

Measurement and characterization of cockroach allergens detected during normal domestic activity

Sandra D. De Lucca, Bsc (Hons), David J. M. Taylor, Bsc (Hons), Timothy J. O'Meara, PhD, Allan S. Jones, PhD, and Euan R. Tovey, PhD *Sydney, Australia*

Background: Cockroach allergen is recognized as a causal factor for asthma. However, airborne cockroach allergen has not been detected in undisturbed conditions, and therefore the behavior and properties of airborne cockroach allergen have been poorly characterized. A new aeroallergen sampling method and sensitive system of immunoassay have been used to examine cockroach allergen exposure.

Objective: Our purpose was to measure and characterize airborne cockroach allergens during normal domestic exposure in the homes of Sydney, Australia.

Methods: Air sampling with Institute of Occupational Medicine, Edinburgh (IOM) samplers was performed in the living rooms of 10 houses during low- and no-disturbance environments. In addition, inhaled particles were collected by each home occupant during low domestic exposure with use of intra-nasal samplers that impact particles onto an adhesive surface. The particles collected on the IOMs and the intra-nasal samplers were immunostained with Bla g 1 monoclonal antibodies. Particle size, morphologic characteristics, and the relative Bla g 1 content of particles were estimated. Reservoir dust samples from the kitchen, living room, and bedroom were assayed by an ELISA. Two forms of repeatability of IOM air sampling were examined. The first measure tested the repeatability of 2 IOM samples collected simultaneously in the same room during low- and no-disturbance activities. The second measure examined the repeatability of IOM sampling over time on 10 consecutive days.

Results: Bla g 1 was detected in reservoir dust samples taken from all homes (geometric mean 1.5 U/g, range 0.2-9.4 U/g). Inhaled particles containing Bla g 1 were detected during 1 hour of intra-nasal sampling in 8 of 10 homes during low disturbance. Cockroach particles were detected on all of the IOM samples collected for both 4-hour low-disturbance and overnight no-disturbance sampling environments. Particles containing Bla g 1 collected with the IOM samplers during low disturbance ranged in size from 3 to 350 μm . These particles are amorphous and irregular in shape, and a majority of the large particles were described as flakes (flat, transparent particles) and fibers (threadlike). A relationship was demonstrated between the allergen content of cockroach particles and their particle size. The larger particles elute more Bla g 1. The coef-

ficient of repeatability for measurements made during low and no disturbance was 3.62 and 2.09, respectively. For measurements repeated over time at the same site, the coefficient of repeatability was 2.61. This represents the fold range within which 95% of pairs of measurements made at an interval of 1 day would be expected to lie.

Conclusions: Airborne cockroach allergen is present in both undisturbed and low-disturbance environments in homes with relatively low reservoir levels of Bla g 1. In agreement with previous reports, airborne particles containing cockroach allergen (Bla g 1) are mainly associated with particles $>10 \mu\text{m}$. These particles are amorphous and irregular in shape and can be described as flakes and fibers. (*J Allergy Clin Immunol* 1999;104:672-80.)

Key words: Cockroach allergy, aeroallergen, Bla g 1, immunostaining

Cockroach allergy, combined with high exposure, continues to be a strongly associated asthma risk in the United States in some inner-city populations¹⁻⁴ and in some rural populations.⁵ In these studies race, poverty, and living conditions were all interrelated. A similar pattern has been observed in France⁶ and in some Asian countries.⁷⁻⁹ As well as asthma, cockroach exposure has also been associated with accelerated age-related decline in FEV₁.¹⁰

The precise nature and source of allergenic cockroach particles is unclear. Cockroach allergens have been localized throughout the insect, especially in the malpighian tubules, saliva, and gut. High concentrations are also found in the fecal pellets and regurgitated secretions.^{11,12} Monoclonal antibodies to the cockroach *Blatella germanica* major allergens Bla g 1 and Bla g 2 have been produced,¹³ which has permitted immunoassays of the allergens in reservoir dust samples. The monoclonal 10A6 (anti-Bla g 1) reacts with a cross-reacting allergen that is detected in 9 of 14 cockroach species, including *Blatella*, *Periplaneta*, *Blatta*, *Leucophea*, and *Supella*.¹⁴ Although cockroach allergens are found throughout the house, including beds, furnishings, upholstery, and carpets, Bla g 1 levels in reservoir dust are generally highest in kitchens.^{2,13,15}

There is concern that allergen levels in reservoirs of settled dust do not correlate with airborne allergen, and this may not be the ideal proxy measurement to determine allergen exposure.¹⁶⁻²⁰ In addition, aeroallergen may provide an index of recent exposure in a disease characterized by rapid changes in morbidity and physiologic characteristics over time. Such measurements of aeroallergen would also allow the effects of concomitant

From the Institute of Respiratory Medicine, University of Sydney, Sydney, Australia.

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Reprint requests: Euan R. Tovey, PhD, Institute of Respiratory Medicine, Room 461, Blackburn Building (D06), University of Sydney, Sydney, New South Wales, Australia 2006.

