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Dustborne *Alternaria alternata* antigens in U.S. homes: Results from the National Survey of Lead and Allergens in Housing

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Abstract

Background: *Alternaria alternata* is one of the most common fungi associated with allergic disease. However, *Alternaria* exposure in indoor environments is not well characterized.

Objective: The primary goals of this study were to examine the prevalence of *Alternaria* exposure and identify independent predictors of *Alternaria* antigen concentrations in U.S. homes.

Methods: Data for this cross-sectional study were obtained from the National Survey of Lead and Allergens in Housing. A nationally representative sample of 831 housing units in 75 different locations throughout the U.S. completed the survey. Information on housing and household characteristics was obtained by questionnaire and environmental assessments. Concentrations of *Alternaria* antigens in dust collected from various indoor sites were assessed with a polyclonal anti-*Alternaria* antibody assay.

Results: *Alternaria* antigens were detected in most (95-99%) of the dust samples. The geometric mean concentration, reflecting the average *Alternaria* concentration in homes, was 4.88 μg/g (SE=0.13 μg/g). In the multivariable linear regression analysis, the age of the housing unit, geographic region, urbanization, poverty, family race, observed mold and moisture problems, use of dehumidifier, and presence of cats and dogs were independent predictors of *Alternaria* antigen

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Abbreviations used:

NIEHS National Institute of Environmental Health Sciences

NSLAH National Survey of Lead and Allergens in Housing

PSU Primary sampling unit

MSA Metropolitan Statistical Area

ANOVA Analysis of Variance

concentrations. Less frequent cleaning and smoking indoors also contributed to higher *Alternaria* antigen levels in homes.

Conclusion: Exposure to *Alternaria alternata* antigens in U.S. homes is common. Antigen levels in homes are not only influenced by regional factors but also by residential characteristics. Preventing mold and moisture problems, avoiding smoking indoors, and regular household cleaning may help reduce exposure to *Alternaria* antigens indoors.

Keywords

Alternaria alternata; allergen; antigen; indoor; exposure; asthma; allergy

INTRODUCTION

Exposure to the fungus *Alternaria alternata* is an important risk factor for asthma and allergic rhinitis.[1–5] Severe asthma and acute, sometimes life-threatening exacerbations of asthma have been associated with *Alternaria* sensitivity and increased airborne concentrations of *Alternaria* spores.[6–10]

Alternaria spores are common aeroallergens in many regions of the world, especially in warm inland climates, but also in arid regions.[8,9,11] *Alternaria* exposure is often assessed by outdoor spore counts, because most intense exposure is likely to occur outdoors.[12–14] Nonetheless, fungal spores can enter a home from outdoor air via ventilation or infiltration, or they can be carried in by occupants.[15,16] Infiltration may have less importance for *Alternaria* spores because of their large size (23-34 μm x 7-10 μm).[17] The indoor environment may also become a secondary source of exposure, if fungal spores colonize interior or building materials.[15,16] Although indoor fungal levels tend to reflect the levels found outdoors, housing characteristics and occupants' behavior can affect exposure levels considerably.[15,16,18,19]

Because of the complexity of fungal exposure assessment, few studies have assessed exposure to *Alternaria* or other fungal allergens in indoor environments.[20] Fungal allergen extracts have largely remained uncharacterized and non-standardized, unlike other common allergens derived from cat, dog, dust mite, cockroach, or pollens.[21,22] Exposure to fungal allergens is traditionally estimated by indirect methods, considering spores as indicators of the presence of allergens.[23] However, allergen content in spores may vary and fungal allergens may also be carried by means other than intact spores (e.g. hyphael fragments).[24–26] Therefore, spore counts may not accurately reflect allergen exposure levels. Recent advances in molecular biology and immunology have facilitated progress in qualifying and quantifying fungal allergens, especially *Altermaria* allergens.[16,22,27] The National Survey of Lead and Allergens in Housing (NSLAH) is the first population-based study that measured antigenic components of *Alternaria alternata*, including allergens, in U.S. homes using a polyclonal anti-*Alternaria* antibody assay.

