampling area. The same trend was also seen with other allergens (cat, dog, house dust mite, and cockroach) (Table 1-3). However, AA concentration was not associated with either floor type or the amount of fine dust per unit area.

### 1.4. CONCLUSIONS FOR SPECIFIC AIM 1

The association of dustborne AA with home characteristics was examined in Specific Aim 1. More than half of the 777 homes participating in the on-site home visit had some sign of mold/water damage, and 37 (4.8%) homes of those had either visible
mold alone or together with water damage on a surface area of larger than 0.2 m². However, AA level was not significantly associated with visually inspected mold/water damage. In contrast, AA level was significantly correlated with several other home characteristics. One group of these home characteristics included factors that are associated with the penetration of particles from the outdoors to indoors. This suggests that the majority of AA in these homes originates from the outdoor environment rather than indoor mold/water damage. Another group of home characteristics was related with indoor microclimate. Cloth dryer venting into the living quarter in homes and use of dehumidifier may be associated with excessive moisture, which may result in mold growth in the dust/carpet. High temperature and low relative humidity in homes may help mold growth and spore release, respectively. However, the measurement of temperature and relative humidity was conducted for a short time period and did not provide enough information to examine their long-term effect on indoor mold growth. Continuous monitoring of temperature and humidity would be needed to characterize indoor climate in future studies. Carpeted floor was a significant risk factor for increased allergen loading. Carpets or area rugs provide large surface areas and reservoirs for particle accumulation and microclimate for fungal growth. AA loading was significantly associated with the amount of fine dust per unit area. On the contrary, AA concentration did not show a significant association with either floor type or the amount of fine dust per unit area. Thus, the effect of floor type on the AA was only found with the allergen loading.
As outdoor mold can contribute to the presence of fungal particles inside houses, visual observation alone may not be sufficient to assess fungal exposure. Sampling of airborne and/or dustborne allergen is needed in addition to visual observation for a more comprehensive exposure assessment.
SPECIFIC AIM 2

EVALUATE INDOOR FUNGAL EXPOSURE THROUGH AIR AND DUST SAMPLING OF *ALTERNARIA* ALLERGEN IN SELECTED HOMES

2.1. INTRODUCTION

Measurements of allergen levels in dust have been used to assess the level of time-integrated exposure. However, dust allergen level is only a surrogate measure of airborne allergen concentration, and actually inhaled doses are difficult to extrapolate. Moreover, most fungal exposure studies have measured total or culturable spore counts instead of fungal allergen when investigating the association between fungal exposure and allergic respiratory symptoms.

In the CCAAPS, a subgroup of infants was selected in a nested case-control study, which included more detailed exposure assessment for indoor aeroallergens. Cases were defined as having a positive SPT reaction to at least one of the tested aeroallergens. Controls were selected among the infants who did not have any positive SPT and was matched with the cases by birth month (± 2 months). Biannual air and dust sampling was performed in these homes. Some homes were sampled more than once, and the number of sampling sessions in each home is dependent on the infants’ enrollment time in the nested case-control study.

In Specific Aim 2, airborne and dustborne allergen levels of *Alternaria* were evaluated by air and dust sampling, and the relationship between airborne and dustborne allergen levels was investigated. Between spring of 2003 and spring of 2004, 112
CCAAPS homes were selected in the nested case-control study. Among these homes, 7 homes had three separate air and dust samples taken, 39 homes had two separate air and dust samples taken, and the rest 66 homes had one air and one dust sample taken. Total of 165 air samples and 165 dust samples were taken during this time and were included in the analysis.

2.2. MATERIALS AND METHOD

2.2.1. Exposure assessment of airborne and dustborne Alternaria allergen

Air sampling was conducted utilizing a Button Personal Inhalable Sampler (SKC, Inc., Eighty-four, PA) and a BGI-400 personal pump (BGI, Inc., Waltham, MA). The inhalable sampler was chosen to measure inhalation exposure to allergens (Aizenberg et al., 2000). Polycarbonate membrane filters with 25 mm diameter and 3 µm pore size (GE Osmonics, Inc., Minnetonka, MN) were chosen after a pilot study to test performance of several types of filters (see 2.2.2.). The pump was kept in a portable carrying box with insulating material to prevent noise and vibration. The Button sampler was attached to the front side of the box and connected to the pump inside.

The sampling period was approximately 48 hours, and the sampling air flow rate was set at 4 L/min in the beginning of the sampling. Time and air flow rates were recorded at the beginning and at the end of the sampling. The sampling was started in the PAR, and the parents were asked to keep the air sampler in the same room where the infants stayed inside the homes in order to estimate the infant’s exposure as closely as possible. The detailed protocol for the air sampling is presented in Appendix F. A
questionnaire was utilized at the conclusion of the sampling session in order to determine the approximate amount of time that the sampler was in the same room as the child (Appendix G). It was determined that the average time that the infants spent in the home during the 48 hour sampling session was 39 hours (81% of time), and during those 39 hours, the sampler was in the same room as the infants for 35 hours (73% of time). In 50% of the homes, the sampler stayed most of the time in the PAR, and the average time that the infants spent in the PAR during the sampling session was 38 hours. In the rest of the homes, the average time that the sampler stayed in the PAR was 16 hours. After sampling, the membrane filters were taken out of the Button samplers in the laboratory, and stored at -20°C until analysis. Before analysis, allergens were extracted into 2 mL of extraction buffer (0.05% Tween 20 in phosphate buffered saline with thiomerosol). The extract was analyzed for Alternaria allergen with the same ELISA method as used in Specific Aim 1. The sampling volume was calculated by multiplying the average air flow rate by the sampling time and was used to estimate airborne allergen concentrations in µg/m³.

A dust sample from the PAR was taken in the same home at the time when the air sampling started. The dust sampling and allergen analysis were conducted by the same protocol as described in Specific Aim 1. The allergen level in dust samples was converted to concentration in µg/g and loading in µg/m² as described in Specific Aim 1.

2.2.2. Pilot study on performance of different filters
An essential requirement of air sampling in our study was to collect airborne particulates efficiently maintaining a consistent flow rate with a minimum pressure drop for 48 hours, and using filter material that does not interfere with the ELISA analysis. A pilot study was conducted to evaluate the flow rate and pressure drop of six different types of filters with three different materials and different pore sizes:

polytetrafluoroethylene (PTFE) membrane filter with pore size of 1 and 2 µm (Omega Specialty Instrument Co., Houston, TX); polycarbonate (PC) membrane filter with pore size of 1, 2, and 3 µm (GE Osmonics, Inc., Minnetonka, MN); and mixed cellulose ester (MCE) membrane filter with pore size of 1.2 µm (Millipore Corp., Billerica, MA).

Each membrane filter was placed in the Button sampler that was connected to a personal sampler pump, and the pressure-drop was measured during operation. The pump was initially set at an air flow rate of 4 L/min.

2.3. RESULTS AND DISCUSSION

Table 2-1 presents the difference in porosity and pressure drop of different filter types. In general, filters showed different pressure-drops due to different porosities of the filters, which represent the portion of open area for a filter. Among six filters, the PC membrane filter with a pore size of 3.0 µm and the PTFE membrane filter with a pore size of 2.0 µm showed the lowest pressure-drops. Between these two filters we chose the PC filter as it has been shown to perform better than the PTFE filter in the ELISA analysis (Schmechel et al., 2003). The collection efficiency for the PC filter with pore size of 3.0 µm was found to be 99% for *Bacillus subtilis* spores with a diameter of 1 µm.
(Burton et al., 2005), which is much smaller than fungal spores of *Alternaria spp.* (aerodynamic diameter of about 18 µm; Adhikari et al., 2003). Therefore, the PC filter with a pore size of 3.0 µm was chosen for the further use.

