

A Side-by-Side Comparison of Sampling Methods for Settled, Indoor Allergens

Mona Mansour,* Bruce P. Lanphear,* Richard Hornung,† Jane Khoury,‡ David I. Bernstein,§
William Menrath,‡ and Joji Decolongon*

*For the Cincinnati Lead and Allergen Sampling Study (CLASS) with Children's Hospital Medical Center, *The Department of Pediatrics, †Institute for Health Policy & Health Services Research, and the ‡University of Cincinnati Departments of Environmental Health and §Internal Medicine, Cincinnati, Ohio 45229*

Received July 20, 2000

Exposure to indoor allergens is associated with asthma, but there is no standardized sampling method for measuring allergens. We compared the association of measured allergen exposure and serum-specific IgE levels and the precision of three sampling methods (Cyclone, Mighty Mite, and Readivac II) to identify a standardized sampling method for indoor allergens. A random sample of 72 children, 5 to 17 years old, with doctor-diagnosed asthma who lived in the same residence ≥ 2 years were enrolled. Composite, side by side floor samples were obtained with all three methods. Dust allergen concentrations and serum-specific IgE levels were measured for *Der f I*, *Fel d I*, and *Bla g I*. Mean allergen concentration did not differ significantly by sampling method. Cat allergen was significantly correlated with serum-specific IgE for Cyclone ($P=0.003$) and Mighty Mite ($P=0.008$), but only marginally for Readivac II ($P=0.07$). Dust mite allergen was significantly correlated with serum-specific IgE for Readivac II ($P=0.02$) and Cyclone ($P=0.038$), but not for Mighty Mite ($P=0.12$). Cockroach allergen was not correlated with serum-specific IgE for any sampling method. In multiple linear regression, cat allergen was associated with serum-specific IgE for Cyclone ($P=0.007$) and Mighty Mite ($P=0.02$), but not for Readivac II ($P=0.06$). In contrast, dust mite allergen was marginally associated with serum-specific IgE for Readivac II ($P=0.07$), but not for Mighty Mite ($P=0.64$) or Cyclone ($P=0.27$). The Cyclone and Mighty Mite were more precise than Readivac II for cat allergen, but there was no difference for dust mite allergen ($P>0.05$). No single method is superior for measurement of indoor allergens. In general, cat allergen collected with the Cyclone was a better predictor of serum-specific IgE levels to *Fel d I*, whereas dust mite allergen collected with the Readivac II was a better predictor of serum-specific IgE levels to *Der f I*. © 2001 Academic Press

Key Words: dust; IgE; sampling; indoor allergens; asthma; children; environment; pediatrics.

INTRODUCTION

Asthma, a disease characterized by inflammation and reversible airway obstruction in response to a variety of stimuli, is rapidly emerging as the most prevalent and serious environmental health problem among children (National Heart, Lung, and Blood Institute, 1991; Weitzman *et al.*, 1992; Taylor and Newacheck, 1992; Weiss *et al.*, 1993). Currently, asthma is estimated to afflict over 4.8 million children in the United States (Adams and Marano, 1995). There is clearly a genetic predisposition to develop asthma, but numerous observational studies, both prospective and cross-sectional, suggest that exposure and sensitization to aeroallergens during early childhood, including dust mite, pet, and cockroach allergens, are necessary for allergy-mediated asthma to develop (Pope *et al.*, 1993; Platts-Mills *et al.*, 1992; Colloff *et al.*, 1992; Sporik *et al.*, 1990).

Despite an extensive literature on the relationship of asthma and exposure to indoor allergens, there is no standardized sampling method for such allergens. Previous studies have used a variety of dust sampling methods (Arlan *et al.*, 1982; Kuehr *et al.*, 1994; Cole *et al.*, 1996; Rosenstreich *et al.*, 1997; Gold *et al.*, 1993). Moreover, whereas there is high correlation for dust mite allergen (*Der p I*) concentrations taken from the same households over a 12-month period (Kuehr *et al.*, 1994), there is little known about the precision of existing sampling methods or the spatial variability of settled allergens. Until a standardized allergen sampling

method is developed, it will be difficult to compare the results of various studies. Moreover, unless sampling methods are accurate and precise, the relationship between allergens and symptoms of asthma will be obscured (Platt-Mills *et al.*, 1995; Lanphear *et al.*, 1995; Emond *et al.*, 1997). Finally, definition of exposure thresholds for various allergens is critical for the eventual prevention and control of asthma, but such thresholds are likely to vary by the surfaces that are sampled and by the sampling methods that are utilized (Pope *et al.*, 1993; Lanphear *et al.*, 1995).

The purpose of this study was to identify a standardized sampling method for settled, indoor allergens. Two criteria were selected to compare three existing allergen sampling methods: precision and whether allergen collected by each of these three methods was a better predictor or more highly associated with sensitization (serum-specific IgE levels to *Der f I*, *Fel d I*, and *Bla g I*). Precision was measured by comparison of the replicate error of side by side samples taken with the three methods.

MATERIALS AND METHODS

A cross-sectional design was employed to measure levels of settled allergens in floor dust and serum-specific IgE levels among children with asthma. All children with doctor-diagnosed asthma between the ages of 5 and 17 years who were evaluated within the last 2 years at one of three allergy clinics in the Greater Cincinnati area were identified. After the presence of errors and duplications was assessed and accounted for, proportional random sampling with stratification by clinic was performed. In addition to the above criteria, children had to live in the same house for at least 2 years, their residence had to be within a 30-mile radius of Cincinnati, and carpeting could not have been removed or replaced in the child's bedroom, principal play area, or other areas involved in the environmental sampling during the past 2 years. Children were excluded from the study if they were taking a prescribed oral steroid on a daily basis.

