

Control strategies for aeroallergens in an animal facility

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Background: Prevalence of the occupational disease laboratory animal allergy could be reduced if aeroallergen reduction strategies are identified.

Objective: To reduce worker exposure to *Mus m 1*, an allergen from laboratory mice, the effect of filter cage tops, increased room ventilation, negatively pressurized ventilated cages, and ventilated cage-changing tables were evaluated.

Methods: Aeroallergen was collected in the ambient air and in the breathing zone and quantified by using a competitive immunoassay.

Results: When mice were housed in unventilated cages, ambient allergen was reduced from 5.1 ng/m³ with no cage top to 1.3 ng/m³ with a simple filter-sheet top and 0.8 ng/m³ with a fitted filter-bonnet top ($P < .05$). Room ventilation was increased from 6 to 10, 15, and 20 air changes per hour and had little effect on aeroallergen levels and no impact on airborne particulate matter. When mice were housed in ventilated cages, ambient allergen was significantly reduced from 1.1 ng/m³ at positive cage pressure to 0.3 ng/m³ at negative cage pressure ($P < .05$). Negative cage pressure combined with handling animals under a ventilated table reduced breathing zone allergen from 28 ng/m³ with neither control strategy in place to 9 ng/m³ ($P < .05$). Use of a ventilated table controlled bacterial contamination, measured as colony forming units, found in negatively pressurized cages. **Conclusion:** Three aeroallergen control strategies are use of filter cage tops, operation of negatively pressurized cages, and use of ventilated changing tables. (*J Allergy Clin Immunol* 1999;103:139-46.)

Key words: Laboratory animal allergy, aeroallergen, animal facility, breathing zone exposure, ventilated cages, cage tops, room ventilation, cage changing, mice

Allergy to laboratory animals is a significant occupational hazard. Over 90,000 personnel in the United States handle animals in research or industrial facilities,^{1,2} and approximately 38,000 personnel in Sweden and the United Kingdom handle laboratory animals.³ The prevalence

Abbreviations used

ACH:	Air changes per hour
CFU:	Colony-forming unit
HEPA:	High-efficiency particulate air
LAA:	Laboratory animal allergy

of laboratory animal allergy (LAA) has been estimated in at least 19 independent research studies and ranged from 11% to 46%, with a pooled average prevalence rate of 21%.⁴ The economic impact of LAA can be extensive. Employees that become allergic can result in reduced productivity and trained personnel, cascading workloads, increased group health insurance rates, and increased worker compensation costs.

The principal route of worker exposure is inhalation of airborne particulate matter contaminated with animal allergens. Additional exposure routes may include skin contact (urticaria or hives), eye contact (conjunctivitis), and ingestion. Inhaled particles greater than 10 μ m in size typically deposit in the nasal-pharyngeal region (upper respiratory tract) and may cause allergic symptoms, such as rhinitis. Particles in the range of about 4 to 10 μ m in size will deposit in the tracheal-bronchial region, and those less than approximately 4 μ m in size will deposit in the alveolar (respirable) region of the lung. Allergens deposited in the lung have the potential to cause asthma.

Rodent allergens are found in a wide range of particle sizes, and there is documentation that both small- and large-size allergen-laden particulates can migrate throughout a facility. Two studies have found rat and mouse allergens predominantly carried on particles 6 μ m or larger,^{5,6} whereas a third study found rat allergen predominantly carried on smaller particles of approximately 2 to 5 μ m in size.⁷ Ohman et al⁸ characterized mouse allergen in public areas of an animal facility. In 4 rooms that were connected by corridors to an animal facility but did not contain mice, allergen was predominantly detected on particles 0.4 to 3.3 μ m in size.⁸ This finding suggests that facility ventilation can carry allergen on small particles over long distances. Additionally, in a free-standing, independently ventilated cafeteria not connected to the mouse facility, mouse allergen was found predominantly on particles greater than 10 μ m in size.⁸ This suggests that the workers themselves may carry large allergen-laden particles throughout a facility.

