

ORIGINAL ARTICLE

Performance of dust allergen carpet samplers in controlled laboratory studies

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Allergens and other pollutants in house dust are collected using a variety of dust samplers that are assumed to operate similarly. This factorial design study compared sampler performance under controlled environmental conditions. House dust with known particle sizes (212–90, 90–45, and <45 μm) and allergen concentrations were sampled from new carpet squares with varying denier, pile height and pile densities. Dust mass and allergen recovery for total dust mites (Der p 1 and Der f 1), cat (Fel d 1) and cockroach allergen (Bla g 1) were assessed using the Eureka Mighty Mite (EURK), the High Volume Small Surface Sampler (HVS), or the American Industrial Hygiene Association (AIHA) method. Allergen concentrations were quantified using enzyme-linked immunosorbent assays, and dust mass results were corrected for carpet fiber shedding. Samplers were compared by exploring mass collection efficiency (CE = fiber corrected dust mass/applied dust mass) and concentration ratio (CR = allergen concentration in collected sample/allergen concentration in test dust). Test dust allergen concentrations varied by particle size fraction due to varying laboratory performance over time. The EURK and HVS samplers had CEs of 41–63% in the small and medium particle size fractions, and collected less than 20% of the available dust from the large size fraction. The AIHA CE ranged from 10% to 17% in the medium and small particle size fractions, but collected little dust in the largest particle size fraction. The AIHA and HVS samplers were more likely to acquire more representative and less variable allergen CRs compared with the EURK method. Health studies that use allergen concentration as an exposure metric need to consider the implications of sampler performance when interpreting links to health outcomes and development of health-based standards for allergens in house dust.

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INTRODUCTION

Dust samples are important for assessing exposure in epidemiology, exposure reduction intervention, surveys and clinical studies of indoor allergens.^{1–4} The indoor allergens dust mite (Der f 1 and Der p 1), cat (Fel d 1) and German cockroach (Bla g 1) have been associated with the development and/or exacerbation of asthma in children and adults.⁵ Carpets are important reservoirs and sources of allergens due to repeated deposition and resuspension. Dust samples taken from carpeted floors or upholstered furniture by vacuuming are a useful tool to estimate long-term human exposure to indoor allergens.⁶

For commonly used vacuum samplers, the average collection efficiency (CE) for bulk carpet dust ranges between 40–90%.⁷ Within a given particle size range, CE depends on carpet properties such as pile height, density, and fiber density (denier), the amount of dust loaded, relative humidity and sampling velocity.^{7,8} There is evidence of substantial variability in allergen CE by sampler and allergen type: the average CE for cat allergen, for example, is ~10% under different carpet conditions, and also is driven by environmental factors.⁹ Dust mite allergens originate from cells lining the mite intestinal tract and are mainly found in 10–40 μm fecal particles.^{10,11} Cockroach allergen, which is submicronic, is typically associated with relatively large particles (a majority are >10 μm in diameter).¹² Both the environmental variables and the properties of allergens change the nature of the

interaction between dust particles and carpet fibers, with smaller particles much more likely to adhere to surfaces than larger dust particles.⁵ The relationship between these various factors is important for characterizing the magnitude of uncertainties in sample collection.

Most field studies of allergens done to date in the United States have used one of the three samplers to characterize allergen exposure by collecting dust from carpets and beds. Although names and model numbers have changed over the years, these samplers fall into three basic types, each with its own collection media or capture device. The Eureka “Mighty Mite” method (EURK), for example, has been most widely used primarily in studies looking at dust mite and roach exposures^{13,14} The CE of these samples under variable environmental conditions, however, is poorly defined, and has been evaluated in only two studies, neither of them definitive.^{1,15} In contrast, the High Volume Small Surface Sampler (HVS or cyclone) method has only been used in smaller scale studies due to its higher relative cost and less convenient ease of use, but has a well-characterized CE and thus, is considered the “gold standard” for sampling house dust.^{16,17} We and others have used the American Industrial Hygiene Association (AIHA) method in past field studies, but it has not been systematically tested against the other methods.^{2,18,19} Because of the use of a high velocity sample pump and efficient collection media, the AIHA method is expected to have a CE similar to the

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HVS.¹⁸ This makes it attractive because of its ease of use, relatively small surface area and wide availability.