Each family was sent a letter describing the study and inviting them to participate. Interviewers then contacted families by telephone in random order to collect basic demographic information and to determine eligibility. Each telephone number identified was called until the family was contacted or at least six calls were made.

Once a family was determined to be eligible and agreed to participate, an appointment was scheduled for an environmental health team to visit their home, obtain a venipuncture blood sample from the

child, conduct an interview, visually inspect the home, and collect environmental samples. All environmental sampling occurred between February 1, 1998 and May 31, 1998. Households received monetary incentive for participation. Approval for the study was received from the Children's Hospital Medical Center Institutional Review Board.

Biologic and Environmental Samples

Venous samples for children's serum-specific IgE levels were obtained during the home visit by a certified phlebotomist. If the child was unavailable at the time of the home visit, a phlebotomist visited the home within 1 week of environmental sampling to obtain IgE levels from the child. Serum-specific IgE levels were measured at Mayo Medical Laboratories and any values above 0.35 kU/L were considered positive or indicative of sensitization to cat, dust mite, or cockroach allergen.

Inclusion criteria for vacuum sampling methods include (1) the frequency of use in ongoing research, (2) flow rate, and (3) the ability to be able to standardize measurement and reduce measurement error as assessed by visual examination of the sampling devices. Frequency of use in other studies was used as a criterion to capitalize on existing research. Flow rate was included as a criterion because many investigators have noted problems in obtaining adequate samples with lower flow rates. The third criterion included characteristics such as potential loss of sample during dust extraction. The three methods selected for this analysis were the Readivac II (Model 6735; Douglas Manufacturing, Walnut Ridge, AZ), the high-velocity, portable Cyclone sampler (CS-3 Industries, OR), and the Mighty Mite (Model No. 3670, 7 amp; Eureka).

Each of the selected sampling methods includes the individual device in conjunction with a specific protocol for that device. The Cyclone, a modified version of the HVS-3 cyclone sampler, is a high-flow vacuum that employs a Dirt Devil vacuum. The Readivac II collects dust in a sleeve (DACI Laboratories, Baltimore, MD) and utilizes a Douglas vacuum. Finally, the Mighty Mite captures dust in a cellulose thimble (Whatman No. 2800199) and utilizes a Eureka vacuum. The purpose of this study was to compare existing sampling methods, not to develop or modify such methods. Thus, the equipment and supplies used and the time vacuumed were as described in the protocols of the original investigators. The sampling time for the Mighty Mite and Readivac II was 30 s for each 18-square-inch area sampled. The sampling time for the Cyclone was 2 min for

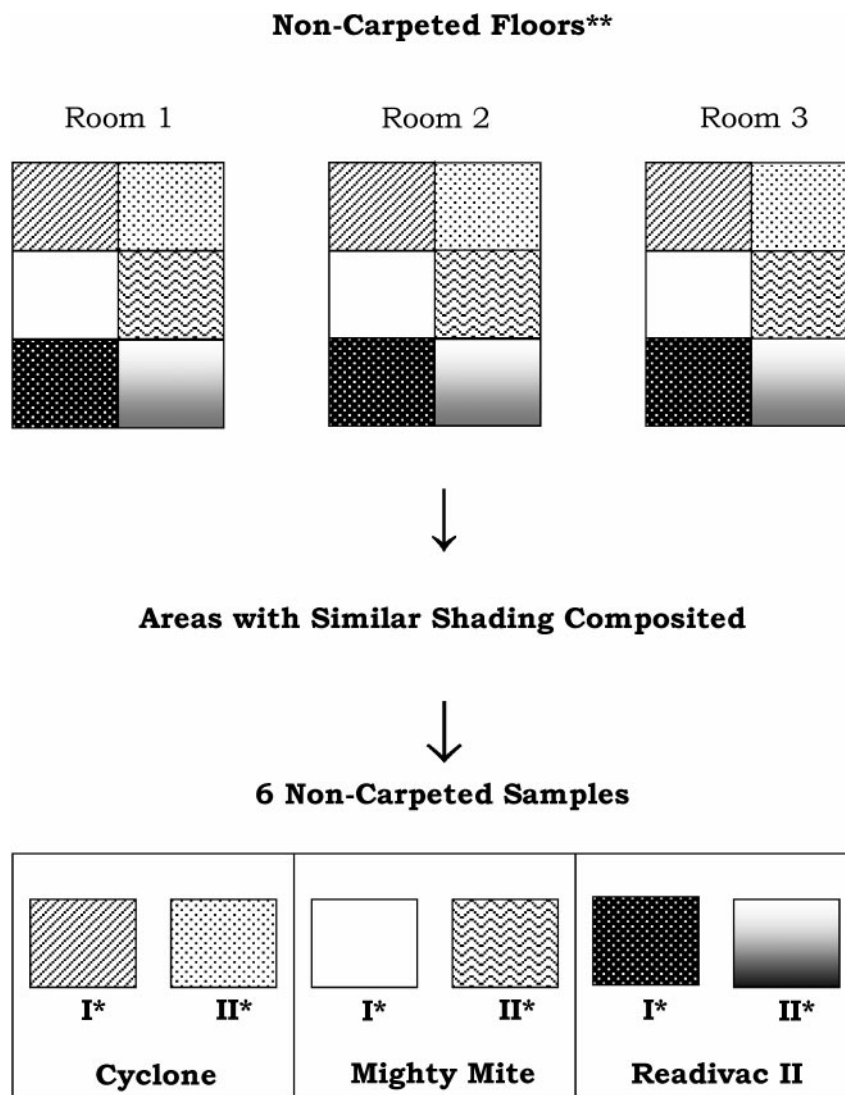


FIG. 1. Sampling schema for noncarpeted and carpeted floors. *I and II refer to replicates one and two. ** Same schema used for carpeted surfaces.