Studies on rodents have shown that increased levels of ambient room allergen correlate with decreased relative

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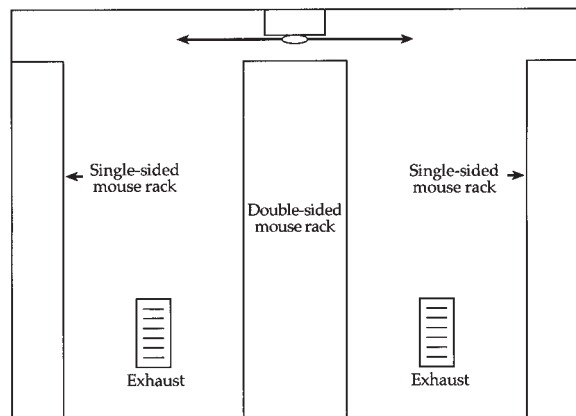


FIG 1. Schematic diagram of conventional mixing ventilation in the experimental mouse room when it housed unventilated cages. Outdoor air is passed through HEPA filters and supplied through ceiling aspirating diffusers. Air is exhausted from the room through grills located low along one side wall.

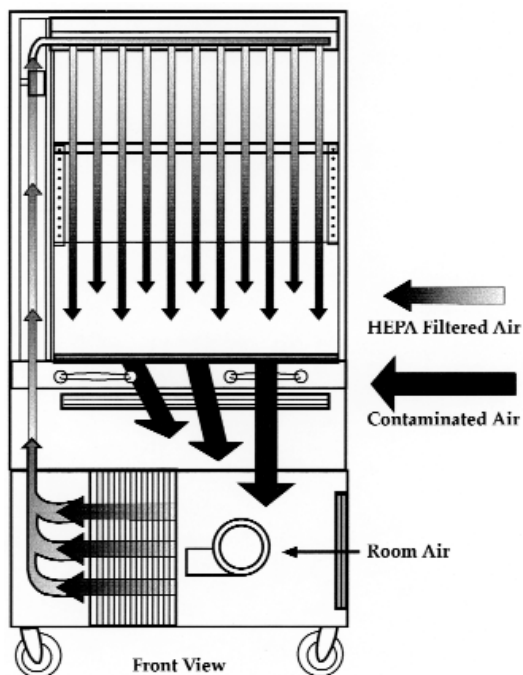


FIG 2. Schematic diagram of airflow pattern in mobile ventilated change station. Room air can be drawn in at the bottom of the table. The air passes through a HEPA filter, is routed to the top of the table, and washes down over the entire work area at a low velocity. Approximately 10% of the recirculated air is positively pressurized toward the worker.

humidity,^{9,10} increased animal density,^{5,6,11} and personnel activities, such as cage changing, room cleaning, and animal handling.^{5,6,11-13} In this study we tested the efficacy of several aeroallergen-reduction methods: the use of filter tops on unventilated cages, increasing the room ventilation rate, maintaining negative pressure in venti-

lated animal cages, and the use of mobile ventilated cage-changing tables. Allergen was quantified from both ambient air and the workers' breathing zone. Allergen in the breathing zone, the volume of air within a 1-ft radius of the head, has been reported to be up to 30 times higher than ambient levels.^{5,6,8,13-15}

METHODS

Experimental room

All experiments were conducted in a previously described mouse room,^{10,16} which initially housed unventilated cages and was later renovated to house ventilated cages. The room design provided conventional mixing ventilation as depicted in Fig 1. Fresh outdoor air passed through high-efficiency particulate air (HEPA) filters and was then supplied to the room through 2 central bidirectional aspirating ceiling diffusers and exhausted through 2 exhaust grills located between the racks near the floor. Room ventilation rates were calculated with a factory-calibrated thermo anemometer (Model 8565; Alnor Instrument Co, Skokie, Ill) to determine the cubic feet per hour of airflow through the air supply duct, and this value was then divided by the room volume. Dampers in the supply and exhaust ducts allowed the ventilation rates to be increased from 6 to 20 air changes per hour (ACH). Ventilated cage pressure was set by adjusting dampers in the supply and exhaust ducts to individual racks. The room temperature was $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and humidity was $45\% \pm 5\%$.

