

Monitoring Personal Allergen Exposure

Tim O'Meara* and Euan Tovey

*Royal Prince Alfred Hospital, Institute of Respiratory Medicine,
University of Sydney, New South Wales, Australia*

Introduction

Personal exposure to airborne allergens indoors is a dynamic and complex interaction between the environment and the patient that results in the deposition of allergen-carrying particles in the upper and/or lower respiratory tract. This exposes various components of the immune system to soluble allergens, which may have a range of clinical consequences including sensitization and the induction of acute or chronic symptoms of disease. Many factors determine this exposure; environmental and physical factors influence the diversity, total amount, and size of particles that can be airborne at any time. Meanwhile, personal factors, such as the level of activity and proximity to the allergen sources, influence the amount of allergens in the breathing zone of the patient and the proportion of these that are inhaled.

Measurement of personal allergen exposure allows the exploration of the relationship between allergen exposure and allergic disease. A range of collection devices and immunoassays have been used to estimate the concentration or amount of allergens in either reservoir dust, or the air, in a variety of occupational and domestic settings. From these proxy measurements of doses of exposure, relationships have been sought for clinical outcomes ranging from sensitization to the changes in acute and chronic indices of such diseases as asthma and hay fever.

Many of the systems used to quantify allergen exposure only provide some measurement of allergens in a standardized sample recovered from a component of the individual's environment. It is difficult to know the relationship between such measurements and what is actually deposited in the upper and/or lower respiratory tract of an individual. This is further complicated by the nature of the allergen

*Author to whom all correspondence and reprint requests should be addressed.

exposure, which may be either over a short period in one location or during an activity, or cumulatively over extended time-frames. At present we are unable to determine allergen exposure profiles and therefore the relevance of certain types of exposure. Continual development of both sampling and assay technology recently has made it possible to both collect and examine samples in a way that more closely allows the analysis of actual exposure that an individual receives. This provides new insights into the nature of such exposure, enables its relationship to disease to be better established and also allows more relevant, simple sampling methods to be validated. This review examines the characteristics of personal allergen exposure, the methods for assessing exposure, and the data available on personal allergen exposure to indoor allergens.

Measuring Personal Allergen Exposure as a Rational Strategy for Prevention and Management of Allergic Disease

It is important for the management of allergic disease not only to establish that the patient has an allergy, but also the presence of an allergen in the patient's environment. A rational approach to reducing allergen exposure and assessing its effect on patient health can only be developed through the measurement of personal allergen exposure.

Allergen Exposure, Sensitization, and Symptoms

Sensitization to a specific allergen remains the strongest risk factor for the development of allergic diseases (1–3). It is clear that there is a strong relationship between allergen exposure and the level of sensitization in a community (3,4). The earlier proposals of allergen “thresholds” for sensitization within the population have recently been refined. Wahn and colleagues (4) quantified the increasing incidence of sensitization to cat and mite allergens with increasing exposure both in a group of infants with atopic parents and in another group without atopic parents.

The relationship of allergen exposure and symptoms of asthma has been more difficult to define directly than the more measurable and simple outcome of sensitization. Support for the role of allergens in asthma comes from a number of sources and fulfills all of Bradford-Hills criteria for proof of an association between a disease and an environmental cause (5). Experimental supportive data for the role of natural exposure in causing symptoms comes in several forms. There is data from provocation of acute symptoms with natural aeroallergen exposure (6–9), data from the worsening of airways hyper reactivity

with either season fluctuations (9,10) or with differences in community allergen levels (11,12), and data from studies where asthma symptoms improve when allergen exposure has been reduced (*see, for example, Custovic (13).*

The model of how allergen exposure “causes” the symptoms of asthma needs to be considered in terms of chronic and acute asthma. Allergen exposure initially stimulates a genetically predisposed person to become sensitized (that is, to produce allergen-specific IgE), usually as a young child. Further allergen exposure gradually leads to the development of nonspecific airways hyper-reactivity in a subgroup of such individuals. Such hyper reactive airways are a common feature of asthma and will respond to a range of stimuli—such as exercise, cold, some viral infections, and additional allergen exposure—to produce the acute symptoms of asthma. It is the ongoing background stimulation by allergens that is important in maintaining the state of hyper-reactivity (14) and there will not necessarily be a link between discrete episodes of asthma and identified episodes of exposure. Such a model is strongly supported by observations of occupational exposure (15), including the experience that the hyper-reactivity may become persistent if allergen exposure is maintained. Acute asthma from allergen exposure is a subset of this; it occurs when there is sufficient exposure of particles that directly interact with the airways, as in the inhalation of animal allergens (6) or small granules derived from burst pollen (16), that are in sufficient dose and are small enough to directly enter the airways.

In a recent editorial, Platts-Mills (17) considered three factors to obscure the relationship between the measurement of allergens in houses and asthma symptoms. These were:

1. The lack of accuracy of reservoir measurements in providing information about allergen exposure;
2. Individual differences in the local inflammatory responsiveness of the lungs (measures based on specific IgE are not very useful for quantifying lung inflammation); and
3. The existence of multiple factors, in addition to allergens, that can trigger an attack of asthma, including chemicals, cold air, and respiratory viral infections.

It is likely that the prevalence of sensitization can be linked to an exposure level based on reservoir dust levels multiplied by time in years (18). It is unlikely that this index will be of sufficient sensitivity to the pathological events involved to be a useful index for asthma symptoms. The improved measurement of personal allergen exposure will greatly facilitate the understanding of its role in asthma. Modeling the quantity, particle size, and timing of such exposure will provide the

only logical foundation for a measure that can be related to clinical effects. It will also be very useful for designing and validating measures to reduce allergen exposure.

Allergen Exposure, Diagnosis, and Disease Reduction

The relationship between a patient's allergen exposure and symptoms of allergic disease is currently explored by skin-prick testing with allergen extracts or measuring the level of specific IgE in the patient's serum. The utility of this information is highly dependent on the patient's history and the correct choice of allergens for screening the patient against. The information that both these tests provide is limited in that it does establish whether a patient is currently exposed to any of the allergens to which they are sensitized, but the level of exposure to the allergen, and therefore whether this allergen is responsible for the patient's current symptoms, cannot be determined. It is known that IgE is persistent and that circulating IgE may remain high in the absence of exposure. In patients who are sensitized to multiple allergens, allergy testing cannot determine current exposure to relevant allergens. A second limitation of allergy testing is that it cannot detect unknown or unsuspected allergens. In Australia, epidemiological studies have found that 8% of patients who have symptoms of allergic disease are skin-prick test negative to a panel of 14 inhalant allergens (19,20). These people do not have specific IgE to any of the standard test-panel allergens used in skin-prick testing and thus are classified with unknown atopy. In a recent survey across California, it was found that 57 different inhalant allergens were required to detect 90% of allergies (21); however, patients are rarely tested against such an extensive panel and even if they are, the question of current exposure still remains. Clinically, undiagnosed atopy may be very important because unsuspected allergens may contribute to current acute or chronic symptoms.

A number of recent publications have reviewed the role of allergen avoidance in reducing both symptoms of allergic disease and the rate of sensitization to aeroallergens (13,22). The most dramatic demonstrations of the effects of allergen reduction on disease severity have come from observations in low-allergen environments, such as hospital rooms (23) or temporary residence in the European mountains (24). Clinical studies in houses have met with more mixed success, as was recently pointed out in the Cochrane Review of allergen avoidance (25). However several well-designed, case-controlled, and prolonged studies in houses have demonstrated gradual improvements in clinical outcomes (26,27). The major difficulty with such trials is that it is unclear to what extent personal aeroallergen exposure was actually

reduced by the avoidance methods employed, despite some reductions in reservoir levels. Several large studies in progress worldwide are examining the effect of allergen reductions on sensitization and the development of allergic diseases. Meanwhile allergen-avoidance strategies have been endorsed by a number of researchers and health organizations worldwide (3,13,22,28–30). It is of critical importance that methods are developed to assess the level of personal allergen exposure that is likely to facilitate meaningful allergen-avoidance strategies.

Components of Personal Exposure to Aeroallergens

An indoor environment contains large reservoirs of allergens and a relatively small amount of this allergen is airborne at any point in time. The contribution of individual sources within an environment is likely to be highly variable and to influence the amount of allergen that is airborne, the size of particles in the air carrying allergen, and therefore the dose of allergen inhaled by an individual in that environment.

The many components of personal exposure to aeroallergens are illustrated in Fig. 1. The sources of most domestic aeroallergen are the large quantities of accumulated mite, pet, and other allergens in the dust of beds, carpets, soft furnishings, and clothing. The contribution of each source to airborne allergen levels varies with time, disturbance, ventilation, and source characteristics, such as the materials the sources are made from. All these factors influence the amount of allergen that is airborne and the characteristics of the particles carrying allergen. Further, this is a dynamic equilibrium where allergen exposure is changing as determined by its generation from sources and removal by ventilation and settling.

It is worth noting that the fraction airborne (nanograms) is of the order of millionths of the total in reservoirs (milligrams) in the house. It is also likely that the fraction of smaller particles that become and remain airborne are only partly representative of all the particles that carry allergen in the settled dust reservoirs. The amount and sizes of the particles that are inhaled will also depend on the characteristics of the airborne particles carrying allergen, the proximity of the patient to the source, the level of activity, the rate of inhalation, and the amount of air inhaled. The site of deposition is in turn influenced by the size of particles that are inhaled.

The Physical Nature of Aeroallergens: Amount, Size, and Shape

The size and shape of particles carrying allergen affects their behavior when disturbed, when airborne, and when inhaled. Many

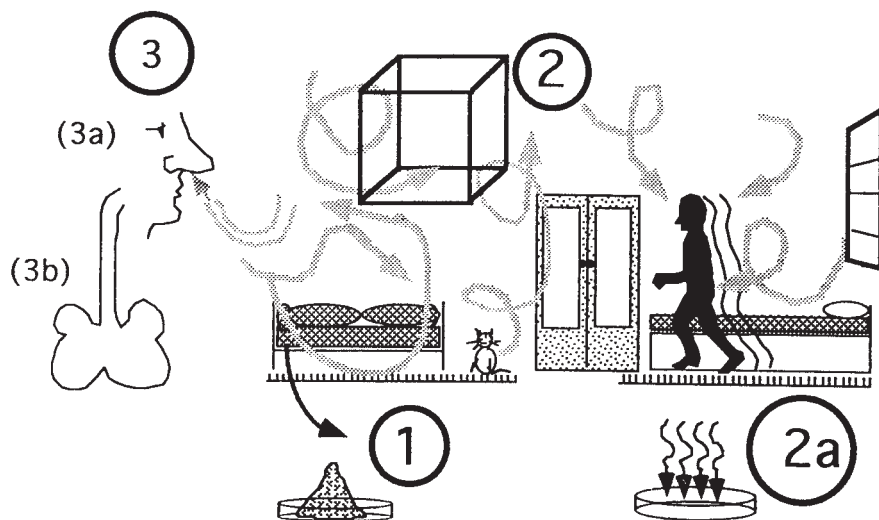


Fig. 1. Personal exposure to airborne allergens. Large reservoirs of allergens exist in normal indoor environments, including bedding, soft furnishings, and pets. Allergens become airborne from a range of reservoirs and sources and are influenced by the level of activity and dust disturbance in the environment. Methods for measuring allergen exposure include: reservoir measurements from carpets and soft furnishings (1); measurement of allergen in the ambient air (2) using a static or breathing zone air samplers, or through the collection of allergen settling from the air using settling plates (2a); measurement of true personal exposure to allergens (3), through the collection of inhaled allergens (3a) or the measurement of allergen deposited into the lung (3b).

studies have examined the amount and particle-size distribution of airborne allergens. Aeroallergens are very heterogeneous both in terms of the quantities that can be found in the air during normal domestic activity and the particles on which they are carried. Mite and cockroach allergens are reported to be carried on large particles and during normal domestic activity can only be detected in airborne samples using highly sensitive assay techniques or after dust disturbance. Dog and cat allergens are carried on a smaller particles and can readily be detected in airborne samples. Personal sampling has consistently demonstrated large variations in individual exposure to aeroallergens.

In order to develop an appreciation of personal allergen exposure, it is essential that we attempt to understand the nature of airborne allergenic particles, including the amount of allergen that is airborne, the size of particles carrying the allergen, the shape and source of particles carrying allergen, and their behavior. The amount of allergen that is inhaled is one of the factors that determines the development of symptoms in sensitized individuals; this is most readily demonstrated

in allergen-exposure challenge rooms or occupational settings, but has only been indirectly examined in normal domestic circumstances (6,8). In addition to the total amount of allergen inhaled, particle size and shape have strong influences on the site of deposition in the respiratory tract and the resultant symptoms. Some studies suggest that few particles above 10 μm in diameter enter the lungs (31), whereas others have shown that particles larger than this clearly do enter the lung (32). Thus particles greater than 10 μm may make an important contribution to the amount of inhaled allergen, even if only a small fraction is deposited in the lung.

A number of studies have examined airborne levels of the major indoor allergens (Tables 1–5). These studies have found that there is a large variation in the amount of a specific allergen that is airborne in different environments and the size of particles that carry airborne allergens. In addition, the shapes and sizes of the particles that carry aeroallergens differ within and between allergens, which affects their behavior when disturbed, when airborne, and when inhaled. It is surprising that for domestic allergens there have been few studies of personal exposure using breathing-zone samplers (*see* section on “Personal Breathing Zone Samplers”), whereas for occupational exposures they have been widely used.

House Dust Mite

For the house dust mite allergens Der p 1, Der p 2, and Der f 1, the levels that can be detected in the air are low, highly variable, and often undetectable by conventional assays in the absence of disturbance (Table 1). This apparently is not a universal finding because others have been able to detect airborne Der p 1 during normal domestic activity (33), after prolonged sampling (34) and have found that levels taken 1 yr apart are highly correlated (33). During dust disturbance, there is often a 50-fold difference in the concentrations of airborne allergen detected in different homes within the one experiment.

Based on reported levels of airborne Der p 1 (Table 1), and assuming a minute volume of 20 L, the average individual would be exposed to the order of 0–3000 pg of allergen (Der p 1) in a 1-h period in an undisturbed environment. This is clearly an enormous range in allergen exposure, but is consistent with measurements of personal exposure to Der p1 made with breathing zone samplers by Sakaguchi (35) (91.1–643 pg/m³), Oliver (36) (0–2500 pg/m³), and Sakaguchi (34) (6–485 pg/m³). Given the reported vast differences in personal exposure to mite allergen, it is clearly impossible to speculate on an individual's personal exposure and therefore such exposure should be

Table 1
Measurement of Airborne Mite Allergens Indoors

Allergen	Collection type and assay	Time, location, and activity	Number of positive results	Level of allergen (pg/m ³)	References
Der 1	Static: 17 L/min and RAST inhibition	45 min, homes, during disturbance	16/18	0–30000	(93)
Der 1	Static: 3.5–17 L/min	2 h-O/N, homes, nondisturbed	0/18		(93)
Der	and RAST inhibition				
	Static: 180 L/min	24–72h, homes, normal domestic disturbance	12/12	370–5570	(80)
Der f 1	and RAST inhibition	109–124h, living room normal domestic activity	10/10	0.4–82.3	(38)
	Static: 6 L/min and RIA	40 min, bedroom, bed-making, and disturbance	9/10	150–12,400	
Der f 1	Static: 200 L/min and monoclonal RIA	24h, bed and living rooms, nondisturbed	36/40	0–78	(78)
Der f 1 ^a	Breathing zone: 6 L/min and RIA	4.9–9.3 h, 30cm above bed during sleep	4/6	9.8–20.7	(35)
Der p 1	Static: 6 L/min & RIA	109–124h, living room normal domestic activity	10/10	2.8–107	(38)
		40 min, bedroom, bed making, and disturbance	10/10	5810–253,000	
Der p 1	Static: 200 L/min and Monoclonal RIA	24 h, bed and living rooms, nondisturbed			
	Rabbit RAST inhibition		18/20	0–53	
	IgE RAST inhibition		20/20	0–164	
Der p 1	Static: 2 L/min and ELISA	3 h, homes, normal domestic activity	28/30	496–1251	(78)
		20 min–2 h, homes, undisturbed	46/68	0–63,000	(33)
Der 1 (Der p 1 and Der f 1)	Static: 18–19 L/min and monoclonal ELISA		0/17		(85)
Der 2	Static: 18–19 L/min and monoclonal ELISA	20 min–2 h, homes, undisturbed	0/17		(85)
Der 1	Static: 18–19 L/min and monoclonal ELISAs	15–45 mins, homes, disturbed	16/16	68,000	(85)
Der 2	Static: 18–19 L/min and monoclonal ELISA	15–45 mins, homes, disturbed	16/16	35,000	(85)

