

ENVIRONMENTAL DETERMINANTS

Allergens in Household Dust and Serological Indicators of Atopy and Sensitization in Detroit Children with History-Based Evidence of Asthma

ANN HOUSTON WILLIAMS, PH.D.,^{1,*} JAMES TRAVIS SMITH, PH.D.,¹ EDWARD E. HUDGENS, B.S.,¹
SCOTT RHONEY, M.S.P.H.,¹ HALÛK ÖZKAYNAK, PH.D.,² ROBERT G. HAMILTON, PH.D., D.ABMLI.,³
AND JANE E. GALLAGHER, PH.D.¹

¹*Epidemiology Branch, National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC, USA.*

²*National Exposure Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC, USA.*

³*Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.*

Background. Home exposure to allergens is an important factor in the development of sensitization and subsequent exacerbations of allergic asthma. We investigated linkages among allergen exposure, immunological measurements, and asthma by examining (1) reservoir dust allergen levels in homes, (2) associations between presence of allergens in homes and sensitization status of resident children, and (3) associations between asthma status and total IgE, atopy (by Phadiatop), and positive allergen-specific tests. **Methods.** The study protocol was approved by Institutional Review Boards (IRBs) of the University of North Carolina Chapel Hill; Westat, Inc.; and the US Environmental Protection Agency Human Research Protocol Office. Data were collected from questionnaires, serum analyses, and household vacuum dust. Children ($n = 205$) were predominately African American (AA) (85.4%) and 51.6% were asthmatic. Sera from 185 children and home dust samples ($n = 141$) were analyzed for total and specific IgE antibodies to allergens from cat and dog dander, cockroach, dust mites, mice, rats, and molds. **Results.** Sixty percent of the homes had detectable levels of three or more dust allergens. The proportions of children with positive allergen-specific IgE tests were dust mite (32%), dog (28%), cat (23%), cockroach (18%), mouse (5%), rat (4%), and molds (24–36%). Children testing positive to a single allergen also had positive responses to other allergens. Those children with positive serum tests for cat, dog, and dust mite lived in homes with detectable levels of cat (51%), dog (90%), and dust mite (Der f 1) (92%) allergens. Correlations between children's specific IgE levels and dust levels were linearly related for dog ($p < .04$), but not for cat ($p = .12$) or dust mite (Der f 1) ($p = .21$). Odds ratios (95% CI) for the associations between asthma and serum-specific IgE were over 1.0 for cat, dog, dust mite (Der f 1), cockroach, and four types of molds. House dust allergen exposure levels, however, exhibited no differences between asthmatic and non-asthmatic homes. **Conclusions.** Both the co-occurrence of multiple allergens in dust and the high frequency of multiple allergen sensitizations indicate that a broad-based intervention aimed at reducing multiple allergens (pets, pests, and molds) would be more successful than any approach that aimed at reducing one type of allergen.

Keywords Immunological measurements, IgE, home exposure, intervention

INTRODUCTION

Asthma is an increasingly important health problem worldwide, especially among children. In the United States, the Centers for Disease Control and Prevention estimate that between 2001 and 2008 asthma increased by 23% in the total population and by 14% among children aged 5–14 years (1). In addition to public health concerns, this increase has economic costs which are estimated to have increased from \$6.2 to \$10.7 billion during the first half of the 1990s (2). Allergic disease, which includes allergic asthma, has been shown to be influenced by diet, environmental, and genetic interactions (3). Because environmental factors (e.g., allergen exposure, infection, chemicals, drugs, physical factors) contribute to an increase in asthma incidence, a significant proportion of disease may be preventable through alteration of the environment and reduced exposure (3). In order to

understand the role that allergens play in the sensitization and exacerbation of asthma in children who spend a large proportion of time indoors, the relationships between indoor air exposures, both at home and in school environments, and asthma, need to be further examined (4).

Thus, the relationships between indoor air quality and human respiratory disease pose a fundamental concern especially for children's health. Numerous studies have examined this question by studying the associations between one or a limited number of indoor allergens and asthma prevalence and severity in children (5–7). Reductions in indoor air quality result from the presence of common allergens that are produced by cats and dogs, cockroaches, mice and rats, dust mites, and mold (6). The proteins associated with these allergens exist as individual particles or they become associated with dust particles in the residential environment (7,8).

This article reports results from a portion of the Mechanistic Indicators of Childhood Asthma (MICA) study that was conducted in the Detroit, Michigan area during fall to early winter 2006–2007. MICA is a cross-sectional study based on a stratified sample of children using two strata: children with asthma and

*Corresponding author: Ann Houston Williams, Ph.D., National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC 27711, USA; Tel: +919 843 4833; Fax: +919 966 0655; E-mail: williams.annh@epa.gov

children without asthma, selected in an approximately 1:1 ratio (9). A total of 205 children aged 9–13 years old participated in the clinical study.

We investigated linkages among the levels of eight indoor allergens using reservoir household dust ($n = 141$) as a specimen with evidence of sensitization based on serum IgE antibody measurements and the clinical history of asthma. Specifically, we compared (1) dust allergen levels in the homes of asthmatic versus non-asthmatic children, (2) the proportion of homes with detectable dust allergens versus the proportion of subjects with detectable allergen-specific IgE levels, (3) associations between concentrations of dust allergens and allergen-specific IgE in the children's serum, and (4) the likelihood of a concomitant history-based asthma diagnosis and allergen sensitization as identified by the presence of allergen-specific IgE antibodies in serum.

METHODS

Study Subjects

The study protocol was approved by the IRBs of the University of North Carolina Chapel Hill School of Medicine; Westat, Inc.; and by the US Environmental Protection Agency (US EPA) Human Research Protocol Office. The parent or guardian of each child provided written consent before the child's participation in this study.

This study was conducted between October 2006 and January 2007 in Detroit, Michigan. Asthmatic and non-asthmatic children 9–12 years of age were identified from the respondents to a children's respiratory health questionnaire (9) and recruited by staff of the Henry Ford Health System. Two hundred and five subjects agreed to participate. Of these children, 51.6% had been previously diagnosed by a clinician as asthmatic as indicated on a respiratory health questionnaire. Eighty-seven percent of the asthmatic subjects were current asthmatics (e.g., reported having asthma symptoms within the previous 12 months) and 86% of the asthmatic subjects were currently taking asthma medications either occasionally or daily.