This article presents nationally representative estimates of dustborne *Alternaria alternata* antigen levels at multiple household sites and identifies independent predictors of *Alternaria* antigen concentrations in U.S. homes.

METHODS

Study data

The data for this study were collected as part of the National Survey of Lead and Allergens in Housing (NSLAH). This cross-sectional study, which was conducted from 1998 to 1999 by the NIEHS and the U.S. Department of Housing and Urban Development, used a complex, multistage design to sample the U.S. population of permanently occupied, non-institutional

housing units that permit children. The study protocol was approved by the NIEHS Institutional Review Board in 1998. The sampling frame of 1,404 primary sampling units (PSUs) consisted of Metropolitan Statistical Areas (MSAs), counties, or groups of counties. MSAs included areas with a large population nucleus and adjacent communities having a high degree of economic and social integration with the area. Every area in the 50 states and the District of Columbia was assigned to a PSU. A nationally representative random sample of housing units was drawn from 75 randomly selected primary sampling units. In all, 831 housing units were surveyed. A detailed description of the methodology for the survey has been previously published.[28]

At each home, a trained interviewer obtained information on housing characteristics and the occupants' household via questionnaire. A copy of the questionnaire can be found on-line at http://www.niehs.nih.gov/airborne/research/risk.html. Environmental data were also acquired by inspection and sample collection. Detailed, well-defined protocols for all aspects of data and sample collection were utilized throughout the study.[28] Briefly, single surface dust samples were collected from a bed (all bedding layers, pillow, and mattress or mattress cover), a sofa or a chair, and from bedroom, living room, and kitchen floors as previously described.[28] Vacuumed dust samples were collected using a Eureka Mighty-Might® 7.0-ampere vacuum cleaner (Eureka Company, Bloomington, Illinois) modified to collect dust into a 19 × 90 mm cellulose extraction thimble (Whatman International, Ltd., England). Each sampling site was vacuumed for 5 minutes. For bedding samples, all bedding layers were vacuumed for a total of 2.5 minutes, the primary sleeping pillow for 30 seconds, and the mattress or mattress cover for 2 minutes.

At the laboratory, dust samples were sieved through 425 μm pore grating and divided into 100 mg aliquots of fine dust. Dust aliquots were extracted in borate buffered saline (pH 8.5), 2 ml per 100 mg dust extracted. After extracts were centrifuged, supernantants were decanted and stored at -20°C. Concentrations of the *Alternaria alternata* antigens were measured with a competitive inhibition enzyme-linked immunosorbent assay (ELISA) using a commercially prepared polyclonal rabbit anti-*Alternaria* antibody (Greer Laboratories, Inc., Lenoir, North Carolina; lot# ZA4-4L) and *Alternaria* antigen standard (Greer Laboratories, Inc., Lenoir, North Carolina; lot# XPM1-X10).[20,24] Briefly, antigen standard at 1 μg/ml in bicarbonate buffer, pH 9.6, was added to 96-well Immunlon 4HBX plates (VWR Scientific) overnight at 4°C. Unbound antigen was washed away (with phosphate buffered saline, pH 7.4) and the plate was blocked with bovine serum albumin. After washing, anti-*Alternaria* antibody, along with either dilutions of unknown samples or dilutions of the antigen standard, were combined in the wells and incubated overnight at 4°C. Unbound material was washed away and peroxidase-labeled goat anti-Rabbit IgG (Sigma Chemical, St. Louis, Missouri) was added to the wells and incubated for 1 hour. Excess antibody was washed away and substrate added; color change was measured kinetically at 405 nm using an OptiMax plate reader (Molecular Devices, Sunnyvale, California). Reaction rates of the unknown sample were plotted against those of the antigen standard to determine concentration. Optimal assay dilutions were determined empirically using dilution matrices. The assay detects major *Alternaria* antigens, including the most common allergen, Alt a1.[29] For most samples, the lower limit of detection of the assay was 0.14 μg/g/sieved dust.