Der p 1 ^a	Breathing zone: 6 L/min and RIA	4.9–9.3 h, 30cm above bed during sleep	6/6	91.1–643	(53)
Der p 1 ^a	Breathing zone and monoclonal ELISA	Nightly for 2 wk, attached to child's pillow	18/45	0–2500	(36)
Der p 1	Settling plates	2 wk, homes, normal domestic activity	52/61	440 (0.0–3700) pg/d	(36)
Der 1	Static: 6 L/min and ELISA	5–7 d, living rooms, normal domestic activity	13/13	18–89.6	(53)
Der 1	Static: 18–22 L/min and monoclonal ELISA	30 min, homes, no disturbance	0/18		(79)
		15 min, same homes, during disturbance: carpets	16/16	4400–190,000	
Der 1 ^a	Breathing zone: 1 L/min and monoclonal ELISA	No carpets 44–61h, homes, normal domestic activity: old bedding	0/2 12/12	29.6–485	(34)
Der p 1	Static: 60 L/min and monoclonal ELISA	New bedding 4 h/d for 10 d, outpatient	10/12 0/10	6–52.2	(54)
Der 2	Static: 6 L/min & RIA	109–124 h, living room, normal domestic activity	10/10	6.3 (1.7–35.1)	(36)
Der 2 ^a	Breathing zone: 6 L/min and RIA	40 min, bedroom, bed-making, and disturbance	10/10	12,600 (3900–180,000)	(35)
Der 2	Static: 6 L/min and ELISA	4.9–9.3 h, 30 cm above bed during sleep	6/6	87.1 (24–362)	(53)
Der p 2	Static: 18–19 L/min and monoclonal ELISAs	5–7 d, living rooms, normal domestic activity	13/13	5.1–24.5	(85)
Der p 2	Static: 18–19 L/min and monoclonal ELISAs	20 min–2 h, homes, undisturbed	0/27		(85)
Der p 2	Static: 18–19 L/min and monoclonal ELISAs	20 min–2 h, homes, undisturbed	0/27		(85)
Der p 2	Static: 18–19 L/min and monoclonal ELISAs	15–45 mins, homes, disturbed	18/18	26,000	(85)
Der p 2	Static: 18–19 L/min and monoclonal ELISA	15–45 mins, homes, disturbed	18/18	25,500	(85)
Der p 2 ^a	Breathing zone and monoclonal ELISA	Nightly for 2 wk, attached to child's pillow	2/45	0–3000	(36)
Der p 2	Settling plates	2 wk, normal domestic activity	43/61	210 (0.0–1400) pg/d	(36)

^aSamples collected with personal samplers.

Table 2
Particle Size Distribution of Airborne Cockroach and Mite Allergens

Allergen	Size range (mm)	%	Conditions	Reference
Bla g 1	>10	80	Disturbed, impinger @ 17.5 L/min (<i>n</i> = 7)	(42)
	1.5–10	10		
	<5	<10		
Bla g 2	>10	74	Disturbed, impinger @ 17.5 L/min (<i>n</i> = 7)	(42)
	1.5–10	16		
	<5	<10		
Der p 1 (Der 1)	>20–6	88.8	Domestic activity	(93)
	15–2	6.7		
	5–1	1.8		
	2.5–0.3	1.4		
	<0.5	1.3		
Mite	>4.1	11	Disturbed (bedmaking) Andersen @ 1130 L/min	(80)
	<–2.3	22		
	<2.3–1.4	24		
	<–0.8	30		
	<0.8	13		
Mite	>4.1	14	Nondisturbed Andersen @ 1130 L/min	(80)
	<4.1–2.3	30		
	<2.3–1.4	13		
	<1.4–0.8	27		
	<0.8	17		
Der 1	>20–6	76.1	During disturbance (Vacuum), cascade @ 17 L/min (<i>n</i> = 6)	(37)
	15–2	13.2		
	5–1	5.5		
	2.5–0.3	3.9		
	<0.5	1.2		
Der p 1	>20–6	78	Disturbed, cascade @ 18–19 L/min (<i>n</i> = 16)	(85)
	15–2	18		
	5–1	1		
	<2.5	2		
Der p 2	>20–6	62	Disturbed, cascade @ 18–19 L/min (<i>n</i> = 18)	(85)
	15–2	21		
	5–1	11		
	<2.5	5		
Der p 1	Flakes 5–50 µm	30	Natural disturbance, IOM sampling @ 2 L/min, microscope	(40)
	Fibers 10–150 µm	34		
	Feces 15–40 µm	36		

assessed using a personal sampling device (*see* sections on “Personal Breathing Zone Samplers” and “Nasal Air Samplers”).

In most homes, during dust disturbance, 80–95% of airborne mite allergen is carried on particles >5 µm diameter (Table 2). After 15 min,

Table 3
Measurement of Airborne Cockroach and Dog Allergens Indoors

Allergen	Collection type and assay	Time, location, and activity	Number of positive results	Level of allergen (pg./m ³)	Reference
Cockroach	Static: 180 L/min RAST inhibition	24–72 h, homes, normal domestic disturbance	5/7	<1000–43,430	(80)
Bla g 2	Static: 60 L/min Monoclonal ELISA	4 h/d for 10 d, outpatient department, normal activity	0/10		(54)
Bla g 1	Static: 20 L/min Monoclonal ELISA	3 h, homes, undisturbed	0/9		(42)
Bla g 1 ^a	Breathing zone: 2 L/min Monoclonal ELISA	8 h, homes, normal domestic activity	0/3		(42)
Bla g 2	Static: 20 L/min Monoclonal ELISA	3 h, homes, undisturbed	0/9		(42)
Bla g 2 ^a	Breathing zone: 2 L/min Monoclonal ELISA	8 h, homes, normal domestic activity	0/3		(42)
Bla g 1	Static: 20 L/min Monoclonal ELISA	45 min, homes, disturbed (vacuum)	7/7	0.9–26.8 U/m ³	(42)
Bla g 2	Static: 20 L/min Monoclonal ELISA	45 min, homes, disturbed (vacuum)	7/7	0.4–4.9 U/m ³	(42)
Can f 1	Static: 6 L/min Monoclonal ELISA	5–7 d, homes, normal domestic activity	3/3 with dogs 0/10 no dogs	2880 (1110–10,500)	(53)
Can f 1	Static: 6–9 L/min Monoclonal ELISA	8 h, homes, normal domestic activity (non-dust raising)	28/28 with dogs 11/36 no dogs	300–99,000 400–1100	(47)
Can f 1	Static: 60 L/min Monoclonal ELISA	4 h/d for 10 d, outpatient department, normal activity	10/10	120–560	(54)

^aSamples collected with personal samplers.

Table 4
Measurement of Airborne Cat Allergens Indoors

Allergen	Collection type and assay	Time, location, and activity	Number of positive results	Level of allergen (pg/m ³)	Reference
Cat	24–72h @ 180 L/min and IgE RAST inhibition	24–72 h, homes, normal domestic activity	?/11	<1000–92,000	(80)
Fel d 1	Static: 200 L/min and IgE RAST inhibition	24 h, homes, nondisturbed	21/30	0–314	(78)
Fel d 1	Static: 18–22 L/min and monoclonal ELISA	<1 h, homes, nondisturbed	1/1	11,000	(81)
Fel d 1	Static: 18–22 L/min and monoclonal ELISA	<1h, homes, nondisturbed: cats	9/9	8900 (2900–19,700)	(81)
Fel d 1	Static: 18–22 L/min and monoclonal ELISA	No cats	0/5	5700 (2400–8000)	(81)
Fel d 1	Static: 18–22 L/min and monoclonal ELISA	<1h, home, nondisturbed		117,000 (66,700–183,000)	(81)
Fel d 1	Static: 17.7–19 L/min and monoclonal ELISA	<1h, same home disturbed (vacuum cleaner)		7700	(85)
Fel d 1	Static: 17.7–19 L/min and monoclonal ELISA	1 h, home, undisturbed, rug on floor, ACH = 1.6		41,000	(85)
Fel d 1	Static: 17.7–19 L/min and monoclonal ELISA	1 h, home, undisturbed, carpeted room, ACH = 0.3		17,400 (2800–51,600)	(86)
Fel d 1	Static: 18–19 L/min and monoclonal ELISA	20 min–2 h, homes, undisturbed	?/8	212,000	(86)
Fel d 1	Static: 18–19 L/min and monoclonal ELISA	20 min, same homes, disturbed	8/8	5960	(53)
Fel d 1	Static: 6 L/min and ELISA	5–7 d, living rooms, normal domestic activity: cats	6/6 cats	(1110–23,000)	(48)
Fel d 1 ^a	Breathing zone: 3–4 L/min and monoclonal ELISA	No cats	0/7	2000–468,500	(36)
Fel d 1	Settling plates	1–2 h, homes, normal domestic activity: cats	15/15	2510 (0.0–64,000) pg/d	
Fel d 1		2 wk, homes, normal domestic activity	4/5		

Fel d 1	Static: 4 L/min and immunogold	24 h, homes, undisturbed: cats	5/5	(51)
Fel d 1 ^a	Breathing zone: 3–4 L/min and monoclonal ELISA	No cats	5/5	(6)
		1 h, homes, normal domestic activity: cats	37/37	
Fel d 1	Settling plates and monoclonal ELISA	No cats	10/40	(126)
		2 wk, normal domestic activity in living room: cats	?/13	
		No cats	?/13	
Fel d 1	Settling plates and monoclonal ELISA	2 wk, normal domestic activity in bedroom: cats	?/13	(126)
		No cats	?/13	
		No cats (<i>see ref. 6</i>)	30/40	(45)
Fel d 1 ^a	Breathing zone: 3–4 L/min and amplified ELISA	4 h/day for 10 d, outpatient department normal activity	7/10	(54)
Fel d 1	Static: 60 L/min and monoclonal ELISA	5 min, range (low-allergen room, grooming cat)	25/25	(50)
Fel d 1 ^a	Nasal sampling and particle immunostaining	5 min, range (low-allergen room, grooming cat)	1/15	(50)
Fel d 1 ^a	Breathing zone: 2 L/min and monoclonal ELISA	5 min, range (low-allergen room, grooming cat)	5/25	(50)
Fel d 1 ^a	Nasal sampling and monoclonal ELISA	8 h overnight, attached to pillow: cats	7/7	(49)
Fel d 1 ^a	Breathing zone: 2 L/min and monoclonal ELISA	No cats	0/20	
Fel d 1	Static and monoclonal ELISA	3–4.3 m ³ , homes, undisturbed: cats	50/50	(49)
		No cats	22/75	

^aSamples collected with personal samplers.

Table 5
Particle Size Distribution of Airborne Cat and Dog Allergens

Allergen	Size (µm)	%	Conditions	Reference
Can f 1	>9	41	Normal domestic, Andersen @ 28.3 L/min	(47)
	5.8–9	22		
	4.7–5.8	13		
	3.3–4.7	11		
	2.1–3.3	7		
	1.1–2.1	3		
	0.65–1.1	2		
Cat	>4.1	29, 18	Disturbed (bed making), undisturbed Andersen @ 1130 L/min (same home)	(80)
	<4.1–2.3	16, 17		
	<2.3–1.4	6, 12		
	<1.4–0.8	16, 15		
	<0.8	30, 39		
Fel d 1	>20–6	39	Undisturbed, cascade, @ 18–22 L/min (<i>n</i> = 1)	(81)
	15–2	27		
	5–1	8		
	<2.5	26		
Fel d 1	>10–3	42	Undisturbed, impinger @ 18–22 L/min (same house as above)	(81)
	10–1.5	35		
	<5	24		
Fel d 1	>20–6	23, 10	Disturbed, undisturbed cascade @ 18–22 L/min (<i>n</i> = 1, same home)	(81)
	15–2	29, 12		
	5–1	8, 16		
	<2.5	41, 62		
Fel d 1	>20–6	33	Undisturbed, cascade @ 18–22 L/min (<i>n</i> = 9)	(81)
	15–2	34		
	5–1	8		
	<2.5	26		
Fel d 1	>20–6	30	Undisturbed ACH = 1.6, cascade @ 18–19 L/min (<i>n</i> = 3)	(86)
	15–2	32		
	5–1	24		
	<2.5	14		
Fel d 1	>20–6	15	Undisturbed ACH = 0.3, cascade @ 18–19 L/min (<i>n</i> = 3)	(86)
	15–2	45		
	5–1	15		
	<2.5	25		
Fel d 1	>20–6	47, 26	Disturbed, undisturbed, cascade @ 18–19 L/min (mean of same 8 samples)	(85)
	15–2	25, 34		
	5–1	11, 18		
	<2.5	16, 22		

Table 5 (continued)

Allergen	Size (μm)	%	Conditions	Reference
Fel d 1	>28	6-53	Normal domestic, Anderson cascade @ 3-3.4 L/min (<i>n</i> = 15)	(48)
	17-28	8-80		
	7-17	0-36		
	4-7	4-33		
	2.6-4	0-20		
	1.5-2.6	0-6		
	<1.5	0-12		
Fel d 1	>20-6	14	Undisturbed, cascade @ 18 L/min (<i>n</i> = 4)	(79)
	15-2	64		
	5-1	15		
	<2.5	11		
Fel d 1	<1	?	Undisturbed, 4 L/min (<i>n</i> = 10)	(51)
	1-10	?		
Fel d 1	>9	46	<i>n</i> = 10, Andersen @ 28.3 L/min, normal domestic	(49)
	5.8-9	22		
	4.7-5.8	14		
	3.3-4.7	15		
	2.1-3.3	3		
	1.1-2.1	2		
	0.65-1.1	3		

10-20% remains airborne and is mainly associated with smaller particles (37), whereas hours later, <0.1% may remain airborne (38).

Using new and more sensitive techniques that identify individual particles carrying allergens (*see* section on "Particle Immunostaining: A New Technique"), Poulos (39) detected inhaled particles carrying Der p 1 under nondisturbed conditions during 10-min sampling periods. Der p 1 was inhaled while lying in bed and in a room with low levels of reservoir allergens and no soft furnishings. These new techniques have also demonstrated that airborne Der p 1 is not only carried on mite feces, but is also carried on amorphous flakes and fibers of a wide range of sizes (39,40). The equivalent aerodynamic diameter of such particles is considerably less than their largest diameters, and because their orientation in air-streams may confound their collection by inertial impaction systems, it is difficult to predict their behavior on inhalation, but it could be anticipated they would carry allergen into the lung.

Cockroach

Despite the emerging importance of cockroach allergens, airborne Bla g 1 and Bla g 2 have not been detected in public buildings (41) or in homes under normal domestic circumstances (42) (Table 3). So far, they have only been detected by conventional techniques in the same homes following massive dust disturbance (42). From these studies it has been inferred that cockroach allergens are carried on particles that are a similar size to those carrying house dust mite allergens (Table 2); however, particle size distribution for cockroach allergens has not been investigated. Using IRM nasal air sampling (*see "Nasal Air Samplers"*) and particle immunostaining (*see "Particle Immunostaining: A New Technique"*) airborne cockroach has been detected under nondisturbed conditions in a domestic living room and kitchen (43).

Cat and Dog

In contrast to the results for mite and cockroach allergens, most studies that have collected and measured airborne cat allergen have found detectable levels in undisturbed rooms (Table 4). The development of an amplified enzyme-linked immunosorbent assay (ELISA) for the detection of Fel d 1 (44) has further enhanced the detection of Fel d 1 from airborne samples. Using this enhanced assay Bollinger (45) re-analyzed samples taken from homes without cats (6) and found that in all 30 that were previously negative detectable, Fel d 1 was present (Table 4). For allergens such as cat and dog it has been suggested that airborne measurements are better than reservoir measurements as they are easy to detect and give a better index of exposure (46,47). However, this does not mean that these levels are static because massive weekly fluctuations in airborne Fel d 1 have been described (0–880 ng/m³), especially in homes without cats (6). Assuming a minute volume of 20 L, the average individual in a 1-h period would inhale 1200 L of air, containing in the order of 100–50,000 pg of allergen in an undisturbed room. Once again, this is a very large range and is supported by personal exposure measurements made with breathing zone samplers by Wood (48) (2000–468,500 pg/m³), Bollinger (6,45) (32) (1000–578,000 pg/m³), and Custovic (49) (0–28,000 pg/m³). It is likely that a 500-fold difference in personal allergen exposure would have consequences for the atopic patient and therefore such exposure should be assessed using a personal sampling device (*see "Personal Breathing Zone Samplers"* and *"Nasal Air Samplers"*).