Few published studies have systematically explored the precision and accuracy of various allergen samplers.^{15,20} For example, Mansour *et al.*¹⁵ compared dust mite, cat and cockroach allergen measurement results from the Eureka and cyclone methods to allergen-specific IgE levels in children. Although they showed that different samplers provided moderately correlated results, the major drawback of the study was that the various samplers gave inconsistent results when compared with biomarker measurements. The likely reason for this is the CE of the samplers varied and important environmental, dust and vacuum variables were not controlled. These findings suggest the CE varies significantly with particle size collected, which "has important implications for the normalization of exposure results using the mass of bulk dust collected as the denominator".⁸

In this study the AIHA, EURK and the HVS were used to sample carpet dust from new carpet squares in a factorial experimental design. The three sampler's dust mass CE and allergen concentration estimates were compared using common indoor allergens (dust mites, cat and cockroach), while controlling for the impact of carpet properties, dust size fraction, electrostatic charge, and relative humidity during sampling. In this study we tested the hypothesis that dust mass and/or allergen collection efficiencies differ by particle size fraction for three commonly used samplers. We also explored the implications of these results for developing standardized protocols for allergen collection and health-based allergen standards.

MATERIALS AND METHODS

Study Design

The study was a randomized factorial design exploring the effect of varying samplers (AIHA, EURK and HVS), dust particle size fraction (large (>90 µm), medium (>45–90 µm) and small (<45 µm)), carpet type and properties (denier, pile height and pile density; see Table 1) and relative humidity (RH, target values of 20%, 50%, and 80%) on allergen and dust mass CE. The order of experiments was developed by combining all the relevant factors, then randomizing the order and incorporating replicates. Experiments were designed in two phases, with Phase I performed to test whether some carpet properties could be dropped from the full factorial design. As a result of a preliminary analysis the main effects described above were kept to inform the Phase II design, but two carpet types (carpets E and T; Table 1) were dropped from further consideration because their key experimental design factors were already covered by other carpets, whereas other carpets (e.g., S) were included more frequently because they had a range of fiber denier, pile height and pile density that represented key design features to be assessed.

Experimental Procedures

All experiments were conducted in a 1.2 m by 1.2 m by 1.5 m metal and plexiglas chamber. A fan circulated air through the chamber and a humidifier and feedback system was used to maintain the RH as needed (Vicks Ultrasonic humidifier, KAZ INC., New York, NY, USA, with a RHCN-1F controller, Omega, Stamford, CT, USA). Environmental conditions inside the chamber were monitored using a Q-trak (Model 8554, TSI, Shoreview, MN, USA). For each experiment, a known mass of dust (~1–1.5 g) containing a known size fraction and concentration of allergens was embedded on a selected precleaned (to remove loose carpet fibers) 46 × 46 cm new carpet square in a controlled environment at the RH level selected. In total, 138 carpet experiments were conducted (Table 2). One of the three samplers were chosen, based on the experimental randomization: (1) a HVS (HVS3, CS3, Sandpoint Idaho) with a cyclone that is 99% efficient in collecting particles down to five microns;²¹ (2) an EURK (Model 972B, 11amp, Electrolux Home Care Products, Bloomington, IL, USA) with Duststream collection insert (40 micron nylon mesh; Indoor Biotechnologies, Charlottesville, VA, USA); or (3) an AIHA (Gilian AirCon 520 Air Sampling Pump, Synsidyne LP, Clearwater, Florida, USA) with a 37 mm polycarbonate filter cassette (0.8 µm pore size MCE filters, Model 738 MCE, Zefon Analytical Accessories, St. Petersburg, FL, USA). The cut-point of the HVS cyclone is estimated to be less than 5 µm at 566 l/min, while the other two

Table 1. Properties of new carpets used in experiments.