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Abbreviations used

BCIP/NBT:	5-Bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium
CV:	Coefficient of variation
GM:	Geometric mean
IOM:	Institute of Occupational Medicine, Edinburgh
PVDF:	Polyvinylidene difluoride

exposures to cockroach allergens and other environmental factors such as air pollution to be studied.

Recently in Strasbourg (France), airborne cockroach allergens Bla g 1 and Bla g 2 were measured.⁶ However, this was only possible when reservoir dust was artificially disturbed by use of a vacuum cleaner for 45 minutes without a bag, and air was sampled at a flow rate of 20 L/min. The allergens were collected on filters during 30- to 45-minute sampling periods, 1 m away from the vacuum cleaner. Under undisturbed conditions airborne cockroach allergens were not detectable. No cockroach allergens could be detected with use of filters fitted to the subject's lapels operating at 2 L/min either during natural disturbance for 8 hours or in the absence of disturbance for 3 to 8 hours. An impinger used to size-fractionate the aeroallergen during artificial dust disturbance showed that more than 80% of allergen was associated with particles >10 µm diameter and less than 10% with particles <5 µm. It is questionable to what extent allergenic cockroach particles detected in the air after artificial disturbance is representative of the particles occurring during natural exposure.⁶

Although measurement of aeroallergen may be desirable, most previous studies have not been able to measure airborne cockroach allergens successfully during low disturbance or have chosen to measure settled dust levels.^{4,15} Consequently, the properties of airborne cockroach allergens are poorly understood.

A novel and very sensitive system of immunoassay has been developed that identifies individual particles that function as allergen sources.²¹ Airborne particles are collected on a protein-binding membrane (polyvinylidene difluoride [PVDF]) by air filtration and are then fixed in position by overlaying with an adhesive tape. The sandwich is wetted, and the allergens elute and bind to the membrane, where they can be detected with an immunoassay with conventional enzyme staining. Particles, which function as allergen sources, are identified by the halo of immunostain around each of them. In an alternative format particles are collected by inertial impaction onto the adhesive tape, which is then laminated with the protein binding membrane, followed by immunostaining.

These systems of immunostaining have been applied to determine whether cockroach aeroallergens can be detected in houses in Sydney during normal domestic activity.

MATERIAL AND METHODS

During October and November (spring) 1998, sampling was performed in 10 houses where the occupants had reported the occa-

sional presence of cockroaches, usually at night. None of the houses could be categorized as infested and no cockroaches were visible at the time of sampling. A sticky trap (Zoro Zoro, Taisho Pharmacy, Tokyo, Japan) was placed on the floor of the living room and left for 24 hours in each of the 10 homes to sample cockroach populations.

Separate dust samples were collected from a total area of 1 m² in the kitchen, living room, and a bedroom in each house with use of a hand-held vacuum cleaner (Makita Electric Works, 4071D) modified for this purpose. The same protocol for all reservoir sampling was used so that samples from similar sites in different houses or from the same house collected at different times could be compared with each other. Samples of fine dust were extracted (100 mg/mL buffer) and assayed for Bla g 1 with use of an established monoclonal-based ELISA.¹³

Air sampling

Two samples were simultaneously collected at 2 sites in the living room in each house with an Airchek 52 sample pump unit (SKC, Eighty Four, Pa) operating with an air flow of 2 L/min and equipped with an Institute of Occupational Medicine, Edinburgh (IOM) (SKC, Blandford, UK), sampling head. Each sampler was placed facing horizontally 1.5 m above the floor. Particles were collected on a dry protein-binding membrane (PVDF) (1 µm pore size), and after sampling the membrane was overlaid with a transparent adhesive tape (IRM Technologies, Sydney, Australia) to prevent particles from moving.

Three sets of air samples were collected (Fig 1):

1. *Low disturbance sampling:* During the evening the samplers were operated for 4 hours while normal quiet domestic activity was conducted in the living room. Subjects were free to move around the living room and carry out their usual domestic activities. This included watching television, reading, eating, talking, etc. No dust raising or cleaning activities were undertaken.

2. *No disturbance sampling:* Sampling was performed with no one in the living room for 8 hours during the night. The IOM samplers were turned on as the occupant went to bed and turned off again in the morning.

3. *Intra-nasal samplers:* Samples were collected for 1 hour in the living room with use of intra-nasal samplers (IRM Technologies, Sydney, Australia) during the period of low-disturbance activity. Subjects were seated for the entire 1 hour of nasal sampling. These samplers collect most particles >5 µm that are inhaled during normal respiration.²² Inhaled particles were collected within the nasal sampler onto a transparent tape adhesive (IRM Technologies) that was overlaid with PVDF membrane (0.45 µm pore size) after sampling was completed. The membrane-adhesive sandwich from IOM and nasal samplers were wetted with methanol and incubated in borate buffer overnight to allow allergens from the particles to bind to the membranes.