Fel d 1 is carried on a wide range of particle sizes and the distribution of particles carrying Fel d 1 varies markedly between homes (48). Generally 40–80% of Fel d 1 is carried on particles above 5 µm

in diameter (Table 5). The subfraction of particles $<5\text{ }\mu\text{m}$ may remain airborne for prolonged periods (days) (49) and this persistence of aeroallergen may explain why cat-allergic people may develop asthma symptoms on entering a building without any dust disturbance. In addition to the role of traditional reservoirs, it is now clear that clothing can be a major source of inhaled Fel d 1 (50).

Airborne Fel d 1 and Can f 1 are found on carbon aggregates (soot), not only in homes with a cat or dog, but also in homes where no pet is present (51,52). However, Fel d 1 appears to be largely associated with flakes, which again may behave as smaller particles in an air stream, which is likely to assist their entry into the lung. Inhaled particles containing cat allergen that were trapped in the nose during nasal air sampling show a similar distribution to those collected from the ambient air (Fig. 2; Table 2).

Airborne dog allergen has been detected in all homes with dogs that have been sampled, whereas in homes without dogs, Sakaguchi (53) was unable to detect Can f 1, and Custovic (47) found detectable levels in one-third of the homes. Using high volume sampling, Custovic (54) was able to detect Can f 1 in the outpatient department of a hospital on 10 consecutive sampling days.

Few studies have examined the size distribution of airborne particles carrying dog allergen. These studies would suggest that Can f 1 is carried on a similar range of particles to those carrying cat allergens (Table 5).

Airborne Fungi

Airborne levels of fungi have been examined in a number of indoor environments (Table 6). Generally fungi are easy to detect (within 5–10 min) using personal air samplers. The most abundant species found indoors are: *Basidiospores*, *Alternaria*, *Cladosporium*, *Penicillium*, yeasts, *Aspergillus*, and Smut spores (55–57). The concentration of airborne fungi indoors was related to the concentration outdoors, although this has a seasonal confounder and is not related to the concentration in reservoir samples (55,57,58). Large variations in different rooms within the one building and between different buildings occur (55,56,58).

Rodent

Airborne guinea pig, mouse urinary, rat urinary and Rat n1 allergens have been examined in either domestic or occupational environments (Table 6). Occupational sampling has concentrated on comparisons of allergen exposure between various occupations associated either directly or indirectly with rodents and activities

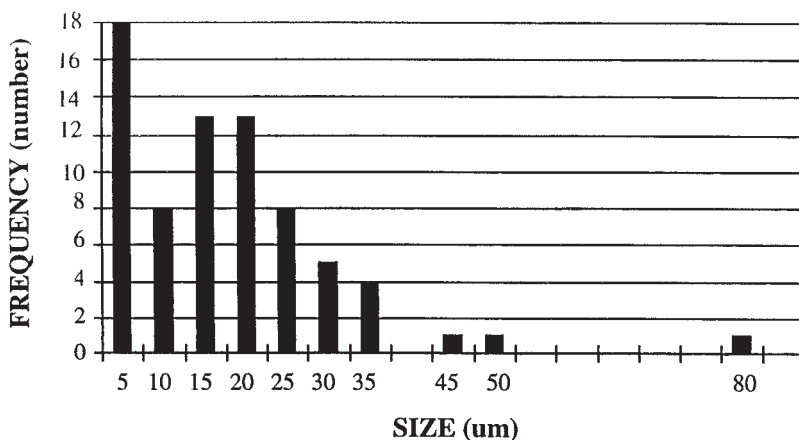


Fig. 2. Histogram of particle-size distribution of inhaled particles eluting Fel d 1 that were collected in IRM nasal air samplers (see section on "Nasal Air Samplers"). Size is the averaged diameter, which is the square root of the length by width. The number given for each frequency bin is the upper limit for that bin; for example, 20 μm includes all particles in the range 15.1–20 μm .

performed within a specified occupation (37,59–64). There were large differences in rodent allergen exposure between occupations with animal handlers/technicians exposed to the highest levels (59–62). In addition, there were large differences in task related exposures, which some studies have found be of the order of four orders of magnitude difference in people performing similar job descriptions (60). Tasks that involve cleaning cages or handling nonsedated animals result in the highest allergen exposures.

Few studies have examined the particle sizes associated with airborne rodent allergens (Table 7). During disturbance it has been found that about 90% of rat allergen is on particles above 5 μm (37), whereas during "normal" levels of activity, about 60% of rat allergen is on particles above 5 μm when collected using a 300 L/min sampler (65).

Pollen

Although pollen exposure is usually considered to occur outdoors, it is recognized that the pollen that remains aloft in outdoor turbulent air will settle in the still air of houses and can be found in house dust. Such penetration of pollen is facilitated by summer ventilation with open windows and unfiltered fans. A Finnish study found a 3-wk lag between outdoor and indoor dust pollen peaks and suggested that traffic of outdoor dust on clothing and footwear also

contributed (66). In a study of the houses of subjects with emergency room asthma admissions in northern California, grass pollen allergy was the major risk factor, and 13 of 15 houses examined had very high seasonal levels of grass pollen allergen in their house dust (67). It was concluded by the authors that, as subjects spent much more time indoors than outdoors, indoor exposure could make an important contribution to their pollen exposure. The levels of pollen allergen ($>10 \mu\text{g dust}$) were 10–100-fold higher than those recorded indoors in the Virginia pollen season (68). In a Texas study of mobile homes, airborne levels of pollens were found to be 4–27-fold less than the simultaneous outdoor levels (69).

It has been recognized since the 1870s (70) that pollen grains may fragment in humid conditions to produce granules, which more recently have been associated with outbreaks of “thunderstorm asthma” (16). Because of their small size, such particles are more likely to remain airborne and to penetrate buildings during regular air exchange. Such studies of nontraditional indoor allergens demonstrates the advantages of personal sampling to identify both the circumstances of exposure and the importance of personally relevant allergens.

Flour Proteins

Wheat proteins and α -amylase have been measured in the air of bakeries using static samplers, and personal exposure has been assessed using breathing zone samplers worn by workers (Table 6). A range of bakeries have been examined and a number of occupations within each bakery (71–75). Wheat antigen was detected in personal samples from many bakery workers (85%); in contrast, α -amylase was detected in only 15% of samples. Individuals performing different jobs were exposed to vastly different amounts of wheat antigen ($33\text{--}252,407 \text{ ng/m}^3$) and α -amylase ($0\text{--}40 \text{ ng/m}^3$); generally, dough makers were exposed to the highest levels of both allergens. Once again it was evident that there were large differences in individual exposure between employees performing the same job and that daily fluctuations in exposure were high (72–74).

α -Amylase is found mainly on particles above $5 \mu\text{m}$ in diameter (Table 7), with more than half present on particles greater than $9 \mu\text{m}$ in diameter.

Latex

Airborne latex allergens have been measured in a medical center, physicians' offices, and a range of rooms in different hospitals (76,77). Levels range from $400\text{--}205,000 \text{ pg/m}^3$ and were found to be 100-fold higher in areas where latex gloves were worn frequently, compared to

Table 6
Measurement of Airborne Fungi, Rodent, and Flour Allergens Indoors

Allergen	Collection type and assay	Time, location, and activity	Number of positive results	Level of allergen (pg/m ³)	Reference
Fungal spores	Static: 10 L/min and total counts	9 min, homes, undisturbed	80/80	667–118,222 spores/m ³	(57)
Fungal spores	Static: 1.5 L/min and total and viable counts	4 h, normal classroom activity	96/96	total 7–360 × 103/m ³	(141)
Latex ^a	Static: 180–60 L/min and IgE RAST inhibition Breathing zone: 5 L/min and IgE RAST inhibition	1–4 h, medical center rooms and staff	96/96 19/19	Viable 0.1–4.5 × 103/m ³ 13,000–121,800	(76)
Latex	Static: 47–3750 L/min and inhibition		12/12	8000–978,000	
Mouse urine	Static: 180 L/min and RAST inhibition	18–20 h, hospital rooms and physician offices	20/32	400–205,000 pg/m ³	(77)
MUA ^a	Breathing zone: 2 L/min and polyclonal ELISA	24–72 h, homes, normal domestic disturbance	?/14	<3–2960	(80)
MUA ^a	Breathing zone: 2 L/min and polyclonal ELISA	6 h, laboratory animal houses, various jobs	149/171	580–12,100	(61)
Rat allergen	Static: 17 L/min and IgG RIA	5–341 min, laboratory animal houses, various tasks	101/123	2400–74,800	(61)
Rat n 1 ^a	Breathing zone: 4.2 L/min and IgG RIA	20 min, rat room undisturbed 20 min, same room, disturbed 1 h, animal handling room, cage cleaning quiet sitting	14/15 14/14 4/4	900–24,000 2000–70,000 19,000–310,000	(37) (59)
RUA ^a	Breathing zone: 2 L/min and IgE RAST inhibition	8 h, animal handling room, various jobs	3/4 ?/271	<1.500–9700 <10,000–>200,000,000	(60)
RUA ^a	Breathing zone: 2 L/min and polyclonal ELISA	6 h, animal handling room, various jobs	172/251	770–16,000	(61)

RUA ^a	Breathing zone: 2 L/min and polyclonal ELISA	5–341 min, animal handling room, various tasks	74/196	800–56,000	(61)
RUA ^a	Breathing zone: 2 L/min and polyclonal ELISA	30–185 min, animal handling room, cage cleaning	26/26	18,000	(62)
RUA	Static: 2 L/min and monoclonal ELISA	60–146 min, undisturbed rat room	38/44	200–900	(62)
Guinea pig	Static: 180 L/min and RAST inhibition	24–72 h, homes, normal domestic disturbance	?/6	<1000–495,000	(80)
Rat n 1 ^a	Breathing zone: 2 L/min and monoclonal ELISA	15 min–4 h, cage cleaning in rat room	2/2	600,000	(64)
RUA ^a	Breathing zone: 2 L/min and RAST IgE inhibition	Same sample	2/2	80,000,000	(64)
Rat n 1 ^a	Breathing zone: 2 L/min and monoclonal ELISA	15–96 min, cage cleaning in rat room	5/5	80,000	(64)
RUA ^a	Breathing zone: 2 L/min and RAST IgE inhibition	Same sample	5/5	100,000,000	(64)
Wheat antigen ^a	Breathing zone: 2 L/min and IgG inhibition ELISA	6–8 h, various bakeries and jobs	379/449	33,000–252,407,000	(72)
α-amylase ^a	Breathing zone: 2 L/min and polyclonal ELISA	6–8 h, bakeries, workers in different jobs and at different bakeries	93/507	<200–221,800	(73,74)
Wheat antigen	2 L/min and monoclonal ELISA	Single bakery	?/12	744,000	(75)

^aSamples collected with personal samplers.

Table 7
Particle Size Distribution of Airborne Flour, Latex and Rodent Allergens

Allergen	Size (μm)	%	Conditions	Source
α -amylase	>9	14–90	normal activity, cascade @ 28.3 L/min ($n = 7$)	74
	9–5.8	9–49		
	5.8–4.7	0–75		
	4.7–3.3	0–8		
	3.3–< 0.4	0–4		
Latex	14	67	normal laboratory environment, cascade	76
	7	14		
	4	7		
	2	6.5		
	< 2	6.5		
Rat allergen	> 20–6	27	non disturbed/some disturbance, cascade @ 17 L/min ($n = 5$)	37
	15–2	45		
	5–1	12		
	2.5–0.3	6		
	< 0.5	10		
Rat allergen	> 20–6	44	during disturbance (dust raising), cascade @ 17 L/min ($n = 8$)	37
	15–2	46		
	5–1	5		
	2.5–0.3	3		
	< 0.5	3		

areas where latex gloves were seldom used (Table 6). Personal exposure to latex aeroallergens has also been measured using breathing zone samplers and it was found that anesthesia personnel had the highest level of exposure (mean 411 ng/m³; range 8–978 ng/m³), whereas exposure of different individuals performing the same job varied by over 100-fold.

Environmental Factors that Influence Personal Exposure

Reservoir levels of allergens are not good markers for short-term personal allergen exposure. The major determinants of personal exposure to airborne allergens are the levels of disturbance of reservoir in a room, the proximity of the individual to the allergen source(s), and the nature of the allergen source.

Reservoir Levels

A review of the data on correlations between reservoir levels of allergens—many collected from the carpet and bed—and airborne allergen concentration suggests that for some studies a relatively small number of samples have been taken, and the correlations found have

largely been owing to a single outlier in the data rather than a clear trend. Generally the data suggests that reservoir concentrations of Der p 1, Der f 1, Der p 2, Fel d 1, and Can f 1 are poor predictors of airborne concentrations taken from the same location (6,33,34,36,38,45,47,78,79). In these studies, six significant correlations were found out of 29 tested, with correlation coefficients in the order of 0.3–0.92; however, four of these strong correlations came from a single study where an outlier appears to have greatly influenced the correlation coefficients. In contrast to these findings, Bollinger (6) found that airborne Fel d 1 could be detected in the absence of detectable levels of Fel d 1 in reservoirs. Some studies have found that airborne Fel d 1 or other animal allergens correlates with the number of cats or other pets living in the house (6, 80), whereas others have found it does not (48,81).

Reservoir concentrations of allergens do not account for much of the variation in airborne concentrations. A confounder in these studies may be that the level of allergen measured in reservoir samples is expressed as μg of allergen/gram of dust, according to recommendations of the Second International Workshop (82). A more appropriate measure of reservoir allergen for the purposes of predicting airborne levels may be the level of allergen per unit of collection area ($\mu\text{g}/\text{m}^2$), which would also reflect the total level of dust in the reservoir.

Dust Disturbance

The level of dust disturbance in a room has a large influence on the amount of allergen that is airborne as well as the particle size distribution of airborne allergens. Tovey (83) reported that mite allergen could not be detected in the absence of disturbance, whereas during disturbance the levels of Der p 1, Der f 1, and Der 2 have been shown to increase by over 1000-fold to levels that can readily be detected in relatively short sampling periods (38,83,84). Swanson (80) found that bed making resulted in a 50-fold increase in airborne mite allergen and a 100-fold increase in airborne cat allergens. Others have found more modest increases in airborne cat allergen (81,85).

Although it has rarely been examined in domestic environments, in occupational environments there is not a strong relationship between the total levels of airborne dust and the level of airborne latex (77) and wheat antigens (72,75), and there is much greater variation in exposure to airborne allergen than total airborne dust.

The authors thoroughly agree with de Blay (42) that “it is difficult to establish whether the allergen particles detected in the air following disturbance correspond to those inhaled during natural exposure, and this continues to be a problem for the interpretation of experiments on aeroallergens.”

Humidity/Air Conditioning/Ventilation

In two houses with air exchange rates of 0.3 and 1.6 air changes per hour (ACH), the house with the higher ACH had less Fel d 1 associated with particles less than 2.5- μ m, and it has been suggested that higher air exchange rates remove small particles from the air (86). This has been confirmed in cat challenge rooms; however, increasing the ACH appears to have little effect on the total amount of allergen that is airborne (48,81,86).

A considerable number of studies have examined the effect of humidity and central heating on the levels of reservoir Der p 1; in contrast, there has only been one report of the effect on airborne Der p 1 where such environmental factors were not important (33).

Types of Reservoirs: Different Types of Carpets, Different Fabrics

Allergen reservoirs consist of carpets, bedding, soft furnishings, curtains, clothing, and for pet allergen the pet itself. There have been few studies that have examined the properties of reservoirs and their influence on aeroallergen. It is likely that different reservoirs accumulate allergens at different rates, release different levels of allergen into the air on disturbance, and also effect the particle-size distribution of allergens released into the air (33,81,85,86). It has been reported that airborne Der p 1 was higher above wool carpets than synthetic carpets or hard floors, although the same concentration of allergen was recovered from these reservoirs (33).