Carpet model	Denier (dpf ^a)	Pile height (cm)	Pile density (stitches/inch in fiber length)
A	18	0.53	13
D	18	0.9	10
E	10	0.59	14
H	12	0.53	10
P	12	1	10
S	17	0.59	10
T	18	0.56	9

Abbreviation: dpf, denier per filament.

^aFiber mass in grams per 9000 m.

Table 2. Total number of dust samples collected in all experiments stratified by sampler and particle size, with number of replicate and non-replicate samples.

Method	Particle size ^a	Total (N)	N, non-replicates	N, replicates
AIHA	Total	49	38	11
	Large	19	16	3
	Medium	10	7	3
	Small	20	15	5
EURK	Total	50	37	13
	Large	19	16	3
	Medium	11	8	3
	Small	20	13	7
HVS	Total	39	34	5
	Large	17	14	3
	Medium	8	6	2
	Small	14	14	0
All		138	109	29

Abbreviations: AIHA, American Industrial Hygiene Association method; EURK, Eureka Mighty Mite method; HVS, high volume surface method; N, number of experiments or replicate experiments with the same treatment.

^aSize fraction: large = 90–212 µm; medium = 45–90 µm; small = <45 µm.

samplers were routinely calibrated without sampling media to confirm flow rates. Test dust collected from inner city homes from a previous study with known allergen levels was used in all experiments.² Test dust was separated into three dust particle size fractions using a sieve shaker (Meinzer II sieve shaker, CSC Scientific Company, Fairfax, VA, USA) to obtain large-, medium- and small-size fractions. Sampling media and dust were desiccated for a minimum of 12 h in a low-humidity chamber before each experiment. Test carpets squares were conditioned at the experiment's proscribed RH level for a minimum of 16 h in a temperature and humidity controlled chamber and monitored using a data logger (RHCN-1F, Omega, Stamford, CT, USA).¹⁶ Dust from each size fraction was then deposited and embedded on one of the seven selected carpets using established methods.^{16,22} After 30 minutes the embedded dust was vacuumed from the carpet squares using the selected sampler. The vacuum was passed over the carpet squares by moving it in an up and down, side by side and left and right diagonal direction four times for each experiment over ~5 min. During the sampling process electrostatic intensity on the carpet was measured using a portable electrostatic field meter (Model 257D Monroe Electronics, Lyndonville, NY, USA).

Quality Control (QC) and Assurance

Measurement devices, including vacuums, scales and data loggers, were calibrated before all experiments, and carpet fiber correction factors (CFCF) were developed for each carpet type based on repeated vacuuming of new carpet squares until fiber mass released was negligible. Allergen

concentration limits were determined using field and laboratory blanks. One blank and one certified sample with known allergen concentrations provided by the granting agency (hereafter, HUD QC samples) was submitted for analysis in each batch of 20 collected dust samples. Approximately 5% of the experiments were randomly assigned as replicates, and data from these experiments were used to estimate precision.

Laboratory Analysis

Collected samples were sent to Air Quality Sciences (Marietta, GA, USA) for analysis using established procedures and protocols.^{23,24} Concentrations of the allergens Der f 1 ($\mu\text{g/g}$), Der p 1 ($\mu\text{g/g}$), Fel d 1 ($\mu\text{g/g}$) and Bla g 1 (U/g) were measured with monoclonal-antibody-based enzyme-linked immunosorbent assays (ELISA).^{23,24} The HUD QC samples were used to track laboratory performance over time. Samples were processed in analytical lots, and mean %CV across all allergen analytical batches was ~30%. Overall laboratory performance was judged to be acceptable for purposes of this analysis as long as batch effects were accounted for, as described in the statistical analysis section.