Animals

To maximize allergen production, we used male C57BL/6J mice in all experiments because this strain produces high levels of Mus m 1 (J. Ohman, unpublished data, 1998) and more allergen is produced by males than females.¹⁷ Colony size was 1088 mice in experiments with unventilated cages and 1440 mice in those with ventilated cages. Because testosterone levels vary with mouse age,¹⁸ the average age was maintained at 9 weeks. The colony was established with 10 groups between 4 and 13 weeks of age and was maintained by weekly replacements of 10% of the oldest mice with 4-week-old mice. Animal husbandry followed the guidelines stated in *Guide for the Care and Use of Laboratory Animals*.¹⁹

Caging systems and cage-changing table

For all experiments, we used square duplex polycarbonate cages ($31 \times 31 \times 14$ cm divided into 2 pens). A wire-rod metal top covered each pen and held feed pellets and a water bottle. Each pen housed 4 mice, with free access to Old Guilford 911 diet (Emory Morse Co, Guilford, Conn) and bottled acidified water (pH 2.8 to 3.1). Bedding consisted of white pine shavings (Crobb Box Co, Ellsworth, Me).

Cages in both the ventilated and unventilated systems were held on a central double-sided rack and 2 single-sided racks on opposite walls. The building's central ventilation system supplied ventilated cages (Thoren Caging Systems, Inc, Hazleton, Pa) with air that first passed through a HEPA filter. Exhaust air from the cages was routed outside the building, and no air was recirculated. Each ventilated cage was covered with a polyester filter top, approximately 7 to 10 μm in pore size (Reemay 2033; Thoren Caging Systems, Inc). The ventilated cages were designed to be operated with either positive, negative, or neutral cage pressure and are described in detail by Clough et al.²⁰ An electronic micromanometer (Airdata Multimeter ADM-870; Shortridge Instruments, Inc, Scottsdale, Ariz) was used at the face of selected cages to determine cage air pressure. Cage changing typically took place on a standard, unventilated changing table (Tripp Wire, Frankfort, Me). Experiment 4 evaluated the use of a mobile, ventilated changing table (Maxi-Miser, Thoren Caging Systems). The self-contained table provides HEPA-filtered, positively pressurized, down-drafted air over the work

space (Fig 2). A caretaker changed one third of the cages each day from 1 PM to 3 PM, Tuesday through Thursday. Soiled cages were removed from the animal room and cleaned in a separate facility.

On days when cage changing took place, the cleaning schedule included wet cleaning and sanitizing of all external surfaces of the cage-changing table. The floor was vacuumed at the end of each day, and on days when the ventilated change table was used, the HEPA filter within it was vacuumed. In addition, animal racks and shelves were cleaned and sanitized at the time cages were removed for changing. Fridays were reserved for extensive room cleaning, which included emptying and sanitizing the grain hopper within the changing table and wet cleaning and sanitizing the floor each week. Every other week, one of the following areas was also cleaned and sanitized: animal room walls and exhaust grills or animal room ceiling and lights.

Effect of cage tops on unventilated cages: Experiment 1

We investigated the effect of different cage tops on the ambient level of Mus m 1. Aeroallergen was measured when cages had either a fitted filter-bonnet top, a flat filter-sheet top (Fig 3), or no filter top. Both filter tops were comprised of the same filter material, a nonwoven filter of natural and synthetic fibers (model FF 8039; Negus Container and Packaging, Madison, Wis), with an air permeability of 140 cfm and a filtration capability of approximately 5 μ m or less. The fitted bonnet tops were assembled locally and were comprised of the filter material fastened to a cardboard band 32 cm² $\times$ 6 cm (International Paper, Putnam, Conn). The flat filter-sheet top was comprised of the filter material only, measuring 31 $\times$ 14.5 cm. The flat tops covered the cage top surface and were laid on the wire-rod cage cover and held in place by the overlying water bottle. For quality assurance, aeroallergen samples were collected in duplicate. Aeroallergen results for the duplicates were found to be within 1% of each other ($n = 4$). The room ventilation rate was 10 ACH throughout the experiment, and cages were changed on an unventilated table.

Effect of room ventilation rate: Experiments 2 and 3

We investigated the effect of room ventilation rates of 6, 10, 15, and 20 ACH on ambient aeroallergen, real-time particulate matter, and CO₂ in rooms containing either filter bonnet-covered unventilated cages (experiment 2) or positively pressurized ventilated cages (experiment 3). We measured aeroallergen, particulate matter, and CO₂ in experiment 2 and only aeroallergen in experiment 3.