each area sampled. The sampling time was determined by conversion of the area per unit time used by the original investigator (square feet or square meters) into a comparable sampling time for the area in this study (square inches). The size of the area sampled differed from those of the original protocols because typical room size was too small to allow side by side sampling of three different methods with a larger template.

Household dust sampling was conducted to characterize the potential exposure of children to indoor allergens from settled dust as measured by allergen concentration (ng/g). In each housing unit, we attempted to collect a maximum of 12 composite dust samples (6 from carpeted surfaces and 6 from

noncarpeted surfaces) that were most accessible to the child. A metal grid containing six templates (3×2) each measuring 18 inches was used to define the floor areas to be sampled (Fig. 1). Each sampling method was randomly assigned to one of the three rows in the grid. Rooms that were too small to accommodate the template were excluded from sampling. All samples obtained within a single room were from the same surface type (e.g., either carpeted or noncarpeted surfaces).

The order in which rooms were to be selected for sampling was standardized as follows: the participant child's bedroom, the child's principal play area, the family room, the kitchen, the living room, the bathroom, and other rooms. A maximum of three

carpeted and three noncarpeted rooms were selected. The midpoint or largest area in the room was selected for sampling, unless the child had a specific play area in the room. In that case, the play area was sampled. Results were adjusted by the area sampled (square foot) because some of the homes had fewer than three carpeted or noncarpeted rooms available for sampling. To ensure that each dust collection method had an equal chance of being used to collect dust from the midpoint of the floor, the location of the sampling methods with respect to each other was determined for individual floors by use of preassigned lists of random numbers.

Laboratory Analyses

Dust mite allergens in dust samples were quantitated for *Der f I* by use of a double-antibody sandwich as previously described (Chapman *et al.*, 1984, 1987a,b; Chapman, 1988; Ford *et al.*, 1985; Heymann *et al.*, 1986; Luczynska *et al.*, 1989; Platts-Mills and Chapman, 1987; Platts-Mills *et al.*, 1992). For cockroach allergens, *Bla g I* (Pollart *et al.*, 1991a,b; Call *et al.*, 1992; Gelber *et al.*, 1993) was assayed by the use of the capture antibody 10A6mAb. The method was similar to that described for *Der f I* except that a second antibody was rabbit anti-cockroach antibody. For assaying cat allergen *Fel d I* (Chapman *et al.*, 1988; Luczynska *et al.*, 1990; DeBlay *et al.*, 1991; Vailes *et al.*, 1994), the method was identical to that described for *Der f I*.

Statistical Analyses

Descriptive statistics (means, medians, numbers of samples, plots) were generated to compare the performance of the three vacuum methods across both carpeted and noncarpeted floors. These statistics were computed for four different response variables: concentrations for cat (*Fel d I*), dust mite (*Der f I*), and cockroach (*Bla g I*) allergens and sieved dust weight (300 μ m). The number and percentage of samples that were detectable and undetectable (allergen levels below the detection limit) for each sampling device and floor surface type were determined. All pertinent analyses included imputed values for undetectable samples (Hornung and Reed, 1990).

After examination of the descriptive statistics for outliers, statistical tests were done to compare levels of allergen collected with the three sampling methods. When the means of collected allergen or dust were compared, a mixed-effect analysis of variance was used in which the houses for each set of

measurements were random effects and the three vacuum methods and two floor surfaces (carpeted vs noncarpeted) were fixed effects. The design was a randomized block design with houses as the blocks for a total of three factors (houses, vacuums, floor surfaces) with two replicate measurements for each combination of houses, vacuums, and floor surfaces. The design was not completely balanced since many of the dust samples collected on noncarpeted surfaces were so small that the two replicates had to be combined to obtain enough material for the allergen assays. All response variables were log-transformed for the analysis of variance since residuals followed a lognormal distribution. Correlations between pairs of sampling methods for the collection of each of the allergens were also performed.

Simple correlation coefficients were calculated to examine bivariate relationships between settled dust allergens and serum-specific IgE levels. The primary analysis was a multivariate linear model with the log of IgE levels as the dependent variable. The primary independent variable of interest was the amount of settled allergen dust as measured by each of the three vacuum methods. Also examined in this model were floor surfaces, household income, smokers in the household, cats in the household, cockroaches visible in the household, age, use of bursts of oral steroid in the past 12 months, race, and allergy shots. The strength of the relationship between IgE levels and the settled dust allergen for each of the vacuum methods was measured by the *F* test associated with each vacuum method.