Collection and Allergen-Specific Analysis of Dust Samples

Subject-owned vacuum cleaners were used by the parents/guardians of participants to collect house dust. The vacuum bags represented collections that ranged from days to weeks. Parents/guardians were asked to categorize their vacuum dust sample as representing collections of (1) 1 day or less; (2) less than 1 week, but more than 1 day; (3) more than 1 week, but less than 1 month; (4) more than 1 month; and (5) I do not know. Vacuum cleaner bags were brought to clinic on the day of the child's visit. Vacuum dust samples were transferred into Ziploc bags and shipped at ambient temperature overnight to EPA (Research Triangle Park, NC, USA). Vacuum dust samples were weighed upon arrival and stored at ambient temperatures. Within 1

month, dust samples were delivered to Research Triangle International (Research Triangle Park, NC, USA) for sieving. Clean nylon 300 μm sieves were equipped with clean polypropylene bottom collection pans and lids. This sieve assembly was placed in a W.S. Tyler RX-86 Sieve Shaker (W.S. Tyler, Mentor, OH, USA) and sieved for at least 5 minutes or until the entire sample was processed. One-gram samples were sent to the Johns Hopkins University Dermatology, Allergy and Clinical Immunology Laboratory for extraction and allergen analysis. Extraction of dust was performed by mixing 100 mg of the fine dust overnight at room temperature with 2 mL of borate-buffered saline containing 5% bovine serum albumin. Extracts were then clarified by centrifugation and supernatants were decanted and stored at -20°C . The dust samples were analyzed for levels of cat (Fel d 1) and dog (Can f 1) epithelial dander allergens, cockroach allergens (Bla g 1 and Bla g 2), house dust mite (*Dermatophagoides pteronyssinus* (Der p 1), *Dermatophagoides farinae* (Der f 1)) allergens, mouse, and rat urine protein allergens (Mus m 1 and Rat n 1, respectively) following the methods described in Wood et al. (10) and Hamilton (11).

The mite allergens were assayed by ELISA (enzyme-linked immunosorbent assay) for (Der p 1) and (Der f 1) (12). Samples were assayed for (Fel d 1), (Can f 1), and (Bla g 1) using two-site monoclonal antibody-based ELISA (13–15). Dust pellets remaining after extraction and any remaining extract were stored at -70°C . Results are reported in $\mu\text{g/g}$ fine dust for (Der p 1), (Der f 1), (Fel d 1), and (Can f 1) and U/g of dust for (Bla g 1) and (Bla g 2). The lower limits of detection were as follows: 0.1 $\mu\text{g/g}$ for (Der p 1) and (Der f 1), 0.12 $\mu\text{g/g}$ for (Fel d 1) and (Can f 1), 0.25 $\mu\text{g/g}$ for (Mus m 1) and (Rat n 1), and 0.6 U/g for (Bla g 1) and (Bla g 2). However, because of limited amounts of dust in some samples, (Der f 1) and (Der p 1) assays had lower limits of detection that ranged from 0.05 to 0.425 $\mu\text{g/g}$. Concentrations less than the lower limits of detection were considered non-detectable.

Quality control of allergen levels in dust was performed in each assay as described by Pate et al. (16) using characterized dust specimens.

Total and Allergen-Specific IgE Analysis

Whole blood (2 mL) was collected into EDTA-containing Vacutainer tubes (BD, Franklin Lakes, NJ, USA) and a complete blood count with differentials was performed by the Henry Ford Health System clinical laboratory. Serum specimens were prepared by collecting whole blood (8 mL) in a Vacutainer serum clot, red/gray tube (BD) for each child. Serum was separated following centrifugation, and a 1.7 mL aliquot of serum was frozen at -80°C and sent to Johns Hopkins University School of Medicine Dermatology, Allergy and Clinical Immunology Reference Laboratory. Total IgE and a multi-allergen screen for allergen-specific IgE antibody specific for a panel of allergens (Phadiatop) were quantified in the ImmunoCAP250 autoanalyzer (Phadia, Kalamazoo, MI, USA). The total serum IgE analysis was calibrated to

the World Health Organization's second international reference preparation for human IgE (75/502) (17,18). Tri-level control sera were analyzed in each assay to assess intra-assay and interassay variation. The working range of the total serum IgE assay was 2–2000 kU/L.

The multi-allergen screen (Phadiatop) is a single analysis that detects the presence (positive/negative) and measures the relative level of IgE antibody specific for a panel of 10 common allergens. Phadiatop levels ≥ 0.35 kUa/L were used in this study to indicate the presence of IgE antibody and provide a general assessment of atopic status.

Each subject's serum was tested for IgE antibody to the same allergen specificities as measured in surface dust. Allergen-specific IgE antibodies were measured against dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), German cockroach, mouse and rat urinary allergens, and cat and dog dander measured by the ImmunoCAP method (Phadia, Portage, MI, USA). Children were classified as sensitized (atopic) if they had specific IgE ≥ 0.35 IU/mL to any of the 11 allergen specificities tested or to a panel of 10 common allergens. Daily quality control was performed with Levey–Jennings plots for human sera that contained known amounts of IgE antibody.

Statistical Analysis

Summary statistics were calculated for all variables for the subject classes, asthmatic and non-asthmatic. Asthmatic status was determined by the parent's or guardian's response to the question "Has your child ever been diagnosed with Asthma?" Of the 205 children, 20 were excluded from these analyses based on lack of response to this question or a blood draw that was not achievable. These exclusions resulted in an overall sample size for blood analyses of 185 children. One hundred and eighty-five subject-collected vacuum dust samples were obtained from the total 205 subjects participating in the MICA study (90.2% participation rate). However, due to limited resources, only 150 vacuum samples of the total 185 dust samples were selected for dust allergen measurements. If analyses required looking at associations between both vacuum dust and allergen-specific IgE serum test results, the analyses included only those subjects who had both serum and dust samples. For example, of the 150 vacuum dust samples with allergen tests, only 141 of the vacuum dust samples had matching serum samples. To ascertain whether we introduced bias by the selection of the subset of vacuum dust samples from the full set, we used a stepwise logistic regression model; the dependent variable in the model was a flag variable (yes/no) to indicate whether the child had dust allergen measurements for the dust collection analysis reported in this article ($N = 141$) relative to the 185 dust samples that we had collected. The candidate predictors included in the model were asthma status (based on questionnaire responses), age, race, and gender; we found no evidence that the selection of the subset of

dust samples, relative to the full dust sample set, was biased relative to asthma status, age, race, or gender.

Summary statistics were calculated based on raw values and did not include values that were below detection (i.e., imputed values). The percent detected is reported for each variable.

Comparative analyses between asthmatics and non-asthmatics were performed using imputed values for measurements below the detection limit. For this study we used a value of one-half the detection limit as the imputed value. Comparative analyses were conducted using a *t*-test of the logged values and were repeated using the non-parametric Wilcoxon signed-rank test. The results did not differ between test types and the more conservative non-parametric test results are reported here. Correlation analyses were computed using logs of measured values. Measurements below the detection limit were excluded. Odds ratios and 95% confidence intervals were calculated using the detection limit value of the measurement in question. The exceptions to this were the analyses of Phadiatop in which the data for Figure 3 were analyzed by quartiles and the total serum IgE where the median value (96.9 kU/L) was used as the cutoff. Phadiatop was analyzed in quartiles: Q1 (1.39–6.22 kUa/L), Q2 (6.8–22.8 kUa/L), Q3 (23.6–51.1 kUa/L), and Q4 (53.2–101 kUa/L), with each quartile being compared individually with subjects with Phadiatop levels below the detection limit (0.35).

RESULTS

Environmental Dust Allergen Levels and Sensitization

Racial and ethnic information was provided by 192 (93.6%) of the 205 children recruited to this study. The children were predominately male (59.9%) and African American (AA; 85.4%) and 9.4% white only (without other race or ethnicity heritage). The mean age of the participants was 10.59 ± 1.37 years. Of these children, 51.6% reported a previous asthmatic diagnosis as indicated on a respiratory health questionnaire. Of the asthmatic children, 87.9% were AA, 63.6% were male, and 5.1% were identified as either mixed race (both AA and white races reported) or other (e.g., Asian, Hispanic, Native American).

