Journal of Exposure Science and Environmental Epidemiology (2014) 24, 3 – 8
© 2014 Nature America, Inc. All rights reserved 1559-0631/14
www.nature.com/jes

ORIGINAL ARTICLE

Performance of the halogen immunoassay to assess airborne mouse allergen-containing particles in a laboratory animal facility

Félix E. Rivera-Mariani[1], Elizabeth C. Matsui[1,2,3] and Patrick N. Breysse[1]

Airborne mouse allergen is a risk factor for respiratory diseases. Conventional assessment techniques provide mass-based exposure estimates that may not capture completely the inhalation risk of airborne allergen particles. In contrast to mass-based estimates, the halogen immunoassay (HIA) combines immunoblotting and microscopy to directly assess allergen-containing particles. We evaluated the HIA for the assessment of airborne mouse allergen and compared the results to the enzyme linked immunosorbent assay (ELISA). Particulate matter $(PM)_{10}$ and $PM_{2.5}$ samples (30 min, 4 l/m) were collected in a mouse facility before, during, and after disturbance of soiled bedding. Concentrations of Mus m 1-positive particles (haloed particles (HPs)) and intensities of the haloes were determined with the HIA. Although HPs/m^3 were positively correlated with mass concentration (statistically significant only with Mus m 1 concentration on PM_{10}), replicates of mass concentration showed higher variability than HPs/m^3. After disturbance, most of the HPs were in the $PM_{2.5}$ fraction. Mean haloes intensities were similar before, during, and after disturbance. The HIA was able to measure allergen-containing particles with less variability than the ELISA, detected the shift of HPs to smaller particles after disturbance, and may suggests similar halo intensity by particles detected during and after disturbance. Our findings suggest that the HIA can be used to assess indoor concentrations of mouse allergen particles and their morphological characteristics.

Journal of Exposure Science and Environmental Epidemiology (2014) **24,** 3–8; doi:10.1038/jes.2012.76; published online 18 July 2012

Keywords: halogen immunoassay; haloed particles; mouse allergen

INTRODUCTION

Exposure to airborne mouse allergen, and its impact on allergic respiratory diseases have been described in occupational, inner-city, suburban, and school settings.[1–4] These studies have shown that these environments can serve as sources for mouse allergen exposure, which could contribute to the initiation and persistence of allergic respiratory diseases.[5]

Exposure assessment to mouse allergen has been traditionally performed by extracting allergen from air and dust samples and measuring the mass concentration with enzyme-linked immunosorbent assay (ELISA). Reservoir (dust) allergen is often an index of allergen source, but does not necessarily measure what is being inhaled by an individual as exposure can occur away from the reservoir allergen as it can be aerosolized with disturbance and thereby become airborne.[6,7] With air samples, during normal and undisturbed conditions, detectable levels of allergen often require long sampling times.[8,9] Long-term sampling may not provide a measure of acute exposure, which is important to determine in diseases known for rapid physiological changes such as with allergic diseases.[10] In addition, incomplete allergen extraction may lead to underestimation of allergen exposure.[9] Mass concentration–based exposure assessments also do not fully capture the distribution of the aerodynamic sizes of allergen-carrying particles, which is important as different-sized particles deposit in different parts of the airway, and therefore elicit different airways responses.[11] Therefore, strategies that measure not only the allergenic protein, but can also directly assess the allergen-containing particles may provide better measures of inhalation risks.

The halogen immunoassay (HIA) is a method that combines immunoblotting and microscopy to detect allergen-containing particles. It has been used to document indoor (i.e. cat, dust mite, cockroach),[6,7,12] occupational (i.e. latex allergens),[13] and outdoor allergens (i.e. pollen, fungi).[14,15] In addition, the sensitivity of the HIA has been demonstrated with previously undocumented fungal allergens.[16,17] More importantly, studies that measured indoor allergens with the HIA were able to detect allergen-carrying particles within short sampling times (10–15 min) or at low concentrations that are difficult to detect with traditional ELISA methods. Cockroach allergen, for example, is difficult to detect in air samples in home environments using ELISA. De Lucca et al.[18] was able to detect airborne cockroach allergen during both normal disturbance (vacuuming) and undisturbed conditions in home environments using the HIA. This was the first study to detect airborne cockroach allergen without creating artificial environmental disturbance. In addition, the HIA provides the ability to determine a particle number-based (numbers of allergen-containing particles per unit volume of air) concentration estimate of exposure over short or long periods of time. Expert groups reviewing air pollution research have identified the need to explore number or count-based exposure metrics for particulate matter (PM) exposure assessments a research priority.[19] In the

[1]Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA; [2]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA; [3]Department of Pediatrics, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA. Correspondence to: Dr. Félix E. Rivera-Mariani, Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe St, E6628, Baltimore, Maryland 21205, USA.
Tel.: +787 463 6862. Fax: +410 955 9334.
E-mail: frivera2@jhmi.edu
Received 10 December 2011; accepted 29 May 2012; published online 18 July 2012

case of allergens, number-based estimates of allergen-containing particles may be a better predictor of inhalation risk than mass-based exposure (i.e. ELISA) by providing concentration and particle-size distribution of allergen-containing particles that have the potential to deposit at different anatomical sites of the respiratory tract. Equal masses of allergen may have different health effects depending on the total number of particles and the location in which they may deposit.