Allergens eluting from the particles captured on both the IOM and nasal samplers were bound to the membranes and immunostained with anti-Bla g 1 monoclonal 10A6 (Indoor Biotechnologies, Charlottesville, Va) followed by an anti-mouse alkaline phosphatase conjugate (Sigma, St Louis, Mo) and developed by use of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) substrate (Sigma).

Positive particles (as determined by the presence of a stained halo of Bla g 1) were counted on both the IOM and intra-nasal membranes under ×100 magnification. The number of positive particles collected on the 2 IOM filters collected at different sites of the room were added together. Likewise, the number of positive particles collected on the left and right sides of the intra-nasal sampler were combined. On the basis of their appearance, allergen-containing particles were characterized under ×100 magnification. Fibers were threadlike in appearance and flakes were flat, translucent par-

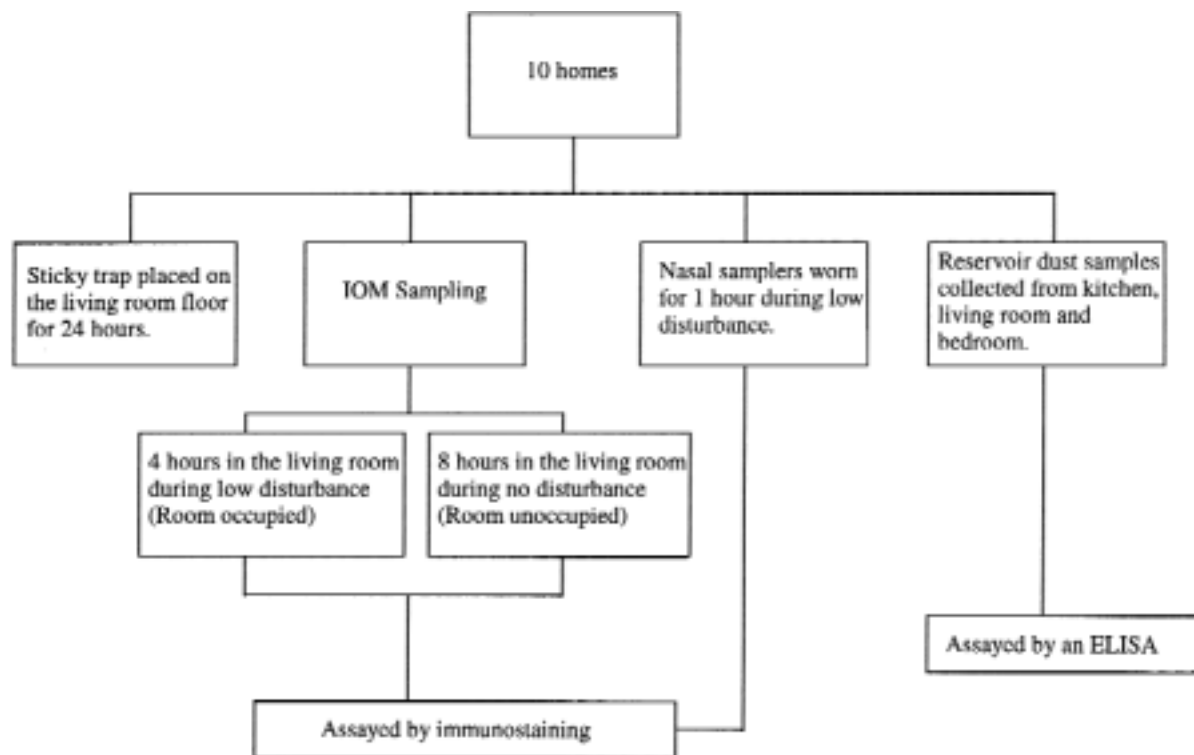


FIG 1. Procedure used for collecting and measuring cockroach allergen.

ticles. The maximum diameter of each particle was measured with an eyepiece graticle. For fibers, their lengths were measured. The relative allergen content of a sample range of positive particles was estimated by measurement of the brightness intensity of the associated immunostained halo. Because halo intensity is a measure of the amount of light absorbed by the stain that is distributed over the area occupied by the halo, all intensity measurements were measured as integrated OD (IOD) according to the following formula:

$$\text{IOD} = A \times \log_{10}(I_o) - \sum \log_{10}(I_{(x,y)})$$

where A is the area of halo staining, I_o is the average background intensity, and $I_{(x,y)}$ is the intensity of halo staining.

All measurements were taken under the same optical conditions and with the camera calibrated so that a clear field (ie, no halo staining) gave the maximum intensity reading. Background intensity measurements were taken for each stained halo from an annulus surrounding the halo but not part of it. This ensured that measurements were not affected by non-uniformities in background illumination levels (ie, shading).

Equipment used for these measurements comprised a Pulnix TM1001, progressive scan, monochrome video camera mounted on an Olympus BH2 microscope, an XPG1000 frame grabber (Dipix, Vancouver, BC), and the "WiT" commercial image analysis software (Logical Vision, Coreco, Burnaby, Canada), running on a Pentium PC (DanCom, Sydney). Statistical analysis was conducted with Minitab statistical software (Minitab, State College, Pa).