Statistical analyses

Statistical analyses were conducted using SUDAAN (Version 8.0, Research Triangle Institute, Research Triangle Park, NC), and Taylor series linearization methods were used to adjust standard errors for the complex survey design. Sample weights were applied to all estimates to account for housing selection probabilities, non-response, and poststratification. A detailed description of statistical weighing for the NSLAH is described elsewhere.[28]

In the statistical analysis, *Alternaria* antigen concentrations were log-transformed because the distributions were skewed to the right. Samples with concentrations less than the detection limit were assigned one-half of the value of the detection limit. Samples having insufficient amount of dust for analysis were considered missing (see Fig 1). We used Spearman rank correlation coefficients to evaluate associations between antigen concentrations. We calculated a house index (i.e. the mean of all sampling location concentrations) to represent the average *Alternaria* antigen concentration in the household.

Descriptive statistics of *Alternaria alternata* antigen concentrations were generated. Median and mean (geometric) concentrations were estimated for each level of selected household characteristics. Comparisons of the log-transformed means were assessed with ANOVA using Wald's F statistics. Characteristics in Tables I and II with p-values less or equal to 0.25 were selected to multivariable linear regression. In the data driven modeling approach, backward elimination strategy was used for model selection. All remaining predictors in the final model had p-values less than or equal to 0.05. For each level of independent predictor, geometric mean of *Alternaria* antigen concentration was computed by adjusting for other predictors in the model.

RESULTS

Prevalence and distribution of *Alternaria* antigen levels in U.S. homes

The majority ($\geq$ 95%) of the dust samples had detectable levels of *Alternaria* antigens. Figure 1 shows a statistical summary of *Alternaria* antigen concentrations in U.S. homes. Spearman rank correlation coefficients between *Alternaria* concentrations at the five sampling locations ranged from 0.16 (bedroom bed vs. kitchen floor) to 0.47 (living room floor vs. upholstery). Correlations between *Alternaria* concentrations at each sampling locations and the house index (which reflects the average *Alternaria* antigen concentration across all sampling locations in a home) were between 0.59 and 0.73 ($p<0.0001$). In our analyses, we used the house index as our primary exposure measure.

Alternaria antigen levels and demographic, household, and behavioral characteristics

We investigated associations between *Alternaria* antigens and various demographic factors by comparing the geometric mean concentration of the house index across levels of the characteristics presented in Table I. Higher *Alternaria* antigen concentrations were present in older homes, homes in the Midwest and South census regions, non-urban homes, single family homes, owner occupied homes, homes in impoverished census areas, homes inhabited by white individuals, and homes inhabited by individuals with less education. In contrast to most of the demographic characteristics, the presence of children and the number of occupants were not associated with higher concentrations of *Alternaria* antigens. Table II shows geometric mean concentrations (house index) by additional housing and behavioral characteristics that are thought to be associated with antigen levels. Concentrations of *Alternaria* antigens were considerably lower ($p=0.003$) in homes that used forced air heating systems or radiators as the main heating source than in homes which used other heating sources (e.g. kerosene space heaters, wood burning stoves/fireplaces). *Alternaria* antigen concentrations were consistently higher in homes where either residents or the field team observed signs of mold or moisture related problems, such as mold or water stains, musty or mildew odor, or dampness in the home ($p<0.001$). The use of a dehumidifier was strongly associated with higher *Alternaria* levels ($p=0.001$). The presence of cats or dogs in the home was also associated with higher *Alternaria* antigen levels ($p=0.016$). Smoking indoors increased mean antigen concentrations significantly ($p=0.027$). Antigen concentrations increased with increasing smoking frequency.

We also examined associations between *Alternaria* antigen levels and the household characteristics in each sampling location separately because some of the characteristics are site-specific. Less frequent cleaning was associated with higher *Alternaria* antigen levels in all locations. In particular, *Alternaria* concentrations were significantly ($p<0.05$) higher in living rooms where the floor cleaning frequency was less often than weekly. *Alternaria* levels were also higher in beds where bedding had not been washed within the past week ($p=0.01$). Type of flooring affected *Alternaria* levels differently depending on the site. *Alternaria* concentrations were significantly higher in kitchens with carpeting than in kitchens without carpeting ($p=0.001$). On the contrary, carpeting in bedrooms predicted much lower *Alternaria* antigen concentrations in beds ($p=0.004$).