Effect of ventilated cage pressure and ventilated cage changing tables on aeroallergen levels and contamination of colony-forming units: Experiment 4

We determined the effect of cage pressure and ventilated cage-changing tables on ambient and breathing-zone aeroallergen and on contamination between the cages and the room. Four experimental conditions were established by setting cage pressure either positive or negative to that of the room and by using either a ventilated or unventilated changing table.

The potential transfer of pathogens between cages and the room was assessed by counting the number of bacterial colony-forming units (CFUs) deposited on petri dishes placed inside clean empty cages in the ventilated rack or open to the room. Dishes open to the room air were located approximately 3 ft off the floor between the animal racks. Petri dishes contained tryptose agar base and were exposed from 12 PM to 8 AM; cage changing occurred between 1 PM and 3 PM. Dishes were then incubated at 37°C for 48 hours, after which the number of CFUs in each dish were counted. We conducted 2 trials, first placing 1 petri dish in each of 20 cages and 20 dishes



FIG 3. Mouse cage on the left shows an example of 2 separate flat, filter-sheet tops, 1 covering each half of the duplex mouse cage. Mouse cage on the right shows an example of a fitted, filter-bonnet top containing a plastic view window for observing the level of water in the water bottles.

in the room and then placing 3 dishes in each of 20 cages and 15 dishes in the room.

Collection, extraction, and quantification of Mus m 1 aeroallergen

We measured ambient aeroallergen as Mus m 1 carried on particulate matter. Ambient particles were collected at a standardized room location from 8 AM to 8 PM, Tuesday through Friday, by a 20-L/min air-sampling pump and an impactor (Air Diagnostics, Naples, Me) approximately 1.5 m from the floor. The impactor included a stainless steel plate (Air Diagnostics) that captured particles larger than 10 μ m and a Teflon filter (Chester LabNet, Tigard, Ore) that captured particles smaller than 10 μ m. For quality assurance, 2 impactors were used side by side to collect paired samples in experiment 1. Aeroallergen in the breathing zone was collected by an impactor fastened to a chest harness worn by personnel.

We extracted Mus m 1 by shaking the filters in a mixture of 2.5% Tween and PBS (pH 7.4) at 4°C for 48 hours. The eluate was stored frozen at -80°C and subsequently lyophilized to concentrate the allergen. The lyophilized eluate was resuspended in 1 mL of distilled water and frozen at -20°C until assayed.

Mus m 1 was quantified immunochemically by competitive immunoassay with pooled IgE antibodies from persons highly sensitive to mice (M. Swanson; Allergic Diseases Research Laboratory, Mayo Clinic, Rochester, Minn). Urine from male BALB/c mice was adsorbed onto the surface of Immulon 4-microtiter Removawell strips (Dynatech Laboratories).¹¹ The strips were incubated overnight at room temperature in a humid box and washed 3 times with RAST wash (1% Tween in 0.1 mol/L of phosphate buffer). Dialyzed, unfractionated urine from male BALB/c mice was also used as reference allergen to create a standard curve. Fifty-microliter aliquots of the air-filter eluates or 50- μ L aliquots of reference allergen dilutions were placed in the wells. Fifty microliters of specific human antisera was added to all wells, and the plates were incubated overnight at room temperature in a humid box. They were washed 5 times, after which 100 μ L (20 ng) of ¹²⁵I-anti-human IgE was added to each well. The plates were then incubated for 18 hours in a humid box and washed 5 times. The Removawell strips were broken apart and placed in a gamma scintillation counter for allergen quantification.

Measurement of real-time particulate matter

To count real-time particulate matter in experiment 2, we used a factory-calibrated real-time particle counter (CI-4100; Climet Instruments, Redlands, Calif) mounted on a cage-changing table. The counter provided separate counts for particles larger than 0.5

TABLE I. Effect of cage tops on ambient mouse allergen, Mus m 1

Top type	Mus m 1 (ng/m ³ ± SEM)*	
	<10 µm	>10 µm
No top	5.1 ± 0.4 ^a	1.9 ± 0.8
Flat filter sheet	1.3 ± 0.3 ^b	0.6 ± 0.3
Fitted bonnet-filter top	0.8 ± 0.3 ^b	0.8 ± 0.2

*Values within columns having different superscripts are significantly different (ANOVA, $P < .05$, $n = 4$).