Therefore, the objective of this study was to evaluate the utility of the HIA to detect and quantify airborne mouse allergen particles by collecting air samples in a mouse facility and compare number-based (HIA) and mass-based (ELISA) concentration estimates. We also evaluated the halo-intensity of the airborne mouse allergen particles.

METHODS

Air Samples

Air sampling was performed during two different days in a mouse allergen facility. Air samples were collected with personal environmental monitors (M200 PEM) (MSP, Shoreview, MN, USA) for PM 10 μm or smaller (PM_{10}) and 2.5 μm or smaller ($PM_{2.5}$). All sampling heads were loaded with 37-mm diameter, 0.8 μm pore size mixed-cellulose-ester protein-binding membranes (MCE-PBM; Millipore, Billerica, MA, USA), and attached to a BGI pump (SKC, Eighty Four, PA, USA) calibrated at 4 l/m. The samplers were located 1.5 m from ground level, 2 m from the entrance of the mouse facility, and 1 m from the walls. Air samples were collected for 30 min, before, during, and 1 h after artificial disturbance of mouse allergen particles (shaking a bin with soiled mouse bedding for 1 min every 15 min during 1 h). Mice moved freely inside their cages during all stages of sampling. After the samples were collected, the sampling heads were brought to the laboratory and the filters divided in half: one half was analyzed with the ELISA and the other half with the HIA (Figure 1). The half of the MCE-PBM analyzed with ELISA was kept in sterile Petri dishes at room temperature until analyzed (2 weeks after being collected). The half to be analyzed with the HIA was sealed with strips of ABgene™ QPCR sealing film (Thermo Scientific, Waltham, MA, USA) and pressure applied to remove air bubbles. Sealed membranes were stored in Petri dishes at room temperature until analyzed with the HIA.

ELISA for Mus m 1

MCE-PBMs were extracted with 1.5 ml of 0.15 mM PBS with 0.1% Tween 20 and shaken overnight at 4 °C.[9] The concentration of mouse allergen was quantified using sandwich ELISA with polyclonal rabbit anti-Mus m 1.[2] Concentrations of mouse allergen with the ELISA were expressed as ng/m^3. The limit of detection (LOD) was determined to be 1.4 ng/m^3.

To assess the recovery of Mus m 1 from MCE filters, we applied two different concentrations (2.8 mg and 0.28 mg) of lyophilized Mus m 1 to MCE filters. The spiked MCE filters were extracted and analyzed with ELISA as above. In both cases, the recovery was $>85\%$ (data not shown).

HIA for Mus m 1

Sealed MCE-PBM were cut into 5-mm squares, in triplicate, and transferred into wells of a 12-well Falcon tissue culture plate (Becton Dickinson, Franklin Lakes, NJ, USA) containing 800 μl of borate buffer. The membranes were incubated overnight at 4 °C to allow the elution of allergen onto the surroundings of the particles of origin. The HIA (Figure 1) was performed as previously described[20] to immunodetect Mus m 1-containing particles. Developed membranes were stored in deionized water at 4 °C and microscopically analyzed within 3 days. Field blanks were analyzed, and no immunostained particles were detected (data not shown).

Image Analysis

Image analysis was performed as previously described[17] with additional modifications. Processed MCE-PBMs were fixed onto microscope slides with an adhesive (generously provided by Dr. Euan Tovey) mixed with acetone. The acetone allowed slight clearance of the MCE-PBM membranes, which facilitated the visualization of the haloed particles. Fixed membranes were examined in a Nikon Eclipse E800 (Nikon, Melville, NY, USA) microscope connected to a SPOT RT3 (Diagnostic Instruments, Sterling Heights, MI, USA) camera synchronized to a computer. Ten images/membrane were captured at $\times 200$ magnification with the software SPOT Advanced (Diagnostic Instruments) by scanning through a horizontal transverse through the middle of the sealed MCE.