Independent predictors of *Alternaria* antigen levels in U.S. homes

Multivariable linear regression was used to identify independent predictors of higher mean concentrations of *Alternaria* antigens in U.S. homes. The following predictors remained in the final model: construction year, region, degree of urbanization, poverty, family race, signs of mold and moisture problems, use of dehumidifier, and presence of cats or dogs in the home. The results for the house index are summarized in Figure 2, which shows adjusted mean concentrations of the independent predictors.

DISCUSSION

This survey is the first study that assessed dustborne *Alternaria alternata* antigen concentrations in the U.S. housing stock. *Alternaria* antigens were present in virtually all homes. Both regional and residential characteristics influenced *Alternaria* antigen concentrations. Based on our data driven prediction model, the age of the housing unit, census region, degree of urbanization, poverty, race of residents, observed mold and moisture problems in the home, use of dehumidifier, and presence of cats and dogs contributed independently to *Alternaria* levels. Our results suggest that antigens of *Alternaria* may deposit in house dust from various sources and via multiple mechanisms.

Regional factors were strongly associated with *Alternaria* levels. Homes in non-metropolitan statistical areas had significantly higher *Alternaria* antigen concentrations than homes in metropolitan statistical areas. *Alternaria alternata* is a ubiquitous saprophyte that is found in the soil and on plants, especially on decaying vegetation.[12,30,31] Therefore, *Alternaria* antigen levels likely reflect the abundance of local vegetation. The regional variation in *Alternaria* antigen concentrations was also substantial; antigen concentrations were clearly higher in Midwestern and Southern homes. Region is considered as an important determinant for fungal concentrations, both indoors and outdoors.[32] Our results are consistent with previous findings that *Alternaria* spores are plentiful in grain-growing areas in the Midwest.[6]

Alternaria antigen concentrations were significantly higher in older homes than in newer homes. Because new homes are more tightly built than older ones, fewer spores may be able to penetrate to indoor environments. Furthermore, newer homes are more likely to be better equipped to provide improved control of environmental factors (e.g. temperature, humidity) indoors. Of the demographic factors, race and poverty had the greatest influence on *Alternaria* levels, both white race and low income contributing consistently to higher *Alternaria* levels in U.S. homes.

Mold and moisture related problems in the home were strongly associated with higher *Alternaria* antigen levels. Although outdoor air is considered the dominant source for indoor fungal spores, indoor sources may increase *Alternaria* antigen levels considerably.[15,16] Fungal growth indoors is influenced by various environmental factors. However, the most important factor controlling fungal growth is water availability.[33] Water leaks, defective drainage,

inadequate ventilation and moisture condensation resulting from faulty thermal insulation, and heating, cooling and ventilation (HVAC) systems have been major contributors to moisture related fungal problems in buildings.[34,35] Prolonged high relative humidity has been shown to increase both dust- and airborne fungal populations in indoor environments.[36-38] *Alternaria* antigen concentrations were significantly higher in homes that used dehumidifiers. The presence of a dehumidifier is likely an indication of an ongoing humidity or moisture problem, although dehumidifiers may also become reservoirs for fungi. In this study, increased humidity per se was not associated with *Alternaria* antigen levels (data not shown). However, this is not necessarily surprising because relative humidity was measured at only one point in time. Dehumidifier use may reflect long-term humidity levels or water availability better than a single relative humidity measurement.

Outdoor allergenic spores such as those of *Alternaria* can be carried on residents' hair, skin, clothing, or shoes as well as on their pets' fur. Chew and coworkers found that the presence of a dog increased fungal populations in floor dust.[39] Our results agree with their findings. The presence of dogs was associated with higher *Alternaria* concentrations especially in living room floor and upholstery dusts. Dogs may spend more time in living rooms than in other rooms in the home. In our data, the presence of cats was also associated with higher *Alternaria* antigen levels.