Of the 185 children providing blood samples, 64% the children tested positive (≥ 0.35 kU/L) for IgE antibody to a panel of allergens (Table 1, Figure 1A). Prevalence of sensitization to specific allergens can be deduced from the proportion of children's serum samples with allergen-specific IgE levels above detection limits. Detectable levels of rat allergen-specific IgE were found in only 4.3% of the children's sera, while the most commonly detected allergen-specific IgE specificity was the mold, *Alternaria alternata*, found in 35.7% of children's serum specimens. Increasing frequencies of other allergen-specific IgEs were as follows: mouse (5.4%), cockroach (18.4%), cat dander (22.7%), *Cladosporium herbarum* (24.3%), *Penicillium notatum* (27%), dog dander (28.1%), *Dermatophagoides pteronyssinus* (31.4%),

TABLE 1.—Summary statistics for allergen-specific serum IgE and allergen-specific dust levels for asthmatic and non-asthmatic children and their homes.

Serum (kUa/L)	N	Percent detected	Mean	Standard deviation	Geometric mean	P-value (Wilcoxon test)
Total serum IgE	185	100	281.51	509.83	99.5	.0049
Asthmatics	96	100	338.89	442.62	130.77	
Non-asthmatics	89	100	219.63	569.67	71.10	
Phadiatop	185	64.3	31.37	29.81	17.72	.0056
Asthmatics	96	69.8	37.34	31.20	20.41	
Non-asthmatics	89	58.4	23.16	25.98	9.67	
Pets						
Cat (Fel d 1)	185	22.7	10.35	20.03	3.25	.0021
Asthmatics	96	31.3	12.12	22.82	3.73	
Non-asthmatics	89	13.5	5.93	9.60	2.30	
Dog (Can d 1)	185	28.11	7.89	18.33	2.90	.0004
Asthmatics	96	38.5	10.05	21.27	2.96	
Non-asthmatics	89	16.9	2.56	4.26	1.22	
Household pests						
Dust mite (Der f 1)	185	31.9	20.34	25.32	5.96	.0987
Asthmatics	96	38.5	17.90	26.83	4.50	
Non-asthmatics	89	24.7	24.44	22.54	9.55	
Dust mite (Der p 1)	185	31.4	13.06	17.28	4.32	.1555
Asthmatics	96	36.5	11.82	17.50	3.53	
Non-asthmatics	89	25.8	14.94	17.17	5.86	
German cockroach	185	18.4	4.03	5.90	2.11	.0362
Asthmatics	96	24.0	4.63	6.94	2.32	
Non-asthmatics	89	12.4	2.78	2.50	1.73	
Mouse	185	5.4	15.26	31.37	2.85	.0710
Asthmatics	96	8.3	18.04	34.92	2.73	
Non-asthmatics	89	2.2	4.13	3.45	3.33	
Rat	185	4.3	4.38	8.88	1.59	.1863
Asthmatics	96	6.3	5.35	10.28	1.70	
Non-asthmatics	89	2.2	1.45	0.83	1.32	
Molds						
<i>Alternaria alternata</i>	185	35.7	14.45	15.22	8.34	.0037
Asthmatics	96	45.8	15.33	16.20	8.30	
Non-asthmatics	89	24.7	12.69	13.21	8.40	
<i>Aspergillus fumigatus</i>	185	32.4	5.27	7.07	2.38	.0003
Asthmatics	96	42.7	6.63	7.90	3.29	
Non-asthmatics	89	21.3	2.34	3.47	1.18	
<i>Cladosporium herbarum</i>	185	24.3	4.04	4.18	2.61	<.0001
Asthmatics	96	36.5	4.34	4.20	2.92	
Non-asthmatics	89	11.2	2.98	4.15	1.75	
<i>Penicillium notatum</i>	185	27.0	3.29	3.95	1.81	<.0001
Asthmatics	96	39.6	3.75	4.20	2.17	
Non-asthmatics	89	13.5	1.85	2.70	1.02	
Dust	N	Percent detected	Mean	Standard deviation	Geometric mean	P-value (Wilcoxon test)
Pets						
Cat (Fel d 1) (ng/g)	141	50.4	93,900	371,230	2,010	.8102
Asthmatics	74	48.6	61,315	200,199	2,732	
Non-asthmatics	67	52.2	127,417	490,092	1,467	
Dog (Can d 1) (ng/g)	141	78.7	28,803	101,934	1,040	.4067
Asthmatics	74	75.7	18,799	38,058	1,017	
Non-asthmatics	67	82.1	38,989	139,575	1,064	
Household pests						
Dust mite (Der f 1) (ng/g)	141	83.0	7,028	17,250	1,691	.9209
Asthmatics	74	82.4	8,296	21,089	1,771	
Non-asthmatics	67	83.6	5,647	11,777	1,609	
Dust mite (Der p 1) (ng/g)	141	7.1	1,570	2,386	415	.5937
Asthmatics	74	8.1	2,087	2,887	634	
Non-asthmatics	67	6.0	794	1,363	220	
Cockroach 1 (U/g)	141	9.9	2.43	2.43	1.47	.7898
Asthmatics	74	10.8	2.1	3.09	1.04	
Non-asthmatics	67	9.0	2.87	1.27	2.34	
Cockroach 2 (U/g)	141	8.5	13.48	21.36	6.02	.6979
Asthmatics	74	9.5	17.01	27.99	5.56	
Non-asthmatics	67	7.5	8.52	5.16	6.74	
Mouse (ng/g)	141	53.2	1,570	6,542	110	.4667

(continued)

TABLE 1.—Continued

Dust	N	Percent detected	Mean	Standard deviation	Geometric mean	P-value (Wilcoxon test)
Asthmatics	74	56.8	1,207	3,649	112	
Non-asthmatics	67	49.3	2,033	9,028	108	
Rat (ng/g)	141	0	—	—	—	—
Asthmatics	74	0	—	—	—	
Non-asthmatics	67	0	—	—	—	
Molds						
Mold spores cfu/g	140	100	12,610	13,432	7,057	.9318
Asthmatics	74	100	13,048	13,397	6,877	
Non-asthmatics	66	100	12,219	13,542	7,221	

Notes: Means and standard deviations were calculated using only detectable levels; *N* = sample size. Data are presented for all measurements and separated by asthmatic and non-asthmatic children. *P*-values are for Wilcoxon non-parametric significance comparisons of dust and serum allergen-specific IgE levels between asthmatic and non-asthmatic children; cfu/g: Colony forming units/gram.