Haloed particles (HPs) on each image were detected and counted using the image analysis software ImageJ (National Institutes of Health, Bethesda, MD, USA; Figure 2). This open-source software has been previously used with the HIA.[17] To detect and count HPs, each image was

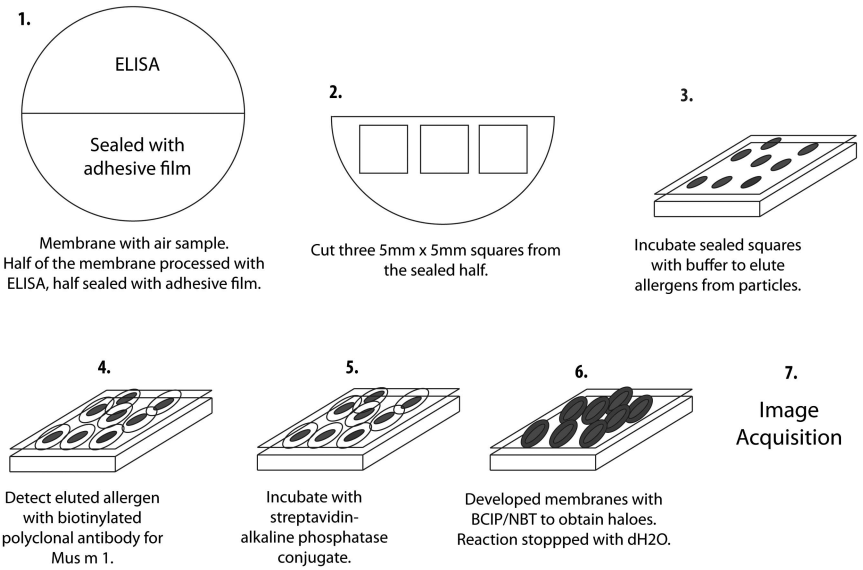

Figure 1. Protocol for the analysis of collected air samples. (1) Membranes with air samples were divided for enzyme-linked immunosorbent assay (ELISA) and halogen immunoassay (HIA) analysis. (2) The half for the HIA was sealed and squares prepared, which were (3) incubated with buffer to elute allergens from the immobilized particles. (4) The eluted allergen was detected with polyclonal antibody (Ab), and (5) after incubating with conjugate, (6) the membranes were developed with the conjugate's substrate to obtain haloes (allergen–antibody complexes). (7) Images were acquired and image analysis performed. BCIP/NBT, 5-bromo-chrloro-3′-indolyphosphate/nitro-blue tetrazolium.

© 2014 Nature America, Inc.

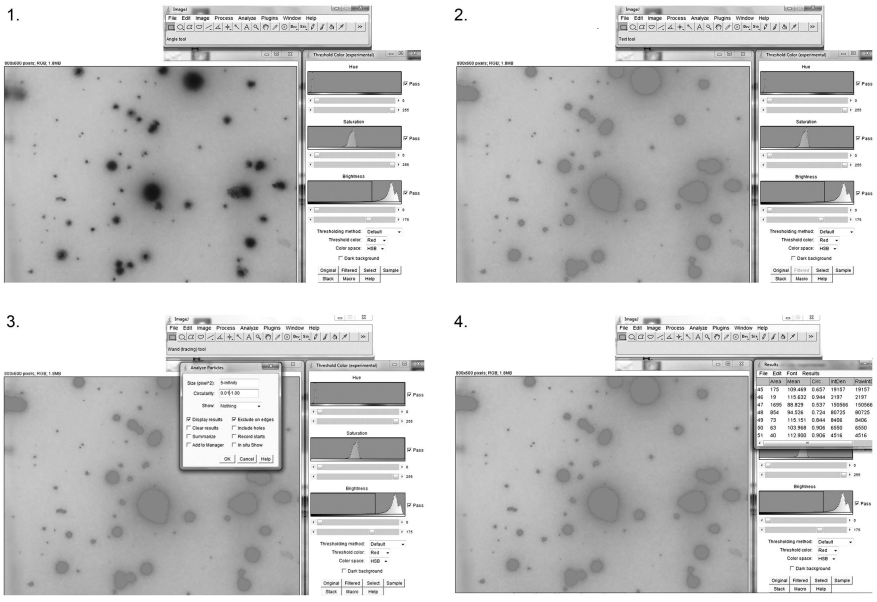

Figure 2. Image analysis protocol. Acquired images were analyzed with ImageJ. (1) Haloed particles on the image were isolated (2) based on their difference in brightness from that of the background. (3) After determining the size range of the particles to include, (4) the concentration of haloed particles in the image was automatically determined by the software, together with integrated densities of each halo.

color-segmented to isolate the particle of interest (particle with purple haloes) from the background. Overlapping haloes were separated by applying the special segmentation feature of the software known as watershed separation. With this function, the software estimates the distance from the center to the edge based on equidistance (i.e. uses points on same particle that are not overlapping with another particle) (http://www.macbiophotonics.ca/imagej/particle_analysis.htm). Given that the integrated density of the haloes is a measure of the color intensity of the stain and the size of the halo,[18,21] the software was set to determine both the total number of HPs and the integrated density (intensity of the haloes) of the halo surrounding each particle. The integrated density of the haloes was reported as the product of size of the halo (pixels^2) and the color intensity (pixels).

