

Role of Ventilation, Air Filtration, and Allergen Production Rate in Determining Concentrations of Rat Allergens in the Air of Animal Quarters¹⁻³

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Introduction

In coping with occupational allergens, or any allergen for that matter, three strategies are available. Generation of allergen can be reduced or eliminated at its source. Ventilation can be increased to carry the allergen away. Finally, the allergen can be filtered out of the air, either with personal respirators or with general area filtration. In coping with laboratory animal allergy, the first option is obviously not available. Standard animal care procedures already specify a high ventilation rate, 15 changes of air per hour, and in cold northern winters much greater ventilation rates are not feasible, even with heat exchange ventilation systems. Therefore, we undertook a study of air filtration in controlling the level of rat urinary protein allergen suspended in rat quarters.

The steady-state concentration of any substance is determined by the ratio between the rate of production and the rate of removal (1). It can be predicted by the general mass balance equation

$$C_{ss} = \frac{PQ_v C_o + S}{E_v Q_v + K + NE_d Q_d}$$

where C_{ss} represents steady-state concentration; the numerator represents the rate of generation of allergen; and the denominator is the rate of removal. $PQ_v C_o$ is the outdoor source term; P is an empirically derived penetration factor, which for respirable particles can be assumed to be 1. Q_v equals ventilation rate; C_o is outdoor concentration. S is indoor source substance generation rate. It has two components, *de novo* generation into the air and resuspension of settled allergen. $E_v Q_v$ is removal by ventilation. E_v is ventilation efficiency, the ratio of the concentration in the exhaust space divided by a mixing factor. In spaces where air is recirculated and mixing is good, E_v can reasonably be as-

SUMMARY Concentration of any substance in the air of a room is determined by the ratio between the rates of its production and removal. Thus, high concentrations can result from high production, low removal, or a combination of the two. Air filtration can supplement ventilation in removing allergens from the air. Control of aeroallergen exposure at any particular location would be more rational and effective if the contribution of production, ventilation, and filtration were known. Using immunochemical assays for rat urinary protein allergen, we measured the rate of production by individual rats and the concentration of allergens in the air of rat animal quarters where the ventilation rate was about 15 changes per hour. We introduced two different high-efficiency filtration systems that provided additional clean air changes up to a maximum of 127 changes per hour. Male rats shed about 20 ng/min of allergen. Because of this high rate of production, substantial reduction in rooms that housed large numbers of animals required the very high air-exchange rates that were achieved with laminar flow small animal isolator racks. Measured concentrations agreed well with concentrations calculated from a mass balance equation whose terms included numbers of animals in the room, production rate by individual animals, and rates of ventilation and filtration. We suggest that this principle of considering both production and removal rates applies not only to devising means of reducing levels of rat allergens in rat rooms, but generally to other indoor allergens as well.

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sumed to be 1. Q_v is ventilation rate. K is natural decay rate (settling for particulates). $NE_d Q_d$ is removal rate by air cleaning; N is cleaner removal efficiency; E_d is device ventilation efficiency, analogous to E_v ; and Q_d is device flow rate.

For rat rooms, the outdoor source term, of course, is zero; the other terms need to be determined. We report here experiments on measurements of the other factors and comparison of the calculated value of C_{ss} with measured values.

Methods

Air-sampling Equipment and Accessories

The principles and assay techniques for quantification of indoor allergens have been described (2). Air Sentinels® (Quan-Tec-Air, Inc., Minneapolis, MN) were used for the collection of airborne particulates. Polytetrafluoroethylene (PTFE) membranes (Quan-Tec-Air, Inc.), which retain 0.3 µm and larger particles at 99.8% efficiency, were used as filtration media. The sampler flow rate was calibrated using a Kurz model 1510 digital flow meter (Kurz, Inc., Monterey, CA) at 1 L/s. Particulates were collected for 24 h, and the volume of air samples was calculated from the flow rate and duration of sampling. Airborne particulate sizing was accomplished by

operating the Air Sentinel® equipped with an Andersen cascade impaction head at 5 L/s (Andersen Samplers, Inc., Atlanta, GA). At this flow rate the impaction head separates particles into five mean mass aerodynamic diameter size ranges (> 9.4, 9.4 to 4.5, 4.5 to 2.7, 2.7 to 1.6, and < 1.6 µm). Except as specified subsequently, the air sampler was centrally located in each room.