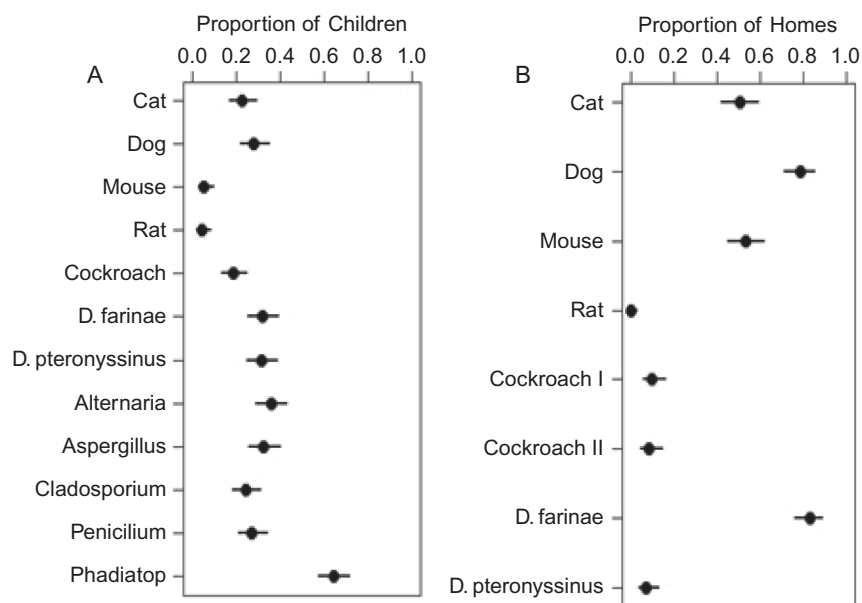


FIGURE 1.—(A) Mean proportions of children (95% CI) with positive allergen-specific IgE tests. (B) Mean proportions of homes (95% CI) that tested positive to cat (*Fel d 1*), dog (*Can f 1*), mouse (*Mus m 1*), rat, cockroach (*Bla g 1* and *Bla g 2*), and house dust mite (*Der f 1* and *Der p 1*).

Dermatophagoides farinae (31.9%), and *Aspergillus fumigatus* (32.4%), (Table 1, Figure 1A).

Vacuum dust samples for the 141 households were collected over the following time periods in the following proportions: 1 day or less (7.4%); less than 1 week, but more than 1 day (14.1%); more than 1 week, but less than 1 month (37.1%); more than 1 month (38.5%); and 2.0% of the parents were unsure of the length of time involved. Of the eight allergens tested in the vacuum dust samples, dust mite (*Der f 1*) and (*Der p 1*) were detectable in 83.0% and 7.1% of the homes, respectively; dog (*Can f 1*) in 78.7%; mouse (*Mus*) in 53.2%; cat (*Fel d 1*) in 50.4%; Cockroach (*Bla g 1* and *Bla g 2*) in 9.9% and 8.5%, respectively; and rat (0%) (Table 1, Figure 1B).

The number and the distribution of dust allergens and allergen-specific IgE serum levels were compared among asthmatic and non-asthmatic children and their households (Figure 2A and B). Over 35% of the asthmatics, as compared with 15% of the non-asthmatics,

tested positive to at least 5 of the 11 different allergens (Figure 2A). Approximately 60% of the asthmatic and non-asthmatic homes had 3 or more detectable dust allergens (Figure 2B).

The distributions of IgE antibody levels were significantly different between asthmatic and non-asthmatic children (Figure 2A), χ^2 test, *df* = 9, *p* < .0001). In particular, more asthmatics had greater than three serum-specific allergens than the non-asthmatics. This contrasts with the distribution of dust allergens in the asthmatic versus non-asthmatic homes that were not significantly different (Figure 2B, χ^2 test, *df* = 9, *p* = .13).

Table 1 presents the means, geometric means, and other related statistics describing the children's Phadiatop, total IgE, and allergen-specific IgE levels and the respective allergen levels of their vacuum dust samples. The data are subdivided by asthmatic versus non-asthmatic status and the mean data were computed using only those

values above the detection limit of the assays. Total serum IgE and Phadiatop levels (Table 1) were significantly greater ($p < .005$, Table 1) in asthmatic as compared with non-asthmatic children by about 50%. Asthmatic children exhibited significantly higher allergen-specific geometric mean antibody levels to cat (Fel d 1) ($p < .002$), dog (Can d 1) ($p < .0004$), German cockroach ($p < .036$), *Aspergillus* ($p < .0003$), *Cladosporium* ($p < .0001$), and *Penicillium* ($p < .0001$), while non-asthmatic children exhibited significantly higher geometric mean antibody levels to *Alternaria* ($p < .0037$).

The percentages of children exhibiting IgE antibody positivity to only one specific allergen were very small. These ranged from 0.5% of the children with a positive test for dog Can f 1 allergen to 5.9% of the

children sensitized to only one mold (*A. alternata*) (data not shown). More commonly, children were sensitized to multiple specific allergens (Figure 2A, Table 2). The highest percentages of multiple allergen sensitivity included 29.7% of the children found to be allergic to both house dust mites (Der p 1 and Der f 1) and 25.9–28.1% sensitized to combinations of two molds (*Penicillium* and *Aspergillus* or *Aspergillus* and *Alternaria*, respectively). These children typically were sensitized to up to eight dust allergens.

Relationships among IgE Levels and House Dust Allergen Levels

Total IgE levels and Phadiatop frequencies did not differ significantly in relation to age or gender (data not shown). Due to the small number of children's samples that exhibited both positive dust allergen and serum-specific IgE for rat, mouse, cockroach, and house dust mite (Der p 1) (i.e., $n = 0, 4$, or 5), we only examined relationships between dust allergens and children's specific IgE levels with larger sample sizes, for example, cat ($n = 17$), dog ($n = 34$), and house dust mite (Der f 1) ($n = 42$). A significant correlation between dust allergens and children's specific IgE levels was only observed for dog allergen (Table 3, $r = 0.35$, $p < .04$) but not for cat ($r = 0.39$, $p = .12$) or house dust mite (Der f 1) ($r = 0.196$, $p = .21$).

Positive cutoff levels for allergens in dust samples thought to represent an increased risk for sensitization (lower) and increased risk for asthma exacerbation (upper) were separately analyzed with regard to an association with asthma status of the children (Table 4).

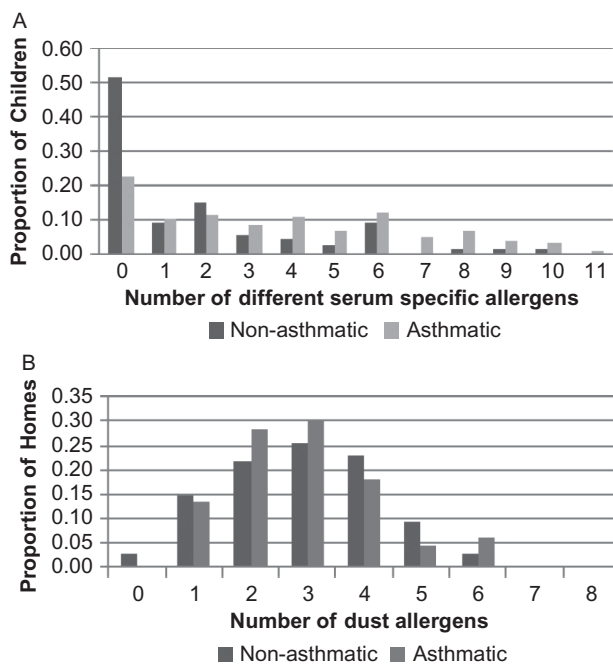


FIGURE 2.—Proportions of asthmatic and non-asthmatic children with a given number of allergen-specific antibodies in serum (A) and dust allergens in the home surface dust (B). The histograms show proportions of children with 1–11 different specificities of IgE antibody. In non-asthmatic children the proportion of serum samples with 8–10 allergens was significantly lower ($p < .011$) than for the asthmatic children.