The AA level ranged from 7.6 to 489.0 µg/m³ (geometric mean of 58.2 µg/m³) for air samples, and 4.9 to 694.2 µg/g (geometric mean of 56.2 µg/g) and 0.1 to 1322.5 µg/m² (geometric mean of 19.1 µg/m²) for dust samples. The widest range, four orders of magnitude, was seen in the dust loading of AA. Detectable levels of airborne AA were found in 60% of homes. On the other hand, airborne *Alternaria* spores were identified in 24% of homes in a concurrent study (Osborne et al., 2005). Figure 2-1 demonstrates the correlation between the air samples (Air$_{conc}$) and dust samples in two different units of concentration (Dust$_{conc}$) and loading (Dust$_{load}$). Although these correlations were statistically significant, correlation coefficients (R) were 0.47 (Air$_{con}$ vs. Dust$_{conc}$) and 0.28 (Air$_{con}$ vs. Dust$_{load}$) showing poor linear relationship of allergen level between air samples and dust samples. In contrast to AA, a good correlation between air and dust samples was found for cat allergen (Table 2-2). Custovic et al. (1998) showed that cat allergens (Fel d 1) are associated with small particles (< 4.8 µm) and can remain airborne for several days. In contrast, the aerodynamic size of *Alternaria* spores is about 18 µm (Adhikari et al., 2003). Thus, AA is expected to be associated with large particles, which do not stay in the air for a long time. Choe et al. (2000) showed that the airborne concentrations of particles of 7.5-10 µm decrease to 1% in about 1.5 hours. Larger particles can be expected to settle down even faster. Air sampling represents a much smaller particle size range than dust sampling. The largest particles in dust samples,
which may contribute most of the AA mass, may be airborne only for very short time periods after activity and may never reach the breathing zone.

As described in Specific Aim 1, floor material affects the retention of dustborne particles and can be expected to influence the aerosolization efficiency of those particles. Therefore, the correlation of AA level was investigated between air and dust samples separately in carpeted and non-carpeted homes. For airborne concentration and dustborne concentration, similar correlations were found in non-carpeted homes and carpeted homes ($R = 0.44$ and $0.48$, respectively). For airborne concentration and dustborne loading, a better correlation was found in non-carpeted homes than in carpeted homes ($R = 0.43$ and $0.24$, respectively) (Figure 2-2). Thus, the effect of floor type on the aerosolization of AA was only found with the dustborne allergen loading.

2.4. CONCLUSIONS FOR SPECIFIC AIM 2

The level of airborne allergen was poorly correlated with that of dustborne allergen. This was attributed to the different particle size ranges of AA in dust and air samples resulting in different amount of AA in these sample types. The allergen level in dust samples is considerably affected by large allergen particles, which may become easily airborne but will settle down quickly. Therefore, dust sampling might not be a good surrogate for inhalation exposure of *Alternaria* allergen. Dust samples are believed to represent cumulative exposure, but the represented exposure time frame is unclear as the age of dust is not known and varies from home to home. The effects of home characteristics, such as the floor material, that may influence the aerosolization
efficiency of dustborne allergens would need to be examined with a larger number of samples in a future study to investigate the relationship between airborne and dustborne allergens.

Long-term 48-hour air sampling was conducted in this study. Conventional air sampling is usually performed for 15-30 minutes. Short-term air sampling often results in under or overestimation of airborne contaminants due to their temporal variation. The 48-hours sampling in this study was selected to assess infants’ inhalation exposure minimizing bias to either peak or off-peak exposures. However, this long-term air sampling did not result in a good correlation with dust sampling, and may still not be long enough when investigating non-acute health outcomes. Therefore, both air and dust sampling may be needed in order to investigate the relationship between cumulative exposure of fungal allergen and respiratory illnesses. Future analysis of the CCAAPS health outcome data with the allergen data obtained by both dust and air sampling is expected to give more information on the value of different sampling strategy.
SPECIFIC AIM 3
ESTIMATE RESPIRATORY DEPOSITION OF AIRBORNE FUNGAL PARTICLES IN INFANTS

3.1. INTRODUCTION

An increase in allergic lower respiratory diseases such as asthma has been found to be associated with mold exposures or damp houses (Wickman et al., 2003; Jaakkola et al., 2005). However, a cause-and-effect relationship has not been established with traditional exposure assessment techniques based on spore count or biomass detection. On the other hand, recent studies have shown that fungal fragments, which are smaller than spores, contain fungal antigens (Górny et al., 2002) and mycotoxins (Brasel et al., 2005). This suggests that fungal fragments may potentially contribute to allergic symptoms by penetrating deeper into the lower airways of the lung. However, little is known about fragment exposure, and future characterization of fungal fragments with respect to their aerodynamic behavior is needed.

In Specific Aim 3, the aerodynamic characteristics of three different types of fungal particles released from contaminated surfaces under controlled laboratory conditions were analyzed using an Electrical Low-Pressure Impactor (ELPI; Dekati Ltd., Tampere, Finland). To achieve this goal, three different sampling conditions were tested for their effectiveness in preventing spore bounce in the ELPI. Based on the size distribution of the airborne fungal particles, the respiratory deposition of fungal particles was calculated and compared between fragments and spores.
3.2. MATERIALS AND METHOD

3.2.1. Measurement of aerodynamic size of fungal particles

3.2.1.1. Test microorganisms and their preparation for measurements

The experiment was conducted with three different species of fungi: *Aspergillus versicolor*, *Penicillium melinii*, and a non-toxic strain of *Stachybotrys chartarum*. *A. versicolor* and *S. chartarum* are among the species that are commonly found in moldy materials (IOM, 2004). *Penicillium* species are common both in non-problem and problem buildings (Meklin et al., 2002; Shelton et al., 2002). These species were selected to represent different aerodynamic sizes of fungal spores: 2.5 µm (*A. versicolor*), 3.0 µm (*P. melinii*) and 5 µm (*S. chartarum*) (Reponen et al., 1995, 1996 and 2001; Sorenson et al., 1987). *A. versicolor* and *P. melinii* are the same strains used in previous studies (e.g., Reponen et al., 2001; Górny et al., 2002). The non-toxic isolate of *S. chartarum* was isolated from an environmental sample and characterized as isolate JS5105 by Jarvis et al. (1998). All fungal species were grown on malt extract agar (MEA) plates at 24°C and a relative humidity of 97-99% for one month to obtain abundant fungal growth.

3.2.1.2. Aerosolization and analysis of the size distribution of fungal particles

The experimental setup used in this study is schematically shown in Figure 3-1. It consists of two parts, one for the aerosolization and the other for the collection and quantification of fungal particles. Fungal aerosolization was conducted by a recently developed Fungal Spore Source Strength Tester (FSSST), which has been designed to aerosolize fungal particles from contaminated surfaces by high speed jet-air using HEPA-filtered air (Sivasubramani et al., 2004ab). The collection and quantification was
performed using the ELPI. It is a real-time particle size analyzer consisting of a corona aerosol charger, real-time multi-channel electrometers measuring real-time particle concentrations, and a low-pressure cascade impactor classifying and collecting particles into 12 size fractions within the size range of 0.03-10 µm. The corona charger of the ELPI charges incoming particles positively, and the charged particles induce positive current on the impactor stages at the time of collection. Then, the electrometers in the ELPI measure the currents on all 12 impactor stages and the data processor of the instrument converts them to particle number concentrations. The entire set-up was placed inside a Class II biosafety cabinet (SterilchemGard; Baker Company, Inc., Sanford, ME). The exit flow from the ELPI was filtered with a HEPA-filter (12144 HEPA capsule filter; Pall Corporation, Ann Arbor, MI) and redirected into the biosafety cabinet to prevent contamination of the room environment. The aerosol background level was measured before starting the aerosolization tests by placing an open agar plate without fungal growth in the FSSST. The background concentrations were found to be negligibly low. After one month of incubation, fungal particles were released from a sporulating agar plate by operating the FSSST for 5 minutes at a flow rate of 30 L/min. The aerosolization of fungal particles was repeated three times for each fungal species.

3.2.1.3. Testing the three methods for preventing spore bounce inside the ELPI

During the pilot operation of the experimental setup with *A. versicolor*, a negative current in the ELPI was observed in the particle size range of 0.1-1 µm. This was attributed to bounce-off of fungal spores from the stages above this size range. The particle bounce and reentrainment is one of the limitations of an inertial aerosol impactor.
In order to minimize the particle bounce problem, a sticky material has been traditionally used for coating the impaction plates. However, when utilizing certain analytical techniques, this coating material may interfere with the measurement of target substances. Alternatively, the bounce of spherical atmospheric particles can be decreased with increasing relative humidity because these particles are hygroscopic and moist particles do not bounce significantly (Vasilious et al., 1999). For this study, three different methods were tested for preventing the particle bounce: humidification of the incoming air and coating the impactor stages with two different sticky materials, DS-515 (Dekati Ltd., Tampere, Finland) and ZEF-X10 (Zefon international, Inc., Ocala, FL). DS-515 is a spray-type of Apiezon-L grease, which is recommended by the ELPI manufacturer to minimize particle bounce. Therefore, the ELPI data obtained using DS-515 coating served as a reference. However, DS-515 is not water soluble, which is a limiting factor for further analysis of particles collected on the impactor stages. In contrast, air humidification as well as coating plates with the water-soluble ZEF-X10 are compatible with microscopic analysis and could potentially be used with other analytical techniques based on aqueous sample processing, such as immunoassays.