In addition, multiple linear regression methods were utilized to examine adjusted models for each specific allergen: cat, dust, and cockroach. Adjusted analyses were performed to examine relationships between log serum-specific IgE and settled dust for cat, dust mite, and cockroach allergens. Only those variables that statistically significantly altered the association between measured allergen and serum-specific IgE levels were included in the final models. For cat allergen, the adjusted model included age and race. For dust mite allergen, the model included age, steroid use in the last 12 months (as a marker of asthma severity), and whether the child had received allergy shots (yes/no). Family income, presence of cockroaches in the home, and smokers present in the home were included in the model for cockroach allergen. Comparisons of the individual beta values from the bivariate and multivariate regression equations were also performed. These comparisons help to determine whether there was a statistically significant difference between the association of allergen collected by a particular method

and sensitization, as measured by serum-specific IgE levels.

We also compared the precision of the three methods, as measured by replicate error. To examine the estimates of precision for each sampling method, we calculated the variance (mean square error) in replicate measurements for each of the three methods. The smaller the mean square error the greater the precision of a particular method. We then compared these estimates in a pairwise fashion for each of the four response variables using *F* tests on the log-transformed data. A significant *F* test for a given comparison indicates that one method had significantly better collection precision than the other. Direction of the difference was noted whenever a significant test result was found.

RESULTS

Seventy-two families of children with asthma participated in the study. Seventy percent of the children in the study were White and 57% were male. The average age of the children was 12.6 years. Thirty-two percent of families had incomes below \$25,000. Other demographics and characteristics of families and children in the study are shown in Table 1. Forty-six percent of children sampled had measurable antibodies to cockroach allergen, 58% had antibodies to cat allergen, and 61% had antibodies to dust mite allergen.

The number and percentage of samples with detectable allergen levels by floor type and method are shown in Table 2. The percentage of detectable samples was higher for carpeted surfaces than for noncarpeted surfaces for all three sampling methods. The number of detectable samples, however, was not significantly different between sampling methods for either carpeted or noncarpeted floors. The maximum number of total samples for the study is 72 homes X 12 composited samples (two replicates for each of three methods for carpeted and noncarpeted surfaces) or 864 samples. For each sampling method by floor type combination there would be a maximum possible number of samples of 144. Due to types of floor surfaces available for sampling, 2 homes had only carpeted surfaces sampled and 9 homes had only noncarpeted surfaces sampled. The difference between the maximum and the actual number of total samples for noncarpeted floors is largely due to the number of replicate samples that were composited for analysis due to the low mass of the total dust collected.

Geometric mean allergen concentration values for the three different methods and the three different

TABLE 1
Demographics and Characteristics of Participants
in the CLASS Study

Variable	Percentage
Race of child	
White	69.4
African American	27.8
Other	2.8
Gender of child	
Male	56.9
Female	43.1
Age of child (years)	Mean age 12.6 ± 3.0
Educational level of parent	
High school	41.7
College	47.2
>College	11.1
Income (\$)	
<25,000	31.6
>25,000	66.7
Marital status	
Married	72.2
Nonmarried	27.8
Triggers by parental report	
Cat	68.1
Dust mite	75.0
Cockroach	15.3
Cat	
Present in home	6.9
Not present in home	93.1
Cockroach	
Visible	25.0
Not visible/noticed	75.0
Oral steroids used in last 12 months	
Yes	47.2
No	52.8
Allergy shots current	
Yes	45.8
No	54.2
Smokers in home	
Yes	28.0
No	72.0
Hours spent at home/week	Hours 118 ± 19.1

allergens are shown in Table 3. In general, the *F* tests demonstrate that there were no statistically significant differences in the amount of allergens detected by these three methods. There were no statistically significant differences in the sieved dust weight for the three sampling methods for noncarpeted surfaces. However, there was a larger sieved dust weight collected by the Cyclone than by the Mighty Mite and the Readivac II for carpeted surfaces.

The range of the correlations for pairs of sampling methods for cat, cockroach, and dust mite allergens

TABLE 2
Percentage of Samples with Detectable Allergen, by Dust Sampling Method and Type of Floor

Dust measure	Cyclone			Readivac II			Mighty Mite		
	Carpet	Noncarpet	Total	Carpet	Noncarpet	Total	Carpet	Noncarpet	Total
Cat antigen (<i>Fel d I</i>)	64% (<i>n</i> = 81)	32% (<i>n</i> = 27)	51% (<i>n</i> = 108)	50% (<i>n</i> = 60)	35% (<i>n</i> = 28)	44% (<i>n</i> = 88)	62% (<i>n</i> = 75)	23% (<i>n</i> = 25)	49% (<i>n</i> = 100)
Dust mite (<i>Der f I</i>)	73% (<i>n</i> = 91)	38% (<i>n</i> = 32)	59% (<i>n</i> = 123)	69% (<i>n</i> = 83)	36% (<i>n</i> = 29)	56% (<i>n</i> = 112)	76% (<i>n</i> = 93)	36% (<i>n</i> = 30)	60% (<i>n</i> = 123)
Cockroach (<i>Bla g I</i>)	79% (<i>n</i> = 99)	65% (<i>n</i> = 55)	73% (<i>n</i> = 154)	81% (<i>n</i> = 97)	73% (<i>n</i> = 59)	78% (<i>n</i> = 156)	79% (<i>n</i> = 96)	75% (<i>n</i> = 62)	77% (<i>n</i> = 158)

was 0.72–0.89, and, as expected, the amounts of allergen collected by the different methods were highly correlated.