TABLE 3.—Correlations between dust allergens and children's specific IgE levels.

Allergens	N	r	r ²	P
Cat	17	0.390	0.152	.1218
Dog	34	0.354	0.126	.0398
Dust mite (<i>D. farinae</i>)	42	0.196	0.038	.2138

Notes: N, sample size limited to those samples that had both positive tests for allergen dust and serum allergen-specific IgE levels. Bold p-value represents significance at $< .05$ for Pearson's correlation (r).

TABLE 2.—Total percentages of children with co-occurring serum antibody levels.

Serum	Cat	Dog	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Penicillium</i>	Rat	Mouse	Cockroach 1	Dust mite (Der f)	Dust mite (Der p)
Cat ($n = 43$)	—										
Dog ($n = 52$)	20.5%	—									
<i>Alternaria</i> ($n = 66$)	15.7%	18.4%	—								
<i>Aspergillus</i> ($n = 60$)	16.2%	20.0%	28.1%	—							
<i>Cladosporium</i> ($n = 46$)	14.6%	16.8%	24.3%	24.3%	—						
<i>Penicillium</i> ($n = 50$)	15.7%	18.4%	24.3%	25.9%	22.7%	—					
Rat ($n = 8$)	2.7%	3.2%	2.7%	2.2%	2.2%	2.2%	—				
Mouse ($n = 10$)	3.8%	4.9%	4.3%	3.8%	3.8%	3.8%	3.2%	—			
Cockroach 1 ($n = 34$)	8.1%	13.0%	9.7%	11.4%	8.1%	10.3%	2.2%	3.2%	—		
Dust mite (Der f) ($n = 59$)	14.6%	18.4%	12.4%	15.1%	11.9%	13.5%	3.8%	4.3%	14.1%	—	
Dust mite (Der p) ($n = 59$)	13.5%	17.8%	13.0%	15.7%	11.4%	13.5%	3.2%	3.8%	14.1%	29.7%	—

TABLE 4.—Proportions of children's homes exceeding either the lower or upper cutoff levels for allergenic sensitivity (lower) or exacerbation (upper) and odds ratio results for significance.

Dust	Cutoff levels	Percent above lower	Percent above upper	O/R* (lower cutoff)	O/R* (upper cutoff)
Cat	>1.0 and >8.0	22.0	13.5	0.886	0.603
Dog	>2.0 and >10.0	27.7	20.6	0.927	1.039
Cockroaches (2)	>1.0 and >4.0	8.5	6.4	0.772	1.412
Dust mites (2)	>2.0 and >10.0	41.8	12.8	0.886	0.508

Note: *Not statistically significant.

These lower and upper threshold levels have been established for environmental cat allergens (>1.0 and >8.0) (19,20), dog allergens (>2.0 and >10.0) (20,21), cockroach allergens (>1.0 and >4.0) (20,22), and house dust mite allergens (>2.0 and >10.0) (20,23). Threshold levels for both allergenic sensitization and exacerbation to house dust mite (both species summed) were exceeded in 54.6% of the homes sampled, in 48.3% of homes for dog allergen, and in 35.5% of homes for cat allergen (Table 4). Threshold levels for cockroach (summed group 1 and 2 specificities) were exceeded in only 14.9% of homes. These levels, however, did not approach significant associations with dust allergen levels for either sensitivity or exacerbation (Table 4, Odds Ratio test, $p > .05$).

In general, the proportions of dust samples from households that were above the allergen detection limit exceeded the proportions of households' health questionnaire reports of the presence of pets, mold, mice, or molds (data not shown). Dog allergen was found in 79% of dust samples while only 30% of these households reported ownership of dogs. Only 14% of households reported that cats were present in the home but 50.4% of the household dust samples indicated the presence of cat allergen. Parents indicated that mice were present in only 13% of their homes, but 53% of household dust samples tested positive for mouse. Those children with positive serum tests for cat, dog and house dust mite (Der f 1) lived in homes which also tested positive to allergens of cat (52%), dog (90%), and house dust mite (Der f 1) (92%) (Table 5).

Association between Asthma Status and Total IgE, Atopy and Serum Allergen-Specific IgE

Unadjusted odds ratios (95% CI) for the associations between asthma and serum-specific IgE were greater than 1.0 for cat, dog, house dust mite (Der f 1), cockroach, Phadiatop levels (at the third quartile), and total IgE levels

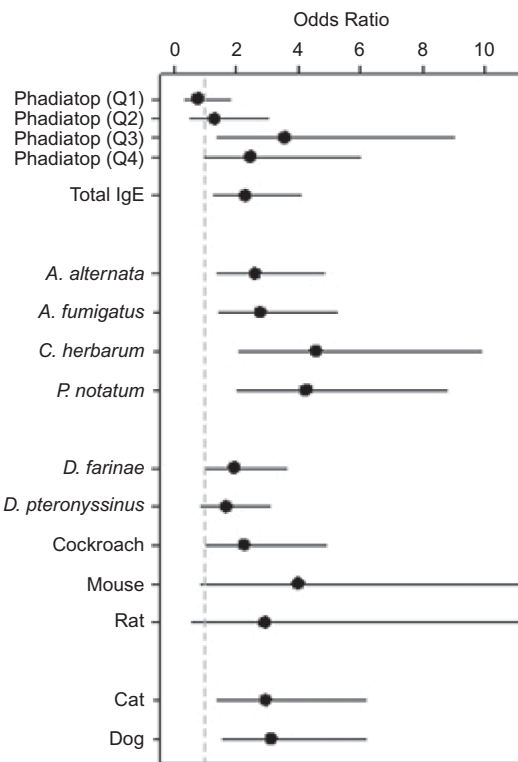


FIGURE 3.—Unadjusted odds ratios for asthma status (error bars denote 95% confidence intervals). Phadiatop was analyzed in quartiles, with each quartile being compared separately to the subjects with Phadiatop levels below the detection limit (≤ 0.35). Associations between serum allergen levels were statistically significant where the 95% confidence intervals do not cross the horizontal axis at 1.0 (Phadiatop Q3, all four allergen-specific molds, cat, and dog).

(using the cut off median IgE level) (Figure 3). The most significant associations with asthma status were found to be with levels of the molds, *C. herbarum*, *P. notatum*, *A. fumigatus*, and *A. alternata*, in descending order.

DISCUSSION

The US EPA recently conducted the MICA study to investigate genomics data, together with phenotypic measurements, to predict the key events associated with asthma severity or symptomology and bioindicators associated with these key events (24). Indoor and outdoor air pollution levels (NO_2 , volatile organic compounds, and polycyclic aromatic hydrocarbons (PAH)) monitoring (25) were collected for approximately 50.0% of the homes. Household vacuum dust metals and PAH were

TABLE 5.—Number and proportions of children's sera and homes with positive tests for both sera and dust allergens.