Concentration of HPs

To calculate the number of total HPs per filter (N_t), the following equation was used,[22]

$$N_t\left(\frac{HPs}{filter}\right) = \frac{N_f}{A_f}At$$

in which N_f is the average particles per field, A_t is the area of the filter (962 mm^2), and A_f is the area of the microscopic field at 200 × (0.2954 mm^2). To convert the number of HPs per filter into HPs per m^3 of air (C_{total}) the equation below was used, in which Q is the flow rate (4×10^{-3} m^3/m) and t is the sampling time (30 min).

$$C_{total} = N_{total}/(Q \times t)$$

Based on counting 1 particle in 10 fields (0.1 particles/field), the LOD for the HIA at ×200 would be 2.8×10^3 HPs/m^3 from a 30-min sample collected at a flow rate of 4 l/min.

Statistical Analysis

The statistical analysis was performed with Minitab 16.1. 1 (Minitab, State College, PA, USA). The means of the triplicates for the concentration of HPs/m^3 were plotted against the mass concentration of Mus m 1 determined with ELISA. An Anderson–Darling normality test was performed to determine normal distribution of the concentrations of HPs/m^3, mass-concentration of Mus m 1, and integrated densities of haloes. HPs/m^3 were graphed with mass-concentration of Mus m 1 (ng/m^3) in a scatterplot. The Spearman rank correlation was calculated to determine the level of correlation between the concentrations of HPs/m^3 and ng/m^3 of Mus m 1. Before testing for significant differences between the geometric means with one-way ANOVA, integrated densities of haloes were log-transformed owing to their non-parametric distribution. $P < 0.05$ was considered statistically significant.

RESULTS

Concentration of HPs and Mass Allergen Concentration

Airborne particles were collected in a mouse facility before, during, and after artificial disturbance on 2 separate days. The concentration of mouse allergen particles was determined with the HIA (HPs/m^3) and the mass concentration of mouse allergen by ELISA (ng/m^3 of Mus m 1). For PM_{10} samples (Table 1), the concentrations of HPs before disturbance were at the LOD (2.8×10^3 HPs/m^3, equivalent to counting a single particle in 10 fields). The concentration of HPs increased by 2–3 orders of magnitude during disturbance and decreased by a factor of about 2 after disturbance. A similar temporal pattern of mouse allergen concentration was observed for ELISA-based mass concentration results. Mouse allergen was undetectable by ELISA in one of the two pre-disturbance samples. In addition, ELISA-based allergen concentrations were low in the after-disturbance samples compared to the change in concentration estimated by the HIA. The concentration of HPs detected with the HIA and ELISA-based mass allergen concentrations for PM_{10} were significantly correlated (Figure 3a; $r = 0.90$, $P = 0.02$). Nevertheless, the percent difference between samples taken on different days, but under the same disturbance conditions, was higher for mass concentrations (61% and 86%) than for HPs (10% and 24%).

The estimates of allergen particle number and mass concentrations in the $PM_{2.5}$ fraction (Table 2) were lower than the PM_{10} results. However the overall temporal pattern is the same as determined for PM_{10}. During disturbance, the concentration of HPs in $PM_{2.5}$ comprised approximately 30% (34% in day 1, 27% in day 2) of total HPs, but after-disturbance HPs in $PM_{2.5}$ comprised a majority of the mouse-positive particles (58% in day 1, 85% in day 2).

© 2014 Nature America, Inc.

Table 1. PM_{10} concentration of HPs/m^3 and Mus m 1 (ng/m^3) before, during, and after disturbance, percentage of differences between both days, and the percentage of total HPs or mass allergen.

PM_{10}	*HIA (HPs/m^3)*			*ELISA (ng/m^3)*		
	Day 1	*Day 2*	*% Difference between days*	*Day 1*	*Day 2*	*% Difference between days*
Before disturbance	2.8×10^3	2.8×10^3	—	<0.5	83.0	—
During disturbance	1.0×10^6	9.0×10^5	10%	666.7	250.0	61%
After disturbance	1.7×10^5	1.1×10^5	24%	116.7	16.7	86%

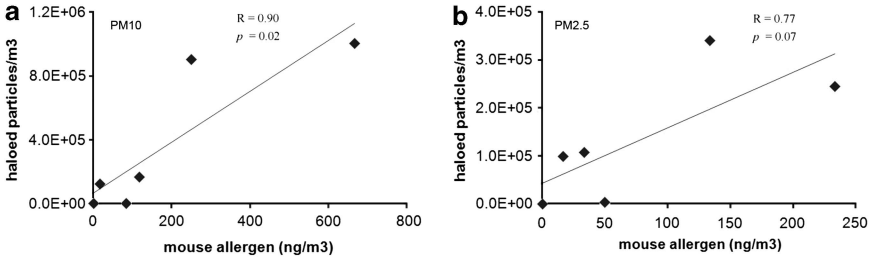

Figure 3. Correlation of haloed particles (HPs)/m^3 and Mus m 1 (ng/m^3). (**a** and **b**) Concentration of HPs and mass concentration of allergen for PM_{10} and $PM_{2.5}$ samples were plotted and Spearman rank correlation determined. $P<0.05$ was considered statistically significant.