Predictors of *Alternaria* antigen concentrations may vary by location because the activities of occupants, humans and pets, can affect each location in the home differently. For example, having carpeting in bedrooms predicted lower *Alternaria* antigen concentrations in beds, whereas kitchens with carpeting had significantly higher *Alternaria* antigen levels than kitchens without carpeting. Carpeting in the kitchen may provide favorable environment for fungal growth because of availability of nutrients (e.g. food and beverage residues). Frequent cooking may also result in higher temperature and humidity levels in kitchens. While carpeting may reduce particle resuspension, possibly explaining the reduced levels in the beds of carpeted bedrooms, it can be a reservoir or an amplification site for fungi.[19,39] Furthermore, the presence of children predicted higher *Alternaria* antigens levels in beds ($p=0.015$) but not in floor or upholstery dusts (data not shown). Children are more likely exposed to potential outdoor sources of the fungus than adults, because they usually spend more time outdoors than adults.[40] Spores and fungal fragments could be carried into bed on children's hair and/or clothing. Although indoor smoking did not reach statistical significance in the final model for the house index, it was a strong predictor for higher *Alternaria* levels in bed dust. Smoking was also significantly ($p=0.03$) associated with higher *Alternaria* antigen levels in living rooms where smoking most likely occurs.[41]

Less frequent cleaning contributed to higher *Alternaria* antigen levels in floor and upholstery dusts, especially in living rooms. Correspondingly, levels of *Alternaria* antigens in beds were lower if bedding was washed more frequently. Washing temperature (cold, warm, hot) did not influence antigen levels (data not shown).

In cross-sectional studies, the temporal sequence of cause and effect cannot necessarily be determined. It is possible that the results from a dust sampling conducted at a single point in time may not represent exposure throughout the entire year because fungal exposure is prone to temporal, particularly seasonal, and spatial variations.[37,39] However, settled dust samples are often used as surrogate measures for long-term exposure because they are considered less influenced by temporal and spatial variability than air samples.[42,43] Sampling in the study was conducted throughout summer, fall, and winter months in each geographic region in order to capture the seasonal variability in the data. We acknowledge that one limitation of the study is that we cannot determine the variablity in *Alternaria* antigen exposure in different geographic regions in the U.S. because of limited number of homes in each region. Although our approach

does not allow for assessments of seasonal variability in antigen levels in individual homes, this is the most cost-effective method of sampling for a large-scale national survey that requires in-person home visits. For other common indoor allergens, measurement of allergen concentration in reservoir dust has generally been used as the standard index of exposure. The presence of missing values is another limitation of the study. Insufficient amount of dust to assay *Alternaria alternata* antigens contributed most to the missing values. We maximized the number of samples in our analyses by using imputed values for the samples that had concentrations less than the detection limit. To evaluate potential bias, we conducted our analyses excluding the imputed values. The final prediction model remained the same in both analyses.

Although monoclonal antibody-based assays are more sensitive and specific for a single allergenic protein, for example for Alt a 1, an important advantage of a polyclonal assay is that it captures the allergen variability that is characteristic to fungal allergen exposure. Allergenic fungi, including *Alternaria alternata*, express great variability in allergen profiles depending on the environmental conditions under which they grow.[24, 29] For example, Barnes et al.[24] observed substantial discrepancies in GP70 and Alt a1 levels over time when these two glycoproteins were measured in air samples. The life cycle of fungi can also affect the different patterns of allergen release; germination has been shown to increase allergen release from *Alternaria* spores.[44] We acknowledge that polyclonal antibody-based assays have their disadvantages; polyclonal antibodies are directed only toward antigens recognized by source species, they can be variable in composition and potency, and cross-reactivity is possible.[16] Because there is a finite supply of the polyclonal antibody that we used, it may not be possible to reproduce the findings of this study in the future. However, some previous studies that have used monoclonal antibody-based assays have had difficulties to detect *Alternaria* allergens in environmental samples,[27] even among populations where *Alternaria* sensitivity and exposure to *Alternaria* spores are known to be common.[27, 45, 46] Furthermore, *Alternaria* species - sensitive subjects elicit positive skin test reactions to other *Alternaria* allergens,[47, 48] not only to Alt a 1.