For rat urinary allergen production rate measurements, the sampler drew air through a vented 10-L Lucite® chamber containing two male rats. Sample time was at least 2 h to allow collection of sufficient quantities of rat urinary protein for accurate assay. Air entering the chamber contained no rat allergen.

Rat Room Descriptions

During the period of these studies, more than

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90% of the rats were males. Room 1034 housed 300 rats, had a volume of 100 m³, and exchanged the air 172 times/h [45 by house ventilation plus 127 by laminar flow high-efficiency particle absolute (HEPA)-filtered small animal isolators; Forma Scientific, Mallinckrodt, Inc., Marietta, OH]. Room 1023 housed 300 rats and had a volume of 80 m³ and a stated 43 air exchanges/h (30 by house ventilation and 13 by HEPA filtration; CRSI 600, Control Resource Systems, Inc., South Bend, IN). Actual air exchange was less because the ventilation ducts were inadvertently occluded during the period of the studies. Room 1031C housed 30 rats and had a volume of 34 m³ and 36 air exchanges per hour (18 by house ventilation and 18 by CRSI 600 HEPA filtration). Room 1022 housed 275 rats and had a volume of 80 m³ and up to 56 air exchanges per hour (30 by house ventilation coupled with 13 and 26 changes by CRSI 600 HEPA filtration). Ventilation was measured by direct determination of airflow in exhaust ducts with an anemometer.

Air Sample Preparation for Analysis

Airborne particulates were collected by filtration or, in the case of the Andersen sampler, by impaction on PTFE membrane. The filter medium is a bilaminate of PTFE and a polyester spun support backing. The sample is delaminated and the PTFE membrane placed into a 13 × 100 mm test tube along with 1 or 2 ml of 50% glycerinated diluent containing 0.2% bovine serum albumin (BSA), Tween® 20, and phosphate buffer, pH 7.4. The support backing is discarded. The tube is vigorously mixed to wet the entire sample and allowed to stand overnight at 4° C. The following day the sample is mixed again and centrifuged, and the supernatant is stored in racks at -20° C until analyzed.

Immunochemical Assay of Rat Urine

Newman-Taylor and coworkers reported the major rat allergens to be in urine (3). A rat urine allergen reference standard was prepared from Sprague-Dawley rat urine collected in metabolic cages. The urine was dialyzed in Spectrapor® 3500 MW cutoff (Spectrum Medical Industries, Los Angeles, CA) casing and lyophilized. Protein determinations by the method of Lowry and coworkers (4) indicated 925 µg/ml of protein.

The radioallergosorbent (RAST) inhibition assay has been described elsewhere (2). Briefly, Immulon II microtiter plate wells are coated with 1 µg rat urinary protein. Either standard

TABLE 2
DISTRIBUTION OF RAT URINARY ALLERGEN
IN PARTICLES OF DEFINED SIZES

Stage	Size (µm)	Rat Allergen (ng/m ³)
1	> 9.4	151
2	9.4-4.5	16
3	4.5-2.7	33
4	2.7-1.6	14
5	< 1.6	63

dilutions of the rat urinary protein or air samples are mixed in the well with 50 µl human sera containing IgE antibodies to rat urine protein. The assay is validated by parallel inhibition curves between the urine reference standard and the air sample extract. It should be noted that this assay measures several allergens from the crude urine standard, not a single allergenic component.

Results

Individual Rat Allergen Production Rate

In seven separate experiments, the average rate of production was 19.5 ± 9.3 ng (mean $\pm$ SD) of urinary protein allergen per minute per male rat.