Repeatability of measures of cockroach allergen

To interpret single measures of allergen exposure, it is necessary to know the reliability or repeatability of individual measurements. Two forms of repeatability of sampling were examined. The first

measure tested the repeatability of 2 IOM samples collected simultaneously at different sites in the same room. This is a useful index for cross-sectional studies. The second measure examined the repeatability of IOM sampling at a single site over time. This is useful in studies examining the variation of airborne Bla g 1 over short periods of time within a home.

Variation between sites within a room. Local variation was assessed by examining the agreement between pairs of measurements made simultaneously in the same room. This measure of repeatability is useful in interpreting the reliability of observed differences between cockroach allergen measurements made at different sites (that is, cross-sectional observations). Replicate samples taken during both low- and no-disturbance conditions in 10 rooms (that is, in 10 houses) were used for this analysis.

Variation over time within homes. The variation over time was assessed by examining the agreement between measurements made at the same site but at different times. This measure of repeatability is useful in interpreting the reliability of observed changes in cockroach allergen measurements at a single site. This was estimated from a series of 10 daily measurements sampled during periods of low activity in the 2 houses with the highest levels of cockroach allergen. The same IOM samplers were used on each day, and flow rates were adjusted to achieve a rate of 2 L/min with the filter in place.

Because the distribution of particle counts was skewed and the variation between replicates increased with the mean, the values were log transformed before analysis.

The assessment of variation between samples collected simultaneously at 2 sites in the same room was quantified as the SD of the differences between replicate pairs of log-transformed counts. The anti-log of this SD multiplied by 2 is known as the coefficient of repeatability.²³ This represents the fold range within which 95% of

TABLE I. Cockroach allergen levels in reservoir dust

Home	Concentration of Bla g 1 in fine dust (U/g fine dust)			No. of captured cockroaches (24 h)
	Kitchen	Living room*	Bedroom	
1	7.1	4.5	1.1	7
2	1.6	0.6	0.6	0
3	1.2	0.5	0.5	0
4	4.8	2.7	0.8	1
5	3.6	2.5	1.0	1
6	0.9	0.5	0.3	0
7	2.4	0.5	0.6	0
8	9.4	4.0	1.6	4
9	2.6	1.7	0.3	0
10	0.7	0.6	0.2	0

*Location of air sampling.

pairs of measurements within the same room would be expected to lie.

The variation over time was quantified by analysis of variance in which the within-home SD among the 10 daily measurements in both homes was determined. The coefficient of repeatability for this analysis was calculated as 2 multiplied by the square root of 2 and then multiplied by the within-home SD.²⁴ This represents the fold range within which 95% of pairs of measurements at the same site, repeated at an interval of 1 day, would be expected to lie.

Other allergen exposure

For the 10th sample taken in the repeatability experiment within homes, the filters were cut into 4 segments. One segment was stained for cockroach (Bla g 1), one for mite (Der p 1) (5H8), another for cat (Fel d 1) (6F9), and the last one acted as a control (no monoclonal). Positive particles counted on the IOM samples taken at the 2 sites of the room were added together. The number of positive particles stained on the cockroach sections were multiplied by 4 for the 10th sample.

Correlations between airborne and reservoir measurements

Regression analysis was used to examine the relationships between airborne cockroach allergen collected by IOM and nasal sampling and between low- and no-disturbance conditions.

RESULTS

Reservoir dust

Cockroach allergen Bla g 1 was detected in all reservoir dust samples taken from the 10 homes in Sydney (Table I). The highest level of Bla g 1 was detected in the kitchens, with geometric means (GM) of 2.5 U/g (range 0.7-9.4 U/g). This was twice the Bla g 1 levels detected in living rooms (GM 1.2 U/g, range 0.5-4.5 U/g) and 5 times the Bla g 1 levels detected in bedrooms (GM 0.6 U/g, range 0.2-1.6 U/g). The overall mean allergen level detected in reservoir dust samples from the 10 homes was GM 1.5 U/g (range 0.2-9.4 U/g).

Cockroaches were only captured in sticky traps in the houses with the 4 highest reservoir concentrations of Bla g 1. Cockroaches captured were identified as German (*Blattella germanica*), American (*Periplaneta americana*), and Smoky brown (*Periplaneta fuliginosa*).

TABLE II. Total number of airborne particles eluting cockroach allergen for each type of air sampler

Home	IOM low disturbance (4 h)	IOM overnight no disturbance (8 h)	Nasal filter 1 h
1	74	90	7
2	9	8	1
3	4	7	1
4	12	20	2
5	8	7	2
6	5	2	0
7	58	22	5
8	67	33	5
9	61	40	4
10	8	5	0

Air sampling

Cockroach particles eluting Bla g 1 allergen (Fig 2) were detected on all of the IOM samples collected for both 4-hour low-disturbance and 8-hour (overnight) no-disturbance sampling. In addition, inhaled cockroach allergen- (Bla g 1) eluting particles were detected during 1 hour of intra-nasal sampling in 8 of 10 houses (Table II).