Individual cats differ in the levels of Fel d 1 that can be washed from their skin and male cats produce more Fel d 1 than female cats (3,87,88). Higher Fel d 1 production by the cat results in higher levels of airborne Fel d 1 (87), because it has been shown that airborne Fel d 1 levels rise when a cat enters a room and repeated washing of the cat reduces the airborne level of Fel d 1 (81,86).

Proximity to Sources

Proximity to the source and level of disturbance of the immediate source have large effects on the level of personal allergen exposure. Within a single room a person inhales threefold more particles containing Fel d 1 when grooming a cat sitting on their lap than when sitting in the room beside the cat or walking around the room where the cat is sleeping. In addition there is a strong correlation between the total number of particles inhaled and the number inhaled containing Fel d 1, when grooming or sitting beside the cat,

but not when the person is at a distance from the cat (39,50). Similarly, clothing is a very important source of allergen owing to its ability to accumulate allergens (89–91) and its proximity to the breathing zone of the wearer. Very high levels of Fel d 1 can be inhaled from clothing in environments where other reservoirs are very low in Fel d 1 (39,50). In a recent study where cats were removed from houses, total cat aeroallergen decreased by 67% within 2 d, owing predominantly to the reduction in large particles, which fell to 13% of baseline (49). The amount of cat allergen associated with smaller particles ($>4.8 \mu\text{m}$) decreased more slowly over 14 wk to 33% of baseline. Interpretation is confounded by the direct contribution made by the animal by shedding and from the contribution made by it disturbing dust. Consistent observations of differences in particle size and quantities of aeroallergen were demonstrated in a study of mouse allergen (Mus m 1) (92). Here, in the room housing the mice, the airborne level was 0.5–15 ng/M³ and associated with particles of a mean diameter of 3.3–10 μm , whereas in the adjacent room in the facility, the airborne level was only 0.2–1.5 ng/m³ and mean particle size 0.43–33 μm .

The bed is commonly cited as the most important domestic site for mite allergen exposure. This is owing to the relatively high concentrations of allergen at this site, the proximity of the head to the allergen source, the proportion of indoor time spent at this site, and the opportunity for dust disturbance. These are probably true, although this has never been modeled definitively. The studies of mite allergen exposure that have been performed in and around beds (34,35,93,94) would all support the importance of this site. Sakaguchi (35) measured the mean level of mite aeroallergen occurring near sleeping subjects as 223 pg Der 1 allergens/m³ (range 91–653), which was 10-fold higher than during normal domestic life in the living room of the same house. When the bedding was replaced with new allergen-free bedding, the airborne level dropped by 20-fold, indicating that the source was the bed rather than the bedroom floor and demonstrating the importance of proximity and disturbance for exposure. Based on our calculations of the data, there was no correlation between the level in the bedding (in $\mu\text{g/g}$) and the associated aeroallergen ($R^2 = 0.129$, $p = 0.485$), reinforcing the need to measure relevant exposure.

Physiological Factors that Influence Personal Exposure

The amount of allergen that is deposited in the upper and lower respiratory tract is influenced by a number of physiological factors that are unique to the individual. These factors include the amount of

air inhaled, the rate of inhalation, breathing pattern, and partitioning between nasal and oral breathing.

Personal exposure to allergen involves the inhalation of particles from the ambient air into the respiratory system, generally via the nose. With increased respiratory demand, driven for example by a higher level of activity, there is a transition to mainly mouth breathing, although some component of mouth breathing occurs in a small proportion of the population at all times. Even when the nose is partly occluded, for example, during a respiratory viral infection, there is still a strong tendency to nose breath despite the increased load.

The drawing of particles into the nose from the ambient air during inhalation is a complex local event. The acceleration of particles with the inhaled airstream depends on their size, shape, and aerodynamic behavior. This is further complicated by any movement of the airstream relative to the nose itself, for example, air currents, thermal body drafts, and movement of the head during activity. Compared to isokinetic sampling (where the sampler is moved into the air at the same rate as the air is drawn into the sampler, in effect "sweeping" up a "tube" of particles), during inhalation most small particles ($<10\text{ }\mu\text{m}$) are accelerated and inhaled, whereas about 30% of large particles may have too much inertia to be accelerated and are not inhaled.

Once inhaled into the nose, most of the particles are deposited at various sites within the respiratory system, although a few smaller particles not deposited will be exhaled, as observed with the exhalation of cigarette smoke.

Minute Volume

The total volume of air inhaled by an individual (termed the "minute volume" and measured in units of L/min) varies both with the individual and with their level of activity. The mean value determined by Silverman (95) for healthy young men when sedentary was 10.3 L/min, gradually rising with increasing intensity of exercise, up to a maximum mean of 113.8 L/min at 1660 kg-m/min (extreme) exercise. The accompanying respiration rate rose from 14.6 to 47.6 breaths/min. Similar figures have been published elsewhere. Another study gave rates of 19, 37, and 61 L/min for slow, medium, and fast exercise, the fast being what would be performed by an average person for 2 min/day. The values for women were 16, 24, and 32 L/min, respectively (96). A cohort study of 15 men and 5 women, aged 19–50, gave group means of 7 ± 3 L/min for sleep, $12\text{ L/min} \pm 7$ for slow activity, $14\text{ L/min} \pm 8$ for medium activity, and 44 ± 36 L/min (highly skewed) for fast activity (97).

A commonly used figure for minute volume regarded as representative of an adult performing light manual work is 20 L/min (98). However in reality, such rates are highly variable depending on both the individual and the activity, and any communal mean used to represent exposure could be expected to contain a significant error if used to extrapolate an exposure dose from data on allergen concentration per unit volume of air.

Breathing Pattern

The shape of the inspiratory airflow wave occurring during sedentary activity is approximately sinusoidal, but changes to approximate a more energy-efficient rectangular wave form with increasing levels of exercise (99). It would not be anticipated that the differences in the wave form would affect the intake of particles as much as other accompanying factors such as nasal-oral partitioning and minute volume.

Nasal-Oral Partitioning of Breathing

Normal subjects at rest generally breath through their noses (100). However, in subjects with rhinitis and asymptomatic asthma, some proportional distribution of breathing through both the mouth and the nose at rest is more common. This distribution of oronasal airflow does not correlate with the level of inspiratory nasal airflow resistance, in both subjects with and without an allergic disease (101). As the minute ventilation increases with exercise, there is a switch from nasal to oronasal breathing. This switch in adults occurs at a mean minute ventilation value of 35 ± 6 L/min (100,101). Once again, this change-over is not heavily influenced by the levels of nasal airflow resistance encountered in moderate nasal obstruction. In all subjects during such exercise, both oral and nasal breathing will be present and exclusive oral breathing is uncommon. There is a wide variation between individuals in the distribution of their oro-nasal ventilation and some subjects approximate their resting distribution even during exercise. In addition, naive normal subjects who are observed with their mouth open during quiet breathing, on investigation were exclusively nasal breathing, as the soft palate closes the oropharyngeal isthmus (102). It has been proposed that the tidal volume increases with oral breathing compared to nose breathing (103), but others have not confirmed this and report no difference in the breathing pattern components in people with and without rhinitis and asymptomatic asthma (101).

Distribution of Airflow Between Nostrils

It has long been recognized that there is fluctuation in nasal patency, a phenomena known as the "nasal cycle," which is driven by

alternating partial congestion and decongestion of the region around the nasal turbinates. More recent objective measurements have detected this phenomena in slightly under half the adult population, whereas the rest change randomly and a few change in parallel. Where it does occur, the length of the periods, their repeatability, and the magnitude of the proportional distribution of airflow all differ between individuals. Further, most young children exhibit a random or parallel airflow rather than a reciprocal pattern and the distribution of the characteristic adult pattern emerges around 7–10 yr of age (104). Overall the major pattern is thus not cyclic, but is random or reciprocal.

Deposition of Particles in the Airways

The site of deposition of differently sized inhaled particles within the respiratory system has long been of interest. This is because the airways are a principle route for many toxic, irritant, and radioactive substances, which provide an occupational exposure hazard, as do the many particles with biological activity (e.g., allergens, fungi, viruses, bacteria, and so forth) of public health importance. Extensive reviews have been published elsewhere, and only a summary of relevant aspects will be provided here.

For definition, the regions of the human respiratory tract are described as follows (105). The “head” region consists of the nose or mouth, which together form the nasopharynx, plus the larynx. The remainder is termed the “thoracic” (or “lung” region), which consists of the trachea. The thoracic region leads to the branching airways known as the bronchi, then the conducting or terminal bronchioles, and finally to the alveolar region, consisting of the respiratory bronchioles, alveolar ducts, and alveolar sacs (where gas exchange occurs).

The behavior of inspired particles is rather complex. During breathing, particles are transported with the inspired and expired air. Not only are these particles affected by airway surfaces, but they are also deposited in the airway by a variety of mechanisms including gravitational sedimentation, inertial impaction, diffusion, and electrostatic forces. Deposition by each of these is determined by different particle characteristics. Inertial impaction occurs mainly in the upper airways and is determined by particle diameter, velocity, density, and shape. Deposition by diffusion and sedimentation is determined by particle size, density, and residence time in the airways (as determined by breathing cycle period) (106) and mainly occurs in the lower airways.

Deposition with Oral and Nasal Breathing

The route of entry into the body provides obvious large differences in deposition site for the larger particles but only minor differences for the deposition of small particles. In both cases the total deposition (the total amount of the entire inhaled sample) has two maxima of 100% for large particles (i.e., $>10\ \mu\text{m}$) and $>80\%$ for very small particles ($<0.01\ \mu\text{m}$). Perhaps surprisingly, for some particles of an intermediate size (in range $0.2\text{--}1.0\ \mu\text{m}$), only $\sim 30\%$ are deposited.

In the case of oral breathing most large particles ($>10\ \mu\text{m}$) are deposited in the laryngeal region whereas particles $1\text{--}10\ \mu\text{m}$ are distributed between the laryngeal, bronchial, and alveolar regions. For nasal breathing, the nose replaces the larynx as deposition site for large particles, whereas particles $1\text{--}10\ \mu\text{m}$ are distributed mainly into the nose with a small component deposited in the larynx and bronchi. Deposition in the alveolar region is bimodal, with high deposition rates for the $1\text{--}5\ \mu\text{m}$ particles that penetrate this far and for an increasing proportion of particles $<0.2\ \mu\text{m}$. The size for minimum deposition is around $0.2\text{--}1\ \mu\text{m}$ particles, the majority of which are exhaled.

Despite the similarities of general theories for particle deposition in the lung, there are many exceptions that question the proportion of large particles that may enter the lung (31,32,56,107,108–110). Although few particles above $10\ \mu\text{m}$ may be expected to enter the lungs (31), particles larger than this clearly do enter the lung (32). Swartengren (32) reported that in healthy nonsmoking men, 21% of a mono-dispersed Teflon particles (density $2.13\ \text{g}/\text{m}^3$) with an aerodynamic diameter of $13.7\ \mu\text{m}$ could deposit in the lungs. Although amount and size of particles are regarded as the most important determinants of personal exposure to allergens, it is clear that additional factors such as particle shape, density, hygroscopicity, airway congestion, and inspiratory flow are important determinants of the amount and site of deposition of inhaled particles.

Sleep and Breathing

The characteristics of breathing during sleep are important because they will affect the inhalation and deposition of allergen at this time when the potential for allergen exposure is high. Indeed most asthmatics report a worsening of asthma at night and it is unclear whether this is entirely owing to pathophysiological mechanisms or whether allergen exposure is exacerbating symptoms.

Breathing patterns during sleep are well-characterized (111). The minute volume is generally similar to that in a sedentary awake state and breathing occurs through the nose unless this is obstructed or the

person is a habitual mouth breather. During sleep onset and during the early phases of non-REM sleep, the breathing is irregular and becomes more regular as sleep deepens. Sighs, involving an approximate doubling of total volume, are not uncommon, occurring 3–4 times/h in adults and teenagers and perhaps 10 times/h in young children. During REM sleep the breathing is less regular, with both the frequency and volume being more variable, including patches of quick shallow breaths and slow deep breaths.

Snoring is only associated with sleep and involves the vibration of the soft pallet and other soft tissues on the pharynx. Snoring occurs with both mouth and nose breathing and varies with body position, being most common in the supine position. Children snore, particularly if there is some upper airways obstruction, and snoring is common in both male and female adults, particularly where alcohol intake and obesity are combined.

The actual quantity of allergen inhaled during sleep has not yet been measured. A possible estimate was provided by Ferguson and Brodie (112) where segments of the lung were lavaged the morning after sleeping at home, but it is speculative that such allergen was inhaled during sleep the previous night. Several studies (Tables 1 and 4) have measured the concentration of allergen in bedroom air under different conditions, but these cure only an estimate of the inhaled amount.

Methods Used for Sampling Allergen Exposure

A number of methods and devices have been developed to collect environmental allergen samples. In most applications, the aim has been to measure not personal exposure to airborne allergens, but rather the amount of allergen that is suspended within a volume of air. The use of personal samplers, such as breathing zone samplers and especially nasal air samplers, should improve our understanding of personal allergen exposure.

Reservoir Measurements

The Distribution and Sampling of Reservoir Dust

The most commonly used index of domestic allergen exposure is the concentration of allergen, in units of weight of allergen per weight of dust, in dust collected with a vacuum cleaner from various sites in the vicinity of the exposure. Commonly used sites are floors (particularly carpets), mattresses, and bedding, although such items as soft furniture and clothing are sometimes sampled. Such collection is simple to perform and has been standardized in terms of area and collection

time as far as is practical (82). This dust is sometimes referred to as "reservoir dust," "settled dust," or sometimes just "dust."

The allergens in such dust may either be produced locally, for example by mites or fungi living at the collection site (as with beds, carpets, or clothing), or may come from sources at other locations, and the allergen will have been ubiquitously distributed by circulating aeroallergen and by other vectors such as clothing. An example of the latter is the distribution of pet allergens to distant places where there are no cats, such as schools and public buildings (41,89,113), anecdotally even to the space shuttle.

Within a house, the level of reservoir allergens vary for different locations in a room (114,115) and with seasons (116,117). Indeed the concentration of allergen in dust collected from carpets can vary markedly (say 10-fold) over short distances (e.g., 1 m) within a room (115). There is no recognized basis to such variation in distribution. By convention, in studies where associations are sought between allergen levels and clinical indices and where multiple samples are taken in a room or house, the highest site has been used as the index of exposure (116).

There are numerous minor differences in the details of protocols used by different research laboratories to collect samples and it is not known to what extent these are important. For example, floor samples may be collected from either the middle or edge of rooms, samples may be collected with mains or battery-powered vacuum cleaners, or the filter units vary in their pore size and air-flow resistance. The dust is either extracted without sieving or after sieving to give a fine fraction, thus avoiding the large fibers but including the fine mineral components. The extraction conditions and buffers used differ and again may influence the amount extracted (117).

Despite these sources of variation, reservoir sampling has formed the backbone of epidemiological studies and contributed tremendously to our general understanding of allergen exposure and allergic disease (3,82).

Aeroallergen Measurement

The measurement of aeroallergen depends on two separate steps that are of equal importance for the accurate determination of personal exposure to airborne allergens. The first essential step is the collection of airborne particles carrying allergen, for which a number of devices have been developed. These devices range in flow rate, portability, cost, and most importantly in their ability to mimic personal exposure to airborne particles carrying allergen. Once airborne particles have been collected, the second step in the process is the measurement of the allergen levels collected by the device. Allergen

measurement is performed largely using a number of different immunoassays, which may generate vastly different results.

Collection Devices

A number of devices have been developed or adapted to collect airborne allergens. Several innovative designs were described by Blackey (1873) (70) including a sticky trap carried aloft by a kite and operated by a clockwork mechanism, as well as a device placed in the mouth, similar to a smoking pipe, which was used to collect inhaled pollens. The latter was the first attempt to examine personal exposure to airborne allergens. Others developed individual passive samplers that were used to collect pollens and fungi (118); however, no methods for determining the volume of air sampled or the relationship between passive exposure and inhaled pollen or fungi were available. Other forms of passive sampling, such as the electrostatic Airpoint sampler (46), would appear to suffer from the same problems.