DS-515 grease was sprayed evenly on aluminum foils of 25 mm in diameter (CF-300, Particle Instruments, LLC, MN) with a 22-mm stencil (DS-125, Particle Instruments) following manufacturer’s instructions. The greased aluminum foils were placed on top of the impactor stages inside the ELPI before the measurement started.
The humidification of fungal particles was achieved by mixing the released fungal particles with water vapor in a specially constructed humidification chamber. The chamber was placed between the FSSST and the ELPI. The final chamber design was selected from three different humidification chambers pretested for their effectiveness in maximizing aerosol mixing while minimizing particle losses (Figure 3-2). The first two (Figure 3-2a, b) were designed to introduce water vapor against the incoming fungal aerosol with or without swirling to maximize aerosol mixing. However, this type of design was found to cause large amounts of particle losses by impacting moistened fungal particles on the interior wall of the chamber. The third type of humidification chamber (Figure 3-2c), which was designed to introduce water vapor in parallel with the fungal aerosol flow, exhibited relatively small particle losses. Therefore, the third design was chosen as the experimental humidification chamber. This was, in turn, compared with the two sticky coatings (DS-515 and ZEF-X10) with respect to the ability to prevent particle bounce. The relative humidity inside the humidification chamber was continuously monitored with a moisture measurement system (Protimeter Plc, Marlow, England). Relative humidity was adjusted to 60-90% to avoid condensation on the chamber wall.

The water-soluble adhesive material, ZEF-X10, was specifically developed for impactor-type air samplers. ZEF-X10 was diluted with water, and the diluted solution was evenly spread on 25-mm aluminum foils. In order to get a thin coating layer with maximum viscosity, the ZEF-X10-coated aluminum foil was dried in an oven (Isotemp
Premium Oven; Fisher Scientific Company, Pittsburgh, PA) for one hour at 40°C and then placed on the ELPI impactor stages for the experiments.

The three different bounce prevention methods were compared by measuring the concentrations of released fungal particles. One-month-old cultures of *A. versicolor* were utilized for this experiment. As described below, the ELPI data obtained with the ZEF-X10 coating and with the DS-515 coating were found to show comparable size distributions of fungal particles. Therefore, the former coating was used for further experiments.

### 3.2.1.4. Size and charge distributions of three fungal species

The concentration, size, as well as the charge distribution of the aerosolized fungal particles were measured three times for each fungal species. After collection, fungal particles were eluted into 2 ml H₂O containing 0.02 % Tween 80 by vortexing individual aluminum foils from each impactor stage for 2 min. Each fungal suspension was filtered onto a separate mixed cellulose ester (MCE) filter of 13 mm diameter and 1.2 µm pore size (Millipore Corp., Bedford, MA). The MCE filters were placed on glass slides and made transparent with acetone vapor for microscopic analysis as described by Adhikari et al. (2003). Spore counting for 12 slides obtained from the 12 impactor stages was conducted from randomly selected 40 microscopic fields using a bright light microscope (Leitz Laborlux S; Leica Mikroskopie und Systeme GmbH, Germany) to confirm the absence or presence of fungal spores on the impactor stages. The concentration of fungal fragments was determined from the ELPI data by combining particle concentrations obtained from all impactor stages where no fungal spores were
observed. The upper size limit for the fragments was determined as the cut-off diameter of the highest impactor stage where no fungal spores were observed. For spores, the size range was determined as the cut-off diameters of impactor stages corresponding to the size of single spores of each species: S. chartarum (3.12-5.11 µm) and A. versicolor (1.99-3.12 µm). The spore concentration was determined from the particle concentration measured at those impactor stages by the ELPI. The concentration and the size of fungal spores and fragments were utilized as input data for fragment exposure for the respiratory modeling.

3.2.2. Computational modeling of respiratory deposition of fungal particles in infants

The deposition of fungal spores and fragments in the human respiratory tract was calculated using a computer-based model, LUDEP 2.07 (ACJ & Associates, Inc., Richland, WA), with parameters representing a typical adult male and a 3-month-old infant. The calculations are based on the concentration data collected within each of the 12 aerodynamic size ranges measured by the ELPI when challenged with each of the two fungal species with distinctly different aerodynamic diameters of spores; S. chartarum (5 µm) and A. versicolor (2.5 µm). For each species, calculations were performed separately for two size fractions; one representing single spores and the other representing fragments. The total intake was expressed as the total number of inhaled fungal particles during the time of exposure, which was one hour in this simulation. The intake was determined by multiplying the concentration of released fungal particles with the time-weighted mean breathing rate and the exposure time (1 hour). Breathing rates and time intervals, which
were different for different breathing patterns, simulate normal indoor activities, but do not include typical outdoor activities such as work or sports activities. For an adult male, the breathing pattern included the following: 0.45 m³/h (sleeping; 55.0% of time), 0.54 m³/h (sitting; 15.0% of time), 1.5 m³/h (light exercise; 30.0% of time); and for an infant: 0.09 m³/h (sleeping; 71.0% of time), and 0.19 m³/h (light exercise; 29.0% of time) (ICRP, 1994).

The model calculates particle deposition into five regions in the respiratory tract: 1) anterior nasal region (ET1), 2) main extrathoracic region comprising the posterior nasal passages, larynx, pharynx, and mouth (ET2), 3) bronchial region (BB), 4) bronchiolar region (bb), and 5) alveolar-interstitial region (AI). The deposition efficiency of inhaled particles into the respiratory regions depends strongly on the particle aerodynamic diameter, \(d_a\). It was calculated for two components: the aerodynamic deposition due to impaction and sedimentation, which is a function of \(d_a^2\), and the thermodynamic deposition due to diffusion, which is an inverse function of \(d_a\). Detailed formulas for calculating deposition in each of the regions are presented in ICRP (1994).

The output data were reported as total or % regional deposition in the respiratory tract and separated for fungal fragments and spores. Likewise, the numbers of deposited particles in specific regions and overall in the respiratory tract were calculated for fragments and spores by multiplying the amount of intake by the % of deposition. The deposition ratio of fragments to spores was obtained by dividing the number of deposited fragments by that of spores.
3.3. RESULTS AND DISCUSSION

3.3.1. Aerodynamic characteristics of fungal particles

The size distributions of *A. versicolor* particles measured by the ELPI when using bounce-preventing methodologies are shown in Figure 3-3. The concentration of released fungal particles, obtained for the 12 size classes utilizing the direct-reading capability of the ELPI, are plotted as a function of the aerodynamic diameter. With the traditional DS-515 coating, the size distribution showed a mode of the particle concentration at the aerodynamic diameter of 1.99-3.12 µm corresponding to the aerodynamic size of *A. versicolor* spores (Reponen et al., 1996). It was also observed that the concentration of particles smaller than 0.80 µm was comparable or higher than that of larger particles confirming our previous studies reporting the release of microbial particles smaller than spores from contaminated surfaces in large quantities (Górny et al., 2002 and 2003). The results obtained with the water-soluble ZEF-X10 coating showed a similar size distribution as the DS-515 revealing a mode for fungal spores at the aerodynamic diameter of 3.12 µm. Humidification, however, resulted in distinctively different particle size distribution as compared with the two other methods used for the particle bounce prevention. No mode was observed in the fungal spore size range, and the concentration of particles with the aerodynamic diameter of 0.20 µm was close to zero as most of the time the ELPI measured negative value of current for that particular stage. When the humidification system was operated at lower relative humidity of 40-50% as compared to the normal operation condition of 60-90%, higher particle concentrations were recorded at upper stages (0.50-8.18 µm), but more zero readings were obtained at small particle size ranges (0.13-0.32 µm). These zero readings appear to be caused by bounce-off of
insufficiently humidified fungal particles. On the other hand, lower humidity reduced the loss of particles, which would otherwise have been deposited on the interior wall of the humidification chamber. In contrast to humidification, we did not observe negative value of current with either DS-515 or ZEF-X10 coatings when testing with *A. versicolor*. This suggests that these two coating media appear to prevent the bounce of *A. versicolor* spores with equal efficiency. As further experiments required preparation of water suspensions from collected particles, the water-soluble ZEF-X10 coating was chosen for further experiments.