Statistical Analysis

Descriptive statistics and univariate general linear regression analyses were performed to compare percent CE and allergen concentration ratio (CR), which are defined as follows for a given size fraction (*i*) and allergen (*j*) and carpet (*k*)

$$\text{CE}_{ijk} = \frac{\text{DR}_{ijk} - \text{CFCF}_k}{\text{DD}_{ijk}} \times 100$$

where DR is mass in grams (g) of dust recovered, CFCF is mean carpet fiber correction factor (g) (see above), and DD is mass (g) of dust deposited.

$$\text{CR}_{ij} = \frac{\text{RAC}_{ij}}{\text{EAC}_{ij}} \times 100$$

where RAC is particle-size and allergen-specific recovered allergen concentration during the experiment, and EAC is the expected allergen concentration, based on replicate measures of the experimental test dust. Phase 1 experiments identified experimental design factors with limited predictive power, and the final data set included data obtained from both phases. Forward selection multivariable linear regression was used to determine if covariates and pair wise interactions were associated with CE or CR at an alpha level of 0.1 after forcing sampler into the model. In the final models, the adjusted associations between outcomes and samplers were evaluated at an alpha level of 0.01. The HUD QC sample results were used to adjust concentrations obtained from each analytical lot. This was done by adjusting the experimental dust allergen concentration results for that specific allergen and particle size fraction combination by the percent recoveries reported for the HUD QC samples for that analytical lot. Analytical lot was included as an independent variable in all ANOVA and regression analyses. Statistical analyses were performed using SAS version 9.3 (SAS Corporation, Cary, NC, USA).

RESULTS

A total of 138 experiments, with replicates, were randomly distributed across the three methods and dust particle size fractions (Table 2). Because of experimental design changes incorporated after Phase I preliminary data analysis that eliminated carpet types E and T, there were a lower number of samples collected with the HVS method (39) compared with those collected with the AIHA (49) and EURK (50) methods. This also resulted in fewer medium dust particle size samples (29) compared with large (55) and small (54) size fractions.

Collection Efficiency

The CE results were available for all 138 samples collected. As shown in Table 3, CE varied widely by sampler and particle size fraction. The AIHA method had the lowest mean CE and the greatest variability for all particle size fractions, collecting very little dust in the large size fraction and less than half the typical mass of dust of the other two samplers for the other two particle size fractions. The EURK method had the highest CE and lowest

Table 3. Summary of CE and allergen CR results by sampling method and dust particle size fraction.

Sampling method	Dust particle size fraction ^a	Collection efficiency (CE)			Concentration ratio (CR)											
		N ^b	Mean ± SD (%)	CV (%)	Bla g1				Fel d1				Total DM ^c			
					n ^d	Det. (%)	Mean ± SD ^e	CV (%)	n ^d	Det. (%)	Mean ± SD ^e	CV (%)	n ^d	Det. (%)	Mean ± SD ^e	CV (%)
AIHA	Large	19	1.5 ± 1.1	71	1 ^f	5.3	75	NA	1 ^f	5.3	1.87	NA	1 ^f	100	6.59	NA
	Medium	10	9.7 ± 8.9	91	10	100	1.15 ± 0.42	37	10	90	1.19 ± 0.9	67	10	100	1.26 ± 0.32	25
	Small	20	17 ± 15	93	18	100	0.85 ± 0.24	28	16	94	1.06 ± 0.52	49	18	100	0.79 ± 0.19	24
EURK	Large	19	47 ± 14	29	19	100	1.8 ± 1.8	101	19	89	0.52 ± 0.42	80	19	16	0.52 ± 0.33	64
	Medium	11	63 ± 10	15	11	100	0.65 ± 0.29	64	11	91	1.01 ± 0.41	40	11	91	0.77 ± 0.31	40
	Small	20	44 ± 17	39	20	100	0.98 ± 0.29	30	20	95	1.14 ± 0.73	64	20	100	0.92 ± 0.54	58
HVS	Large	17	24 ± 13	54	17	100	1.3 ± 0.84	65	17	94	0.45 ± 0.49	110	17	12	0.69 ± 0.78	113
	Medium	8	41 ± 12	29	8	100	0.73 ± 0.42	58	8	63	0.49 ± 0.38	78	8	100	0.84 ± 0.2	24
	Small	14	42 ± 18	44	14	100	0.86 ± 0.26	29	14	93	1.26 ± 1.69	134	14	100	0.76 ± 0.22	29

^aParticle size fraction: large = 90–212 µm; medium = 45–90 µm; small = < 45 µm. ^bN: number of experiments conducted. ^cTotal dust mite = Der p1 + Der f1. ^dn: number of laboratory analytical results. ^eN–n = the number of experiments with insufficient dust mass for allergen quantification. ^fMean and SD calculated using 1/2 the analytical lot detection limit for each batch. ^gOnly one dust sample from the AIHA method large particle size fraction tests had sufficient volume for the allergen analyses.