HIGH-VOLUME STATIC SAMPLERS

High-volume filter samplers with flow rates in the range 60–1100 L/min have been used to collect airborne allergens. These static samplers are normally placed in a fixed position in a room and do not measure personal exposure to allergens. In many cases they have been used for prolonged sampling periods and the amount of allergen collected and measured is likely to be equivalent to 10 d cumulative exposure in an average individual (20 L/min). In addition, the higher volume samplers may fragment particles and therefore reduce the apparent particle size (37,119). When used for prolonged periods of time, high-volume samplers may remove allergenic particles at a faster rate than they are released into the air and therefore underestimate the level of allergen in the air (33). The converse may also be true; because of the high velocity of the air intake, particles larger than would be inhaled are collected. This would result in an overestimate of the amount of allergen compared to the amount that would be inhaled. An additional problem with using high-volume samplers is that they are too noisy for domestic use (120).

Other static filter samplers (i.e., those at a fixed location in a room) have used lower flow rates (6–20 L/min) and more likely approximate the minute volume of humans. This may result in the collection of a more meaningful sample; however, sampler design in addition to flow rate are important considerations because they can greatly influence the relative percentages of different-sized particles sampled (121). The major shortfalls of these types of samplers is their static nature, and the fact that they do not reproduce the changes in flow rates and particle accelerations associated with human respiration.

PERSONAL BREATHING ZONE SAMPLERS

Personal samplers, which are worn by the subject, have some advantages in that they do monitor the air by which the subject is surrounded. These samplers are typically battery-powered, portable, and have constant flow rates of 1–10 L/min. Such samplers are referred to as breathing zone samplers and sample air within a 1-ft zone of the wearer. Breathing zone samplers do not monitor what is actually inhaled and do not take into account the change in respiration associated with activities and fluctuations in a breathing cycle. Mark and Vincent (98) examined a number of sampling heads for use with breathing zone samplers and found that there were wide variations between the efficacy of sampling heads for different particle sizes in the range of 6–74 μm . The Institute of Occupational Medicine (IOM) sampling head was developed to conform to the aerosol sampling characteristics of a mannequin aspirating a “breathing” waveform through the mouth.

There is a vast amount of literature comparing the capture efficiency of different breathing zone samplers (*see review, ref. 122*), and it is apparent that certain samplers perform well over a range of particle sizes and ambient wind velocities, whereas others do not. The efficacy of such samplers for collecting personal exposure to aeroallergens has not been assessed. However, performance criteria for breathing zone samplers has been published (121), and it is likely to be a strong guide for personal allergen sampling. Despite many shortcomings, this form of sampling is vastly superior to static sampling for the determination of personal exposure to aeroallergens.

NASAL AIR SAMPLERS

We have developed intranasal air samplers (IRM nasal air sampler), which is a single-stage, slotted, impaction sampler with inhaled and exhaled air flowing through the same ducts. Each sampler consists of a polyurethane body shaped to fit the nose (Fig. 3). An adhesive tape is placed onto this body, upon which particles are collected. The soft silicon seal is placed around the body of the sampler to secure the tape. Each pair of nasal samplers is attached by a flexible silicon connector. The device is concealed in the nasal vestibule (Fig. 4). It has a nasal resistance of 5.13 cm water/L/s at a pressure of 1 cm water, which is at the low end of noticeable change of nasal flow, similar to that experienced with mild nasal congestion. The particle capture efficiency of the device is influenced by particle size and inspiratory flow. This device has a collection efficiency of 100% for particles with an aerodynamic diameter of 10 μm or greater, and 50% for particles of 5 μm at a flow of 25 L/min. At 40 L/min, 100 and 90% of 10 μm and 5 μm particles are collected. It has been worn for up to 4 h when the limiting factor is nasal irritation owing to the physical pressure of the seals (123).

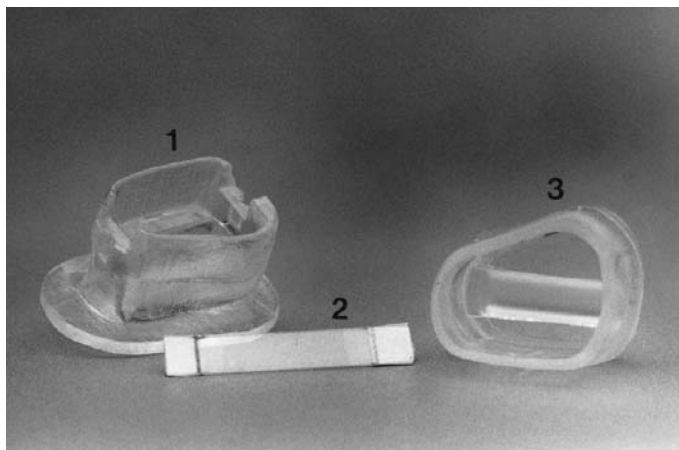


Fig. 3. The IRM nasal air sampler consists of three components: 1) An inner core that accelerates particles toward the adhesive strip; 2) an adhesive strip on which inhaled particles are impacted and remain bound; and 3) a soft outer shell that provides comfort and fit for the wearer.

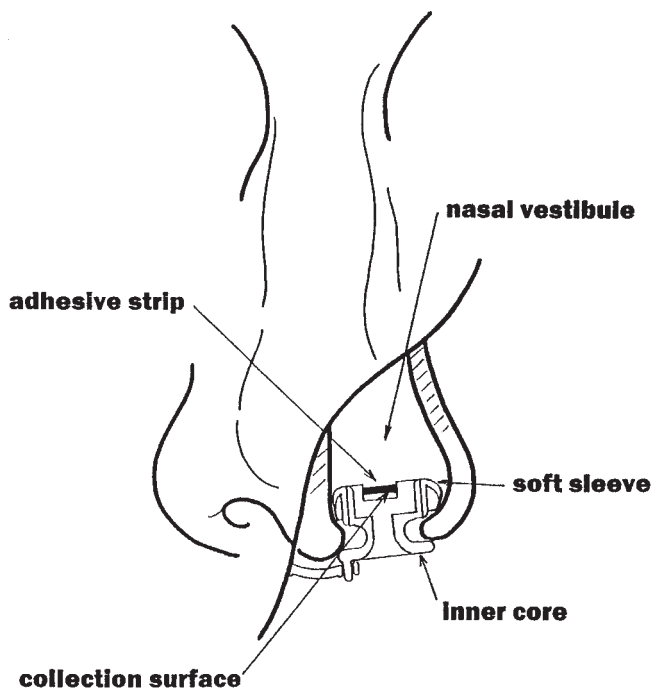


Fig. 4. Cross-section of the nose showing the IRM nasal sampler. The sampler fits into the nasal vestibule and is designed to provide minimum resistance both during inhalation and exhalation. Inhaled particles are impacted onto an adhesive strip inside the sampler where they remain.

Samples collected using this technique may be analyzed by ELISA (see section on "ELISA and RIA") or by particle immunostaining (Fig. 5; see section on "Particle Immunostaining: A New Technique").

SETTLING PLATES AS A PROXY MEASURE OF AEROALLERGEN

Indoor aeroallergen exposure has also been estimated using settling plates (124). This involves placing a pair of large Petri dishes in an open location and measuring the allergen content of the dust that settles over a 7–14 d period. This provides a nonvolumetric index of aeroallergen "turnover" described in units of ng of allergen/m³/d. Because mite aeroallergens become airborne when disturbed by human activity and thus provide exposure, collecting the settling material in a plate provides a longitudinal index of this. The settling plate technique has been used for grab sampling of indoor and outdoor fungi over short time-frames, and is not regarded as particularly accurate for this compared to volumetric sampling (125) because it is biased towards larger spores. However such confounders are less important when applied to sampling of indoor mite allergens, which are mainly associated with large particles.

In climates and houses where reservoir mite levels are high, using standard ELISA assays of approx 1 ng/ml sensitivity, it is possible to detect the quantities of allergen that settle out in a day, although such collections are usually performed for 2 wk. However, when reservoir concentrations are lower, the quantity collected in the plate is often below the limit of detection. Using amplified ELISA assays, the sensitivity can be increased about a 100-fold and low levels of cat allergen can thus be detected in houses without a cat by this method (44).

In two allergen-avoidance interventions, although there was a reduction in the concentration of allergen in reservoir samples collected from the treated sites, there was no significant reduction in aeroallergen as measured by the plate method (27,124) in the same room, questioning the likely clinical effectiveness of the intervention.

Longer-term airborne allergen sampling, through the use of settling plates, suggests a stronger correlation between reservoir and airborne allergens with correlation coefficients of 0.1–0.52 for house dust-mite allergens from a range of reservoirs (mattress, duvet, pillow, and carpet) (36) and 0.56 for *Fel d 1* (126).

How Do the Current Methods Compare?

STATIC VS BREATHING ZONE SAMPLERS

During high levels of dust disturbance, there are minor differences in the amount of allergen collected by a breathing zone sampler carried by a person and the amount of allergen collected by a static sampler with the same flow rate placed in the center of the room

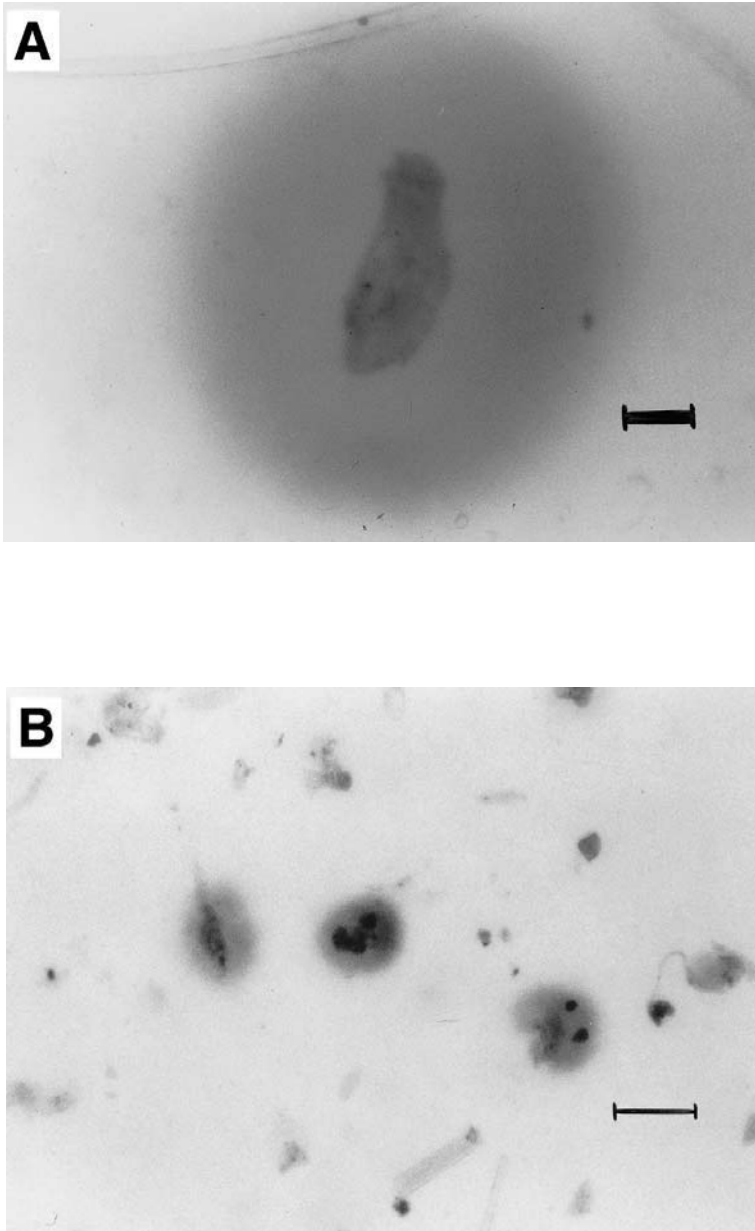
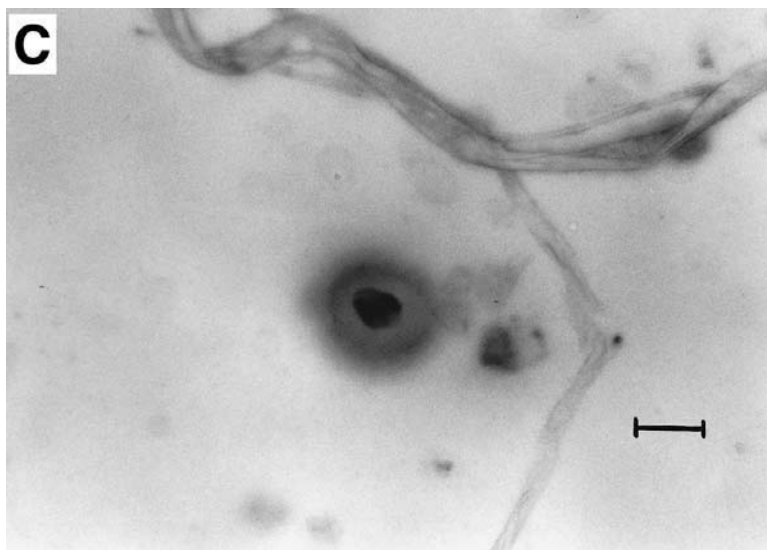


Fig. 5. Particles collected on the IRM nasal air sampler and immunostained with a range of anti-allergen probes. (A) A particle eluting Fel d 1 and immunostained using monoclonal 6F9 (Indoor Biotechnologies), bar = 50 μ m. (B) Particles eluting Der p1 and immunostained using MAb 5H8. Three halos can be seen surrounding particles eluting Der p1, bar = 100 μ m (C) Particles collected by a patient while lying in bed and immunostained with the patient's IgE, bar = 50 μ m.



while an activity is undertaken (62,64). However as the room size or level of activity and dust disturbance changes, it is apparent that static samplers give a poor index of personal exposure to airborne allergens. Price and Longbottom (127) examined three different types of samplers for the collection of mouse, rat, and rabbit allergens in laboratory animal rooms. It was found that a person wearing a breathing zone sampler (2 L/min) collected 2–3 times the amount of allergen that a static samplers, with higher volumes (10 and 140 L/min) collected when placed in the same room. This was not true of all activities and rooms, highlighting the variation in personal allergen exposure. Gordon (60) found that breathing zone samplers (2 L/min) attached to animal technicians collected an order of magnitude more rat allergen than static samplers operating in the same room. Swanson (76) also found that breathing zone samplers worn by medical staff collected fivefold more latex allergen than static samplers in the same room. Renstrom (62) found a median coefficient of variation of 23% for 28 pairs of samples collected by 2 IOM samplers placed 15 cm apart in the same room.

BREATHING ZONE SAMPLING VS NASAL SAMPLING

In order to truly monitor personal exposure to airborne allergens it is essential to take into account the amount of allergen that is actually inhaled by the subject. This was clearly demonstrated by Van Metre (8), who measured the fall in FEV₁ in cat allergic asthmatics when exposed to different challenges with cat allergen. The two types of challenges were “natural” exposure in a cat-challenge room and bronchial provocation using nebulized cat allergen. In the cat-challenge

room, personal exposure was measured using breathing zone samplers. For each challenge the dose of cat allergen causing a 20% fall in FEV₁ was recorded. It was found that there was a strong correlation between doses used in the two methods only if allergen exposure in the cat challenge room was adjusted for mean minute ventilation of the subject while in the cat challenge room.

Nasal air samplers have been compared with breathing zone samplers fitted with IOM sampling heads (39,50), however, the assays used for quantification of the allergens were different for the two forms of sampler. The nasal air sampler uses a more sensitive assay system and detects more allergen. When using the same particle immunostaining assay, the IOM samplers appear to collect more smaller particles carrying allergen than the nasal air sampler. The development of amplified ELISA assays will facilitate measurement of the small quantities of allergen associated with normal domestic exposure and allow comparison of these two methods of personal allergen sampling.

Methods Used for Measurement of Allergen Exposure

A range of assays have been used to quantify the level of allergen exposure. For immunoassays, there is a lack of standardization between assays and for some of the major allergens the conventional assays do not appear to be sensitive enough for the detection of normal levels of allergen exposure. The use of amplified ELISAs and particle immunostaining assays has enhanced our ability to detect airborne allergens and will facilitate the quantification of personal allergen exposure.

The choice of assay has often been dictated by the availability of detection reagents and assay technologies. Initial assays were based on radioallergosorbent (RAST) inhibition, then radioimmunoassay (RIA), ELISA, polymerase chain reaction (PCR), and more recently particle immunostaining and rapid chromatographic assays.