Room Stratification of Allergen

An estimate of removal by settling was obtained by measuring allergen concentration in Room 1022 at three levels in the room: at floor level, and 36 and 90 inches above the floor (table 1). Although we observed a downward trend in allergen concentration as sample height increased, the differences were small, and in further calculations we assumed removal by settling to be negligible in this environment where air mixing is high. To an unknown extent, removal by settling is balanced by generation from resuspension of settled dust. Because rooms have bare floors and are cleaned frequently, we assumed resuspension was also negligible.

Allergen Distribution in Defined Particle Sizes

Particle sizing from samples taken at 36-inch level demonstrated allergen activity in all stages of the impactor (table 2). Scanning electron photomicrographs confirm effective particle size separation (figure 1). Note the short hair impact on the stage holding the smallest pa



Fig. 1. Scanning electron photomicrographs (x500) of PTFE media supporting sized particles from five mean mass aerodynamic diameter size ranges: (1) unexposed; (2) > 9.4; (3) 9.4 to 4.5; (4) 4.5 to 2.7; (5) 2.7 to 1.6; and (6) < 1.6 µm.

TABLE 1
AIRBORNE RAT URINARY ALLERGEN
CONCENTRATION AT DIFFERENT
HEIGHTS

Sampler Location	N	X $\pm$ SD (ng/m ³)
Floor	20	658 $\pm$ 299
Bench (36 inches)	21	468 $\pm$ 278
Suspended (90 inches)	19	380 $\pm$ 207

TABLE 3
CALCULATED AND MEASURED AIRBORNE RAT URINARY
ALLERGEN (ng/m³)

Room No.	Rat Count	Room Size (m ²)	Airflow (m ³ /min)		Air Changes (h ⁻¹)	Calculated X ± SD	Measured X ± SD
			V	F			
1034	300	100	75	212	172	20	18 ± 7.2
1023	300	80	43*	0	30	146	803 ± 282.0
1031C	30	34	16	0	18	57	47 ± 25.7
1031C	30	—	16	17	36	29	26 ± 12.5
1022	275	80	43	0	30	134	216 ± 56
1022	275	—	43	17	43	94	208 ± 217
1022	275	—	43	34	56	72	114 ± 92

Definition of abbreviations: V = ventilation, F = filtration (HEPA)

* Designed ventilation, actual ventilation was much less because exhaust vents were inadvertently occluded.

cles. The fiber oriented itself aerodynamically as a smaller particle. About a third of the allergen was in respirable-sized particles less than 4.5 µm.

Steady-State Allergen Concentration Measurement Calculations

From the production rate, the number of animals housed, the size of the room, and ventilation and filtration rates, we calculated average airborne concentrations of rat urine using the steady-state mass balance equation. Using these assumptions, there is perfect rank-order correlation between calculated and observed concentrations (table 3). The quantity of allergen calculated from the equation and actually measured correlated within the error of measurement in all rooms except one.

The apparent exception, Room 1023, was discovered to be due to an inaccurate value for the stated ventilation rate. After the air samples had been collected, we discovered that the exhaust vents were partially clogged during the collection. The actual ventilation rate at this time is unknown.

Achievement of low allergen levels in Room 1034 despite the large numbers of rats is attributed to the substantially higher air exchange rate in that room. The

animals were housed on five laminar flow racking systems that each moved 1,500 cfm (43 m³/min) of HEPA-filtered air out over the animals. Operation of the units in this mode is intended to provide a pathogen-free environment for the animals. This HEPA-filtered laminar flow air exchange, together with the usual ventilation, also worked to lower the airborne rat allergen compared to Room 1022 or 1023 of similar size and ventilation, housing a similar number of animals in conventional cages (figure 2 and table 3).

Room 1022 was monitored over several months with the CRSI 600 HEPA filter operating at a flow rate of 17 m³/min. The removal efficiency E_d of this device is approximately 90% (table 4). The production of allergen in this room overwhelmed its removal by combined ventilation and HEPA filtration as there was no difference during early November when the HEPA filter was turned off (figure 3).