^aParticle size fraction: large = 90–212 μm ; medium = 45–90 μm ; small = < 45 μm . ^bN: number of experiments conducted. ^cTotal dust mite = Der p 1 + Der f 1. ^dn: number of laboratory analytical results. N—n = the number of experiments with insufficient dust mass for allergen quantification. ^eMean and SD calculated using 1/2 the analytical lot detection limit for each batch. ^fOnly one dust sample from the AIHA method large particle size fraction tests had sufficient volume for the allergen analyses.

Table 4. Univariate regression results for associations between collection efficiency and allergen specific CRss.

Independent variable	Dependent variables			
	Collection efficiency (CE)	Ln concentration ratio (CR)		
		Bla g1	Fel d1	Total DM ^a
<i>Difference in means between methods</i>				
AIHA and EURK	40***	0.09	0.46.	−0.47**
AIHA and HVS	25***	0.16	0.87**	−0.53**
<i>Difference in means between particle sizes</i>				
Small and medium	5.4	0.15	−0.25	0.12
Small and large	−8.8*	0.44**	−0.92***	−0.61***
Difference in means between low and high denier	7.0	0.057	0.42	0.85***
Difference in means between high and low pile height	−0.27	−0.16	0.23	0.064
Difference in means between high and low carpet density	8.4	−0.44**	0.38	0.027
Change in mean for each unit increase in relative humidity	0.026	−0.00020	−0.00018	0.0017
Change in mean for each unit increase in electrostatic intensity	0.57	−0.0026	−0.022	−0.026

*** $P < 0.001$, ** $P < 0.01$, > or = 0.001, * $P < 0.05$, > or = 0.01, $P = < 0.1$, > or 0.05. ^aTotal DM = Der p 1 + Der f 1.

variability for all three particle size fractions, with mean CEs that ranged from 44% to 63%. The mean CEs for the HVS method ranged from 24% to 42%.

Univariate regression analysis indicated significant associations between CE and sampler, particle size fraction and carpet density (Table 4). Multiple linear regression analysis indicated a significant association ($P < 0.0001$) between mean CE and method after adjusting for carpet density and the interaction between sampler and particle size fraction (Table 5). The sampler accounted most of the CE-associated variability (56%). The interaction between sampler and particle size fraction and carpet density accounted for another 11% and 5% of the overall variability, respectively.

The final regression model presented in Table 5 accounts for 72% of the variance in CE, and includes pile density and an interaction term for the sampler and particle size fraction. The final model indicates that the EURK method is the most efficient at collecting dust from the medium and large particle size fractions, the AIHA method is the least efficient at collecting dust from all particle size fractions and that EURK and HVS methods are indistinguishable when collecting dust in the smallest size fraction. The final model also indicates that for all methods and particle size fractions, CEs are higher in high density carpets than low density carpets.

Dust Allergen CRs

Dust allergen results were not available for 20 out of the 138 samples, because there was insufficient dust mass collected for accurate allergen quantification. Allergen detection frequencies ranged from 63% to 100% for all samplers in the in the medium and small dust size fractions, and all samplers had relatively low frequencies of allergen detection in the large size fraction (Table 3).

Cockroach allergen. Mean cockroach allergen CRs did not vary substantially by sampler, but did vary by particle size fraction (Table 3). Mean cockroach allergen CRs ranged from 0.65 to 1.8 across all samplers. Variability in CR increased from the smallest to the largest particle size fraction. The maximum CR of 75 is an outlier calculated from the only AIHA method test result with sufficient mass in the large particle size fraction.

Univariate regression analysis indicated significant associations, at an alpha level of 0.1, between particle size fraction and carpet density for cockroach allergen (Table 4). No significant association between cockroach allergen CR and method was observed.