The fractional particle concentrations and the distribution of induced current measured with the ELPI for three different types of fungi are shown in Figure 3-4. Microscopic counting of fungal spores collected on impactor stages was performed following the ELPI measurement and the results are presented in Figure 3-5. For *S. chartarum*, the number of particles increased with decreasing size and no negative value of current was observed (Figure 3-4a). The microscopic counting data, shown in Figure 3-5a, demonstrated that no spores were found on impactor stage 7 and below (the corresponding aerodynamic diameters $\leq 0.79 \mu m$) indicating that the ZEF-X10 coating effectively prevented spore bounce. Microscopic examination confirmed that the large numbers of particles at lower impactor stages were truly fragments. The average concentration of released *S. chartarum* fragments (0.03-0.79 $\mu m$) obtained in our experiments was 380 particles/cm$^3$, which was about 514 times higher than that of spores in a size range of 3.12-5.11 $\mu m$. This result supports the evidence that *S. chartarum* spores can be difficult to aerosolize. For *A. versicolor*, the ELPI results showed that the
spores and fragments were released in comparable amounts (Figure 3-4b). The highest number of spores counted by microscopy was for stage 10 (3.12 µm) (Figure 3-5b) coinciding with the mode measured by the ELPI. The microscopic examination confirmed that no observable spore bounce occurred onto impactor stages 6 or below.

In contrast, the ELPI measurement conducted with *P. melinii* showed different size and current distributions from those measured for *S. chartarum* and *A. versicolor* (Figure 3-4c). High negative value of current was observed at impactor stage 5 within 30 seconds of the measurement resulting in zero concentration for the corresponding particle size (0.32 µm). Spores were observed on all impactor stages by microscopic counting demonstrating spore bounce-off in the ELPI (Figure 3-5c). In order to confirm the bounce effect, the numbers of fungal spores, which saturate the surface of impactor stages theoretically, and the sampling time to collect those spores were calculated utilizing the particle concentration data measured by the ELPI. The single spore area on the impactor stage, which is occupied by a single spore of *P. melinii*, was calculated with the assumption that *P. melinii* spores have a spherical shape with the diameter of 3.0 µm. The total number of spores saturating the actual impaction surface with a single layer was obtained by dividing the actual impaction surface area by the single spore area. The saturation time was calculated by dividing the number of saturating spores by the measured concentrations of particles in the size range of *P. melinii* spore (1.99-3.12 µm), and dividing it by the ELPI sampling flow rate (30 L/min). The saturation time was compared with the actual sampling time from the beginning of the aerosolization to the appearance of high negative value of current. It was found that the saturation times for
stage 9 (1.99 µm) and 10 (3.12 µm) were 12 seconds and 30 seconds, respectively, similar to the time when the negative value of current in the ELPI started appearing. Therefore, it was concluded that the negative value of current resulted from the particles that bounced from the saturated impactor stages. However, it is still not clear why the negative value of current and spore bounce did not happen for the two other fungal species, *S. chartarum* and *A. versicolor*. The spore bounce could be caused by the slightly higher concentration of *P. melinii* or by a higher aggregation rate of *P. melinii* aerosolized spores compared to two other species. *P. melinii* has a tendency to release spores in chains. These chains could break during impaction resulting in the escape of spores that have been separated during the break off (Trunov et al., 2001). Furthermore, the spiny surface of *P. melinii* spores could prevent a good contact between the spore and the collection surface (Trunov et al., 2001).

It was concluded that the particle distributions of *A. versicolor* and *S. chartarum* showed the real contribution of fragments, but that of *P. melinii* was masked by the spore bounce effect occurring on the upper stages of the ELPI. Based on the microscopic examination, the upper size limit for fragments was set at an aerodynamic diameter of 0.79 µm for *S. chartarum* and at 0.50 µm for *A. versicolor*. No clear fragment-spore border with respect to the particle size could be established for *P. melinii*, and, thus, this species was not considered for the respiratory deposition calculation, which was undertaken using the data on aerodynamic characteristics of the aerosolized fungal particles.
As mentioned earlier, a clear separation of fragments from spores for \textit{S. chartarum} and \textit{A. versicolor} was achieved successfully by the ELPI with the ZEF-X10 coating. The ZEF-X10 medium was found to be compatible with the microscopic analysis used for the quantification of fungal spores on the impactor stages. However, fungal fragments are too small to be quantified by microscopic analysis. Therefore, an attempt was made to test the immunological reactivity of fungal fragments as well as spores from samples collected on the ZEF-X10 medium. To test the compatibility of the ZEF-X10 medium with the immunoassay protocol, fungal suspensions with ZEF-X10 medium were prepared. Fungal particles of \textit{S. chartarum} and \textit{A. versicolor} scraped from the sporulating agar plates were separately mixed in ZEF-X10 solution (110 µl of ZEF-X10 and 2 ml of sterilized filtered water) imitating fungal particles suspended from the ZEF-X10-coated aluminum foil. Enzyme-linked immunosorbent assay (ELISA) was performed for the suspensions as described by Schmechel et al. (2003). It was found that fungal particles remained in suspension inside the ELISA wells even after overnight incubation, and the resulting optical densities of fungal samples were very low. It seems that the high viscosity of the ZEF-X10 medium blocked antigen adsorption to the ELISA well surface resulting in correspondingly low ELISA values. This assay interference emphasizes the need to develop sample processing techniques, which are compatible with sample analysis techniques to ensure the accuracy of the results.

### 3.3.2. Respiratory deposition of fungal particles

The efficiency of particle deposition in specific regions of the respiratory tract strongly depends on the particle’s aerodynamic diameter and the breathing flow rate.
Therefore, the difference between the size distributions of inhaled spores and fragments as well as the difference in breathing rates between an adult and an infant could result in different regional particle depositions. Model simulations were performed with two fungal species, *A. versicolor* and *S. chartarum*, which showed distinctively different concentration ratios of fragments to spores.

Figure 3-6 presents the regional deposition fractions in percentages separately for inhaled fragments and spores representing one hour of exposure. The total intake, which is the total number of inhaled fungal particles during the time of exposure, was calculated based on the concentration of fungal particles measured by the ELPI and modeled for age and activity-specific breathing pattern of an adult male and 3-month-old infant. For *S. chartarum*, which was found to release significantly greater number of fragments than spores, the total one-hour intake was about 500 times greater for fragments than that of spores [for an adult, $300 \times 10^6$ fragments/hour and $0.6 \times 10^6$ spores/hour; for an infant, $453 \times 10^5$ fragments/hour and $0.9 \times 10^5$ spores/hour]. For *A. versicolor*, the intake of fragments was three times higher than that of spores for an adult and an infant. The model calculations indicated that the total respiratory deposition fractions for fragments and spores were 27-46% and 84-95%, respectively. In general, 65-90% of inhaled spores for both fungal species were deposited in ET1 (anterior nasal region) and ET2 (main extrathoracic region), while only 3-15% and 2-5% of spores were deposited in AI (alveolar interstitial region) and BB-bb (bronchial- bronchiolar region), respectively. More than half of the inhaled fungal fragments were exhaled again, and the exhalation was more efficient for an adult male than for a 3-month-old infant. Among fungal
fragments retained in the respiratory system, 60% were deposited in AI, while only 14-15% of fragments were deposited in ET1 and ET2. Total and regional deposition fractions were slightly higher in an infant than in an adult for both fragments and spores. The total deposition of fragments for an adult and an infant were 27-41% and 33-46%, respectively, and those of spores were 84-92% and 93-95%, respectively.