The major strength of this study is that the survey sample is nationally representative. The weighted characteristics of the survey sample, including distributions of housing characteristics, socioeconomic, and demographic factors, did not differ significantly from nationwide characteristics obtained from other national surveys,[28] which strengthens the external validity of our findings. To improve the internal validity, quality assurance was integrated into all components of the study; sampling procedures, data collection and analysis followed detailed, well-defined protocols.[28] The NSLAH is the first study to estimate levels of *Alternaria* antigens in the U.S. housing stock. The NSLAH data provide valuable new information on exposure to *Alternaria* antigens in indoor environments, although further validation of fungal immunoassays is warranted in order to determine clinically relevant exposure levels in the future. Immunoassays that are used to assess fungal allergen exposures have not yet achieved the same reliability as have similar assays for other allergens.[21]

In conclusion, *Alternaria* antigens are commonly detected in U.S. homes. Antigen levels are influenced not only by regional and housing characteristics but also by residents' behavior. The age of the housing unit, census region, urbanization, poverty, family race, signs of mold and moisture problems, use of dehumidifier, and presence of cats and dogs can affect *Alternaria* antigen levels significantly. In addition, the frequency of cleaning activities in the home contributes to *Alternaria* antigen concentrations. Smoking indoors may also increase *Alternaria* antigen levels in home environments. Preventing mold and moisture related problems, avoiding indoor smoking, and regular household cleaning could potentially lower *Alternaria* antigen concentrations in homes.

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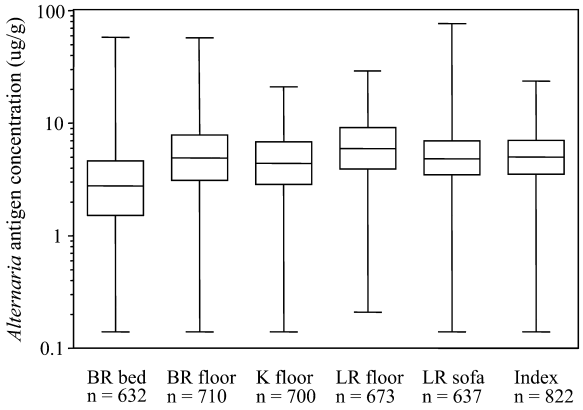

FIG 1.

Distributions of *Alternaria* antigen concentrations in U.S. homes. Box plots display the minimum and maximum values and the 25th, 50th, and 75th percentiles. Bedroom is abbreviated as BR, kitchen as K, and living room as LR. Index refers to the house index.

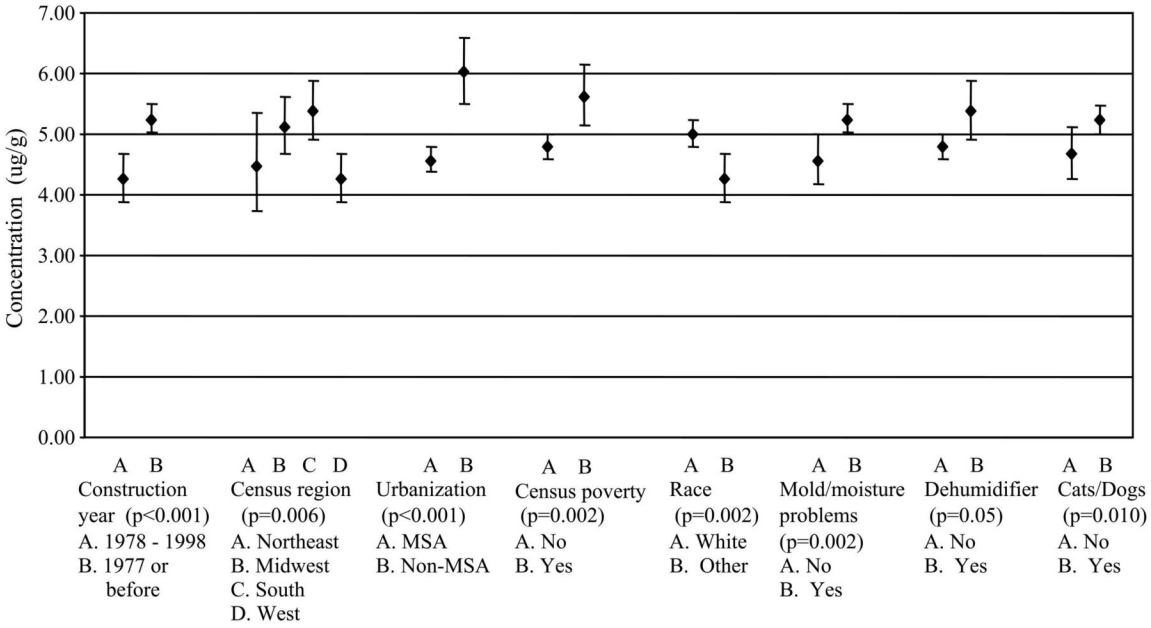

FIG 2.