Multiple linear regression analysis found a significant association between mean cockroach allergen, CR and sampler after adjusting for carpet density and the interaction between method and particle size fraction ($P < 0.0001$) (Table 5). Sampler accounted for less than 1% of the variability observed in the cockroach allergen CRs. The interaction between sampler and particle size fraction accounted for most of the observed variability (50%) in cockroach allergen concentration, while in the final model carpet density accounted for only an additional 3% of the observed variability (Table 5).

The final regression model indicates that the AIHA method is better at collecting cockroach allergen from the medium particle fraction than the other two methods. It also indicates that for all methods and particle size fractions, cockroach allergen is recovered at a higher rate from high-density carpets compared with lower-density carpets.

Cat allergen. Mean cat allergen CRs varied by method and particle size fraction, and were nearest to one for the small and medium particle size fractions collected by the EURK and AIHA methods and the HVS in the small size fraction (Table 3). Greater variability was observed for the large and the medium size fractions using the HVS sampler.

Univariate regression analysis indicated significant associations, at an alpha level of 0.1, between sampler and particle size fraction for mean cat allergen CRs (Table 4). Regression analysis indicated a significant association between mean cat allergen CR and method after adjusting for particle size fraction ($P = 0.0008$) (Table 5). Sampler (8%) and particle size fraction (7%) accounted for most of the variability in cat allergen CRs the in the final model presented in Table 5.

For each particle size fraction, the best fit regression model indicates the AIHA method is the most efficient at recovering cat allergen, and the HSV3 sampler is the least efficient. It also indicates that for each sampler, cat allergen is recovered most efficiently from the smallest particle size fraction.

Total dust mite. Mean total dust mite allergen CRs varied by method and particle size fraction, with the largest size fraction displaying the greatest variability (Table 3). Mean total dust mite allergen CRs were least variable using the AIHA and HVS methods, and more variable but nearest to one for the small particle size fraction collected using the EURK method. The maximum CR of 6.5 is an outlier calculated from the only AIHA method test result with sufficient mass in the large particle size fraction.

Table 5. Coefficients and statistical significance results for best fit multivariable linear regression models for CE and allergen CRs.

Intercept	Main effects						Interaction terms				
	Method: EMM ^a	Method: HSV3 ^a	PS: ^b large ^c	PS: ^b medium ^c	High pile density ^d	High denier ^e	EURK*PS large ^{a,b,c}	EURK*PS medium ^{a,b,c}	HSV*PS large ^{a,b,c}	HSV* PS medium ^{a,b,c}	
Dependent variable: CE: $R^2 = 0.72$, $F = 35.9$, $P < 0.0001$, $N = 138$	0.13	0.27	0.27	−0.15	−0.078	0.10	—	0.19	0.27	−0.030	0.081
Dependent variable: Ln Bla g1 CR: $R^2 = 0.53$, $F = 13.7$, $P < 0.0001$, $N = 118$	−0.28	0.13	0.041	4.3	0.22	0.29	—	−4.0	−0.70	−4.0	−0.41
Dependent variable: Ln Fel d1 CR: $R^2 = 0.15$, $F = 6.1$, $P = 0.0008$, $N = 118$	0.019	−0.22	−0.60	−0.78	−0.25	—	—	—	—	—	—
Dependent variable: Ln total DM ^f CR: $R^2 = 0.46$, $F = 10.2$, $P < 0.0001$, $N = 118$	−0.86	0.12	0.04	2.0	0.36	—	0.71	−2.7	−0.69	−2.7	−0.45
Bold-italic font indicates significant associations at an alpha level of 0.01 or less. ^a Referent: AIHA method. ^b Size fraction: large = 90–212 μm; medium = 45–90 μm; small = <45 μm. ^c Referent: small particle size. ^d Referent: low pile density. ^e Referent: low denier. ^f Total DM = Der p1 + Der f1.											

Bold-italic font indicates significant associations at an alpha level of 0.01 or less. ^aReferent: AIHA method. ^bSize fraction: large = 90–212 μm ; medium = 45–90 μm ; small = <45 μm . ^cReferent: small particle size. ^dReferent: low pile density. ^eReferent: low denier. ^fTotal DM = Der p1 + Der f1.