Bivariate Analysis

Linear regression analysis shows that settled cat allergen collected with the Cyclone and the Mighty Mite was more highly correlated with serum-specific IgE to *Fel d I* ($P = 0.003$ and $P = 0.008$, respectively), than was the Readivac II ($P = 0.07$). In contrast, settled dust mite allergen collected by the Readivac II and the Cyclone ($P = 0.02$ and $P = 0.038$, respectively) was more highly correlated with serum-specific IgE to *Der f I* levels than was the Mighty Mite ($P = 0.12$). For cockroach, none of the methods showed a statistically significant correlation be-

tween settled dust levels of allergen and serum-specific IgE to *Bla g I* (Table 4).

Multiple Linear Regression Analysis

In adjusted analyses, cat allergen collected by the Cyclone and the Mighty Mite ($P = 0.007$ and $P = 0.02$, respectively) was more highly associated with serum-specific IgE to *Fel d I* than was the Readivac II. None of the sampling methods was superior for measuring dust mite allergen, but the association of allergen collected by the Readivac II was close to being statistically significant ($P = 0.07$) (Table 4). None of the methods showed a statistically significant correlation between settled dust levels of cockroach allergen and serum-specific IgE to *Bla g I*.

TABLE 3
Geometric Mean Concentrations of Cat, Dust Mite, and Cockroach Allergen in 72 Houses by Dust Sampling Method and Type of Floor

	Cyclone		Readivac II		Mighty Mite		<i>P</i> values	
	Carpeted	Noncarpeted	Carpeted	Noncarpeted	Carpeted	Noncarpeted	Carpeted	Noncarpeted
Cat antigen (<i>Fel d I</i>) (ng/g)	60.07 13.40 (63)	39.14 8.40 (65)	60.98 13.71 (63)	62.02 8.46 (65)	61.60 14.42 (63)	47.15 8.67 (67)	$X^2 = 1.4063$ $P = 0.4950$	$X^2 = 0.7854$ $P = 0.6752$
Dust Mite (<i>Der f I</i>) (ng/g)	165.15 22.48 (63)	46.87 8.64 (65)	192.14 15.87 (63)	61.79 9.47 (65)	182.96 15.69 (63)	67.49 8.52 (67)	$X^2 = 0.0570$ $P = 0.9719$	$X^2 = 0.0398$ $P = 0.9803$
Cockroach (<i>Bla g I</i>) (IU/g)	0.25 24.40 (63)	0.72 37.01 (65)	0.54 24.17 (63)	1.36 44.05 (65)	0.36 26.41 (63)	1.36 33.58 (67)	$X^2 = 1.1824$ $P = 0.5536$	$X^2 = 1.7612$ $P = 0.4145$
Sieved weight	1.09 2.89 (63)	0.04 6.20 (70)	0.12 2.14 (63)	0.04 4.70 (70)	0.24 2.63 (63)	0.04 4.12 (70)	$P = 0.0001$	$P = 0.8383$

TABLE 4

Unadjusted and Adjusted Models of Log Serum IgE Antibody versus Log Settled Dust Allergens (*Der f I*, *Fel d I*, *Bla g I*) by Dust Sampling Method, for Combined Carpet and Noncarpet Surfaces^a

Settled allergen	Cyclone coefficient (<i>P</i> value)		Readivac II coefficient (<i>P</i> value)		Mighty Mite coefficient (<i>P</i> value)	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Cat allergen (<i>Fel d I</i>)	0.35 (0.003)	0.309 (0.007)	0.19 (0.070)	0.202 (0.058)	0.29 (0.008)	0.256 (0.020)
Dust mite (<i>Der f I</i>)	0.22 (0.038)	0.140 (0.274)	0.26 (0.021)	0.233 (0.071)	0.16 (0.121)	0.065 (0.636)
Cockroach (<i>Bla g I</i>)	0.02 (0.376)	− 0.022 (0.628)	0.003 (0.482)	− 0.041 (0.740)	− 0.02 (0.624)	− 0.065 (0.829)

Note. Adjusted analysis for dust mite included age, oral steroid use in past 12 months, and allergy shots. Adjusted analysis for cockroach included income, presence of cockroaches in the home, and smoking. Adjusted analysis for cat included age and race.

^aAll *P* values are one-tailed.

There were no significant differences in the associations of measured allergen exposure and serum-specific IgE levels for any of the sampling methods. Thus, for example, although the strength of this association for cat allergen as measured with the Cyclone was greater than the strength of this association for cat allergen as measured with the Readivac II, it was not significantly different.

The Cyclone and the Mighty Mite were more precise than the Readivac II for the measurement of cat allergen concentration. There was no significant difference, however, in precision of the three sampling methods for the measurement of dust mite or cockroach allergen concentrations (Tables 5 and 6).

DISCUSSION

Based on data from this study, we conclude that no existing method is superior as a standardized measure for indoor allergens. In general, allergen measured with the Cyclone appeared to be a better predictor of sensitization to cat allergen but it was not statistically significantly different from the other two methods. The Readivac II was marginally superior for predicting sensitization to dust mite allergen, but it also was not statistically different from the other two methods.

No single method was consistently more precise than the other methods for all three allergens. It appears, however, that the ranking of precision of methods (as measured by their mean square errors) exhibited trends similar to those of the preference of devices established by the analyses of correlation between allergen collected and sensitization. The Cyclone and the Mighty Mite, for example, were clearly more precise for the measurement of cat allergen concentration than the Readivac II. Although

the precision estimates of the Cyclone and Mighty Mite were not statistically different, if one ranks them in the order of their mean square errors, the Cyclone ranks above the Mighty Mite and both rank above the Readivac II (MSE = 0.378, 0.425, and 0.652, respectively). In bivariate and multivariate analyses for correlations between allergen and sensitization, the same rank order for devices holds. In bivariate analyses the *P* values for the Cyclone, Mighty Mite, and Readivac II are 0.003, 0.008, 0.070, respectively. This relationship between the correlation of allergen and sensitization, and precision is consistent with findings in the environmental lead literature (Lanphear *et al.*, 1995; Emond *et al.*, 1997).