Allergens	Number of positive sera	Number of positive dust allergens	Positive sera and home dust allergen (%)
Cat	33	17	51.5
Dog	40	36	90.0
Dust mite (<i>D. farinae</i>)	48	44	91.6

also obtained (9). Because exposure to allergens is important in studies of allergic sensitization and the development and exacerbation of allergic asthma (26), we report here relationships and variations in the concentrations of household vacuum dust allergens (house dust mite, cockroach, cat, dog, mouse, and rat) and subject sensitization to these same allergen specificities.

The percentages of both asthmatic and non-asthmatic children with positive allergen-specific IgE antibody tests were mold (25–36%), house dust mite (31%), dog (28%), cat (23%), cockroach (18%), mouse (5.4%), and rat (4.3%). We compared our data to those of the National Cooperative Inner City Asthma Study (26) in which subjects drawn from large metropolitan areas were primarily AA (73.5%) with low average incomes ($\leq \$15,000/\text{year}$) and to the metropolitan Boston longitudinal birth cohort study of home allergens and asthma (27). In that study, 499 children came from a subject population that was largely white (74%) and whose parents, to a great extent (78%), were college educated. Although differing in demographic characteristics, our results are more similar to the metropolitan Boston population with the MICA children exhibiting higher frequencies of sensitivity to cats and dogs and a lower frequency of sensitization to cockroaches. Dust mite and cockroach allergens have been previously shown to have distinct and opposite associations with socioeconomic factors and population density (20).

Dust allergen levels were similar to those reported in other studies (4,28,29). House dust mite (Der f 1) was present in 83% of homes, dog allergen in 79%, mouse urine protein in 53%, and cat dander in 50%. The two allergens from cockroaches (group 1 and 2 allergens), when summed, were detectable in 18% of the home dust samples. Further, proportions of dust samples from households that were positive for allergens exceeded the proportions of households' health questionnaire reports of the presence of pets, mice, or molds. This is consistent with numerous studies that have found that children's homes may exhibit allergens from pets even when pets are not found in the homes or following removals of pets such as cats from homes (11,30). Several studies have found both cat and dog allergens in dust samples from schools at levels sufficient to cause low-grade clinical asthma (31–34) probably because of the ease of portability of these allergens on clothing. Thus, children who live in homes with pets may transfer pet allergens to school and then from the school buildings to homes of children who are not pet owners. It is possible also that previous owners or residents of the homes studied may have been pet owners. The high proportion of homes (83%) with house dust mite (Der f 1) allergen is somewhat surprising given that the vacuum dust sampling was not limited to the bedroom. In a study by Salo et al. (4), the bedroom floor, for example, exhibited approximately 2 times higher levels of dust mite as those from kitchen floors. The high proportion of children with positive dust mite serum tests suggests that exposure to dust mite allergen levels was underestimated.

Although not statistically significant, homes of asthmatics had lower levels of dog ($p = .41$) and cat ($p = .81$) dust allergens but higher levels of house dust mite ($p = .59$ and $p = .92$), cockroach ($p = .79$ and $p = .7$), and mouse ($p = .47$) allergens and generally higher levels of mold spores ($p = .93$). This difference in levels of pet versus pests and mold-derived allergens in asthmatic homes suggests that parents of asthmatic children generally avoid owning cats or dogs. It is not clear as to why there is a higher proportion of homes with pests and mold spores in asthmatic homes as compared with non-asthmatic homes but it may be because these allergens are more difficult to visually detect and reduce by remediation (35).

The only significant association between dust allergen levels and dust allergen sensitization was related to dog allergen exposures. These results are in contrast with results of Huss *et al.* (36) where the higher the level of allergen exposure to dust mite and cockroach, the more likely the patient was to have a positive allergy skin test. The lack of concordance in our study for all but dog allergens might be explained by the variations in the time period over which the dust vacuum samples were collected. For example, in our study the vacuum dust samples represented sampling ranging from several days to less than a month. Time of collection and concentration are important factors to capture in the period of sensitization. Accordingly, a short acute exposure to dust mites (due to, for instance, changing of bedding) might result in immediately elevated levels of dust mite allergen-specific IgE that, following the exposure, could then diminish to a non-detectable level.

Cockroach and dust mite allergens are typically found on larger particles that fall rapidly after disturbance while dander is on smaller particles ($<5 \mu\text{m}$) and may remain airborne for hours and is influenced by ventilation (37). Comprehensive estimates of airborne exposure, thus, require an element of disturbance of the home environment (e.g., vacuum cleaning, bed making), although the concentration of allergen on floors is thought to provide an indirect surrogate measurement of exposure (6). Alternatively, the weak associations (other than dog dander) could relate to the fact that the children spend considerable time indoors in school, an important additional source of allergen exposures. Thirty-six papers from the literature reported concentrations of cat, dog, dust mite, and/or cockroach allergens in settled school dust (37). The collection of the vacuum dust sample provides confirmation of the presence of allergens in the home, but associations between the concentrations of allergen and allergen sensitization could be optimized by sampling for a prescribed time period, and optimally, just prior to the blood draw. A cohort followed over time with repeat blood measures, as in a recent study by Wegienka et al. (35), is needed to understand the complex and temporal relationships between allergen exposure sensitization and the development and exacerbation of asthma.

With regard to the usefulness of using vacuum dust samples as surrogates for indoor short-term

environmental exposures to chemicals, recent studies have shown that house dust nicotine (a marker for environmental tobacco smoke) and house dust polychlorinated biphenyl concentrations may be useful surrogates of more persistent chemical exposures and, thus, may be useful in evaluating associations between chemical exposures and risk of childhood diseases (38,39). Our study, along with others where vacuum dust sampling either was subject-collected or represented vacuum sampling throughout the home (40–42), suggests that subject-collected dust samples should be considered as a cost-effective option for epidemiologic and clinical studies. The Department of Housing and Urban Development recently published a vacuum dust sample collection protocol that has flexibility in sampling techniques because studies often have differing research goals and/or may be resource limited (43).

Causal relationships have been reported in the literature (44) between exposure to the allergens produced by cats, cockroaches, and house dust mites, and exacerbations of asthma in sensitized individuals. There also is evidence of associations between dog and fungal allergen exposures with exacerbation of asthma in individuals specifically sensitized to these allergens. Considering these observations, we examined whether associations existed between asthma status and total IgE, atopy, and positive allergen-specific IgE antibody levels to dog, cat, cockroach, and dust mites. The third quartile Phadiatop measure, total IgE, and positive serum tests for all four molds, dog, cat, cockroach (Bla g 1), and house dust mite (Der f 1) were associated with asthma status. Positive serum tests for mouse were not associated with asthma status, possibly due to the low frequency of positive serum samples for this allergen. No associations between home dust allergen levels and asthma status were observed even when we restricted dust data to those above published threshold levels.

Studies have shown that multiple exposures to allergens increase the probability of a child having asthma symptoms (4). Multiple allergens detected in children's homes in this study were common. Sixty percent of the home dust samples had three or more dust allergens. These results are consistent with studies of Salo et al. (4), Leaderer et al. (20), and Eggleston (26). Salo et al. (4) looked at 831 housing units across the United States and reported that 51% of the homes had at least 6 detectable allergens and 45% of the homes had at least 3. In our study more than 95% of the children who had positive IgE antibody levels to a dust allergen also showed sensitization to multiple dust allergens.