Adjusted mean (geometric) concentrations of *Alternaria* antigens (μg/g) for the house index (N=822) and 95% confidence intervals for levels of the independent predictors identified in the multivariable linear regression model. MSA indicates Metropolitan Statistical Area.

Table I.

Geometric means of *Alternaria* concentrations (house index[*]) by demographic characteristics

Characteristic	Number of homes	GM (SE)[†] (μg/g)	p-value[‡]
Total	822	4.88 (0.13)	--
Construction year			<0.001
1978-1998	216	4.21 (0.17)	
1977 or earlier	606	5.22 (0.17)	
Census region			<0.001
Northeast	151	4.59 (0.46)	
Midwest	195	5.42 (0.30)	
South	276	5.21 (0.22)	
West	200	4.06 (0.19)	
Urbanization			<0.001
MSA[§]	684	4.53 (0.14)	
Non-MSA	138	6.16 (0.20)	
Housing unit type			<0.001
Multi-family	124	4.01 (0.24)	
Single family	698	5.04 (0.15)	
Tenure			0.036
Renter occupied	282	4.58 (0.17)	
Owner occupied	537	5.01 (0.16)	
Number of persons in the household			0.870
1-3	543	4.89 (0.14)	
4-9	279	4.85 (0.24)	
Children (< 18 years)			0.188
No	423	4.97 (0.16)	
Yes	396	4.74 (0.16)	
Census poverty			0.002
No	643	4.76 (0.16)	
Yes	136	5.67 (0.26)	
Race			<0.001
White	603	5.10 (0.16)	
Other	219	4.11 (0.16)	
Education			0.008
Above high school	553	4.67 (0.17)	
High school or less	255	5.38 (0.19)	

[*] House index is the mean of the sample location concentrations

[†] GM indicates geometric mean, (SE) standard error of the mean

[‡] Wald F-test on difference of means across levels of the characteristic

[§] Metropolitan Statistical Area

Table II.

Geometric means of *Alternaria* concentrations (house index) by allergen-related housing and behavioral characteristics

Characteristic	Number of homes	GM (SE)* (μg/g)	p-value†
Total	822	4.88 (0.13)	--
Main heating source			0.003
Gas/Electric forced air	556	4.68 (0.15)	
Radiator	74	4.77 (0.56)	
Other	189	5.64 (0.24)	
Air filtration device used			0.547
No	702	4.87 (0.15)	
Yes	101	5.04 (0.27)	
Mold or moisture problems‡			<0.001
No	398	4.43 (0.15)	
Yes	424	5.38 (0.18)	
Dehumidifier in the home			0.001
No	676	4.70 (0.15)	
Yes	130	5.87 (0.34)	
Cats or dogs currently			0.016
No	455	4.66 (0.18)	
Yes	359	5.17 (0.15)	
Windows/doors kept open in the past month			0.920
No	132	4.91 (0.23)	
Yes	685	4.88 (0.14)	
Season			0.682
Summer	285	4.90 (0.26)	
Fall	344	4.75 (0.23)	
Winter	193	5.05 (0.28)	
Smoking inside the home			0.027
No smoking indoors	536	4.61(0.19)	
Light smoking§	75	4.89 (0.34)	
Heavy smoking‖	208	5.60 (0.28)	

*GM indicates geometric mean, (SE) standard error of the mean

†Wald F-test on difference of means across levels of the characteristic

‡Assessed by observation (occupants, field team)

§Tobacco products smoked indoors < 4 times a day

‖Tobacco products smoked indoors 4 or more times a day