A significant positive relationship ($r^2 = 0.571$, $P < .001$, $n = 10$) was found between samples collected with the IOM at low disturbance and those collected overnight with no disturbance. There was a significant positive relationship ($r^2 = 0.895$, $P < .001$, $n = 10$) between the IOM samples taken from the 2 sites in one room and intranasal samples collected during low disturbance.

No significant relationship ($r^2 = 0.358$, $P = .068$, $n = 10$) could be demonstrated between Bla g 1 concentrations in living room reservoir dust and numbers of allergenic Bla g 1 particles collected at low disturbance by IOM samples. However, significant positive relationships were found between Bla g 1 concentrations in living room reservoir dust and airborne Bla g 1 particles captured in the living room by undisturbed IOM and intranasal samples ($r^2 = 0.561$, $P = .013$, $n = 10$ and $r^2 = 0.512$, $P = .02$, $n = 10$, respectively).

Allergenic particle size and characterization

A total of 306 particles containing Bla g 1 were collected with the IOM samplers during low-disturbance activity. These particles ranged from 3 to 350 μ m in diameter. More than 80% of particles eluting cockroach allergen were greater than 10 μ m during low disturbance (Fig 3). A total of 234 particles containing Bla g 1 were collected during no disturbance. Although the nature and size of the particles were similar, the distribution differed because 40% of particles carrying allergen were associated with particles less than 10 μ m.

Detected allergenic particles existed either as amorphous particles or as larger allergen-carrying fibers (Fig 4). The majority of the larger amorphous particles were determined to be thin, transparent, flake-like particles.

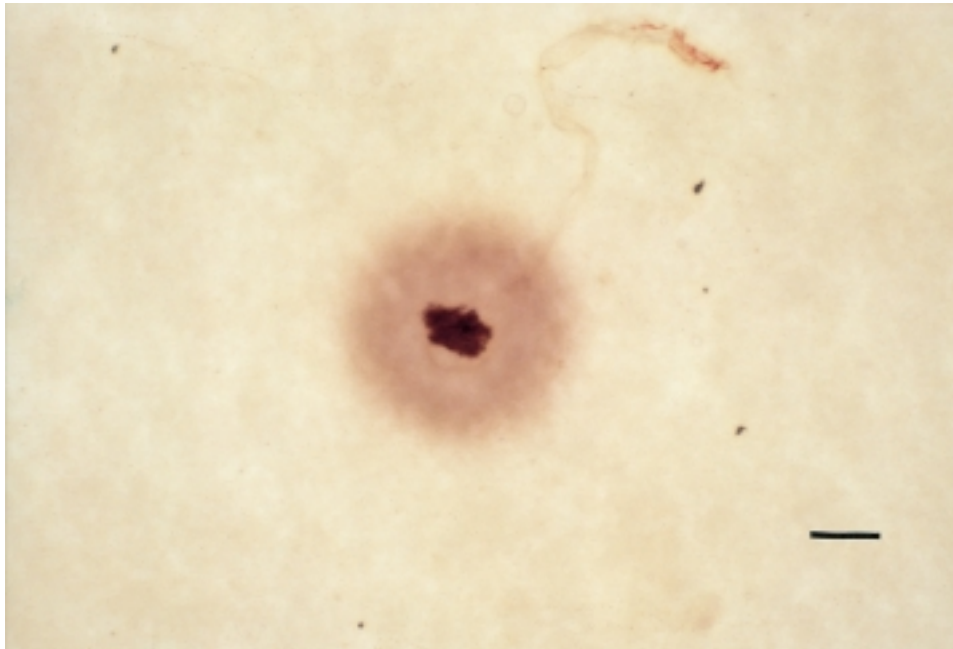


FIG 2. Amorphous particle-eluting cockroach allergen Bla g 1. Bar, 25 μ m.

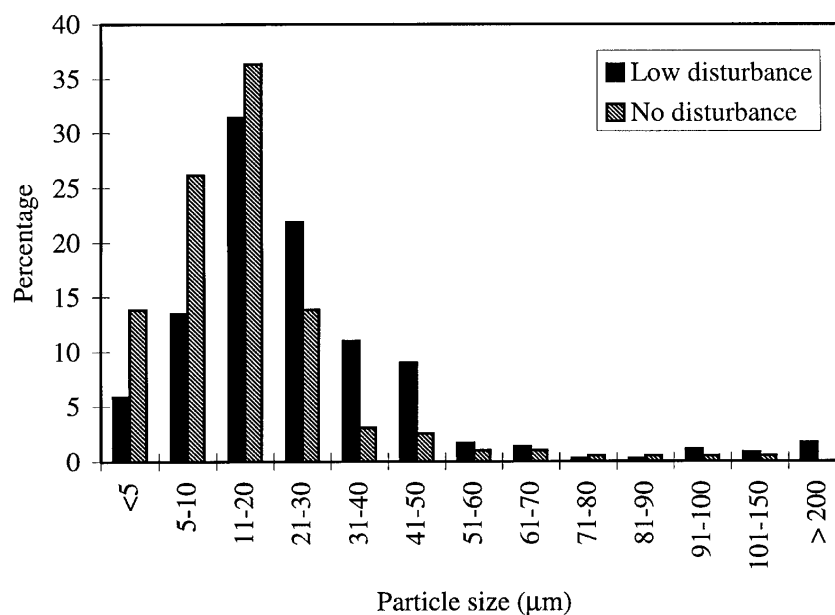


FIG 3. Percentage distribution of particle size of cockroach allergen collected with IOM samplers during low and no disturbance.