Table 2. $PM_{2.5}$ concentration of HPs/m^3 and Mus m 1 (ng/m^3) before, during, and after disturbance, percentage of differences between both days, and the percentage of total HPs or mass allergen.

$PM_{2.5}$	*HIA (HPs/m^3)*				*ELISA (ng/m^3)*			
	Day 1	*Day 2*	*% Difference between day 1 and 2*	*% Total HPs/m^3*	*Day 1*	*Day 2*	*% Difference between day 1 and 2*	*% Total ng/m^3*
Before disturbance	$<2.8 \times 10^3$	2.8×10^3	—	Day 1 (0%) Day 2 (100%)	<0.5	50	—	—
During disturbance	3.4×10^4	2.5×10^5	23%	Day 1 (3%) Day 2 (27%)	133.3	233.3	43%	Day 1 (20%) Day 2 (93%)
After disturbance	9.8×10^4	1.1×10^5	26%	Day 1 (58%) Day 2 (100%)	16.7	33.3	46%	Day 1 (15%) Day 2 (NA)

Abbreviation: NA, not available.
Total percentage of allergen of $PM_{2.5}$ was higher than that of PM_{10}.

The correlation (Figure 3b) between the HIA and ELISA from $PM_{2.5}$ samples yielded a positive coefficient ($r=0.77$), but the correlation was borderline significant ($P=0.07$). In addition, as with PM_{10}, the between-day percent difference was higher for mass concentration compared with HP concentration (23% and 26% for HPs; 43% and 46% for mass concentrations).

Integrated Density of Haloes

The halo density, which is the product of area of the halo and intensity of the stain,[18,21] was measured with the image analysis software ImageJ. For PM_{10} and $PM_{2.5}$ samples (Table 3), there were no significant differences in the geometric means of the $\log_{10}$-transformed integrated densities of the detected haloes.

DISCUSSION

In this study, we present the first assessment of airborne mouse allergen concentrations using the HIA. Although the HIA and ELISA are both immunoassays, the HIA detects number of particles carrying allergen, whereas the ELISA measures the mass concentration. We characterized mouse allergen particle-number concentrations during low- and relatively high-concentration conditions over relatively short-term (30-min) sampling intervals. Although the HIA has been previously used to detect other allergens, in this study we demonstrated the utility of HIA to detect mouse allergen particles even under conditions where the concentration was low (no disturbance condition).

At least for one of the sampling events, the HIA was able to detect allergen-containing particles when the ELISA mass concentration was below the LOD, demonstrating the utility of the HIA for short-term low-concentration settings. In a study by Poulos et al.,[12] air samples were collected for 10 min at a low flow rate (2 l/m) to measure Der p 1-carrying particles in homes during different activities (i.e. dust raising, no disturbance, low allergen facility). Similar to our study, the ELISA was not able to detect airborne Der p 1 in the same samples in which Der p 1-positive particles were detectable with the HIA. The ELISA is a highly sensitive assay commonly used to measure allergen concentrations and while optimizations have been made to further increase its sensitivity,[2,23,24] in low allergen concentration settings, long sampling periods are often still required.[2,9,24–29]

In addition, in low allergen or disturbance scenarios with small loads of allergen-carrying particles, some allergen could remain

© 2014 Nature America, Inc.

Table 3. Integrated densities of haloes before, during, and after disturbance.

Sample	*PM_{10} (pixel^2)*[a]		*$PM_{2.5}$ (pixels^2)*[a]	
	Day 1	*Day 2*	*Day 1*	*Day 2*
Before disturbance	4.02 (3.84–4.37)	4.57 (3.97–4.95)	NA[b]	4.43[c]
During disturbance	3.95 (2.54–5.88)	3.93 (2.04–6.47)	3.94 (2.89–5.59)	3.78 (2.61–5.84)
After disturbance	3.87 (2.44–5.11)	3.77 (2.71–5.26)	3.86 (2.47–4.74)	3.55 (1.90–5.22)

[a]Data presented in geometric means (range).
[b]NA, not available. HPs/m^3 below LOD.
[c]Integrated density for only one HP.

embedded in the filter and fail to be extracted and detected with the ELISA.[9] In our study, this may explain why in day 1 before disturbance of PM_{10} the ELISA did not detect mouse allergen. Another explanation could be a function of sampling in which HIA samples captured a particle, but the samples corresponding to the ELISA did not. For diseases in which the immunological and physiological outcomes can occur during short-time intervals, such as allergic respiratory diseases,[10,18] accurate assessment of recent and short aeroallergen exposures are warranted. There are many cases where shorter-term task-based sampling is needed to identify sources of allergen exposure. Task-based sampling, for example during vacuuming or animal transport, will often require sample times $<$30min. The HIA is well suited for this type of exposure assessment.