Univariate regression analyses indicated significant associations, at an alpha level of 0.1, between the mean total dust mite allergen CRs and sampler, particle size fraction and denier (Table 4). Regression analysis indicated a significant association between mean total dust mite allergen CR and sampler after adjusting for denier and the interaction between method and particle size fraction ($P < 0.0001$) (Table 5). The sampler accounted for relatively little of the observed variability (9%) in the total dust mite allergen CRs. The interaction between sampling method and particle size fraction accounted for 24% of the observed variability, while denier accounted for 13% of the observed variability. The final model presented in Table 5 accounted for 46% of the overall variability in dust mite CRs.

The final model indicates that the EURK method was better at recovering dust mite allergen from the small particle size fraction, while the AIHA method was better at recovering dust mite allergen from the medium particle size fraction. It also indicates similar performance for the HVS and EURK methods for the large particle size fraction.

DISCUSSION

Dust sampler and particle size fraction were the factors most strongly associated with increased dust CE in these experiments, and this effect is much larger than the effect of any of the environmental or carpet variables on sampler performance. The performance of the three samplers varied substantially for both CE and allergen CR across the various particle size fractions tested. The EURK and HVS samplers had the best CEs across the two smaller particle size fractions, and all samplers had relatively low CE for particles in the largest size fraction (i.e., >90 μm). The AIHA sampler collected little dust in the large size fraction, and about half the typical mass of dust of the other samplers for the medium and small size fractions. These data suggest that use of the EURK (and to a certain extent the HVS) sampler(s) tends to increase the variability and underestimate allergen concentrations in house dust. This is because these samplers collect substantial dust mass from the larger particle size fraction, which is likely not important for accurate allergen concentration quantification.

In contrast to the effect of particle size, allergen CR results demonstrated that the AIHA and HVS samplers were more likely to collect representative samples of cockroach and dust mite allergen, as indicated by CRs that had lower %CVs and were closer to unity when compared with the EURK method. In this

study, all samplers had cat allergen CRs that were highly variable: all samplers performed poorly in terms of reporting representative concentration results. As previous research has shown, cat allergen is sticky and appears to affect sampler performance.^{25,26} As a consequence, the dust mite and cockroach results from this study are more important for assessing sampler performance for a range of allergens. Focusing on the medium size fraction results, cockroach CR results were closest to unity ($\pm 15\%$ of unity) with less variability using the AIHA method. The CR results in the small size fraction were similar for all three samplers. In contrast, the total dust mite CR results in the medium and small size fraction were similar for all three samplers, though the AIHA and HVS displayed less variability (<30%) compared with the EURK method (40% and 58%). These data indicate that the AIHA and HVS methods likely provide more accurate concentration measurements for non-cat allergens, though this appears related to the increased particle CE of the EURK method. Although these relative differences are likely important for assessing associations with biomarkers and health outcomes, this factor is not the only consideration when choosing a sampler for use in studies.

Implications for Dust Sampling in Field Studies

One of the goals in assessing sampler performance was to make recommendations for best practices for dust collection in field studies. While our laboratory results provide relevant data on dust CE and allergen concentration, other factors need to be considered in choosing a sampler in homes. Field studies in indoor environments typically have significant time restraints, and must be designed to collect multiple types of information in an efficient manner.^{27–29} Typically they are designed to meet a number of important considerations that are also conflicting goals: efficient subject contact, recruitment, retention and compliance with study protocols; valid and complete sample collection; and compliance with institutional review board ethical research strictures, such as low burden for participants. Samples should be collected with the sampler that allows for meeting these multiple objectives in the most rigorous manner that addresses scientific objectives of the study. When dust sampling is conducted the usual decision rule for a successful sampling event is collection of sufficient mass for accurate concentration quantitation. It is clear from these laboratory-based results that this approach may only insure that the sample mass is sufficient without necessarily improving measurement accuracy, allergen exposure estimation or links to health outcomes.