µm and for particles larger than 5.0 µm; the difference of the 2 counts provides the 0.5 to 5.0 µm values presented here. Ten-minute samples were taken every 15 minutes from 8 AM to 6 PM, Monday through Thursday. Two separate time periods were delineated for data analysis: background levels (8 AM to 12 PM) and cage-changing levels (12 PM to 3 PM). Data were collected by a computer equipped with the software Data Logger for CI-4100, Version 1.25 (Climet Instruments CI-4100) and then transferred to SYSTAT (SPSS Inc, Chicago, Ill) for statistical analysis. Particle counts are expressed as the number of particles in a 10-minute sample.

Monitoring of CO₂

CO₂ was monitored in experiment 2 by an infrared CO₂ detector (model RI-411A and 1058; Gastech, Inc, Newark, Calif) and chart recorder (Primeline model 6723; Soltec Corp, San Fernando, Calif). To determine the amount of CO₂ present in the ambient room air under the conditions of 6, 10, 15, and 20 ACH, we calculated the difference between CO₂ in the supply air (100% fresh outdoor air) and the ambient room air. CO₂ concentrations were measured from 12 AM to 5 AM on Monday, Tuesday, and Wednesday of each experimental condition. The concentrations determined each day were averaged together for each given condition ($n = 3$). Both supply and room air were monitored by using 2 sampling lines, 1 placed in a supply duct and the other in the same location as the particulate monitor. A timeshare device alternately sampled the supply and room air every 10 minutes.

Statistics

Aeroallergen data was analyzed with ANOVA followed by Tukey HSD post-hoc tests. In the instances where the sample number was 5 or sample number was 4, the minimum detectable difference between conditions was calculated at a significance level of .05 and a confidence level of 90%. In all cases in which statistical significance is noted, the difference between the values exceeds the calculated minimum detectable difference. Contamination data, expressed as CFUs, were analyzed by using ANOVA for mean CFUs per plate in the room, and Kruskal-Wallis for mean CFUs per plate in the cage. Chi-square analysis was used to compare the total number of CFUs found inside cages at all conditions.

Statistical analysis of real-time particulate matter was performed on alternate time points as suggested by O'Brien et al.²¹ We used ANOVA to compare particulate levels at the 4 ventilation rates during the following 2 time periods: background conditions (8 AM to 12 PM) and cage-changing activities (12 PM to 3 PM).

RESULTS

Effect of cage tops on unventilated cages: Experiment 1

We questioned the efficacy of filter tops in reducing aeroallergen levels in rooms housing unventilated cages. To

TABLE II. Effect of room ventilation rate on ambient mouse allergen, Mus m 1

	Mus m 1 (ng/m ³ ± SEM)*	
	<10 µm	>10 µm
Unventilated cages, bonnet-filter tops		
6 ACH [†]	2.0 ± 0.2	0.32 ± 0.03
10 ACH	1.2 ± 0.6	0.25 ± 0.02
15 ACH	1.0 ± 0.2	0.31 ± 0.07
20 ACH	1.7 ± 0.1	0.27 ± 0.05
Positively ventilated cages		
6 ACH [†]	1.1 ± 0.2	0.13 ± 0.03 ^a
10 ACH	1.2 ± 0.2	0.30 ± 0.02 ^{a,b}
15 ACH	1.1 ± 0.2	0.24 ± 0.04 ^a
20 ACH	1.5 ± 0.2	0.50 ± 0.09 ^b

*The categories of unventilated or positively ventilated cages were analyzed separately for the effect of room ventilation. For each category, Mus m 1 values for a given size fraction having different superscripts are significantly different (ANOVA, $P < .05$). Columns with no superscripts have no significant differences ($n = 4$ for unventilated cages and $n = 5$ for ventilated cages). For the column in which significant differences are noted, the minimum detectable difference was calculated to be 0.21 ng/m³ at a significance level of .05 and a 90% confidence level. Ventilated cages were ventilated at 60 ACH and positively pressurized at 0.13 Pa.