The data did not help us to draw conclusions about which sampling method should be used for the measurement of cockroach allergen. There was not a significant difference in the amount of allergen collected by the three methods, nor was there a statistically significant relationship between the amount of settled cockroach allergen and the serum-specific IgE levels to *Bla g I*. The reason for this lack of association is unclear. The number of undetectables for cockroach allergen was lower than those for dust mite and cat. It is possible that none of these devices collect cockroach allergen in an optimal manner; alternatively, we may not have sampled the surfaces most likely to contain cockroach allergen. Others have reported that the concentration of cockroach allergen is higher in kitchens (Rosenstreich *et al.*, 1997), and cockroach allergen found in bedrooms was most highly correlated to sensitization of an individual (Eggleston *et al.*, 1998). Compositing the bedroom sample with other rooms in this study may have altered the relationship of exposure and sensitization.

TABLE 5
Pairwise Comparisons of Precision (Replicate Error) for Sampling Methods

Dust measure ^a	Cyclone vs Readivac II	Cyclone vs Mighty Mite	Readivac II vs Mighty Mite
Settled cat allergen	$F = 1.72$ $P = 0.013$	$F = 1.12$ $P = 0.310$	$F = 1.53$ $P = 0.041$
Settled dust mite allergen	$F = 1.11$ $P = 0.332$	$F = 1.09$ $P = 0.364$	$F = 1.21$ $P = 0.222$
Settled cockroach allergen	$F = 1.20$ $P = 0.233$	$F = 1.15$ $P = 0.280$	$F = 1.03$ $P = 0.443$
Total sieved weight (mg)	$F = 2.04$ $P = 0.001$	$F = 1.51$ $P = 0.038$	$F = 3.09$ $P = 0.000$

^a Refer to Table 6 to determine which of two devices is significantly more precise than the other device. Device with the lower mean square error is more precise.

A number of criteria should be considered when a standardized sampling method is selected. We propose that the primary criteria used to designate a standardized sampling method include precision, accuracy, and a measure of whether the amount of allergen collected is correlated with sensitization (i.e., bioavailable). Precision indicates the ability to consistently and reliably sample an allergen. The ability of the sampling method to better predict sensitization or exposure to a particular allergen is also important. Studies of lead-contaminated house dust have demonstrated that the relationship between the lead in settled dust and the blood lead level differs by sampling method (Lanphear *et al.*, 1995). Secondary criteria include cost, ease of use, and minimum technician and respondent burden. Finally, if all other characteristics are equal, the number of studies that are using or have previously used a specific sampling device should be incorporated into the selection criteria.

The Mighty Mite and the Readivac II are clearly superior to the Cyclone for ease of use, cost, and burden to respondent and field workers. Thus, for large-scale sampling, the Readivac II appears superior if one is interested in sampling dust mites. On the other hand, the Cyclone method has been used

to measure lead, endotoxin, and pesticides, in addition to allergens. Thus, while it is more costly and cumbersome, the sampling characteristics for the Cyclone have been reported for a wider range of residential exposures (Lanphear *et al.*, 1995; Farfel *et al.*, 1994). Thus, to measure multiple exposures simultaneously, the Cyclone appears to be superior. Still, the Mighty Mite, which is being used in two or more longitudinal cohorts and in the HUD/NIEHS National Lead and Allergen Survey, also appears to be a reasonable method, especially for investigators who are interested in direct comparison of their results with national survey data.

There are several limitations of this study that should be noted. First, sample size may have limited our ability to identify differences between sampling methods. Second, we measured only children's home environments and IgE levels during one season. Dust mite concentration peaks during the summer months (Arlian *et al.*, 1982), and it is possible that the relationship of IgE and dust allergen varies throughout the year. Seasonal variation is not well described for other allergens. Third, the relationship between settled dust allergens and specific IgE antibody may vary for different allergens. This difference may be mediated by a number of factors,

TABLE 6
Estimates of Precision (Replicate Error) for Sampling Methods

Data measure ^a	Cyclone (C1 vs C2) MSE (<i>df</i>)	Readivac II (R1 vs R2) MSE (<i>df</i>)	Mighty Mite (M1 vs M2) MSE (<i>df</i>)	Rank of precision
Settled cat allergen	0.378 (73)	0.652 (64)	0.425 (69)	C > R, MM > R, C = MM
Settled dust mite allergen	0.230 (72)	0.207 (64)	0.250 (69)	R = C = MM
Settled cockroach allergen	0.278 (72)	0.232 (64)	0.268 (69)	R = MM = C
Total sieved weight (mg)	0.020 (78)	0.042 (68)	0.014 (72)	MM > C > R

^a All settled allergen levels are log transformed. Lower values indicate less replicate error and better precision. To calculate the geometric standard deviation take the antilog of square root of mean square error (GSD = 10 square root MSE).

including particle size, the likelihood of the allergen to be aerosolized, and the location of exposure. Sampling methods that collect smaller amounts of dust or that do not collect certain particle sizes may obscure the relationship. If children are sensitized through other methods of exposure, i.e., bedding, the method's ability to collect allergen may be irrelevant and the use of IgE may be misleading. Studies that have compared sampling devices in laboratory-based settings suggest that different methods have a particle size bias which can impact the use of mass bulk as the denominator for normalization of exposure (Lewis and Breysse, 1998). Fourth, although our subjects were randomly sampled, the sample population may not be representative of children with asthma in the United States. Thus, one cannot assume that the observed relationships are valid for other populations. Finally, we did not measure accuracy of these sampling methods—their ability to collect a known quantity of allergens from a surface—which is better done in a laboratory setting.