Immunoassays

Over time, many immunoassays have been developed by different groups for the measurement of airborne allergens; this has resulted in the development of a number of different antibodies and a process of continual improvement in assay sensitivity, reliability, speed, and accuracy. It is important that we continue to improve our immunoassays and to develop new and novel assays that bring us closer to measuring true personal allergen exposure. It is also important to realize that at present many different assays are being used to measure the same allergens; this makes comparisons of allergen levels virtually impossible. Setting of threshold values for occupational (including

classroom) exposure, defining relationships between symptoms and level of exposure, and assessing the efficacy of allergen avoidance practices is extremely difficult, since a 10-fold reduction in the allergen level in one assay may only be equivalent to a twofold reduction in another. In order to be able to compare allergen levels it is essential that we move toward standard assays.

RAST Inhibition

Initial assays used for the measurement of indoor airborne allergens were RAST inhibition assays, such as inhibition of binding to human IgE, human IgG, or rabbit sera (IgG). RAST inhibition has been used to measure total mite allergen (128), Der p 1 (78,80), Fel d 1 (78,80), flour proteins (71), rat urinary allergens including Rat n 1 (59,60,129,130), mouse urinary allergens (80), cockroach allergen (80), guinea pig allergen (80), and latex allergen (76). In these assays radio-labeled purified or semi-purified allergen was mixed with pooled sera from allergic individuals (or polyclonal rabbit sera) and allergen extracted from the filters of air samplers. This assay does not measure the levels of a specific allergen in an extract; instead, it equates the level of binding to an extract with the level of binding to a standardized solid-phase bound allergen. Therefore antibodies that crossreact with other components in the extract will cause the assay to record higher values of allergen than are present. Swanson (80) reported that the sensitivity of the RAST inhibition assay was highly dependent on the purity of the allergen source.

Swanson (78) found poor correlations between RAST inhibition and monoclonal radioimmunoassays used to quantify Der p 1 and Der f 1 in the one sample. The RIA assays had 10-fold lower detection limits than the RAST inhibition assay and gave values of 30 times lower than the RAST assay, but had coefficients of variation that were twice those of the RAST inhibition assay. Renstrom (64) found that despite strong correlations between RAST inhibition and monoclonal ELISA assay for rat urinary allergen, levels from the RAST inhibition assay were several hundred-fold higher (median 316, range 7–2680). Such differences were largely found to be to the antibody sources used in each assay (63). It was demonstrated that the IgE pool used in the inhibition assay reacted with equal affinity to rat urinary allergen and dust from the rat rooms, despite there being a 700-fold difference in rat urinary allergen between the pure allergen and the rat room dust. Therefore a disadvantage of the RAST and other inhibition assays is the heterogeneity of the antibodies, however, one benefit from such antibodies is that they will bind allergens in a range of forms that may be found in an airborne sample and do not require an intact allergen molecule, which is the basis of some two-site monoclonal assays.

ELISA and RIA

ELISA is the most widely used assay for the detection of airborne allergens. RIAs have also been used for quantification of airborne allergens; however, they have been largely replaced by ELISA. For detection of aeroallergens, the allergen is eluted from the filter used in air or breathing zone samplers. ELISA has been used for the detection of airborne Bla g 1, Bla g 2, Can f 1, Der f 1, Der p 1, Der p 2, Fel d 1, Rat n 1, mouse urinary allergen, rat urinary allergen, and α -amylase inhibitor. These assays generally use two antibodies, one for capture of the allergenic protein and the second for detection of the captured allergen. Such capture assays are highly specific for intact allergenic proteins because the MAbs and PABs must recognize two epitopes on the one allergen molecule for detection. The use of a defined set of MAbs and standards enhances our ability to compare results from different studies. However, the ability of the assays to detect partially degraded allergens is likely to be limited, although such partially degraded allergenic proteins may still present themselves as allergen sources in the patient's environment.

ELISA assays are often not sensitive enough to detect the relatively low amounts of allergen associated with normal short-term personal exposure in both occupational and domestic environments. Recent improvements in assay sensitivity by the use of an amplifying step (44,45,62) has resulted in 10–100-fold improvements in the sensitivity of assays for the detection of rat α_{2u} -globulin and Fel d 1. It is likely that the use of this amplified ELISA and its extension to other allergens will provide a method for accurate measurement of the quantities of allergen associated with personal exposure to aeroallergens.

Conventional sandwich ELISAs used for the detection of Fel d 1 or Der p 1 (81,131) were not sensitive enough to detect consistently the quantities of allergen inhaled in 5–20 min under normal domestic situations (39,50). The use of amplified ELISAs for detection of inhaled allergens are currently being investigated in collaboration with Dr. Anne Renstrom (Institute of Working Life, Solna, Sweden). For use in an ELISA assay, the allergen is eluted from the adhesive material used as the capture surface in the nasal sampler in the same way it would be eluted from the filter of a breathing zone sampler.

Particle Immunostaining: A New Technique

An immunostaining technique for the direct staining of particles collected in the IRM nasal air sampler has also been developed and is similar to the techniques described by Tovey (83) for identifying particles containing mite allergens and more recent press-blotting methods described by Schumacher (132) and Takahashi (133).

Several variants of this assay have been published; all rely on binding the allergens eluting from a particle onto a permanently associated protein-binding membrane. This association between particle and eluted allergen is retained, so that when the bound allergens are visualized by immunostaining, each particle that is an allergen source is surrounded by a halo of immunostained allergen (Fig. 5).

In the first variant of this assay (40,50), the particles are captured on a nitrocellulose membrane that has previously been coated with a very thin layer of a porous adhesive agarose and backed by a humectant-containing layer. After collection of particles, and prior to immunostaining, the membrane is coated again with a second layer of agarose to hold the particles in association with their eluted allergen. In this system the membrane remains moist throughout the collection period and elution of allergens starts as soon as they are captured.

In a second variant, the particles are collected by impaction onto a transparent adhesive tape in the nasal sampling device. The particle-laden tape is removed from the sampler and pressed onto a protein binding membrane, sandwiching the collected particles between the tape and the protein-binding membrane. The adhesive tape forms a strong and permanent bond with the protein-binding membrane and particles are spatially fixed between the two layers. Laminated samples can be stored for at least 6 wk without deterioration. The first step in the immunostaining process is elution of the allergens, which takes place as soon as the tape-particle-membrane sandwich is placed in aqueous buffer. Following elution the assay is similar to other membrane-based immunoassays and involves blocking, primary antibody incubation, washing, secondary antibody (or conjugate) incubation, washing, and substrate development. The immunostaining process occurs through the protein binding membrane. We have optimized the protocols for immunostaining for a range of allergens using different antibodies and conjugates, including allergen-specific monoclonals, polyclonals, and human IgE or IgG. A range of fluorescent and enzyme conjugate systems and substrates may be used.

Once the immunostaining process is complete, samples can be examined under a microscope and the number and sizes of particles with allergenic halos can be enumerated. The density of immunostaining around each particle and the size of halos can be determined using image analysis. As described, the allergen eluting from each particle binds to the membrane in its immediate vicinity, which allows absolute certainty of the source of the allergen and also serves to concentrate the allergen around the particles, therefore enhancing the sensitivity of allergen detection.

Further variants of this general method have been described. With Burkard samplers, the usual silicone grease medium used to

trap airborne particles was replaced by the dry adhesive tape and all particles (both pollen grains and smaller carriers) that were sources of allergens were detected by association with their immunostained allergens (134). The method can also be used in a converse format (39,40) where particles are directly collected by filtration onto a dry protein-binding membrane (e.g., in an IOM sampler filter), which is subsequently sandwiched with adhesive tape; the sandwich is then assayed for allergens as described earlier.

We have found this technique to be extremely sensitive because it allows us to measure a single particle that is inhaled and carrying allergen (Fig. 5). The amount of allergen associated with such particles is likely to be in the order of sub picograms. This has allowed us to examine individual activities and a range of locations within homes to document more fully the nature of personal allergen exposure (39,50). We have been able to measure airborne mite (Der p 1 & Der p 2), cat (Fel d 1), cockroach (Bla g 1), dog (Can f 1), and *Alternaria* in undisturbed indoor environments (39,40,43,50). We have also examined the morphology and particle-size distribution of inhaled particles containing allergen in the absence of dust disturbance. Mite allergens are associated with a range of particle types including feces, fibers, and flakes that range in size from $<10\ \mu\text{m}$ to $>150\ \mu\text{m}$ (40). The particle-size distribution of particles inhaled that contain cat allergen is given in Fig. 2.

The repeatability of this technique in combination with nasal air sampling is high, with similar levels of particles eluting cat allergen recorded when the same activity is repeated in the same location a number of times and over a number of weeks (Fig. 6). There is a moderate-high and highly significant relationship between the total number of particles inhaled and the number of particles inhaled that are carrying a particular allergen from certain environments; however, this relationship is influenced by the proximity of the sampler to the allergen source and the level of dust disturbance. For a range of domestic activities performed in a single home in close proximity to a cat, or wearing a sweater that contains cat allergen, the correlation is strong ($r^2 = 0.74$, $p < 0.0001$); however, it is apparent that in a low cat-allergen environment, no such correlation exists ($r^2 = 0.46$, $p = 0.21$).

Rapid Chromatographic and Dipstick Methods

The increasing demand for cheap, simple, and rapid diagnostic assays has stimulated the development of a wide range of assays for both antigens and antibodies that function along or through a strip of porous membrane. A common example is a pregnancy test available in many supermarkets, but assays for a wide range of diseases (malaria, HIV, tuberculosis) are being transformed to this format. These usually provide a result above or below a threshold value

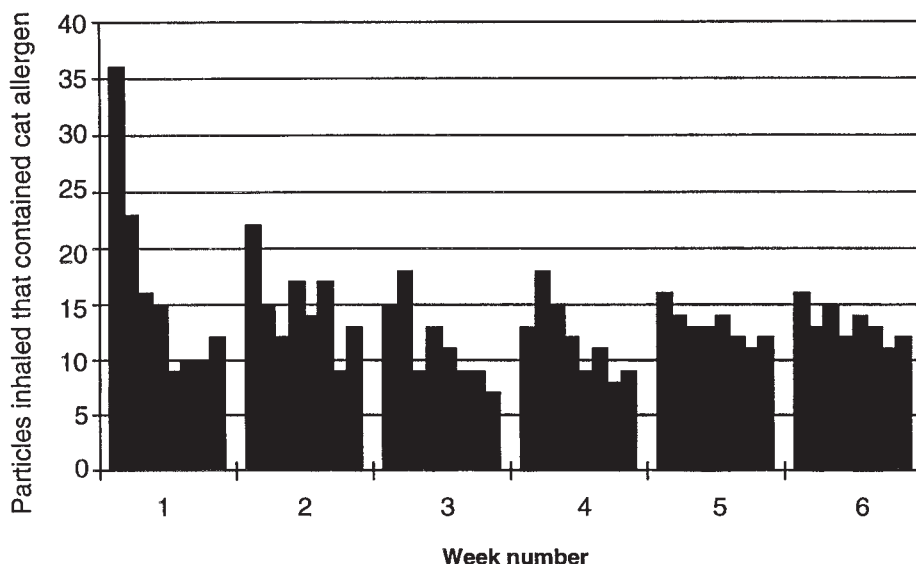


Fig. 6. Repeatability of inhaled allergen sampling using the IRM nasal air sampler. Allergen was collected for eight consecutive, 10-min sampling periods on one day, which was repeated weekly for 6 wk. The sampling activity involved lying on a bed with the cat present in the room. Sample number one in wk 1 was collected under a higher level of disturbance than the other samples and had significantly more cat particles and total number of dust particles. The mean number of particles inhaled containing cat allergen (excluding sample 1, wk 1) was 13.0, the standard deviation was 3.38, and the coefficient of variation was 26%.

although more quantitative determinations can be made based on staining intensity or area. At least two “dipstick” assays that measure allergens have appeared on the market (135), and a more rapid chromatographic assay (136) that provides semiquantitative data within minutes is being proto-typed. All these are only semiquantitative and require dust to be collected with a vacuum cleaner.

Nonimmunoassay

Genetic Probes

Genetic techniques have the potential to provide specific identification of biological particles containing a genome such as pollens and fungi, which are recognized as allergen sources by other methods. Although this technique for the identification of allergen sources is in its infancy, it has a number of advantages. It potentially could be used where it is difficult to make identifications because

the particles are of similar morphology; for example, distinction between fungi of the genera *Aspergillus* and *Penicillium* or between individual species of either of these genera. Although such fungi could potentially be distinguished using specific antibodies, this requires access to the antibodies and the surety that they are neither crossreactive or that common epitopes are not present. Immuno-identification of fungi also is dogged by their inconsistent expression soluble antigens. A third circumstances where genetic systems may be useful is where fungi have lost their viability and so cannot be cultured, but will have retained their genetic material. This is not uncommon for aerospora.

Oligonucleotide probes capable of differentiating between airborne fungi of the genera—*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*—have been developed (137). These probes have been designed from the more variable internal transcribed spacer regions of the ribosomal DNA gene repeat unit. When PCR amplification of these regions with universal primers followed by oligonucleotide probe hybridization is performed, highly specific identification can be achieved. Oligonucleotide probe hybridization following PCR has been used successfully to identify a range of other clinically important fungi (138).

Pollen and Spore Counts

Morphological identification of pollen grains and fungal spores and manual counting under a microscope has been used extensively by aerobiologists for the measurement of airborne exposure to pollens and fungi. There is some evidence that counting of pollen grains may not always give an accurate indication of the level of plant-derived airborne allergens in environments (139,140). Similarly, there is some doubt as to whether fungal spore counts are an accurate representation of the fungal allergenic content of the air (120). The major factors that seems to determine the validity of pollen or spore counts is the amount of allergen carried on particles that are not pollen grains or fungal spores, such as diesel particles, starch granules, and other pauci- and sub-micronic particles. Some studies have suggested that more than half of the airborne allergen is not associated with intact pollen grains or spores, but is carried on pauci- or sub-micronic particles that may readily enter the lung (120,133,139,140). Pollen grains and fungal spores can also contain varying amounts of allergen (132–134), which cannot be assessed by morphological techniques. The development of immunological techniques (132–134), such as particle immunostaining (see section on “Particle Immunostaining: A New Technique”) for the identification of all particles carrying pollen and fungal allergens may be the future direction in measuring exposure to these allergens.

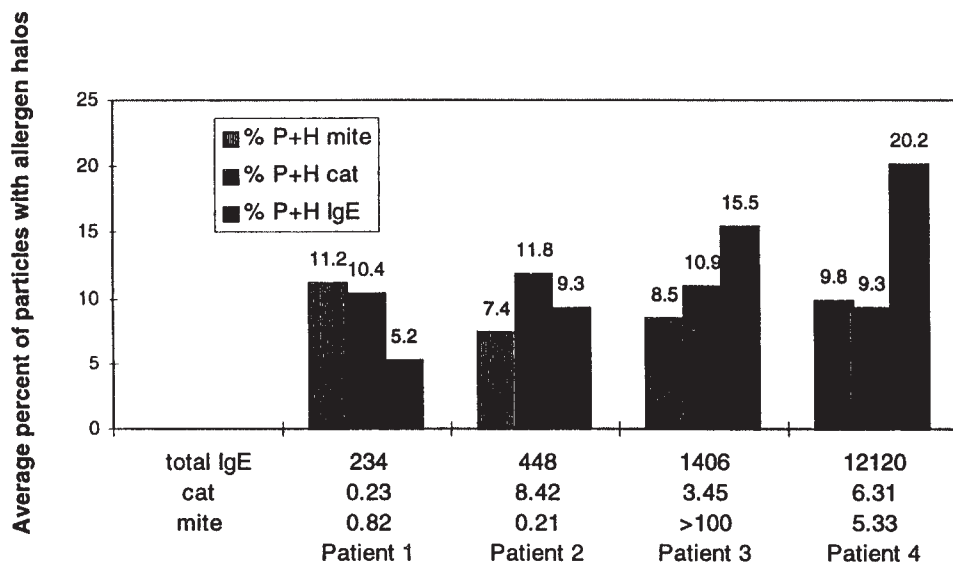


Fig. 7. The percent of all particles inhaled from a given environment carrying allergen (% P + H, is percent of particles inhaled with allergen halos) as determined by specific MAb staining (mite or cat) or by staining using the patient's IgE. Total IgE and specific titers to cat and mite in each of the four patients are given.

Specific Allergen vs Total Allergen Load

In assessing personal allergen exposure, consideration should be given to the source of antibody probe to be used. If allergen exposure is to be related to symptoms it is important to measure all allergens the individual is exposed to in a given environment and not restrict measurement to a specific allergen.