Figure 3-7 presents the fragment/spore deposition ratio, which is the number of fragments divided by the number of spores deposited in the respiratory tract. Generally, the deposition ratio is affected by (i) the intake ratio, which relates the number of fragments to spores inhaled into the respiratory tract, and (ii) the regional deposition ratio, which depends on the breathing rate and the aerodynamic characteristics of the inhaled particles including their size. As shown in Figure 3-6, spores tend to be deposited in the upper airways and fragments in the lower airways, which was observed more prominently for a 3-month-old infant. For *S. chartarum*, the intake values resulted in a total deposition ratio of approximately 230 and 250 for an adult and an infant, respectively (Figure 3-7a). This means that the number of deposited fragments of *S. chartarum* was 230-250 fold higher than that of spores. For *A. versicolor*, which showed comparable concentrations for fragments and spores, the total deposition ratio was less than 1 for both an adult and an infant (Figure 3-7b). The regional deposition ratio was highest at the bronchiolar region (bb) and the alveolar region (AI) and lowest at the main extrathoracic region (ET2) for both age groups and for both fungal species. However, the scale of deposition considerably varied with age and fungal species. The regional deposition ratio of *S. chartarum* varied from 6150 (bb) and 4530 (AI) to 34 (ET2) for an
infant, and from 1350 (bb) and 1340 (AI) to 36 (ET2) for an adult. For \textit{A. versicolor}, the regional deposition ratio varied from 17 (bb) and 9 (AI) to 0.14 (ET2) for an infant, and 4 (bb) and 3 (AI) to 0.14 (ET2) for an adult. This shows that the deposition ratio in the lower airways but not the upper airways for a 3-month-old infant was 4-5 times higher than that of an adult male under the same exposure conditions. This suggests that the tendency of fragments to deposit into the lower airways is amplified 4-5 times for an infant when compared to an adult.

Once fine particles enter into the lower respiratory tract, they are difficult to clear. The clearance rate of fine particles from alveolar region to lymph nodes is reported as $0.00002 \text{ d}^{-1}$ compared to $1 \text{ d}^{-1}$ obtained for few micrometer size particles in the nasal region (ICRP, 1994). Experimental and epidemiological studies have reported that fine particles may increase inflammation and alter macrophage responses (Oberdörster et al., 1992; Renwick et al., 2004) as well as contribute to an increase in chronic obstructive pulmonary disease, pneumonia, and an overall decrease in lung function (Zanobetti et al., 2000; Peters et al., 1997). These studies have also suggested that the pulmonary toxicity of fine and ultrafine particles is due to their considerable total surface area. Normal lung development in children is particularly sensitive to pollutants until age 6-8 years (Burri et al., 1997; Cunningham et al., 1996) and our results suggest that exposure to fungal fragments may represent a greater relative risk than fungal spores during the early stages of childhood.
3.4. CONCLUSIONS FOR SPECIFIC AIM 3

In Specific Aim 3, the size distribution of fungal particles of aerodynamic diameters between 0.03 and 10 µm was measured with a direct-reading instrument, ELPI. The ELPI was found to discriminate fungal fragments and spores size-specifically when the collection surfaces of the impactor stages were coated with either of two adhesives: Apiezon-based DS-515 and water-soluble ZEF-X10. This study revealed that the fungal fragments released from contaminated surfaces outnumber spores and are more effectively deposited in the lower airways for a 3-month-old infant compared to an adult male. This trend was more significant for S. chartarum than for A. versicolor. Thus, the potential health effects associated with fine fragments of S. chartarum could be more pronounced in small children than in adults. While the mechanisms for adverse health effects associated with fungal exposure are not fully understood, recent toxicological characterizations have shown that fragments may contain mycotoxins and antigens.

In conclusion, the high number of released fungal fragments in combination with their potential to deliver harmful antigens and mycotoxins to the alveolar region of the lung, especially for young children, suggest that future exposure measurements need to include fragment measurements. This would complement current measurement strategies based on spore counts or sample cultivation and provide more realistic and comprehensive exposure profiles.
SPECIFIC AIM 4

INVESTIGATE THE RELATIONSHIP BETWEEN MOLD/WATER DAMAGE IN HOMES AND PREVALENCE OF RESPIRATORY SYMPTOMS AND SENSITIZATION IN INFANTS

4.1. INTRODUCTION

Although mold and dust mite growth have been associated with indoor dampness and each has been independently linked to adverse health effects, few studies have studied their combined contribution to respiratory symptoms and allergy (Nafstad et al., 1998; Belanger et al., 2003; Burr et al., 1993 and 1997). Moreover, as indicated by Jaakkola (2005), most home dampness studies were designed as cross-sectional or case-control studies and might not examine accurate exposure-health outcomes.

In Specific Aim 4, a prospective cohort study was conducted to investigate the relative risk of wheezing (persistent wheezing, atopic persistent wheezing, allergic persistent wheezing) and sensitization to aeroallergens in 640 infants in association with mold/water damage in homes. The model included house dust mites (HDM) allergen levels and economic status as confounding factors.
4.2. MATERIALS AND METHOD

4.2.1. Evaluation of indoor exposure and health outcomes in infants

The prevalence of mold/water damage and house dust mite was estimated as potential risk factors to cause wheezing and sensitization in infants. Mold/water damage class (described in Specific Aim 1) was utilized as an independent variable representing indoor mold exposure for the statistical analysis. Sub-samples of the dust collected at on-site home visits (described in Specific Aim 1) were analyzed for house dust mite (*Dermatophagoides farinae*: Der f 1) by ELISA. The lower limit of detection was 0.1-1.6 µg/g. The detailed protocol for the analysis of house dust mite allergen is presented in Appendix E2.

During the infants’ first clinic visit at average age of 13 months, infants underwent skin prick test (SPT) for food (milk and egg) and 15 aeroallergens as described in Specific Aim 1 (LeMasters et al., 2005). Infants, who showed any positive reaction (≥ 3 mm wheel), were classified as sensitized. Meanwhile, medical evaluations for infants were directed at identification of wheezing symptoms. The ISAAC questionnaire for 4-5 year-old children was adapted to develop a wheezing question for our cohort (ISAAC, 1998). At least two episodes of wheezing were defined as persistent wheezing. If the persistent wheeze occurred in an infant with positive SPT to any allergen (either food or aeroallergens) and with positive SPT to at least one aeroallergen (positive SPT to food alone was excluded), it was defined as atopic persistent wheezing and allergic persistent wheezing, respectively. Of 777 homes that had an on-site home visit
for the exposure assessment, 640 families brought their child for a SPT. Therefore, for further health outcome analysis, only these 640 infants were included.

4.2.2. Statistical analysis of the relationship between mold/water damage in homes and the prevalence of health outcomes

Multiple logistic regression analyses were conducted to estimate the relative risk (RR) of persistent wheezing, atopic persistent wheezing, allergic persistent wheezing, SPT(+ to aeroallergen [SPT(+aero)], and SPT(+) to mold [SPT(+)mold] in relation to mold/water damage class. House dust mite (HDM) exposure and economic status were included as confounding factors. Odds ratios were interpreted as risk ratios due to the large sample size (n=640). Mold/water damage class was classified as above (0, 1, and 2). HDM allergen was classified into three groups: \( \leq \) lower limit of detection (LLOD); LLOD-2 µg/g; and \( \geq 2 \) µg/g. Economic status was divided into two groups with the cutoff household income level of $20,000, which is close to the poverty threshold for a family of four based on the data from the U.S. Department of Commerce, Bureau of the Census (2004). In a separate analysis, moldy odor was categorized into two groups: moldy odor detected in at least one room among PAR, baby’s bedroom, and basement; no moldy odor in any of these rooms.

4.3. RESULTS AND DISCUSSION

Two well-known risk factors for respiratory illnesses, indoor mold/water damage and house dust mites, were investigated together. The association of persistent wheezing,
atopic persistent wheezing, allergic persistent wheezing, SPT positivity to at least one aeroallergen [SPT(+aero)], and SPT positivity to at least one mold [SPT(+mold)] during the first year of life was analyzed in relation to home and family characteristics. Infants with atopic persistent wheezing were defined as persistent wheezers with sensitization to any allergens (n=44), and the comparison group was defined as non-wheezers without sensitization to any allergens (n=343). Infants with allergic persistent wheezing were defined as persistent wheezers with sensitization to at least one aeroallergen (n=30), and the comparison group was defined as non-wheezers without sensitization to any allergens (n=343). Persistent wheezing, atopic persistent wheezing, and allergic persistent wheezing were reported in 40%, 20%, and 17% of infants who lived in the mold class 2 homes, respectively. Tables 4-1, 4-2, 4-3, 4-4, and 4-5 demonstrate the relative risk of persistent wheezing, atopic persistent wheezing, allergic persistent wheezing, SPT(+aero), and SPT(+mold), respectively.