CONCLUSIONS

We conclude from these data that no single method is superior as a standardized measure for indoor allergens. Cat allergen as collected by the Cyclone is a better predictor of sensitization to cats but is not statistically significantly better than the other two methods. In contrast, dust mite allergen collected with the Readivac II was marginally superior for predicting sensitization to dust mites, but it was not statistically different from the other two methods. The ranking of sampling methods for precision was consistent with their strength of association for measured allergen and sensitization. We therefore infer that precision is an important criterion to identify sampling methods to be evaluated in population studies. The findings of this study reinforce the need to compare the sampling characteristics of allergen sampling methods, including precision, accuracy, and the association between amount of allergen collected and sensitization in a larger sample of children, including those who do not have asthma.

ACKNOWLEDGMENTS

We give special thanks to the CLASS study staff. We also thank Drs. Brett Kettelhut and Michelle Lierl and their patients from the Cincinnati Asthma and Allergy Center and the Children's Hospital Medical Center. This work was funded, in part, by The United States Department of Housing and Urban Development, the Institutional National Research Service Award (NRSA)

No. 1T32PE10027, and Faculty Development Grant No. 5-DO8-PE50077 from the Bureau of Health Professions, HRSA, PHS, DHHS.

REFERENCES

- Adams, P. F., and Marano, M. A. (1995). "Current Estimates from the National Health Interview Survey, 1994." National Center for Health Statistics, Vital Health Stat **10**(193).
- Arlian, L. G., Bernstein, I. L., and Gallagher, J. S. (1982). The prevalence of house dust mites, *Dermatophagoides* spp., and associated environmental conditions in homes in Ohio. *J. Allergy Clin. Immunol.* **69**, 527-532.
- Call, R. S., Smith, T. F., Morris, E., Chapman, M. D., and Platts-Mills, T. A. (1992). Risk factors for asthma in inner city children. *J. Pediatr.* **121**, 862-866.
- Chapman, M. D. (1988). Allergen specific monoclonal antibodies: New tools for the management of allergic disease. *Allergy* **43**, 7-14.
- Chapman, M. D., Aalberse, R. C., Brown, M. J., and Platts-Mills, T. A. E. (1988). 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.* **140**, 812-818.
- Chapman, M. D., Heymann, P. W., and Platts-Mills, T. A. E. (1987a). Epitope mapping of two major inhalant allergens, Der p I and Der f I, from mites of the genus *Dermatophagoides*. *J. Immunol.* **139**, 1479-1484.
- Chapman, M. D., Heymann, P. W., Wilkins, S. R., Brown, M. J., and Platts-Mills, T. A. E. (1987b). Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. *J. Allergy Clin. Immunol.* **80**, 184-194.
- Chapman, M. D., Sutherland, W. M., and Platts-Mills, T. A. E. (1984). Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P1 by using monoclonal antibodies: Binding to each epitope can be inhibited by serum from dust-mite allergic patients. *J. Immunol.* **133**, 2488-2495.
- Cole, E. C., Dulaney, P. D., Leese, K. E., Hall, R. M., Foorde, K. K., Franke, D. L., et al. (1996). Biopollutant sampling and analysis of indoor surface dusts: Characterization of potential sources and sinks. In "Characterizing Sources of Indoor Air Pollution and Related Sink Effects" (B. A. Tichenor, Ed.), pp. 153-165. Am. Soc. Testing and Materials, PA.
- Colloff, M. J., Ayres, J., Carswell, F., Howarth, P. H., Merrett, T. G., Mitchell, E. B., et al. (1992). The control of allergens of dust mites and domestic pets: A position paper. *Clin. Exp. Allergy* **22**(Suppl. 2), 1-28.
- DeBlay, F., Chapman, M. D., and Platts-Mills, T. A. E. (1991). Airborne cat allergen (Fel d 1): Environmental control with the cat in situ. *Am. Rev. Respir. Dis.* **143**, 1334-1339.
- Eggleston, P. A., Rosenstreich, D., Lynn, H., Gergen, P., Baker, D., Kattan, M., et al. (1998). Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J. Allergy Clin. Immunol.* **102**, 563-570.
- Emond, M. J., Lanphear, B. P., Watts, A., and Eberly, S. (1997). Measurement error and its impact on the estimated relationship between dust lead and children's blood lead: Members of the Rochester Lead-in-Dust Study Group. *Environ. Res.* **72**, 82-92.