Long fibers were collected more frequently during low disturbance.

Relative allergen content in cockroach particles

A relationship was demonstrated between the allergen content of cockroach particles and their particle size (Fig 5). Larger particles generally elute more Bla g 1 allergen.

Repeatability of measures of cockroach allergen

The coefficient of repeatability of pairs of IOM samples taken simultaneously in the same room was 3.62- and 2.09-fold for low- and no-disturbance collections, respectively. This means that if measurements collected simultaneously under conditions of low and no distur-

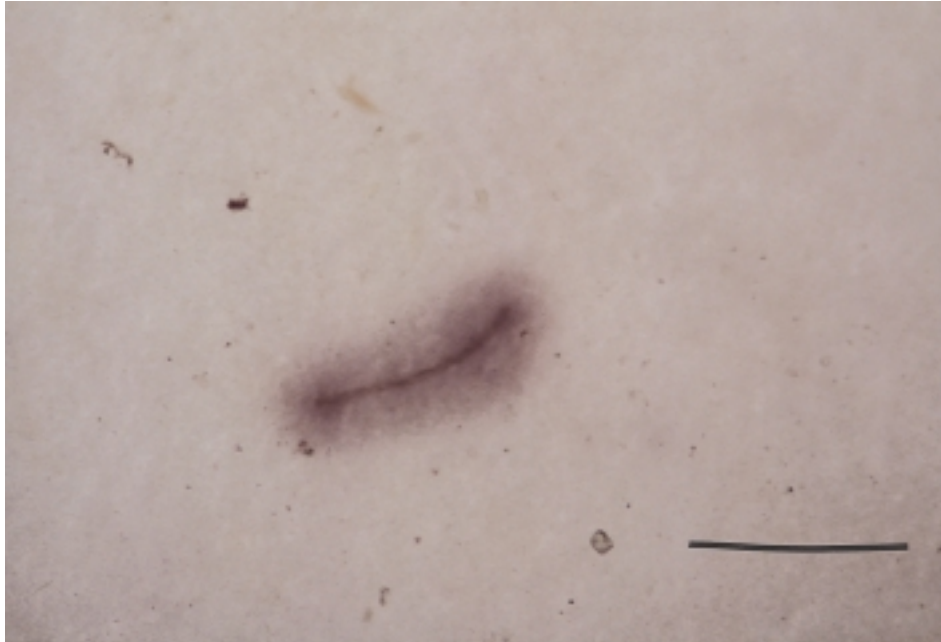


FIG 4. Fiber eluting Bla g 1. Bar, 230 μ m.