An important finding in the current study was the different percentage of allergen-carrying particles on the $PM_{2.5}$ and PM_{10} fractions during and after disturbance. It is known that cat allergen-carrying particles can range in size, with the majority being within 1–20 μm in diameter and small particles being able to remain airborne for long periods.[10,30] Particles carrying dog allergens have been reported to be within this size range, with $>$80% being carried in small particles ($<5\,\mu$m).[31,32] Studies characterizing rodent (i.e. rats, mice) allergens have reported similar particle size distribution, with particles diameters ranging within 3–10 μm in settings with high particle content (i.e. rat or mouse facilities), and smaller particle sizes in areas with no evident source of allergens.[33,34] Comparing the HP concentrations in the PM_{10} and $PM_{2.5}$ size fractions during disturbance indicates that approximately 30% of the allergen particles are in the $PM_{2.5}$ fraction and 70% are in the coarse fraction ($PM_{10-2.5}$). Interestingly, after disturbance the majority of HPs shifted to the $PM_{2.5}$ size fraction. This finding suggests that the HIA can detect small allergen-carrying particles that can remain airborne after a disturbance event. This is important because the size of allergen-carrying particles is a determinant of the early and late bronchial responses of allergen exposure.[11] Therefore, the HIA may be a useful tool for the assessment of indoor inhalation risk of different size of allergen-containing particles during different conditions of particle disturbance.

De Lucca et al.[18] measured and characterized cockroach allergen particles with the HIA and found a correlation between particle size and intensity of the halo. In their study, particles were collected on an IOM sampler and particles' sizes examined microscopically with a graticule. The image analysis software used in the current study (ImageJ) provides for the automatic measurement of morphological characteristics and geometrical measurements of the particle, such as area, diameter, and circularity, among others. Unfortunately, the definition obtained with the image acquisition hardware and software was not sufficient to isolate the particles from the haloes in cases in which the haloes obscured the particle of origin, precluding us from conducting analysis similar to De Lucca et al. Comparing the haloes' integrated densities to that of haloes of known microdotted concentrations of allergen may have provided a better way to examine the relation of allergen concentration carried on each immunostained particles.[35] Nevertheless, it remains to be determined whether the consistent integrated densities seen before, during, and after disturbance in this study suggest that individuals may be exposed to airborne particles with similar allergen-carrying potentials in different size fractions with or without particle disturbance.

A limitation of this study was that we collected samples during a narrow set of conditions making it difficult to extrapolate these finding too broadly. Owing to the restricted access to the mouse facility, we were limited to 2 days of sampling. In addition, we recognize that the MCE-PBM used to collect air samples is not the conventional sampling media for mass allergen concentration measurements. Teflon or glass fiber filters are commonly used for allergen collection and extraction. Because we wanted to examine allergen-carrying particles in the same collection media in which mass concentration was evaluated, we conducted the sampling in the same MCE filter and conducted the analysis on filters divided into equal halves. It is possible that the proteins (allergens) may be more tightly bound to the MCE than Teflon filters making extraction more difficult. However, the extraction procedure based on Hollander et al.[9] using a protein-based buffer plus surfactant should minimize such losses. In addition, we were able to extract $>$85% of mouse allergen from spiked filters, suggesting that a bias associated with filter type is small (data not shown). Future studies will include a comparison of HIA using MCE filters and ELISA using Teflon filters.

We acknowledge that the sampler used in our study may have discriminated against larger particles ($>PM_{10}$) that could carry more allergen, which may have affected the results of image analysis of the haloes. We wanted to sample in particle size ranges commonly assessed in exposure assessment studies and which may be more relevant in bronchial responses.[2,3,10,11]

This study demonstrates the utility of the HIA to measure airborne mouse allergen particles. To our knowledge, this is the first study in which the HIA has been applied to assess airborne mouse allergen. Furthermore, although previous studies with the HIA have used image analysis to detect intensity of the haloes, this is the first study in which image analysis was used to calculate concentration of HPs and measure the halo intensity concurrently. Interestingly, when there was a small concentration of airborne allergen-containing particles, the HIA was able to detect allergen concentrations below the LOD of the ELISA method. In addition, with the aid of size-selective samplers we were able to evaluate HPs in different size fractions (PM_{10} and $PM_{2.5}$) and illustrate the shift of HPs to small particle sizes after disturbance. Taken together, we provide evidence that the HIA can be a useful assessment methodology for mouse allergen exposures in indoor environments. The modifications of the HIA applied to the current study could be expanded for the study of other allergens or biological compounds to which specific antibodies are available. Future studies will expand on this work to compare