4.3.1. Mold damage and health outcomes

Relative risk (RR) of persistent wheezing (Table 4-1) was significantly increased with the exposure to major mold/water damage (class 2) [RR (95%CI): 2.8 (1.2-6.5)]. The exposure to class 2 damage increased relative risk of atopic persistent wheezing nearly seven times [RR (95%CI): 6.8 (2.1-21.6)] (Table 4-2). Furthermore, the class 2 damage increased relative risk of allergic persistent wheezing almost nine times [RR (95%CI): 8.5 (2.4-30.8)] (Table 4-3). Mold/water damage was also associated with increased risk for sensitization to aeroallergens (Table 4-4) and mold (Table 4-5), although this increase was not significant and not consistent with the extent of damage. In
separate data analysis, when using a two-level-mold classification (merging classes 1 and 2 homes together), the associations between existence of mold/water damage and persistent wheezing and atopic persistent wheezing became non-significant. This demonstrated that the cutoff size of visible mold $\geq 0.2 \text{ m}^2$ for class 2 was able to distinguish substantial mold damage in homes. A borderline positive relationship was found between moldy odor and persistent wheezing ($p=0.06$).

It should be noted that our on-site home visit included only observable mold/water damage. Very often microbial colonization exists in enclosed building cavities within walls and ceilings and is not always visually observed. However, our semi-quantitative observation of mold/water problems was able to identify an environmental risk group with significant increase of wheezing in class 2 homes.

### 4.3.2. House dust mites and health outcomes

There was no significant association of HDM exposure with wheezing or sensitization in infants. Only a few infants (16%) were exposed to a HDM allergen concentration $\geq 2 \mu\text{g/g}$, which is a level considered to increase the risk of sensitization (Kuehr et al., 1994). Only 3% of infants were sensitized to house dust mite at the average age of 13 months. The average concentration of mite allergen in these sensitized infants was 0.5 $\mu\text{g/g}$. Of 640 infants, 84% was exposed to lower than 2 $\mu\text{g/g}$ of mite allergen. Similar results were also reported by Lau (2000) and Wahn (1997) demonstrating low prevalence of mite-sensitization in infants under low level of mite exposure (median concentration of 0.2 $\mu\text{g/g}$). Lau (2000) found higher prevalence of wheezing in mite-
sensitized children only after age two. Similarly, other studies, which found a dose-dependent prevalence of sensitization or an increased risk of wheezing and other asthmatic symptoms in mite-sensitized people, targeted older children (Squillace et al., 1997; Sporik et al., 1990; Lau et al., 1989).

Due to a lack of studies that investigated exposure and health outcomes in early infancy, different target populations, and methodologies measuring health outcomes, it is difficult to compare these results with other studies. Many prospective studies did not perform a skin prick test to identify sensitivity to aeroallergens in infants but only after the children were older (Woodcock et al., 2004; Peat et al., 2004). Baseline test for atopy in addition to tests during the follow-up would be needed to identify clear exposure-sensitization relationships for future longitudinal studies. In the CCAAPS study, the future annual SPT and medical examinations will provide important information on the evolution of the children’s atopy in relation to their exposures. Other types of aeroallergens have also been measured and will be used in future analysis to investigate combined health effects of multiple exposures.

4.3.3. Economic status and health outcomes

Most of the homes in this study (86%) belonged to an income group ≥ $20,000. Persistent wheezing was reported in 31% of low-income group (< $20,000) and in 17% of the income group ≥ $20,000 suggesting that low economic status is associated with higher risk of persistent wheezing. The infants in income group ≥ $20,000 were less likely to have persistent wheezing [RR (95%CI): 0.5 (0.3-0.8)]. However, prevalence of
atopic persistent wheezing, allergic persistent wheezing, SPT(+) to aeroallergen, and SPT(+) to mold were not significantly different between groups.

4.3. CONCLUSIONS FOR SPECIFIC AIM 4

As indicated by Newman (2001) and Bornehag (2001), most previous studies have been designed as cross-sectional measuring prevalence not incidence, or case-control studies, which measure neither prevalence nor incidence and have susceptibility to bias. With the prospective cohort study design, it was possible to show the temporal relationship between early exposure and health outcome without selection, reporting, or information bias. The information bias was minimized in the exposure assessment, as mold/water damage was inspected by home visit teams instead of parental reports. Objective measurements on types of damage and size of damaged area in this study improved the exposure assessment compared to qualitative measurement or parental report, as shown by Nafstad et al. (1998). Another unique aspect of this study is that skin prick tests were performed in the entire cohort to unequivocally establish infants’ atopic phenotype.

In conclusion, visible mold was shown to be a significant risk factor for persistent wheezing, atopic persistent wheezing, and allergic persistent wheezing in infants whose parent is atopic. However, the allergen level of house dust mite was not associated with any of the investigated health outcomes. It remains to be seen how environmental exposure affects the development of sensitization and wheezing, and what relationship
exists between the early onset of wheezing and the development of asthma in these infants through their early life.
OVERALL CONCLUSIONS

This study has demonstrated that indoor mold exposure may contribute to the development of persistent wheezing in infants. The exposure assessment, which was conducted with different methods including visual inspection of home characteristics and measurement of dustborne and airborne Alternaria allergen, showed that each method measured a particular type of mold exposure. The results suggest that none of these methods is superior for the assessment of indoor mold exposure. On the other hand, the laboratory experiments and computational modeling showed that nano-sized fungal fragments were released in large quantities from contaminated surfaces and deposited in the lower respiratory tract with greater consequences for infants than adults. Considering their biological origin as well as small size and large quantities, these fungal fragments could cause allergic lower respiratory symptoms and illnesses in infants, including wheezing that was significantly associated with fungal exposure in this study. Although only 24% of homes had airborne Alternaria spores (as analyzed by microscopic counting) and less than 5% of homes had Alternaria fungi growing on damaged surfaces, airborne Alternaria allergen was detected in 60% of homes. This difference could be attributed to the presence of fungal fragments that can be detected with the immunochemical method but not with the microscopic counting method. Mold growth in a home could be a source of substantial fungal fragment exposure, which cannot be estimate based on visible mold alone as mold growth is often hidden inside the building structures. It suggests that future exposure measurements need to include fungal fragment measurements. This would complement current measurement strategies and provide more comprehensive exposure profiles when investigating health outcomes.
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**Table 1-1.** The prevalence of mold/water damage in homes and the criteria for the classification of damage.

<table>
<thead>
<tr>
<th>Class</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| Class 0 | Must not have any of the following:  
  - Water damage  
  - Visible mold  
  - Moldy odor  
  - Water/mold damage history |
| Class 1 | Must have at least one indication from the list above  
  (visible mold should be < 0.2 m²). |
| Class 2 | Must have visible mold.  
  - Mold alone in the entire room ≥ 0.2 m²  **OR**  
  - Combined area of visible mold + water damage on the same surface ≥ 0.2 m² |
### Table 1-2. Home characteristics and their prevalence (n=777 homes).