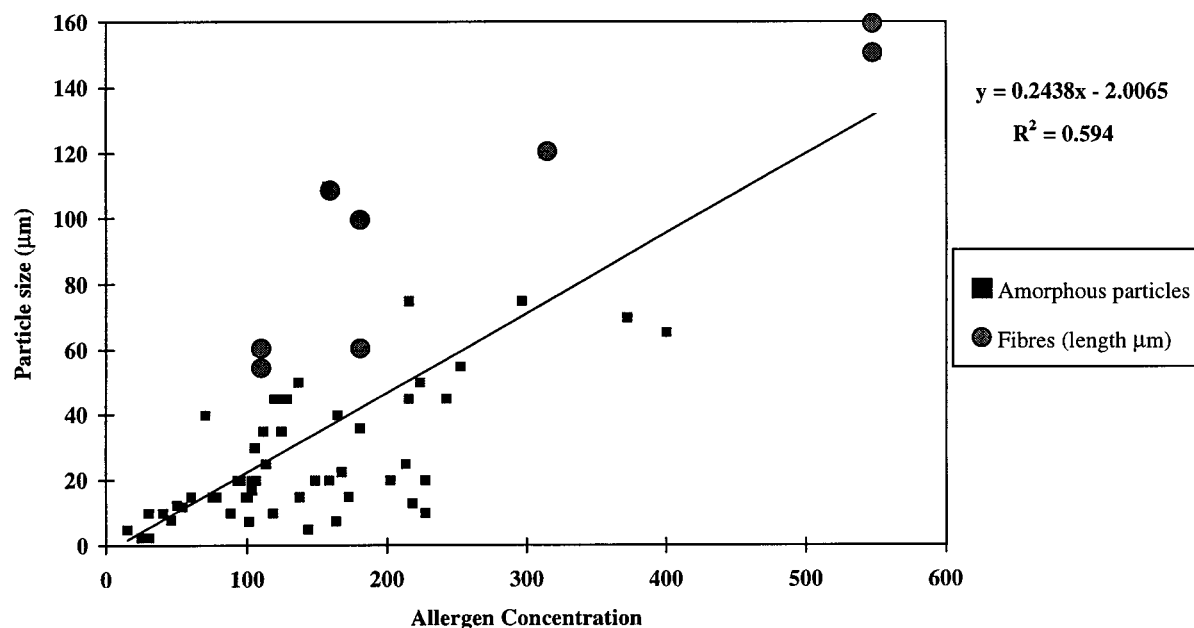


FIG 5. Size of particles eluting Bla g 1 versus allergen concentration of cockroach particles collected with IOM samplers.

bance differ by more than 3.62-fold or 2.09-fold, respectively, then there is a 95% probability that they are really different. Two measurements taken in the same site that differ by less than this cannot be regarded as significantly different.

The coefficient of repeatability for pairs of measurements made at the same site, repeated at an interval of 1 day, was 2.61. Change in allergenic particle count at a

given site greater than this is likely (with 95% probability) to represent a true change. The mean ($\pm$ SD and coefficient of variation [CV]) of allergenic cockroach particles collected per day was 36.1 ($\pm$ 8.44 and CV 23%) and 31.45 ($\pm$ 8.61 and CV 27%) in homes 1 and 2, respectively (Fig 6). The allergenic particles collected during low disturbance were all in the range of 2.6-fold for home 1 and for home 2 and can therefore be regarded as reproducible.

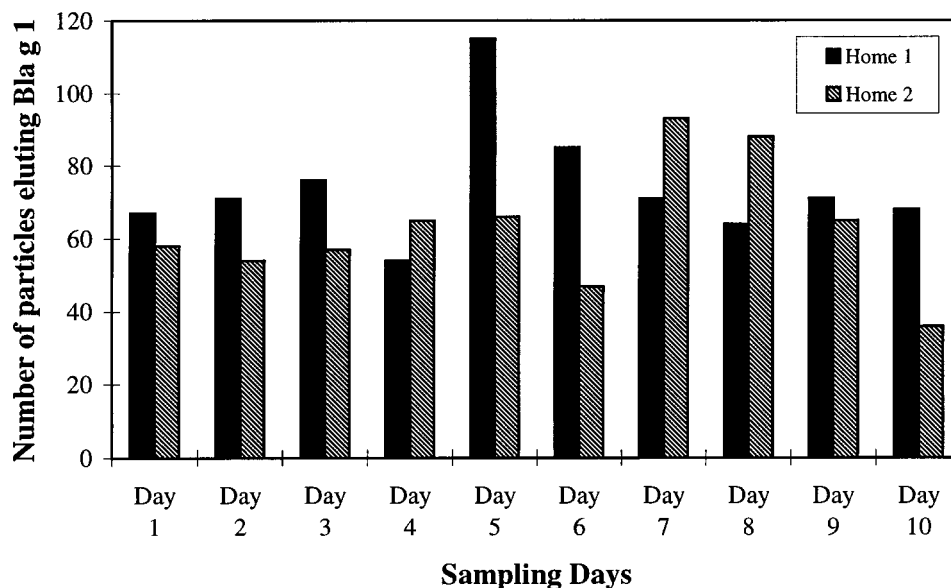


FIG 6. Repeatability of cockroach sampling with IOM samplers in 2 homes.

Other allergen exposure

A total of 135 particles containing allergen were collected with 2 IOM samplers over 4 hours in home 1. Of the total number of positive particles captured, 13% contained Bla g 1, 49% Der p 1, and 38% Fel d 1. In home 2, 116 particles were found to contain allergen. Of these, 8% of allergenic particles captured contained Bla g 1, 29% Der p 1, and 63% Fel d 1. Both homes contained a cat. No positive particles were detected on the control samples for either home.

DISCUSSION

Cockroach allergen is increasingly recognized as an important causal factor for asthma. Despite this, previous studies have been unable to detect airborne cockroach allergens without artificially disturbing the environment.^{6,25} As a consequence, the behavior and properties of airborne cockroach allergen in a normal home environment has been poorly characterized.