© 2014 Nature America, Inc.

count- and mass-based exposure indices in epidemiological studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are very grateful to Dr. Alan Scot and his graduate students of the Department of Microbiology and Molecular Immunology at the Johns Hopkins School of Public Health for granting access to the microscope facility. Also, we would like to acknowledge Dr. Euan Tovey, Research Leader at the Woolcock Institute of Medical Research (Sidney, Australia), for his generosity in providing the adhesive to fix the membranes, and the Dr. Matsui's staff for performing the ELISA analysis and allowing access to the mouse facility. This research was supported by NIESH (T32 ES O7141-28, PO1 ES 018176, P50 ES 015903), U.S. EPA (RD8345101).

REFERENCES

1 Matsui E.C., Wood R.A., Rand C., Kanchanaraksa S., Swartz L., and Eggleston P.A. Mouse allergen exposure and mouse skin test sensitivity in suburban, middle-class children with asthma. *J Allergy Clin Immunol* 2004: **113**(5): 910–915.
2 Matsui E.C., Simons E., Rand C., Butz A., Buckley T.J., and Breysse P., et al. Airborne mouse allergen in the homes of inner-city children with asthma. *J Allergy Clin Immunol* 2005: **115**(2): 358–363.
3 Pacheco K.A., McCammon C., Thorne P.S., O'Neill M.E., Liu A.H., and Martyny J.W., et al. Characterization of endotoxin and mouse allergen exposures in mouse facilities and research laboratories. *Ann Occup Hyg* 2006: **50**(6): 563–572.
4 Sheehan W.J., Rangsithienchai P.A., Muilenberg M.L., Rogers C.A., Lane J.P., and Ghaemghami J., et al. Mouse allergens in urban elementary schools and homes of children with asthma. *Ann Allergy Asthma Immunol* 2009: **102**(2): 125–130.
5 Nelson H.S. The importance of allergens in the development of asthma and the persistence of symptoms. *J Allergy Clin Immunol* 2000: **105**(6 Pt 2): S628–S632.
6 De Lucca S.D., O'Meara T.J., and Tovey E.R. Exposure to mite and cat allergens on a range of clothing items at home and the transfer of cat allergen in the workplace. *J Allergy Clin Immunol* 2000: **106**(5): 874–879.
7 O'Meara T.J., De Lucca S., Sporik R., Graham A., and Tovey E. Detection of inhaled cat allergen. *Lancet* 1998: **351**(9114): 1488–1489.
8 Chew G.L., Correa J.C., and Perzanowski M.S. Mouse and cockroach allergens in the dust and air in northeastern United States inner-city public high schools. *Indoor Air* 2005: **15**(4): 228–234.
9 Hollander A., Gordon S., Renstrom A., Thissen J., Doekes G., and Larsson P.H., et al. Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy* 1999: **54**(2): 142–149.
10 Platts-Mills T.A.E., and Woodfolk J.A. Allergens and their role in the allergic immune response. *Immunol Rev* 2011: **242**(1): 51–68.
11 Casset A., Purohit A., Birba E., Chenard M.P., Uring Lambert B., and Bahram S., et al. Bronchial challenge test in asthmatics sensitized to mites: role of particle size in bronchial response. *J Aerosol Med* 2007: **20**(4): 509–518.
12 Poulos L.M., O'Meara T.J., Sporik R., and Tovey E.R. Detection of inhaled Der p 1. *Clin Exp Allergy* 1999: **29**(9): 1232–1238.
13 Poulos L.M., O'Meara T.J., Hamilton R.G., and Tovey E.R. Inhaled latex allergen (Hev b 1). *J Allergy Clin Immunol* 2002: **109**(4): 701–706.
14 Mitakakis T.Z., Barnes C., and Tovey E.R. Spore germination increases allergen release from Alternaria. *J Allergy Clin Immunol* 2001: **107**(2): 388–390.
15 Razmovski V., O'Meara T.J., Taylor D.J., and Tovey E.R. A new method for simultaneous immunodetection and morphologic identification of individual sources of pollen allergens. *J Allergy Clin Immunol* 2000: **105**(4): 725–731.
16 Green B.J., Sercombe J.K., and Tovey E.R. Fungal fragments and undocumented conidia function as new aeroallergen sources. *J Allergy Clin Immunol* 2005: **115**(5): 1043–1048.
17 Rivera-Mariani F.E., Nazario-Jimenez S., Lopez-Malpica F., and Bolanos-Rosero B. Sensitization to airborne ascospores, basidiospores, and fungal fragments in allergic rhinitis and asthmatic subjects in san juan, puerto rico. *Int Arch Allergy Immunol* 2011: **155**(4): 322–334.