<table>
<thead>
<tr>
<th>Home characteristics</th>
<th>Categories</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong> (direct source or carrier of allergens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mold classification</td>
<td>0</td>
<td>336</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>403</td>
<td>51.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>37</td>
<td>4.8</td>
</tr>
<tr>
<td>Appearance of cockroaches</td>
<td>Yes</td>
<td>89</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>688</td>
<td>88.5</td>
</tr>
<tr>
<td>Number of cats</td>
<td>0</td>
<td>607</td>
<td>80.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>76</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>73</td>
<td>9.7</td>
</tr>
<tr>
<td>Number of dogs</td>
<td>0</td>
<td>521</td>
<td>68.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>179</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>56</td>
<td>7.4</td>
</tr>
<tr>
<td>Number of residents</td>
<td>≤ 4</td>
<td>529</td>
<td>68.1</td>
</tr>
<tr>
<td></td>
<td>&gt; 4</td>
<td>248</td>
<td>31.9</td>
</tr>
<tr>
<td>Number of stuffed toys in the PAR†</td>
<td>0</td>
<td>325</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>≥ 1</td>
<td>443</td>
<td>57.7</td>
</tr>
<tr>
<td>Number of plants in the PAR†</td>
<td>0</td>
<td>564</td>
<td>74.2</td>
</tr>
<tr>
<td></td>
<td>≥ 1</td>
<td>196</td>
<td>25.8</td>
</tr>
<tr>
<td>Relative humidity in the PAR† (%)</td>
<td>&lt; 30</td>
<td>109</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>30-50</td>
<td>587</td>
<td>75.6</td>
</tr>
<tr>
<td></td>
<td>≥ 50</td>
<td>81</td>
<td>10.4</td>
</tr>
<tr>
<td>Temperature in the PAR† (ºF)</td>
<td>&lt; 75</td>
<td>433</td>
<td>55.7</td>
</tr>
<tr>
<td></td>
<td>≥ 75</td>
<td>344</td>
<td>44.3</td>
</tr>
<tr>
<td>Season of dust sample collection</td>
<td>Spring</td>
<td>133</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>240</td>
<td>30.9</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>271</td>
<td>34.9</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>133</td>
<td>17.1</td>
</tr>
<tr>
<td><strong>Group 2</strong> (indirect source or control factors of allergens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insect extermination</td>
<td>Yes</td>
<td>263</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>514</td>
<td>66.1</td>
</tr>
<tr>
<td>Distance of PAR from the kitchen (ft)</td>
<td>≤ 14</td>
<td>308</td>
<td>47.1</td>
</tr>
<tr>
<td></td>
<td>&gt; 14</td>
<td>346</td>
<td>52.9</td>
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<tr>
<td>Cloth dryer vented in the living quarter</td>
<td>Yes</td>
<td>15</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>762</td>
<td>98.1</td>
</tr>
<tr>
<td>Humidifier use</td>
<td>Yes</td>
<td>444</td>
<td>57.4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>329</td>
<td>42.6</td>
</tr>
<tr>
<td>Dehumidifier use</td>
<td>Yes</td>
<td>127</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>639</td>
<td>83.4</td>
</tr>
<tr>
<td>Floor material of the PAR†</td>
<td>Carpeted</td>
<td>709</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td>Non-carpeted</td>
<td>68</td>
<td>8.8</td>
</tr>
<tr>
<td>Vacuuming frequency</td>
<td>≤ 1 time/week</td>
<td>286</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>2 time/week – 1 time/day</td>
<td>371</td>
<td>47.8</td>
</tr>
<tr>
<td></td>
<td>≥ 1 time/day</td>
<td>120</td>
<td>15.4</td>
</tr>
<tr>
<td>Vacuum type</td>
<td>Standard</td>
<td>474</td>
<td>69.1</td>
</tr>
<tr>
<td></td>
<td>HEPA</td>
<td>212</td>
<td>30.9</td>
</tr>
<tr>
<td><strong>Group 3</strong> (general housing characteristics)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area of home (ft²)</td>
<td>&lt; 1000</td>
<td>162</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>1000-2000</td>
<td>270</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td>2000-3000</td>
<td>181</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>3000-4000</td>
<td>106</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>≥ 4000</td>
<td>58</td>
<td>7.5</td>
</tr>
<tr>
<td>Type of the PAR†</td>
<td>Busy room*</td>
<td>705</td>
<td>90.7</td>
</tr>
<tr>
<td></td>
<td>Bedroom</td>
<td>72</td>
<td>9.3</td>
</tr>
<tr>
<td>Heating method</td>
<td>Forced air heating only</td>
<td>391</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>386</td>
<td>49.7</td>
</tr>
<tr>
<td>A/C method</td>
<td>Windows open</td>
<td>171</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>A/C unit only</td>
<td>603</td>
<td>77.9</td>
</tr>
<tr>
<td>Dust amount per unit sampling area (g/m²²)</td>
<td>≤ 0.3</td>
<td>388</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.3</td>
<td>389</td>
<td>50.1</td>
</tr>
</tbody>
</table>

Note: For some homes, data were not available.

† PAR: Primary activity room.

* Busy room: living room, family room, kitchen, dining room.
### Table 1-3. Multiple linear regression model of allergen levels on home characteristics.

<table>
<thead>
<tr>
<th>Home Characteristics</th>
<th>Altern ( \mu g/g )</th>
<th>Altern ( \mu g/m^2 )</th>
<th>Cat ( \mu g/g )</th>
<th>Cat ( \mu g/m^2 )</th>
<th>Dog ( \mu g/g )</th>
<th>Dog ( \mu g/m^2 )</th>
<th>Mite ( \mu g/g )</th>
<th>Mite ( \mu g/m^2 )</th>
<th>Roach ( \mu g/g )</th>
<th>Roach ( \mu g/m^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of cats</td>
<td></td>
<td>↑↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>Y&gt;N</td>
<td>Y&gt;N</td>
<td></td>
</tr>
<tr>
<td>Presence of dogs</td>
<td>↑↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cockroach appearance</td>
<td></td>
<td>↑↓</td>
<td>N&gt;Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mold class</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y&gt;N</td>
<td>Y&gt;N</td>
<td></td>
</tr>
<tr>
<td>No. of residents</td>
<td></td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of plants in the PAR(^\d)</td>
<td></td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative humidity in the PAR(^\d)</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature in the PAR(^\d)</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season of sample collection(^#)</td>
<td></td>
<td></td>
<td>F&gt;SP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SU&gt;W</td>
<td></td>
<td>F&gt;SP</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insect extermination</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance of PAR(^\d) from the kitchen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dryer use (vented inside living quarter)</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehumidifier</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y&gt;N</td>
<td></td>
</tr>
<tr>
<td>Floor type of PAR(^\d) (Carpet vs. Non-carpeted)</td>
<td></td>
<td>↑↓</td>
<td>C=N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuuming frequency</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum type (HEPA vs. Standard)</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S&gt;H</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area of home</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of PAR(^\d) (Busy room vs. Bedroom)</td>
<td></td>
<td>↑↓</td>
<td>BS&gt;BD</td>
<td>BS&gt;BD</td>
<td></td>
<td></td>
<td></td>
<td>BD&gt;BS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/C method (windows Open vs. Closed)</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heating method (forced air use)</td>
<td></td>
<td>↑↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dust amount per unit area</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^\d\) PAR: Primary activity room.

\(^\#\) Season: F (fall), SP (spring), SU (summer), W (winter).

↑ Positive linear relationship.

↓ Negative linear relationship.

↑↓ Non-linear relationship.
### Table 2-1. Test of pressure drop of different types of filter for air sampling.

<table>
<thead>
<tr>
<th>Filters</th>
<th>Porosity (%)</th>
<th>Pressure drop (H₂O in)</th>
<th>Average pressure drop (H₂O in)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Button sampler only with a metal mesh support</td>
<td>NA</td>
<td>0.426</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.000</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>PTFE 1.0 µm</td>
<td>NA</td>
<td>41.232</td>
<td>42.768</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.755</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>46.868</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>42.215</td>
<td></td>
</tr>
<tr>
<td>PTFE 2.0 µm</td>
<td>NA</td>
<td>5.847</td>
<td>4.454</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.657</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>PC 1.0 µm</td>
<td>15.7</td>
<td>22.001</td>
<td>20.677</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.682</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.769</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.257</td>
<td></td>
</tr>
<tr>
<td>PC 2.0 µm</td>
<td>6.3</td>
<td>29.290</td>
<td>27.787</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.537</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.110</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.210</td>
<td></td>
</tr>
<tr>
<td>PC 3.0 µm</td>
<td>14.0</td>
<td>4.968</td>
<td>3.696</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.942</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.429</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.443</td>
<td></td>
</tr>
<tr>
<td>MCE 1.2 µm</td>
<td>NA</td>
<td>15.627</td>
<td>14.328</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.565</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.094</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.025</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2-2. Allergen concentrations and the correlation between air and dust samples (n=165).