In order to examine the relationship between personal allergen exposure and symptoms of allergic disease in individuals, it may be necessary to examine the entire spectrum of allergens in a patient's environment, rather than the level of a single allergen, to which the individual is sensitized. Many studies have examined the concentration of a single allergen in an environment, this measurement may greatly underestimate the allergen challenge an individual with multiple atopies would face in this environment. For example, in an environment high in cat, dog, cockroach, and mite allergen, measuring the concentration of a single allergen is unlikely to give a reflection of the total allergen load to which an individual who is allergic to all four allergens is exposed. Using nasal air samplers, we have commenced experiments to monitor total allergen load, i.e., all allergens in the patient's environment to which they are allergic. Taking nasal samples from the same

environment, we examined the differences in allergen load as determined by staining inhaled particles with either the patient's IgE, mite, or cat-specific MAbs. It was found that an individual patient's total IgE reacts with vastly different number of particles inhaled compared with the number of particles containing a specific allergen. Figure 7 illustrates the results for four patients with a range of concentrations of total IgE, mite, and cat-specific IgE. A similar percentage of particles carrying cat and mite allergens were detected on each nasal sample, which indicated a similar specific allergen exposure. However, Patient 1 only reacted to 5.2% of the particles inhaled, whereas patient 4 reacted to 20.2%. We have found that highly atopic individuals may react to 20–50% of the particles they inhale from their environment, whereas a single allergen, such as cat or mite, is unlikely to account for 10% of the particles in their environment. Thus, in such environments, measurement of a single allergen may underestimate the total allergen load by two to fivefold.

Future Directions

More than ever before there is a public interest in assessing environmental exposure to airborne allergens. This heightened interest is the result of an increase in the prevalence of allergic diseases in our community, which is now around 20–30% for asthma in children, and an increase in the understanding of the likely role that inhalant allergens play in such allergic diseases. Airborne allergens have a recognized role in the aetiology of asthma and maintenance of airway hyperreactivity. There is an increasing emphasis on the role of allergen avoidance in the management of asthma. A number of large-scale allergen avoidance trials are under way throughout the US, UK, and Australia. If these trials demonstrate the efficacy of allergen avoidance for primary reduction of allergy and allergic disease, then it is likely that the community's interest in domestic allergen exposure will be expanded further. It is also recognized that for immunotherapy to be effective it must be targeted at the clinically relevant allergens.

Despite the acknowledged importance of allergen exposure, for both the public and physicians it is difficult to determine the identity and the level of exposure to allergens. Indeed, it seems counter-intuitive that physicians should recommend, and patients should accept advice to practice allergen avoidance based on a skin test or RAST test. Such avoidance practices are not based on knowledge of exposure levels, whether methods to reduce exposure have been effective, what are the dominant allergens, or where in the subject's environment most exposure is occurring. Although their impact has not been adequately tested, again it would seem intuitive that there were massive demand

for methods to determine the identity and level of allergens in an environment that were relevant to the individual patient.

Over the past five years there have been a number of improvements in the methods available to researchers for monitoring personal exposure to aeroallergens. The development of amplified ELISAs has permitted the quantification of the levels of allergen that are normally associated with short-term personal exposure under natural conditions. Particle immunostaining also offers high sensitivity and can be used to examine the size, shape, and morphology of particles carrying allergens. The sources of individual particles carrying allergen in a patient's environment can be determined and therefore the allergens identified.

Collection systems for allergens have also undergone a number of changes over the past 20 years. Reservoir measurements continue to be widely used and their utility for establishing the relationship between sensitization and exposure across communities has been of fundamental importance to our understanding of asthma. Measurement of airborne allergens has advanced from the use of high volume static samplers to personal samplers worn by the individual, which attempt to collect true personal exposure. Personal samplers include a number of breathing zone samplers and more recently the nasal air sampler, which may be the most accurate method for obtaining a true index of personal exposure.

These new developments in sampling and assay technology have resulted in the development of another set of tools for researchers to explore the relationships between allergen exposure and sensitization, symptoms, and remission of allergic diseases. This is likely to result in some fine tuning of our understanding of the role of allergens in allergic diseases. Through the accurate measurement of personal allergen levels, exposure profiles can be determined and can be used to assess the differences in clinical outcomes between, for example, chronic low-level exposure and periodic high-level exposure.

It is apparent that the next challenge for monitoring personal allergen exposure is the development of technologies for the measurement of personal allergen exposure in the wider community. The properties of such technologies could be predicted to provide a system that is safe and simple to use; inexpensive; allows sampling of multiple sites; measures levels in a way that is relevant to personal exposure; is easily accessible; is able to measure the major allergens recognized to be associated with symptoms, or is able to better measure allergens that are relevant to the patient's symptoms. The development of such technologies will provide patients and physicians with clear indications of the most important allergens in a patient's environment and the most effective strategies for reducing these allergens.

Acknowledgments

The authors would like to thank the staff of the Allergen Diagnostic Project for their contributions to this review. Mr. David Taylor performed the nasal sampler repeatability experiments and, with assistance from Ms. Sandra De Lucca and Ms. Leanne Poulos, examined the relationship between total allergen load and specific allergen levels.

References

1. Barnetson R, Wright A, Benton, E. (1989), IgE mediated allergy in adults with severe atopic eczema. *Clin Exp Allergy* 19:321–325.
2. Droste J, Kerkhof M, Demonchy J, Schouter JP, Rijcken B. (1996), Association of skin test reactivity, specific IgE, total IgE and eosinophils with nasal symptoms in a community-based population study. *J Allergy Clin Immunol* 97:922–932.
3. Platts-Mills T, Vervloet D, Thomas W, Aalberse R, Chapman M. (1997), Indoor allergens and asthma: report of the third International workshop. *J Allergy Clin Immunol* 100:s1–s24.
4. Wahn U, Lau S, Bergmann R, Kulig M. (1997), Indoor allergen exposure is a risk factor for sensitisation during the first three years of life. *J Allergy Clin Immunol* 99:763–769.
5. Sporik R, Chapman M, Platts-Mills T. (1992), House dust mite exposure as a cause of asthma. *Clin Exp Allergy* 22:897–906.
6. Bollinger M, Eggleston P, Flanagan E, Wood R. (1996), Cat antigen in homes with and without cats may induce allergic symptoms. *J Allergy Clin Immunol* 97:907–914.
7. Sicherer S, Wood R, Eggleston P. (1997), Determinants of airway responses to cat allergen: comparison of environmental challenge to quantitative nasal and bronchial allergen challenge. *J Allergy Clin Immunol* 99:798–805.
8. Van Metre T, Marsh D, Adkinson N, Fish J, Kagey-Sobotka A, Norman P, Radden E, Rosenberg G. (1986), Dose of cat (*Felis domesticus*) allergen 1 (Fel d 1) that induces asthma. *J Allergy Clin Immunol* 78:62–75.
9. van der Heide S, De Monchy J, De Vries K, Dubois A, Kauffman H. (1997), Seasonal differences in airway hyperresponsiveness in asthmatic patients: relationship with allergen exposure and sensitization to house dust mites. *Clin Exp Allergy* 27:627–633.
10. Altounyan REC. (1970), Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In: *Disodium Chromoglycate in Allergic Airways Disease*, Pepys J, Frankland AW, eds. Butterworths, London, pp. 43–53.
11. Andrae S, Axelson O, Bjorksten B, Fredriksson M, Kjellman N-IM. (1988), Symptoms of bronchial hyperreactivity and asthma in relation to environmental factors. *Arch Dis Childhood* 63:473–478.
12. Peat J, Tovey E, Toelle B, Haby M, Gray E, Mahmic A, Woolcock A. (1996), House dust mite allergens: a major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 152:141–146.
13. Custovic A, Simpson A, Chapman M, Woodcock A. (1998), Allergen avoidance in the treatment of asthma and atopic disorders. *Thorax* 53:63–72.
14. Platts-Mills T, Woodfolk J, Chapman M, Heymann P. (1996), Changing concepts of allergic disease: The attempt to keep up with real changes in lifestyles. *J Allergy Clin Immunol* 98:S297–306.
15. Chan-Yeung M. (1988), Occupational asthma update. *Chest* 98:407–411.

16. Knox RB. (1993), Grass pollen, thunderstorms and asthma. *Clin Exp Allergy* 23:354–359.
17. Platts-Mills TE. (1995), (Editorial) Is there a dose-response relationship between exposure to indoor allergens and symptoms of asthma? *J Allergy Clin Immunol* 96:435–440.
18. Pope A, Patterson R, Burge H. (1993), *Indoor Allergens: Assessing and Controlling Adverse Health Effects*. National Academic Press, Washington, DC.
19. Peat J, van den Berg R, Green W, Mellis C, Leeder S, Woolcock A. (1994), Changing prevalence of asthma in Australian children. *Br Med J* 308: 1591–1596.
20. Peat J, Tovey E, Gray E, Mellis C, Woolcock A. (1994), Asthma severity and morbidity in a population sample of Sydney school children: Part 2—Importance of house-dust mite allergens. *Aust NZ J Med* 24:270–275.
21. Galant S, Berger W, Gillman S, Goldsobel Aea. (1998), Prevalence of sensitization to aeroallergens in California patients with respiratory allergy. *Ann Asthma Allergy Immunol* 81:203–210.
22. Tovey E, Marks G. (1999), Methods and effectiveness of environmental control. *J Allergy Clin Immunol* 103:179–191.
23. Platts-Mills T, Tovey E, Mitchell E, Moszoro H, Nock P, Wilkins S. (1982), Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 25 September:675–678.
24. Boner AL, Peroni DG, Piacentini GL, Venge P. (1993), Influence of allergen avoidance at high altitude on serum markers of eosinophil activation in children with allergic asthma. *Clin Exp Allergy* 23:1021–1026.
25. Hammarquist C, Burr M, Gotzsche P. (1988), House dust mite control measures in the management of asthma (Cochrane Review). in *The Cochrane Library*. Update Software, Oxford, UK.
26. Walshaw M, Evans C. (1986), Allergen avoidance in house dust mite sensitive adult asthma. *Q J Med* 58:199–215.
27. Carswell F, Birmingham K, Oliver J, Crewes A, Weeks J. (1996), The respiratory effects of reduction of mite allergen in the bedrooms of asthmatic children—a double-blind controlled trial. *Clin Exp Allergy* 26:386–396.
28. British Thoracic Society, NAC, Royal College of Physicians of London, et al. (1997), *The British Guidelines on asthma management:1995 review and position statement*. *Thorax* 52:s1–s21.
29. Aas et al. (1997), *European Allergy White Paper: Allergic Diseases as a Public Health Problem*. Van Moerbeke D, ed. UCB Institute of Allergy Braine-L'Alleud, Belgium.
30. AAAAI. (1998), *Position statement: Environmental allergen avoidance in allergic asthma*. American Academy of Allergy Asthma and Immunology.
31. Task G. (1966), Deposition and retention models for internal dosimetry of the human respiratory tract. *Health Physics* 12:173–208.
32. Swartengren M, Falk R, Linnman L, Philipson K, Camner P. (1987), Deposition of large particles in the human lung. *Exp Lung Res* 12:75–88.
33. Price J, Pollock I, Little S, Longbottom J, Warner J. (1990), Measurement of airborne mite allergen in homes of asthmatic children. *Lancet* 336:895–897.
34. Sakaguchi M, Inouye S, Sasaki R, Hashimoto M, Kobayashi C, Yaseuda H. (1996), Measurement of airborne mite allergen exposure in individual subjects. *J Allergy Clin Immunol* 97:1040–1044.
35. Sakaguchi M, Inouye S, Yasueda H, Shida T. (1992), Concentrations of airborne mite allergens (*Der I* and *Der II*) during sleep. *Allergy* 47:55–57.
36. Oliver J, Birmingham K, Crewes A, Weeks J, Carswell F. (1995), Allergen levels in airborne and surface dust. *International Archives of Allergy and Immunology* 107:452,453.

37. Platts-Mills T, Heymann P, Longbottom J, Wilkins S. (1986), Airborne allergens associated with asthma: Particles sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 77:850-857.
38. Sakaguchi M, Inouye S, Yaseuda H, Irie T, Yoshizawa S, Shida T. (1989), Measurement of allergens associated with dust mite allergy 2. Concentrations of airborne mite allergens (Der 1 and der 2 in the house. *Internat Arch Allergy Appl Immunol* 90:190-193.
39. Poulos L, De Lucca S, O'Meara T, Graham J, Sporik R, Tovey E. (1998), Detection of inhaled Der p1 and Fel d 1. *J Allergy Clin Immunol* 101:S158.
40. De Lucca S, O'Meara T, Graham J, Sporik R, Tovey E. (1998), Mite allergen (Der p 1) is not only carried on feces. *J Allergy Clin Immunol* 101:S168.
41. Custovic A, Green R, Taggart S, Smith A, Pickering M, Chapman M, Woodcock A. (1996), Domestic allergens in public places 2: dog (Can f 1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings. *Clin Exp Allergy* 26:1246-1252.
42. de Blay F, Snachez J, Hedelin G, Perez-Infante A, Verot A, Chapman M, Pauli G. (1997), Dust and airborne exposure to allergens derived from cockroach (*Blattella germanica*) in low-cost public housing in Strasbourg (France). *J Allergy Clin Immunol* 99:107-112.
43. Tovey E, Graham J, O'Meara T, Widmer F, Lovborg U, Jones A, Sporik R. (1998), A new in vitro diagnostic method. *J Allergy Clin Immunol* 101:S129.
44. Larsson P, Malmberg P, Wickman M. (1996), Assessment of airborne Fel d 1 concentrations using an amplified ELISA [Abstract]. *J Allergy Clin Immunol*. 97:421.
45. Bollinger M, Wood R, Chen P, Eggleston P. (1998), Measurement of cat allergen levels in the home by use of an amplified ELISA. *J Allergy Clin Immunol* 101:124, 125.
46. Ahlf E, Elfman L, Svensson M, Nybom R, Elfman L-M. (1997), A new method for collecting airborne allergens. *Allergy* 52 (Suppl 37):53.
47. Custovic A, Green R, Fletcher A, Smith A, Anthony C, Pickering C, Chapman M, Woodcock A. (1997), 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* 155:94-98.
48. Wood A, Laheri A, Eggleston P. (1993), The aerodynamic characteristics of cat allergen. *Clin Exp Allergy* 23:733-739.
49. Custovic A, Simpson A, Pahdi H, Green RM, Chapman MD, Woodcock A. (1998), Distribution, aerodynamic characteristics, and removal of the major cat allergen Fel d 1 in British homes. *Thorax* 53:33-38.
50. O'Meara T, De Lucca S, Sporik R, Graham A, Tovey E. (1998), Detection of inhaled cat allergen. *Lancet* 351:1488,1489.
51. Ormstad H, Namork E, Gaarder P, Johansen B. (1995), Scanning electron microscopy of immunogold labeled cat allergens (Fel d 1) on the surface of airborne house dust samples. *J Immunol Methods* 187:245-251.
52. Ormstad H, Johansen B, Gaarder P. (1998), Airborne house dust particles and diesel exhaust particles as allergen carriers. *Clin Exp Allergy* 28:702-708.
53. Sakaguchi M, Inouye S, Irie T, Miyazawa H, Watanabe M, Yasueda H, Shida T, Nitta H, Chapman MD, Schou C, Aalberse RC. (1993), Airborne cat (Fel d 1), dog (Can f 1), and mite (der 1 and Der 2) allergen levels in the homes of Japan. *J Allergy Clin Immunol* 92:797-802.
54. Custovic A, Fletcher A, Pickering A, Francis H, Green R, Smith A, Chapman M, Woodcock A. (1998), Domestic allergens in public places 3: house dust mite, cat, dog and cockroach allergens in British hospitals. *Clin Exper Allergy* 28:53-59.
55. Levetin E, Shaughnessy R, Fisher E, Ligman B, Harrison J, Brennan T. (1995), Indoor air quality in schools: exposure to fungal allergens. *Aerobiologia* 11:27-34.