[†]Room ventilation rates.

answer this question, we measured ambient Mus m 1 when cages had either a fitted bonnet top, a flat filter-sheet top, or no top. Both types of filter tops produced a statistically significant, 4- to 6-fold reduction in the levels of Mus m 1 measured on particles smaller than 10 µm (Table I, $P < .05$). Levels of aeroallergen on particles larger than 10 µm were reduced 2- to 3-fold with filter tops in place, although the reduction was not statistically significant because of the greater variability of large particles (Table I).

Effect of room ventilation rate: Experiments 2 and 3

The primary question was whether increasing the room ventilation rate is effective in reducing the levels of Mus m 1, particulate matter, and gaseous contaminants. Increasing the ventilation rate had little effect on aeroallergen levels in rooms housing either unventilated or positively ventilated cages. Under the condition of unventilated cages, aeroallergen levels at 20 ACH approached the levels found at the lower ventilation of 6 ACH. Increasing the ventilation in rooms housing positively ventilated cages was even less effective; the increased rate not only failed to reduce aeroallergen levels but actually elevated the levels of aeroallergen particles larger than 10 µm when increased from 6 to 20 ACH (Table II). The increase in room ventilation rate from 6 to 15 ACH reduced CO₂ levels almost 3-fold, whereas the increase from 15 to 20 ACH reduced CO₂ only slightly (Fig 4). Increasing the ventilation rate had no significant effect on levels of ambient particulate matter during either background or cage-changing con-

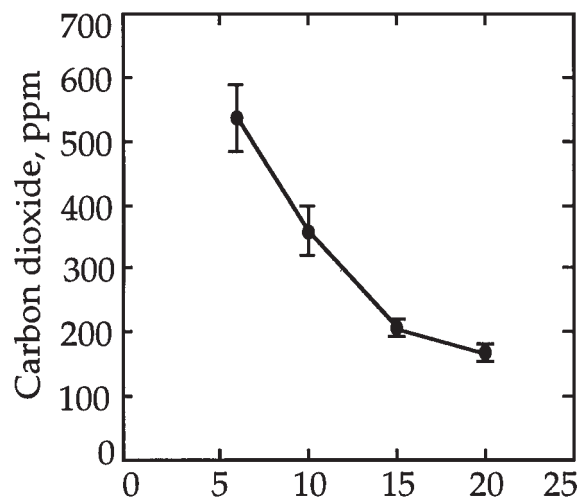


FIG 4. CO₂ was measured at a central room location and in the supply duct from 12 AM to 5 AM, Monday through Wednesday. Background CO₂ levels in the supply duct were approximately 400 ppm; they were subtracted from the room levels, and the difference is plotted in the graph. Values are means $\pm$ SEM. Unventilated cages with fitted-bonnet tops were used during this experiment.

ditions (Fig 5). Particulate concentrations at all ventilation rates peaked after 1 PM during cage changing and declined at 2 PM during a short work break (Fig 5). Levels of particulate throughout the day were similar at all ventilation rates. For clarity in data presentation, only the levels at 6 and 20 ACH are shown in Fig 5.

Effect of cage pressure and ventilated cage-changing tables on aeroallergen levels and contamination by CFUs: Experiment 4

Ventilated cages are typically maintained under positive pressure to control the spread of infectious agents among animals. We measured the impact of both negative and positive cage pressure on ambient aeroallergen and contamination of CFUs.

Ambient aeroallergen on particles less than 10 μ m was significantly reduced when cages were maintained under negative pressure ($P < .05$, Table III) from 1.1 ng/m³ at positive cage pressure to 0.3 ng/m³ or less. Breathing-zone aeroallergen was significantly reduced when a ventilated table was used ($P < .05$, Table III). The combination of negative pressure and a ventilated table reduced breathing-zone aeroallergen 3-fold from 28 ng/m³ at positive cage pressure and an unventilated table to a low of 9 ng/m³ ($P < .05$).

Contamination of CFUs was higher in cages under negative pressure than in those under positive pressure when cage changing took place on an unventilated table ($P < .05$, Table IV). Ventilating tables, however, controlled the spread of CFUs into negatively pressurized cages. Ventilating tables also significantly reduced CFU contamination in the room (Table IV).