- Farfel, M., Lees, P. J., Bannon, D., Lim, B., and Rohde, C. (1994). Comparison of two cyclone-based collection devices for the evaluation of lead-containing residential dusts. *Appl. Occup. Environ. Hyg.* **9**, 212-217.
- Ford, A. W., Rawle, F. C., Lind, P., Spieksma, F. T., Lowenstein, H., and Platts-Mills, T. A. E. (1985). Standardization of *Dermatophagoides pteronyssinus*: Assessment of potency and allergen content in ten coded extracts. *Int. Arch. Allergy Appl. Immunol.* **76**, 58-67.
- Gelber, L. E., Seltzer, L. H., Bouzoukis, J. K., Pollart, S. M., Chapman, M. D., and Platts-Mills, T. A. E. (1993). Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am. Rev. Respir. Dis.* **147**, 573-578.
- Gold, D. R., Rotnitzky, A., Damokosh, A. I., Ware, J. H., Speizer, F. E., and Ferris, B. G., Jr. (1993). Race and gender differences in respiratory illness prevalence and their relationship to environmental exposures in children 7 to 14 years of age. *Am. Rev. Respir. Dis.* **148**, 10-18.
- Heymann, P. W., Chapman, M. D., and Platts-Mills, T. A. E. (1986). Antigen Der f I from the dust mite *Dermatophagoides farinae*: Structural comparison with Der p I from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J. Immunol.* **137**, 2841-2847.
- Hornung, R. W., and Reed, L. D. (1990). Estimation of average concentration in the presence of nondetectable values. *Appl. Occup. Environ. Hyg.* **5**, 46-51.
- Kuehr, J., Frischer, T., Meinert, R., Barth, R., Forster, J., Schraub, S., et al. (1994). Mite allergen exposure is a risk for the incidence of specific sensitization. *J. Allergy Clin. Immunol.* **94**, 44-52.
- Lanphear, B. P., Emond, M., Jacobs, D. E., Weitzman, M., Tanner, M., Winter, N. L., et al. (1995). A side-by-side comparison of dust collection methods for sampling lead-contaminated house dust. *Environ. Res.* **68**, 114-123.
- Lewis, R. D., and Breysse, P. N. (1998). A comparison of the sampling characteristics of two vacuum surface samplers for the collection of dust mite allergen. *Appl. Occup. Environ. Hyg.* **13**, 536-541.
- Luczynska, C. M., Arruda, L. K., Platts-Mills, T. A. E., Miller, J. D., Lopez, M., and Chapman, M. D. (1989). 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* **118**, 227-235.
- Luczynska, C. M., Li, Y., Chapman, M. D., and Platts-Mills, T. A. E. (1990). Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). *Am. Rev. Respir. Dis.* **141**, 361-367.
- National Heart, Lung, and Blood Institute. (1991). "Guidelines for the Diagnosis and Management of Asthma." Expert Panel Report, NIH Pub. No. 91-3042. National Asthma Education Program, Office of Prevention, Education, and Control, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.
- Platts-Mills, T. A. E., and Chapman, M. D. (1987). Dust mites: Immunology, allergic disease, and environmental control. *J. Allergy Clin. Immunol.* **80**, 755-775.
- Platts-Mills, T. A. E., Sporik, R. B., Wheatley, L. M., and Heymann, P. (1995). Is there a dose-response relationship between exposure to indoor allergens and symptoms of asthma? *J. Allergy Clin. Immunol.* **96**, 435-440.
- Platts-Mills, T. A. E., Thomas, W. R., Aalberse, R. C., Vervloet, D., and Chapman, M. D. (1992). Dust mite allergens and asthma: Report of a Second International Workshop. *J. Allergy Clin. Immunol.* **89**, 1046-1060.
- Pollart, S. M., Mullins, D. E., Vailes, L. D., Hayden, M. L., Platts-Mills, T. A. E., Sutherland, W. M., et al. (1991a). Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. *J. Allergy Clin. Immunol.* **87**, 511-521.
- Pollart, S. M., Smith, T. F., Morris, E. C., Gelber, L. E., Platts-Mills, T. A. E., and Chapman, M. D. (1991b). Environmental exposure to cockroach allergens: Analysis with monoclonal antibody-based enzyme immunoassays. *J. Allergy Clin. Immunol.* **87**, 505-510.
- Pope, A. M., Patterson, R., and Burge, H. (Eds.). (1993). "Indoor Allergens: Assessing and Controlling Adverse Health Effects." Committee on the Health Effects of Indoor Allergens, Division of Health Promotion and Disease Prevention, Institute of Medicine. National Academy Press, Washington, DC.
- Rosenstreich, D. L., Eggleston, P., Kattan, M., Baker, D., Slavin, R. G., Gergen, P., et al. (1997). The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N. Engl. J. Med.* **336**, 1356-1363.
- Sporik, R., Holgate, S. T., Platt-Mills, T. A. E., and Cogswell, J. J. (1990). Exposure to house-dust mite allergen (*Der p I*) and the development of asthma in childhood. A prospective study. *N. Engl. J. Med.* **323**, 502-507.
- Taylor, W. R., and Newacheck, P. W. (1992). Impact of childhood asthma on health. *Pediatrics* **90**, 657-662.
- Vailes, L. D., Li, Y., Bao, Y., DeGroot, H., Aalberse, R. C., and Chapman, M. D. (1994). Fine specificity of B-cell epitopes on *Felis domesticus* allergen I (Fel d 1): Effect of reduction and alkylation or deglycosylation on Fel d I structure and antibody binding. *J. Allergy Clin. Immunol.* **93**, 22-33.
- Weiss, K. B., Gergen, P. J., and Wagener, D. K. (1993). Breathing better or wheezing worse? The changing epidemiology of asthma morbidity and mortality. *Annu. Rev. Public Health* **14**, 491-513.
- Weitzman, M., Gortmaker, S. L., Sobol, A. M., and Perrin, J. M. (1992). Recent trends in the prevalence and severity of childhood asthma. *J. Am. Med. Assoc.* **268**, 2673-2677.