56. Consentino S, Palmas F. (1996), Occurrence of fungal spores in the respiratory tract and homes of patients with positive skin test to fungi. *Aerobiologia* 12: 155–160.
57. Garrett M, Hooper B, Cole F, Hooper M. (1997), Airborne fungal spores in 80 homes in the La Trobe Valley, Australia: levels, seasonality and indoor-outdoor relationships. *Aerobiologia* 13:121–126.
58. Menzies D, Comtois P, Pasztor J, Nunes F, Hanley J. (1998), Aeroallergens and work-related respiratory symptoms among office workers. *J Allergy Clin Immunol* 101:38–44.
59. Eggleston P, Ansari A, Ziemann B, Adkinson N, Corn M. (1990), Occupational challenge studies with laboratory workers allergic to rats. *J Allergy Clin Immunol* 86:63–72.
60. Gordon S, Tee R, Nieuwenhuijsen M, Lowson D, Harris J, Newman TA. (1994), Measurement of airborne rat urinary allergen in an epidemiological study. *Clin Exper Allergy* 24:1070–1077.
61. Hollander A, Van Run P, Spithoven J, Heederik D, Doekes G. (1997), Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. *Clin Exper Immunol* 27:617–626.
62. Renstrom A, Larsson P, Malmberg P, Bayard C. (1997), A new amplified monoclonal rat allergen assay used for evaluation of ventilation improvements in animal rooms. *J Allergy Clin Immunol* 100:649–655.
63. Renstrom A. (1997), Allergy to laboratory animals: risk factors for the development of allergy and methods for measuring airborne rodent allergens. PhD Thesis, National Institute for Working Life, Sweden.
64. Renstrom A, Gordon S, Larsson P, Tee R, Newman TA, Malmberg P. (1997), Comparison of a radioallergosorbent (RAST) inhibition method and a monoclonal enzyme linked immunosorbent assay (ELISA) for allergen measurement. *Clin Exp Allergy* 27:1314–1321.
65. Swanson M, Campbell A, O'Hallaren M, Reed C. (1990), Role of ventilation, air filtration, and allergen production rate in determining concentrations of rat allergens in the air of animal quarters. *Am Rev Respir Diseases* 141:1578–1581.
66. Yli-Panula E, Rantio-Lehtimäki A. (1995), Birch-pollen antigenic activity of settled dust in rural and urban homes. *Allergy* 50:303–307.
67. Pollart S, Reid M, Fling J, Chapman M, Platts-Mills T. (1988), Epidemiology of emergency room asthma in northern California: Association with IgE antibody to ryegrass pollen. *J Allergy Clin Immunol* 82:224–230.
68. Platts-Mills T, Hayden M, Chapman M, Wilkins S. (1987), Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. *J Allergy Clin Immunol* 79:781–791.
69. Sterling D, Lewis R. (1998), Pollen and fungal spores indoor and outdoor of mobile homes. *Ann Allergy Asth Immunol* 80:279–285.
70. Blackley CH. (1988), *Experimental Research in the Causes and Nature of Catarrhus aestivus* (Hay Fever or Hay Asthma). Dawson Publishing Co., London. Reprinted from Bailliere Tindall and Cox, Oxford Historical Books, 1873.
71. Sandiford C, Nieuwenhuijsen M, Tee R, Newman TA. (1994), Measurement of airborne proteins involved in Baker's asthma. *Clin Exper Allergy* 24:450–456.
72. Houba R, van Run P, Deederik D, Doekes G. (1996), Wheat antigen exposure assessment for epidemiological studies in bakeries using personal dust sampling and inhibition ELISA. *Clin Exp Allergy* 26:154–163.
73. Houba R, Heederik D, Doekes G, van Run P. (1996), Exposure-sensitization relationships for α -amylase allergens in the baking industry. *Am J Respir Crit Care Med* 154:130–136.
74. Houba R, van Run P, Doekes G, Heederik D, Spithoven J. (1997), Airborne levels of α -amylase allergens in bakeries. *J Allergy Clin Immunol* 99:286–292.

75. Wiley K, Smith M, Allan L, Griffin P. (1997), Measurement of airborne flour exposure with a monoclonal-based immunoassay. *International Archives of Allergy and Immunology* 114:278–284.
76. Swanson M, Bubak M, Hunt L, Yunginger J, Warner M, Reed C. (1994), Quantification of occupational latex aeroallergens in a medical center. *J Allergy Clin Immunol* 94:445–451.
77. Baur X, Zhiping C, Allmers H. (1998), Can a threshold limit value for natural rubber latex airborne allergens be defined? *J Allergy Clin Immunol* 101:24–27.
78. Swanson M, Campbell A, Klauck M, Reed C. (1989), Correlations between levels of mite and cat allergens in settled and airborne dust. *J Allergy Clin Immunol* 83:776–783.
79. Woodfolk J, Luczynska C, de-Blay F, Chapman M, Platts-Mills T. (1993), The effect of vacuum cleaners on the concentration and particle size distribution of airborne cat allergen. *J Allergy Clin Immunol* 91:829–837.
80. Swanson M, Agarwal M, Reed C. (1985), An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: Studies with mite, roach, cat, mouse and guinea pig antigens. *J Allergy Clin Immunol* 76:724–729.
81. Luczynska C, Li Y, Chapman M, Platts-Mills T (1990), Airborne concentrations and particle size distributions of allergen derived from domestic cats (*Felis domesticus*): Measurements using cascade impactor, liquid impinger and a two-site monoclonal antibody assay for Fel d 1. *Am Rev Respir Diseases* 141:361–367.
82. Platts-Mills T, Thomas W, Aalberse R, Vervloet D, Champman M. (1992), Dust mite allergens and asthma: report of a second international workshop. *J Allergy Clin Immunol* 89:1046–1060.
83. Tovey E, Chapman M, Platts-Mills T. (1981), Mite faeces are a major source of house dust mite allergens. *Nature* 289:592,593.
84. Woodfolk J, Hayden M, Ciutire N, Platts-Mills T. (1995), Chemical treatment of carpets to reduce allergen: Comparison of the effects of tannic acid and other treatments on proteins derived from dust mites and cats. *J Allergy Clin Immunol* 96:325–333.
85. de Blay F, Heymann P, Chapman M, Platts-Mills TAE. (1991), Airborne dust mite allergens: comparison of group 2 allergens with group 1 mite allergen and cat-allergen Fel d 1. *J Allergy Clin Immunol* 88:919–926.
86. de Blay F, Chapman M, Platts-Mills T. (1991), Airborne cat allergen (Fel d 1): Environmental control with the cat in situ. *Am Rev Respir Dis* 143:1334–1339.
87. Wentz P, Swanson M, Reed C. (1990), Variability of cat allergen shedding. *J Allergy Clin Immunol* 85:94–98.
88. Jalil-Colome J, de Andrade DA, Birnbaum J, Casanova D, Mege J-L, Lanteaume A, Charpin D, Vervolet D. (1996), Sex differences in Fel d 1 allergen production. *J Allergy Clin Immunol* 98:165–168.
89. Enberg R, Shamie S, McCullough J, Ownby D. (1993), Ubiquitous presence of cat allergen in cat-free buildings: probable dispersal from human clothing. *Ann Allergy* 70:471–474.
90. Tovey E, Mahmic A, McDonald L. (1995), Clothing—an important source of mite allergen exposure. *J Allergy Clin Immunol* 96:999–1001.
91. D' Amato G, Liccardi G, Russo M, Barber D, D'Amato M, Carreira J. (1997), Clothing is a carrier of cat allergen. *J Allergy Clin Immunol* 99:577,578.
92. Ohman J, Hagberg K, MacDonald M, Jones R, Paigen B, Kacergis J. (1994), Distribution of airborne mouse allergen in a major mouse breeding facility. *J Allergy Clin Immunol* 94:810–817.
93. Tovey E, Chapman M, Platts-Mills T. (1981), The distribution of house dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 124:630–635.

94. Carswell F, Clark J, Robinson P, Platts-Mills TAE. (1983), The dose of house dust mite antigen (P1) inhaled by infants aged one month. *Ann Allergy* 51:539–542.
95. Silverman L, Lee G, Plotkin T, Sawyers L, Yancey A. (1951), Air flow measurements on human subjects with and without respiratory resistance at several work rates. *AMA Arch Ind Hyg Occupational Med* 3:461–476.
96. Shamoo D, Linn W, Peng R, Solomon J, Webb T, Hackney J, Gong H. (1994), Time-activity patterns and diurnal variation of respiratory status in a panel of asthmatics: implications for short-term air pollution effects. *J Expos Anal Environ Epidemiol* 4:133–148.
97. Shamoo D, Johnson T, Trim S, Little D, Linn W, Hackney J. (1991), Activity patterns in a panel of outdoor workers exposed to oxidant pollution. *J Expos Anal Environ Epidemiol* 1:423–428.
98. Mark D, Vincent J. (1986), A new personal sampler for airborne total dust in work places. *Ann Occup Hyg* 30:89–102.
99. Lafortuna C, Minetti A, Mognoni P. (1984), Inspiratory flow pattern in humans. *Journal of Physiology* 57:1111–1119.
100. Niinimaa V, Cole P, Mintz S, Shephard R. (1981), Oronasal distribution of respiratory flow. *Respir Physiol* 43:69–75.
101. Chadha T, Birch S, Sackner M. (1987), Oronasal distribution of ventilation during exercise in normal subjects and patients with asthma and rhinitis. *Chest* 92:1037–1041.
102. Rodenstein D, Stanescu D. (1984), Soft palate and oronasal breathing in humans. *J Appl Physiol* 57:651–657.
103. Perez W, Tobin M. (1985), Separation factors responsible for changes in breathing pattern induced by instrumentation. *J Appl Physiol* 59:1515–1520.
104. Mennella J, Beauchamp G. (1992), Developmental changes in nasal airflow patterns. *Acta Otolaryngol (Stockh)* 112:1025–1031.
105. Vincent J. (1994), Measurement of fine aerosols in workplaces a review. *Analyst* 119:19–25.
106. Heyder J. (1982), Particle transport onto human airway surfaces. *Eur J Respir Disease* 119:29–50.
107. Stuart B. (1973), Deposition of Inhaled aerosols. *Arch Intern Med* 131:60–73.
108. Brian J, Valberg P. (1979), Deposition of aerosol in the respiratory tract. *Am Rev of Respir Disease* 120:1325.
109. Heyder J, Gebhart J, Rudolf G, Schiller C, Stahlhofen W. (1986), Deposition of particles in the human respiratory tract in the size range 0.005–15 mm. *J Aerosol Sci* 17:811–825.
110. Kim C, Kang T. (1997), Comparative measurement of lung deposition of inhaled fine particles in normal subjects and patients with obstructive airway disease. *Am J Respir Crit Care Med* 155:899–905.
111. Phillipson E, Bowes G. (1986), Control of breathing during sleep. In: *Handbook of Physiology: Section 3: The Respiratory System*. American Physiological Society, Baltimore, MD, pp. 649–660.
112. Ferguson P, Broide D. (1995), Environmental and bronchoalveolar lavage Dermatophagoides pteronyssinus antigen levels in atopic asthmatics. *Am J Respir Crit Care Med* 151:71–74.
113. Berge M, Munir AK, Deborg S. (1998), Concentrations of cat (Fel d 1), dog (Can f 1) and mite (Der f 1 and Der p 1) allergens in the clothing and school environment of Swedish schoolchildren with and without pets. *Pediatr Allergy Immunol* 9:25–30.
114. Marks GB, Tovey ER, Peat JK, Salome C, Woolcock AJ. (1995), Variability and repeatability of house dust mite allergen measurement: implications for study design and interpretation. *Clin Exp Allergy* 25:1190–1197.

115. Simpson A, Hassall R, Custovic A, Woodcock A. (1998), Variability of house dust mite allergen levels within carpets. *Allergy* 53:602–607.
116. Sporik R, Hill D, Thompson P, Stewart G, Carlin J, Nolan T, Kemp A, Hosking C. (1998), The Melbourne House Dust Mite study: long term efficacy of house dust mite reduction strategies. *J Allergy Clin Immunol* 101:451–456.
117. Siebers R, Luey B, Crane J, Fitzharris P. (1997), The effects of temperature and buffer on the extraction of Der p 1 from dust (let). *J All Clin Immunol* 100:580.
118. Leuschner R, Boehm G. (1979), Investigations with the 'individual pollen collector' and the 'Burkard trap' with reference to hay fever patients. *Clin Allergy* 9:175–184.
119. Emberlin J. (1995), Plant allergens on pauci-micronic airborne particles. *Clin Exper Allergy* 25:202–205.
120. Reed C, Swanson M, Yunginger J. (1986), Measurement of allergen concentration in the air as an aid in controlling exposure to aeroallergens. *J Allergy Clin Immunol* 78:1028–1030.
121. Baron P. (1998), Personal aerosol sampler design: a review. *Appl Occupat Environ Hyg* 13:313–320.
122. Bartley D. (1998), Inhalable aerosol samplers. *Appl Occupat Environ Hyg* 13:274–278.
123. Sercombe J, Pavlicek P, Xavier M, Lea S, Graham J, Tovey E. (1998), Assessment of nasal and pocket air samplers. *J Allergy Clin Immunol* 101:S80.
124. Tovey E, Marks G, Matthews M, Green W, Woolcock A. (1992), Changes in mite allergen Der p I in house dust following spraying with a tannic acid/acaricide solution. *Clin Exp Allergy* 22:67–74.
125. Maunsell K. (1952), Air-borne fungal spores before and after raising dust (sampling by sedimentation). *International Archives of Allergy* 3:93–102.
126. Smith A, Custovic A, Green R, Woodcock A. (1996), Correlation between levels of major cat allergen Fel d1 in reservoir and settling dusts in homes with and without cats. *Thorax* 51 (Suppl 3):A42.
127. Price J, Longbottom J. (1988), ELISA method for measurement of airborne levels of major laboratory animal allergens. *Clin Allergy* 18:95–107.
128. Tovey E, Vandenberg R. (1979), Mite allergen content in commercial extracts and in bed dust determined by radioallergosorbent tests. *J Clin Allergy* 9:253–262.
129. Eggleston P, Newill C, Ansari A, Pustelnik A, Lou S-R, Evans R, Marsh D, Longbottom J, Corn M. (1989), Task-related variation in airborne concentrations of laboratory animal allergens: Studies with Rat n1. *J Allergy Clin Immunol* 84:347–352.
130. Eggleston P, Ansari A, Adkinson N, Wood R. (1995), Environmental challenge studies in laboratory animal allergy: Effect of different airborne allergen concentrations. *Am J Respir Crit Care Med* 151:640–646.
131. Luczynska C, Arruda L, Platta-Mills T, Miller J, Lopez M, Chapman M. (1989), A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p1 and Der f1. *J Immunol Meth* 118:227–235.
132. Schumacher M, Griffith R, O'Rourke M. (1988), Recognition of pollen and other particulate aeroantigens by immunoblot microscopy. *J Allergy Clin Immunol* 82:608–616.
133. Takahashi Y, Nagoya T, Watanabe M, Inouye S, Sakaguchi M, Katagiri S. (1993), A new method of counting airborne Japanese cedar (*Cryptomeria japonica*) pollen allergens by immunoblotting. *Allergy* 48:94–98.
134. O'Meara T, Razmovski T, Hjelmroos-Koski M, Marks G, Tovey E. (1999), New techniques for capture and immunostaining of particles in Hirst type spore traps. *J Allergy Clin Immunol*.

135. Mistrello G, Gentili M, Roncarolo D, Antoniotti P, Ottoboni F, Falagiani P. (1998), Dot Immunobinding assay for detection of mite antigens in house-dust samples. *J Med Entomol* 35:143–147.
136. Chapman MD. (1998), Environmental allergen monitoring and control. In: 30 Years with IgE, von Hage-Hamstem M, Wickman M, eds. Munksgaard, Copenhagen, pp. 53–58.
137. Gaskell G, Carter D, Britton W, Tovey E, Benyon F, Lovborg U. (1997), Analysis of the internal transcribed spacer regions of ribosomal DNA in common airborne allergenic fungi. *Electrophoresis* 18:1567–1569.
138. Sandhu G, Kline B, Stockman L, Roberts G. (1995), Molecular probes for the diagnosis of fungal infections. *J Clin Microbiol* 33:2913–2919.
139. Stewart G, Holt P. (1985), Submicronic airborne allergens. *Med J Aust* 143:426,427.
140. Spieksma F, Kramps J, Van Der Linden A, Nikkels B, Plomp A, Koerten H, Dijkman J. (1990), Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction. *Clin Exp Allergy* 20:273–280.
141. Smedje G, Norback D, Edling C. (1997), Subjective indoor air quality in schools in relation to exposure. *Indoor Air* 7:143–150.