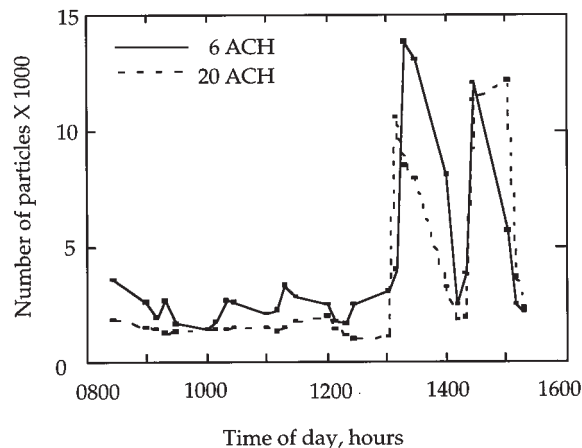


FIG 5. Particles 0.5 to 5.0 μ m in size were measured at ventilation rates of 6, 10, 15, and 20 ACH. There were no significant differences noted ($.05 > P < .01$). A significance of .01 was used to compensate for the multiple statistical comparisons. For clarity, only data from 6 and 20 ACH are plotted.

DISCUSSION

Filter cage tops

We conclude that cage tops are an effective aeroallergen control strategy because both filter top types reduced levels of ambient aeroallergen by up to 84% (Table I). The effectiveness of cage tops in reducing aeroallergen levels has been documented in other studies as well. Filter tops reduced the levels of airborne rat aeroallergen by about 75%⁶ and levels of airborne mouse prealbumin and albumin by 90% and more than 82%, respectively.²² The effectiveness of the flat filter sheet was somewhat surprising because the sheet is not fitted around the cage lip; it rests on top of the cage, pinned in place by the water bottle. These data indicate that even a modest barrier between the cage and room can significantly reduce aeroallergen within the room. The effect that the bonnet top has on the cage microenvironment has been previously reported.²³

Ventilated cages and cage-changing tables

The advantages of ventilated cages include an improved microenvironment and macroenvironment^{16,24} and a reduction in airborne contamination within a rodent colony.^{20,25} The Thoren cage system used in this study can be operated with cage air pressure either positive, negative, or neutral to the room. Positive pressure ensures that only air passed through a HEPA filter enters the microenvironment, substantially reducing the risk of infection from airborne pathogens. Negative pressure provides the highest level of cage containment to reduce the spread of animal aeroallergens into the room. Although neutral cage pressure may be desirable, this cage condition is difficult to reliably maintain.

TABLE III. Effect of cage pressure and ventilated changing table on allergen concentration

Rack pressure	Ventilated table	Mus m 1 (ng/m ³ ± SEM)*	
		<10 μm	>10 μm
Ambient allergen			
Positive	No	1.1 ± 0.1 ^a	0.3 ± 0.02
Positive	Yes	1.0 ± 0.1 ^a	0.2 ± 0.02
Negative	No	0.3 ± 0.1 ^b	BD
Negative	Yes	0.1 ± 0.02 ^b	BD
Breathing zone allergen			
Positive	No	27.9 ± 5.1 ^a	5.3 ± 0.9 ^a
Positive	Yes	12.5 ± 3.1 ^b	3.7 ± 0.9 ^{a,b}
Negative	No	15.0 ± 3.8 ^{a,b}	3.0 ± 0.5 ^{a,b}
Negative	Yes	9.1 ± 2.6 ^b	1.8 ± 0.6 ^b

BD, Below detection.

*Within ambient and breathing zone locations, values within columns having different superscripts are significantly different (ANOVA, $P < .05$; $n = 9$ for ambient allergen and $n = 6$ for breathing zone allergen). Room ventilation was 10 ACH, cage ventilation was 60 ACH, and cage pressure was either 0.13 or -0.07 Pa.

Clough et al²⁰ demonstrated that ventilated cages can reduce the spread of CFUs into rodent cages. CFUs were reduced 99% with positive cage pressure as compared with open, nonventilated cages, and they were reduced 94% with negative cage pressure.²⁰ Our results indicate that the use of a ventilated cage-changing table can control bacterial contamination into negatively pressurized cages. When cages were changed in the ventilated table, the number of CFUs found in negatively pressurized cages was not different from the number found in positively pressurized cages.

One study has shown that aeroallergen levels were reduced approximately 15% when mice were housed in positively pressurized cages compared with open, nonventilated cages.²⁴ Our study contrasted the use of positively pressurized cages versus negatively pressurized cages; the results indicate that a 4-fold reduction in aeroallergen concentrations can be achieved when cages are operated under negative pressure. In addition, the use of a ventilated cage-changing table improved this aeroallergen reduction to 10-fold (Table III).