On the other hand, Room 1031C, a small room with only 30 rats, illustrates what can be achieved by the same HEPA air filtration when the conditions are suitable. The concentration of rat urinary protein allergen was reduced in half when the air exchange rate was doubled, although the allergen production rate remained the same.

Discussion

This study exemplifies the value of considering separately the individual components of the mass balance equation in planning control of allergen exposure. Ventilation in these animal care rooms would be considered excellent under most conditions. In comparison to the 15 to 20 air exchanges per hour in these rat rooms, air exchange in homes or offices with windows closed is at least 10 times less. Rats produce allergen at such a fast rate that large numbers of animals can outstrip the capacity of ordinary ventilation and filtration systems to remove it adequately. For example, in Room 1034 with 300 rats, an air exchange rate of 172 changes per hour was required to achieve reasonable control. This is 1,000-fold greater than the air exchange rate in a typical contemporary energy-efficient American home with the windows closed.

In the rat room with high air exchange rates providing good air mixing, high allergen production rates, and cement floors that are frequently cleaned, settling of allergen contributed little to removal, and resuspension contributed little to production. In other environments, such as homes, this may not be the case. Settling may become the major means of removal in rooms with poor air mixing and exchange rates, and mechanical resuspension from dust may be a major source of production. In such an environment, the particle size distribution making up the steady-state allergen concentration would be skewed toward smaller particles whose settling rate is very slow. In such a situation, improved ventilation and filtration is expected to be a more effective means of reducing allergen.

Our observations in rat rooms, where more than half the allergen was in particles larger than 9.4 µm mean mass aerodynamic diameter (too large to be respirable), are similar to those reported by

TABLE 4
HEPA FILTER (CRSI 600)
REMOVAL EFFICIENCY

	Rat Urinary Allergen (ng/sample)	Efficiency (%)
Intake	35,358	
Exhaust	3,493	90
Intake	15,006	
Exhaust	1,059*	> 93
Intake	25,859	
Exhaust	3,507	86
Intake	25,502	
Exhaust	2,755	89

* Lower limit of detection.

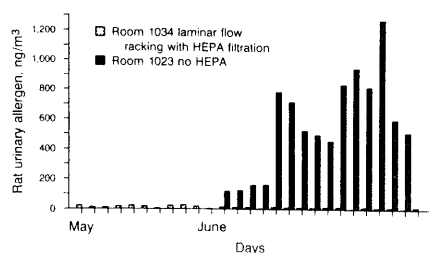
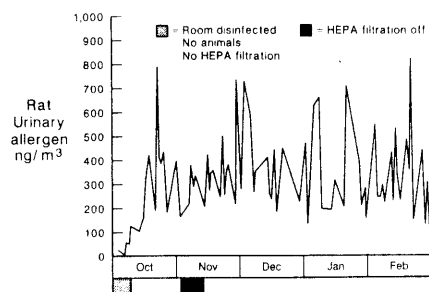


Fig. 2. Room 1034 (hatched) reduced levels of airborne rat urinary allergens over a month (late May through early June). Room 1023 (cross-hatched) daily levels of airborne rat urinary allergen over a 2-wk period. Days 1 to 4 represent predicted levels given air exchange and animal count; however, after Day 5, ventilation was not normal.

Fig. 3. Room 1022 daily levels of rat urinary allergen levels as rats were introduced into the room. Additional air exchange supplied by HEPA filtration at a rate of 17 m³/min made little difference in the allergen concentration.



Platts-Mills and coworkers (5) in a similar environment but with different sampling techniques. There was, however, substantial allergen carried with respirable particles less than 4.5 μ m mean mass aerodynamic diameter as well. Particle size not only determines the rate of settling but also the allergic symptoms. Large particles tend to cause upper airway symptoms; small particles penetrate to the airways and cause bronchial symptoms.

It is understood from previous studies that humidity, caging systems, and air sampler location are all factors contributing to the concentrations measured in the air. These factors, however, were constant

and the variables that were most important to consider for the mass balance equation were animal numbers, room size, and ventilation and filtration rates. These general principles of pollutant production and removal can be extended to any indoor environment for the assessment and improvement of air quality.

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