18 De Lucca S.D., Taylor D.J., O'Meara T.J., Jones A.S., and Tovey E.R. Measurement and characterization of cockroach allergens detected during normal domestic activity. *J Allergy Clin Immunol* 1999: **104**(3 Pt 1): 672–680.
19 Matter C.o.R.P.f.A.P., and Council N.R. *Research Priorities for Airborne Particulate Matter: IV. Continuing Research Progress*. The National Academies Press: Washington, DC, 2004.
20 Green B.J., Mitakakis T.Z., and Tovey E.R. Allergen detection from 11 fungal species before and after germination. *J Allergy Clin Immunol* 2003: **111**(2): 285–289.
21 Tovey E.R., Taylor D.J.M., Graham A.H., O'Meara T.J., Lovborg U., and Jones A.S., et al. New immunodiagnostic system. *Aerobiologia* 2000: **16**: 113–118.
22 Adhikari A., Martuzevicius D., Reponen T., Grinshpun S.A., Cho S.H., and Sivasubramani S.K., et al. Performance of the Button Personal Inhalable Sampler for the measurement of outdoor aeroallergens. *Atmospheric Environ* 2003: **37**(34): 4723–4733.
23 Ferrari E., Tsay A., Eggleston P.A., Spisni A., and Chapman M.D. Environmental detection of mouse allergen by means of immunoassay for recombinant Mus m 1. *J Allergy Clin Immunol* 2004: **114**(2): 341–346.
24 Korpi A., Mantyjarvi R., Rautiainen J., Kaliste E., Kalliokoski P., and Renstrom A., et al. Detection of mouse and rat urinary aeroallergens with an improved ELISA. *J Allergy Clin Immunol* 2004: **113**(4): 677–682.
25 Curtin-Brosnan J., Paigen B., Hagberg K.A., Langley S., O'Neil E.A., and Krevans M., et al. Occupational mouse allergen exposure among non-mouse handlers. *J Occup Environ Hyg* 2010: **7**(12): 726–734.
26 Hollander A., Van Run P., Spithoven J., Heederik D., and Doekes G. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. *Clin Exp Allergy* 1997: **27**(6): 617–626.
27 Hollander A., Heederik D., Doekes G., and Kromhout H. Determinants of airborne rat and mouse urinary allergen exposure. *Scand J Work Environ Health* 1998: **24**(3): 228–235.
28 Jones R.B., Kacergis J.B., MacDonald M.R., McKnight F.T., Turner W.A., and Ohman J.L., et al. The effect of relative humidity on mouse allergen levels in an environmentally controlled mouse facility. *Am Ind Hyg Assoc J* 1995: **56**(4): 398–401.
29 Peng R.D., Paigen B., Eggleston P.A., Hagberg K.A., Krevans M., and Curtin-Brosnan J., et al. Both the variability and level of mouse allergen exposure influence the phenotype of the immune response in workers at a mouse facility. *J Allergy Clin Immunol* 2011: **128**(2): 390–396 e397.
30 Wood R.A. Laboratory animal allergens. *ILAR J* 2001: **42**(1): 12–16.
31 Custovic A., Green R., Fletcher A., Smith A., Pickering C.A., and Chapman M.D., et al. Aerodynamic properties of the major dog allergen Can f 1: distribution in homes, concentration, and particle size of allergen in the air. *Am J Respir Crit Care Med* 1997: **155**(1): 94–98.
32 Luczynska C.M., Li Y., Chapman M.D., and Platts-Mills T.A. Airborne concentrations and particle size distribution of allergen derived from domestic cats (Felis domesticus). Measurements using cascade impactor, liquid impinger, and a two-site monoclonal antibody assay for Fel d I. *Am Rev Respir Dis* 1990: **141**(2): 361–367.
33 Ohman Jr J.L., Hagberg K., MacDonald M.R., Jones Jr R.R., Paigen B.J., and Kacergis J.B. Distribution of airborne mouse allergen in a major mouse breeding facility. *J Allergy Clin Immunol* 1994: **94**(5): 810–817.
34 Platts-Mills T.A., Heymann P.W., Longbottom J.L., and Wilkins S.R. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 1986: **77**(6): 850–857.
35 Tovey E., Lucca S.D., Poulos L., and O'Meara T. The Halogen assay—a new technique for measuring airborne allergen. In: Jones M.G., and Lympany P. (eds.). *Allergy Methods and Protocols*. Humana Press: Totowa, NJ, 2008: **138**: 227–246.

© 2014 Nature America, Inc.