As the rate of asthma among children is higher in AAs, this racial difference in prevalence cannot be accounted for by socioeconomic status alone (26). Two recent genome-wide association studies have confirmed a strong genetic component of asthma (45,46). As reported by Joubert et al. (47,48) we have begun to look at the integration of genetic (single nucleotide polymorphism) and environmental data from the MICA study, for investigations of childhood allergy and asthma.

CONCLUSIONS/KEY FINDINGS

The frequency of positive allergen-specific IgE levels increased with increasing levels of dog dander allergen in the home, but no such relationships could be identified for cat or house dust mite (Der f 1). A high frequency of homes containing multiple allergens also had a high frequency of subjects with positive IgE antibody to multiple allergens. Both the co-occurrence of multiple allergens in household dust and the high frequency of multiple allergen sensitizations indicate that a broad-based intervention aimed at reducing multiple allergens (pets, pests and molds) would be more successful than any approach aimed solely at reducing one type of allergen over another. Serum testing provides a sensitive, integrated measure of the child's allergic response to allergens and represents exposures to multiple allergens that occur in varied indoor environments.

The children in our study were predominately AA, a cohort of particular concern based on their higher prevalence of asthma. Our analyses were similar to other reported indoor dust and serum allergen studies, with the exception that we employed a simple, cost-effective subject-collected vacuum dust sample that was not standardized with regard to vacuum cleaner type, area sampled, or frequency of collection. There is a limited but growing body of literature that suggests that this simple vacuum dust collection may be a useful alternative in epidemiological and clinical studies. Furthermore, given the sufficient quantities of vacuum dust obtained, we are evaluating several potentially modifiable environmental exposures (metals and PAH) in the context of MICA's gene expression, genomics data, and other phenotypic measurements. Taken together with the indoor dust and serum allergen measures reported here, we hope to identify key genetic and phenotypic events that are associated with allergic responses and asthma phenotype.

ACKNOWLEDGMENTS

The authors are grateful to the parents, guardians, and children who participated in this study as well as the Henry Ford Health System, Detroit, Michigan, clinical teams, and laboratory staff. They also acknowledge Research Triangle Institute that conducted the dust sieving, and John Wisenauer and Katie A. Laugh at the Johns Hopkins University Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory who analyzed the total and allergen-specific IgE antibody levels. The authors also thank Drs. Michael Madden and Marsha Ward for their helpful comments and careful review of this manuscript.

Funding. The information in this document has been funded by the US EPA. It has been subjected to review by EPA's National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the agency.

DECLARATION OF INTEREST

The authors claim no conflicts of interest. The work was supported by EPA contracts #EP-D-07-109 to Westat, Inc.; #EP-D-07-009 to RTI; and #EP-D-06-114 to Johns Hopkins University—DACI Laboratory.

REFERENCES

1. CDC. Asthma – data and surveillance: National Health Interview Survey (NHIS) data. Centers for Disease Control and Prevention 2008. Available at: <http://www.cdc.gov/asthma/nhis/default.htm>. Accessed October 2, 2010.
2. Gergen PJ. Understanding the economic burden of asthma. *J Allergy Clin Immunol* 2001; 107(Suppl. 5):S445–S448.
3. Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Birth Defects Res B Dev Reprod Toxicol* 2008; 83(6):547–560.
4. Salo PM, Arbes SJ, Jr., Crockett PW, Thorne PS, Cohn RD, Zeldin DC. Exposure to multiple indoor allergens in US homes and its relationship to asthma. *J Allergy Clin Immunol* 2008; 121(3):678–684.
5. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitization, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357(9258):752–756.
6. Platts-Mills TAE. The role of indoor allergens in chronic allergic disease. *J Allergy Clin Immunol* 2007; 119(2):297–302.
7. Raja S, Xu Y, Ferro AR, Jaques PA, Hopke PK. Resuspension of indoor aeroallergens and relationship to lung inflammation in asthmatic children. *Environ Int* 2010; 36(1):8–14.
8. Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the third international workshop. *J Allergy Clin Immunol* 1997; 100(6):S2–S24.
9. Williams AH, Gallagher JE, Hudgens E, Johnson MM, Mukerjee S, Ozkaynak H, Neas LM. EPA Observational studies of children's respiratory health in Detroit and Dearborn, Michigan. ACE2009 Air & Waste Management Association's 102nd Annual Conference & Exhibition; June 16–19, 2009; Detroit, MI.
10. Wood RA, Eggleston PA, Lind P, Ingemann L, Schwartz B, Graveson S, Terry D, Wheeler B, Adkinson NF, Jr. Antigenic analysis of household dust samples. *Am Rev Respir Dis* 1988; 137(2):358–363.
11. Hamilton RG. Assessment of indoor allergen exposure. *Curr Allergy Asthma Rep* 2005; 5(5):394–401.
12. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p I and Der f I. *J Immunol Methods* 1989; 118(2):227–235.
13. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TA. Monoclonal antibodies to the major feline allergen Fel d I. II. Single step affinity purification of Fel d I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess Fel d I exposure. *J Immunol* 1988; 140(3):812–818.
14. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TA. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol* 1989; 83(5):875–882.
15. Schou C, Hansen GN, Lintner T, Lowenstein H. Assay for the major dog allergen, Can f I: investigation of house dust samples and commercial dog extracts. *J Allergy Clin Immunol* 1991; 88(6):847–853.
16. Pate AD, Hamilton RG, Ashley PJ, Zeldin DC, Halsey JF. Proficiency testing of allergen measurements in residential dust. *J Allergy Clin Immunol* 2005; 116(4):844–850.
17. Hamilton RG, Adkinson NF. Immunological tests for the diagnosis and management of human allergic disease: total and allergen-specific IgE and allergen specific IgG. In: Rose HR, de Macario EC, Fayey JL, Friedman H, Penn GM, eds. *Manual of Clinical Laboratory Immunology*. Washington, DC: American Society of Microbiology, 1997:881.
18. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol* 2010; 125(Suppl. 2):S284–S296.
19. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TA. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993; 147(3):573–578.
20. Leaderer BP, Belanger K, Triche E, Holford T, Gold DR, Kim Y, Jankun T, Ren P, McSharry JE, Platts-Mills TA, Chapman MD, Bracken MB. Dust mite, cockroach, cat, and dog allergen concentrations in homes of asthmatic children in the northeastern United States: impact of socioeconomic factors and population density. *Environ Health Perspect* 2002; 110(4):419–425.
21. Ingram JM, Sporik R, Rose G, Honsinger R, Chapman MD, Platts-Mills TA. Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among children living in Los Alamos, New Mexico. *J Allergy Clin Immunol* 1995; 96(4):449–456.
22. Gelber L, Seltzer L, Pollart S, Chapman M, Platts-Mills T. Specific IgE ab and exposure to cat and cockroach allergens as risk factors for acute asthma. *J Allergy Clin Immunol* 1991; 87(1 Pt. 2):233.
23. Platts-Mills TAE, de Weck AL, Aalberse RC, Bessot JC, Bjorksten B, Bischoff E, Bousquet J, Van Bronswijk JEMH, ChannaBasavanna GP, Chapman M, Colloff M, Goldstein RA, Guerin B, Hart B, Hong C-S, Ito K, Jorde W, Korsgaard J, Le Mao J, Miyamoto T, Lind P, Lowenstein H, Mitchell EB, Murray AB, Nolte D, Norman PS, Pauli G, Ranganath HR, Reed C, Reiser J, Stewart G, Turner KJ, Vervloet D, Wen T. Dust mite allergens and asthma – a worldwide problem. *J Allergy Clin Immunol* 1989; 83(2 Pt. 1):416–427.
24. Gallagher J, Hudgens E, Williams AH, Inmon J, Rhoney S, Andrews G, Reif D, Heidenfelder B, Neas L, Williams RM, Johnson M, Ozkaynak H, Edwards S, Cohen-Hubal E. Mechanistic indicators of childhood asthma (MICA) study: piloting an integrative design for evaluating environmental health. *BMC Publ Health* 2011; 11:344.
25. Johnson M, Hudgens E, Williams R, Andrews G, Neas L, Gallagher J, Ozkaynak H. A participant-based approach to indoor/outdoor air monitoring in community health studies. *J Expos Sci Environ Epidemiol* 2008; 19(5):492–501.
26. Eggleston PA. Environmental causes of asthma in inner city children. The National Cooperative Inner City Asthma Study. *Clin Rev Allergy Immunol* 2000; 18(3):311–324.
27. Kitch BT, Chew G, Burge HA, Muilenberg ML, Weiss ST, Platts-Mills TA, O'Connor G, Gold DR. Socioeconomic predictors of high allergen levels in homes in the greater Boston area. *Environ Health Perspect* 2000; 108(4):301–307.
28. Arbes SJ, Jr., Cohn RD, Yin M, Muilenberg ML, Friedman W, Zeldin DC. Dog allergen (Can f 1) and cat allergen (Fel d 1) in US homes: results from the National Survey of Lead and Allergens in Housing. *J Allergy Clin Immunol* 2004; 114(1):111–117.
29. Salo PM, Yin M, Arbes SJ, Jr., Cohn RD, Sever M, Muilenberg M, Burge HA, London SJ, Zeldin DC. Dustborne *Alternaria alternata* antigens in US homes: results from the National Survey of Lead and Allergens in Housing. *J Allergy Clin Immunol* 2005; 116(3):623–629.
30. van der Brempt X, Charpin D, Haddi E, da Mata P, Vervloet D. Cat removal and Fel d I levels in mattresses. *J Allergy Clin Immunol* 1991; 87(2):595–596.
31. Munir AKM, Einarsson R, Schou C, Dreborg SKG. Allergens in school dust: I. The amount of the major cat (Fel d I) and dog (Can f I) allergens in dust from Swedish schools is high enough to probably cause perennial symptoms in most children with asthma who are sensitized to cat and dog. *J Allergy Clin Immunol* 1993; 91(5):1067–1074.
32. Perry TT, Vargas PA, Bufford J, Feild C, Flick M, Simpson PM, Hamilton RG, Jones SM. Classroom aeroallergen exposure in Arkansas head start centers. *Ann Allergy Asthma Immunol* 2008; 100(4):358–363.
33. Almqvist C, Larsson PH, Egmar A-C, Hedrén M, Malmberg P, Wickman M. School as a risk environment for children allergic to cats and a site for transfer of cat allergen to homes. *J Allergy Clin Immunol* 1999; 103(6):1012–1017.