Current guidance for dust allergen sampling does not specify a vacuum sampler but does recommend that studies use one of two types of in sampling train collection media, sample a large surface area for at least 5 minutes, obtain samples so that area concentrations can be calculated and sieve the dust sample to $<300\text{ }\mu\text{m}$ before analysis.^{1,30} Our results suggest that further refinement of sampling guidance should recommend vacuum properties, such as minimum CE for small particles and maximum large particle size capture. In addition, collection of some carpet factors, such as denier and pile density, is worthwhile. These results suggest that in most cases measurement of other carpet and environmental factors, such as pile height, RH and EI, are not necessary.

Health guidelines developed to date for allergens have focused on concentrations in house dust, and there have been calls to develop health-based standards for indoor environments.⁵ Suggested health benchmarks include $>8\text{ }\mu\text{g/g}$ for cat (acute symptoms), $>2\text{ }\mu\text{g/g}$ for cockroach (sensitization) and $>2\text{ }\mu\text{g/g}$ for dust mite (sensitization).⁵ Given the effect of sampler on allergen concentration estimation in this study, future research aimed at establishing health-based standards need to specify sampler and incorporate QA/QC methods that make allergen concentration measurements interpretable. Future studies of the effect of indoor environment, such as the National Children's study in the United States, are considering using samples from resident vacuum bags.^{29,31,32} Given the recent introduction of bagless vacuums to the broad consumer market and that resident ownership of a vacuum varies considerably by income, allergen concentration results obtained from resident vacuums needs careful evaluation in light of these laboratory results.

Strengths and Limitations

The principle strengths of this study include (1) a randomized, controlled design, (2) the use of a well-characterized size-fractionated house dust collected from inner city homes and (3) controlled environmental test conditions that mimic the range of conditions found in the field. Nonetheless, the use of real world house dust also leads to related limitations, principally because of variability in dust concentrations related to agglomeration of allergen-laden particles, which likely lead to considerable batch to batch variability in measured allergen concentrations. This variability, coupled with variability in ELISA performance, lead to substantial between analytical batch variability in these experiments. This shortcoming was identified using QA/QC samples, and addressed in our analysis by controlling for analytical batch. This underscores the ongoing need for reference dusts with known allergen concentrations to assess the performance of both field and laboratory methods over time.² The use of size-fractionated test dust is both a strength and limitation of this work, because field studies collect bulk dust samples and relate them to their outcome of interest. Although these results are specific to the size-fractions tested, these results suggest that more stable allergen concentration measurements will be achieved if bulk dust samples are sieved to $<90\text{ }\mu\text{m}$ s, though doing so may exclude agglomerated particles. Lastly, these results are limited to allergens and carpets types tested and do not address sampler performance on hard surfaces or worn carpets. The existing literature shows that carpet wear affects dust mite and cat allergen retention.^{33–35} Further experimental work will be needed to address these issues and limitations.

The best sampler for these allergens and these conditions may not be the best for other biological, metals or semivolatile or persistent organic compounds. Nonetheless, given, what is known about where those pollutants partition in dust, that is, most are present in the $<100\text{ }\mu\text{m}$ size fraction, it is likely that these results are widely applicable to similar samplers for these other compounds of interest.^{5,36–38}

In conclusion, while CE is typically used to assess sampler performance and insure sufficient dust for quantification in field studies, allergen concentration is also an important consideration in evaluating sampler performance, as overall mass collected is less important than allergen concentration for health-based standards and assessing the effectiveness of exposure reduction interventions. Future research in this area should focus on identifying methods that optimize sampling of allergens, and other pollutants in indoor environments on both carpets and hard surfaces.

ABBREVIATIONS

AIHA, American Industrial Hygiene Association (vacuum sampling method); Bla g 1, Major allergen of the German cockroach; CE, Collection efficiency; CR, Concentration ratio; CV, Coefficient of variation (%); Der f 1, *Dermatophagoides farinae* allergen; Der p 1, *Dermatophagoides pteronyssinus* allergen; ELISA, Enzyme-Linked ImmunoSorbent Assay; EURK, Eureka Mighty Mite; Fel d 1, *Felis domesticus* allergen; HVS, High volume surface sampler; RH, Relative humidity

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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