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Airborne allergen Concentration (Airconc, µg/m³)</th>
<th>Dustborne allergen</th>
<th>Correlation between air and dust samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM</td>
<td>CV</td>
<td>&lt;LLOD (% of samples)</td>
</tr>
<tr>
<td>Allergen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternaria</td>
<td>58.17</td>
<td>0.94</td>
<td>32.73</td>
</tr>
<tr>
<td>Cat</td>
<td>0.12</td>
<td>3.65</td>
<td>67.27</td>
</tr>
<tr>
<td>Dog</td>
<td>10.24</td>
<td>3.34</td>
<td>29.70</td>
</tr>
<tr>
<td>Dust mite</td>
<td>0.10</td>
<td>1.61</td>
<td>97.58</td>
</tr>
<tr>
<td>Cockroach</td>
<td>0.0008</td>
<td>0.98</td>
<td>86.67</td>
</tr>
</tbody>
</table>

GM: Geometric mean.  
CV: Coefficient of variance.  
LLOD: lower limit of detection.  
R: Correlation coefficient.
### Table 4-1. Relative risk (RR) of persistent wheezing in relation to home and family characteristics.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Unadjusted RR</th>
<th>Adjusted RR&lt;sup&gt;(3)&lt;/sup&gt;</th>
<th>95%CI&lt;sup&gt;(4)&lt;/sup&gt;</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mold class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>330</td>
<td>1.24</td>
<td>1.27</td>
<td>0.83-1.93</td>
<td>0.27</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>3.31</td>
<td>2.83</td>
<td>1.24-6.46</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>HDM&lt;sup&gt;(1)&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ LLOD&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>395</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOD-2 µg/g</td>
<td>132</td>
<td>1.12</td>
<td>1.12</td>
<td>0.68-1.85</td>
<td>0.65</td>
</tr>
<tr>
<td>≥2 µg/g</td>
<td>110</td>
<td>1.15</td>
<td>1.16</td>
<td>0.68-1.98</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20,000</td>
<td>549</td>
<td>0.48</td>
<td>0.49</td>
<td>0.30-0.81</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Note: For some homes, allergen data were not available.

<sup>(1)</sup> HDM: house dust mite.

<sup>(2)</sup> LLOD: lower limit of detection.

<sup>(3)</sup> Adjusted for all independent variables in the model, mold class, HDM, and income.

<sup>(4)</sup> 95%CI: 95% of confidence interval.
Table 4-2. Relative risk (RR) of atopic persistent wheezing in relation to home and family characteristics.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Unadjusted RR</th>
<th>Adjusted RR$^{(3)}$</th>
<th>95%CI$^{(4)}$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mold class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>168</td>
<td>1.48</td>
<td>1.41</td>
<td>0.70-2.85</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>203</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>6.60</td>
<td>6.78</td>
<td>2.13-21.60</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>HDM$^{(1)}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ LLOD$^{(2)}$</td>
<td>241</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOD-2 µg/g</td>
<td>75</td>
<td>0.98</td>
<td>0.98</td>
<td>0.42-2.31</td>
<td>0.97</td>
</tr>
<tr>
<td>≥2 µg/g</td>
<td>69</td>
<td>1.40</td>
<td>1.50</td>
<td>0.67-3.34</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20,000</td>
<td>338</td>
<td>0.91</td>
<td>0.98</td>
<td>0.38-2.54</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Note: For some homes, allergen data were not available.

$^{(1)-(4)}$ The same as in Table 4-1.
Table 4-3. Relative risk (RR) of allergic persistent wheezing in relation to home and family characteristics.

<table>
<thead>
<tr>
<th>Molds class</th>
<th>n</th>
<th>Unadjusted RR</th>
<th>Adjusted RR$^{(3)}$</th>
<th>95% CI$^{(4)}$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>164</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>194</td>
<td>1.29</td>
<td>1.29</td>
<td>0.54-3.11</td>
<td>0.57</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>7.70</td>
<td>8.53</td>
<td>2.36-30.81</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HDM$^{(1)}$</th>
<th>≤ LLOD$^{(2)}$</th>
<th>234</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HDM-2 µg/g</td>
<td>71</td>
<td>0.68</td>
<td>0.72</td>
<td>0.23-2.29</td>
<td>0.58</td>
</tr>
<tr>
<td>HDM-2 µg/g</td>
<td>66</td>
<td>1.34</td>
<td>1.68</td>
<td>0.64-4.37</td>
<td>0.29</td>
</tr>
</tbody>
</table>

| Income      | < 20,000       | 91  | 0.64            | 0.73          | 0.25-2.14| 0.57    |
|-------------|---------------|-----|----------------|---------------|---------|
| ≥ 20,000    | 549           | 0.64| 0.73            | 0.25-2.14     | 0.57    |

Note: For some homes, allergen data were not available.

$^{(1)}$-(4) The same as in Table 4-1.
Table 4-4. Relative risk (RR) of skin prick test positivity to aeroallergens [SPT(+)-aero] in relation to home and family characteristics.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Unadjusted RR</th>
<th>Adjusted RR(^{(3)})</th>
<th>95%CI(^{(4)})</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mold class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>330</td>
<td>0.84</td>
<td>0.82</td>
<td>0.54-1.24</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>1.79</td>
<td>1.90</td>
<td>0.82-4.41</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>HDM(^{(1)})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ LLOD(^{(2)})</td>
<td>395</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOD-2 µg/g</td>
<td>132</td>
<td>1.07</td>
<td>1.10</td>
<td>0.66-1.83</td>
<td>0.71</td>
</tr>
<tr>
<td>≥2 µg/g</td>
<td>110</td>
<td>1.14</td>
<td>1.20</td>
<td>0.70-2.05</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20,000</td>
<td>549</td>
<td>0.98</td>
<td>1.05</td>
<td>0.59-1.87</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Note: For some homes, allergen data were not available.

\(^{(1)}\)-(\(^{(4)}\)) The same as in Table 4-1.
Table 4-5. Relative risk (RR) of skin prick test positivity to mold [SPT(+)mold] in relation to home and family characteristics.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Unadjusted RR</th>
<th>Adjusted RR (3)</th>
<th>95% CI (4)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mold class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>330</td>
<td>1.65</td>
<td>1.76</td>
<td>0.92-3.38</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.57</td>
<td>0.58</td>
<td>0.07-4.57</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>HDM (1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ LLOD (2)</td>
<td>395</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOD-2 µg/g</td>
<td>132</td>
<td>0.80</td>
<td>0.80</td>
<td>0.37-1.74</td>
<td>0.58</td>
</tr>
<tr>
<td>≥2 µg/g</td>
<td>110</td>
<td>0.52</td>
<td>0.52</td>
<td>0.20-1.38</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20,000</td>
<td>549</td>
<td>0.89</td>
<td>0.83</td>
<td>0.36-1.94</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Note: For some homes, allergen data were not available.

(1)-(4) The same as in Table 4-1.
Figure 2-1. Correlation of the level of *Alternaria* allergen between airborne concentration (µg/m³) and a) dustborne concentration (µg/g) and b) dustborne loading (µg/m²).
Figure 2-2. Correlation of the level of *Alternaria* allergen between air and dust samples in homes with different floor types. Airborne concentration (µg/m³) and dustborne concentration (µg/g) in homes with a) non-carpeted floor and b) carpeted floor. Airborne concentration (µg/m³) and dustborne loading (µg/m²) in homes with c) non-carpeted floor and d) carpeted floor.
Figure 3-1. Experimental set-up for the release and collection of fungal fragments.
Figure 3-2. Three different designs of the humidification chambers. The upper panel illustrates vertical cross-sectional view of the three chambers, and the lower panel shows horizontal cross-sectional view of them.
Figure 3-3. The effect of particle bounce preventing methods on particle size distribution. The particle size distribution of aerosolized *A. versicolor* particles as measured by the ELPI with DS-515 coating on the impactor stages (○), humidification of the incoming air (△), and ZEF-X10 coating of the impactor stages (■). Each data point represents the mean ± SD of three repeats.
Figure 3-4. The particle concentration (○; left y-axis) and induced current (□; right y-axis) measured by the ELPI for aerosolized particles of three fungal species: a) *S. chartarum*, b) *A. versicolor*, and c) *P. melinii*. ZEF-X10 was used as the coating material on the ELPI impactor stages. Each data point represents the mean ± SD of three repeats.
Figure 3-5. The ELPI measurement vs. microscopic counting. The particle concentration measured by the ELPI (○; left y-axis) and the number of spores collected onto each ELPI impactor stage counted under a microscope (■; right y-axis) for aerosolized particles of three fungal species: a) *S. chartarum*, b) *A. versicolor*, and c) *P. melinii*. The ELPI data are the same as in Figure 3-4.
Figure 3-6. Modeling the deposition of fragments and spores (%) into different regions of the respiratory tract for one hour of exposure. ET1 indicates the anterior nasal region; ET2 indicates the main extrathoracic region; BB indicates the bronchial region; bb indicates the bronchiolar region; AI indicates the alveolar interstitial region; ND indicates not deposited particles. The total and fractional depositions were calculated for an adult male and a 3-month-old infant.
Figure 3-7. The ratio of respiratory deposition of fragments to that of spores (F/S) for fungal particles of a) *S. chartarum* and b) *A. versicolor*.