34. Anyo G, Brunekreef B, de Meer G, Aarts F, Janssen NAH, van Vliet P. Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children. *Clin Exp Allergy* 2002; 32:361–366.
35. Wegienka G, Johnson CC, Havstad S, Ownby DR, Zoratti EM. Indoor pet exposure and the outcomes of total IgE and sensitization at age 18 years. *J Allergy Clin Immunol* 2010; 126(2):274–279, 279.e1–e5.
36. Huss K, Adkinson NF, Jr., Eggleston PA, Dawson C, Van Natta ML, Hamilton RG. House dust mite and cockroach exposure are strong risk factors for positive allergy skin test responses in the Childhood Asthma Management Program. *J Allergy Clin Immunol* 2001; 107(1): 48–54.
37. Tranter DC. Indoor allergens in settled school dust: a review of findings and significant factors. *Clin Exp Allergy* 2005; 35:126–136.
38. Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P, Buffler PA. Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. *Environ Health Perspect* 2009; 117(6):1007–1013.
39. Whitehead T, Metayer C, Gunier RB, Ward MH, Nishioka MG, Buffler P, Rappaport SM. Determinants of polycyclic aromatic hydrocarbon levels in house dust. *J Expo Sci Environ Epidemiol* 2011; 21(2):123–132.
40. Arbes SJ, Jr., Sever M, Vaughn B, Mehta J, Lynch JT, Mitchell H, Hoppin JA, Spencer HL, Sandler DP, Zeldin DC. Feasibility of using subject-collected dust samples in epidemiologic and clinical studies of indoor allergens. *Environ Health Perspect* 2005; 113(6): 665–669.
41. Colt JS, Gunier RB, Metayer C, Nishioka MG, Bell EM, Reynolds P, Buffler PA, Ward MH. Household vacuum cleaners vs. the high-volume surface sampler for collection of carpet dust samples in epidemiologic studies of children. *Environ Health* 2008; 7:6.
42. Niemeier RT, Sivasubramani SK, Reponen T, Grinshpun SA. Assessment of fungal contamination in moldy homes: comparison of different methods. *J Occup Environ Hyg* 2006; 3(5):262–273.
43. Ashley PJ, Dewalt G, Hamilton RG, Jones J, Pinzer E. Vacuum dust sample collection protocol for allergens. In: Program HsHH, editor. 2.0 ed: HUD Office of Healthy Homes and Lead Hazard Control; 2008.
44. Institute of Medicine: Committee on the Assessment of Asthma and Indoor Air DoH, Prevention PaD. Clearing the Air: Asthma and Indoor Exposures. Washington, DC: National Academy Press, 2000.
45. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufer A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007; 448(7152):470–473.
46. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, Lester LA, Gern JE, Lemanske RF, Jr., Nicolae DL, Elias JA, Chupp GL. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. *N Engl J Med* 2008; 358(16):1682–1691.
47. Joubert B, Reif D, Edwards S, Waidyanatha S, Leiner K, Hudgens E, Egeghy P, Gallagher J, Cohen-Hubal E. Integration of genetics (SNP) and environmental (PAH urinary metabolites) data for investigations of childhood allergy and asthma. American Association for Aerosol Research (AAAR) Conference, “Bridging the gap from sources to health outcomes”; San Diego, CA, 2010.
48. Joubert BR, Reif DM, Edwards SW, Leiner KA, Hudgens EE, Egeghy P, Gallagher JE, Hubal EC. Evaluation of genetic susceptibility to childhood allergy and asthma in an African American urban population. *BMC Med Genet* 2011; 12:25.

Copyright of Journal of Asthma is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.