This article demonstrates for the first time a method for sampling, detecting, and analyzing airborne cockroach allergen in both undisturbed and low-disturbance environments. In addition, cockroach allergen sampling was performed in October and November (spring) when cockroach activity is not at its peak in Sydney and most allergen present would have been residual material from the previous summer when populations were higher. Airborne sampling was conducted in an area of the house (living room) where the incidence of cockroach allergen has not been previously recorded as high, and the houses that were sampled were not heavily infested with cockroaches. In support of this, the GMs of Bla g 1 detected in 10 homes in Sydney were lower than those found in studies conducted in other countries around the

world (GM 1.5 U/g, range 0.2-9.4 U/g). De Blay et al⁶ measured high levels of Bla g 1 in low-cost public housing in Strasbourg (France) with a GM of 3919 U/g (range 530-14,306 U/g). Similarly, Rosenstreich et al¹ found a median Bla g 1 concentration of 8.2 U/g (n = 476) in bedrooms of 8 inner-city US neighborhoods. Only 4 houses captured cockroaches in the sticky traps overnight. These cockroaches were identified as German, American, and Smoky brown, all of which liberate Bla g 1 allergen. Although all 3 species of cockroaches are common in Sydney, only the German species was caught in all 4 homes. The same 4 houses had Bla g 1 concentrations higher than 2 U/g present in the living room, which has been suggested as a sensitization threshold for atopic patients in previous Bla g 2 studies.^{2,26,27} The threshold level for Bla g 1 is not known and may differ from Bla g 2.

The link between reservoir samples and airborne allergen exposure has always been a tenuous one. More pertinent from an exposure perspective is the number and size of allergen particles that are airborne and therefore able to be inhaled and the quantity of allergen per particle. In this study no significant correlation could be demonstrated between reservoir dust and airborne IOM samples collected at low disturbance, and a weak correlation was found between reservoir dust and undisturbed aeroallergen. Yet there was a strong correlation between the low-disturbance IOM samples and the number of particles actually inhaled and captured on intra-nasal devices. This indicates that a major determinant of the amount of cockroach allergen inhaled under low-disturbance conditions was the amount airborne as measured by IOM sampling, and this was not related to the level of reservoir allergen. Under no-disturbance conditions a stronger relationship between IOM sampling and reservoir Bla g 1 was found, which may be due to the longer

sampling time and very low activity that may result in equilibration between reservoir and airborne *Bla g 1*.

Airborne cockroach particles have many similarities to airborne dust mite particles.²¹ Many are irregular-shaped amorphous particles, the majority captured being greater than 10 μm in diameter. Most of the large amorphous particles captured by the IOM sampler were thin translucent flakes with a large surface area-to-width ratio. Fibers were also shown to carry large amounts of cockroach allergen. The biologic origin of these particles is unknown. Such "flake-like" particles could be expected to behave aerodynamically as particles of much smaller diameter than their 2 dimensional measurements would suggest, and many would probably be inhaled into the lung. The more efficient collection of airborne cockroach particles by the IOM samplers compared with the intranasal samplers may be because the nasal samplers are less efficient for collecting particles $<10\ \mu\text{m}$, and many of the particles carrying cockroach allergens could be expected to behave as smaller particles.

A relationship was demonstrated between the cockroach allergen content and the size of the cockroach-positive particle that was collected by the IOM samplers. Allergen concentration is reported in this study as relative units that are related to the maximum intensity measurable with the image analysis system used and assumes linearity of absorbance and staining with concentration of allergen. Ideally, allergen concentration for the particle halos could be reported in absolute units such as nanograms per liter of inhaled air by use of a suitable standard containing allergen concentrations at levels comparable to those of the naturally occurring particles. These standards should be measured under the same conditions as the natural particles, which dictate that they be of comparable size. Delivery of very small volumes into very small areas is currently under development for use in fields such as neurophysiology and microdotting for genome mapping. Adoption of similar microdotting methods is currently being studied as a way of providing standards (ie, halos of known allergen concentration) that will allow reporting of allergen concentrations as absolute units with use of the techniques detailed in this article.

Repeat sampling performed in 2 homes that had high levels of cockroach allergen demonstrated that all samples were in the range of 2.61-fold. These results indicate that cockroach sampling under the same environmental conditions on different days is reasonably reproducible and that changes in levels that exceed these ranges represent significantly different levels. Such information can be used to examine the efficiency of allergen avoidance practices.

The coefficient of repeatability across homes was higher for low disturbance than no disturbance (3.62 vs 2.09). Houses had different numbers of occupants, and it is likely that different levels of activity would have occurred in different areas of each living room. Thus low-disturbance samples were more variable between homes than no-disturbance samples were. This suggests

that for comparisons no-disturbance samples may be more reliable than low-disturbance samples.

Immunostaining for other indoor allergens (*Der p 1* and *Fel d 1*) in the 2 homes with the highest levels of airborne cockroach suggested that cockroach allergen is present on fewer particles than mite or cat allergen.

In summary, cockroach allergen has been detected in both an undisturbed environment and under normal domestic activity. Effectively, airborne cockroach allergen has been detected in environments that were thought to contain insufficient concentrations for detection. In agreement with previous reports, airborne particles containing cockroach allergen (*Bla g 1*) are associated with particles $>10\ \mu\text{m}$. These particles are amorphous and irregular in shape. A majority of the large particles were described as flakes (flat, transparent particles) and others as fibers (threadlike). Although cockroach allergen is of lesser concern in the homes of Sydney compared with other indoor allergens (dust mite and pet allergen), these results allow the behavior and properties of airborne cockroach allergen to be characterized during normal domestic activities.

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