Because aeroallergen concentrations can be relatively high during cage changing,^{5,6,8,13,14} the control of airborne particulate matter at the changing station is essential. It is clear that while handling animals, the use of ventilated tables, biosafety cabinets, and ventilated surgery tables can play a significant role in controlling the spread of infectious disease and reducing personnel exposure to aeroallergen. Our aeroallergen results are significant given that the ventilated changing station used for these studies was positively pressurized to the worker. The table was designed to protect the animals placed within the table's enclosure and not the worker. Additional worker protection would be expected with the use of a negatively ventilated changing station. Use of such a table, in combination with negatively pressurized cages,

could allow for enhanced simultaneous protection of personnel and animals.

Clinical significance

The most effective control strategies identified here can reduce allergen exposure 10-fold in ambient air and 3-fold in the worker's breathing zone (Table II). The important question is whether such a reduction will result in fewer individuals becoming sensitized or experiencing allergic symptoms.

Hollander et al²⁶ studied a group of 86 individuals never exposed to rat allergen and found that 3.5% had specific IgE to that allergen. This finding suggests that some sensitization among exposed individuals will therefore always be present. Renström et al²⁷ found this to be true in a study investigating the effect of improved work practices and the risk of sensitization. In an improved and "clean" facility, a population having less than 4 years of laboratory animal experience and assigned to work at all times at ventilated benches or cabinets were studied. Of 26 atopic individuals working with male rodents, 23% reported symptoms to rodents, and 12% were found to be sensitized. However, individuals who were not atopic or who were not working with male rodents were not sensitized.

Although it may not be feasible to achieve 0 cases of sensitization, managing for occupational LAA could be made easier if a threshold level could be identified below which only a minority of individuals experienced symptoms. Eggleston et al²⁸ found that persons exposed to less than 5 ng/m³ of rat n I had fewer allergic responses than persons exposed to higher concentrations of rat allergen.²⁸ In a follow-up study, however, individuals exposed to concentrations less than 5 ng/m³ did exhibit allergic symptoms, and thus this rat allergen threshold exposure level has not been confirmed.²⁹ To the authors' knowledge, no studies have been conducted on threshold levels for exposure to mouse allergens. Threshold limit values are difficult to establish for animal allergens because the allergic response depends on many factors, including individual sensitivity and the duration, intensity, and frequency of exposure. Furthermore, allergen collection methods and assay techniques are not standardized worldwide or within countries, making it difficult to compare results between different institutions.

The experience of the occupational health staff at this institution has shown that symptomatic individuals can show improved health when relocated to work in positions associated with less allergen exposure. For example, one symptomatic animal care technician showed significant clinical improvement by using a ventilated table to change animal cages. The individual remained healthy and continued employment for 2 years in the same job and was later promoted to a position that required less animal handling. In addition, a 1992 animal room survey (unpublished data) at this institution indicated that rooms associated with the lowest frequency of allergy symptoms also had the lowest ambient allergy concentrations. Average ambient allergen levels in these rooms were 0.2 ng/m³ compared with the mean of 2.0 ng/m³ for rooms

TABLE IV. Effect of cage pressure and ventilated changing table on the number of CFUs found on petri dishes placed in the room or inside cages

Condition	Cage pressure (Pa)	Ventilated table	Total CFUs*		Mean CFUs/plate†	
			Room	Cages	Room	Cages
Standard	0.13	No	1481	31 ^a	42 ± 1.3 ^a	0.4 ± 0.1
Test	0.13	Yes	634	37 ^a	18 ± 0.9 ^b	0.5 ± 0.1
Test	-0.07	No	718	54 ^b	20 ± 0.8 ^b	0.7 ± 0.1
Test	-0.07	Yes	276	34 ^a	8 ± 0.4 ^c	0.4 ± 0.1

To convert pascals to inches of water, multiply by 0.0040218.

*Values within a column having different superscripts are significantly different (chi-square analysis, $P < .05$).

†Values within a column having different superscripts are significantly different (mean room data: ANOVA, $P < .05$; cage mean data: Kruskal-Wallis one-way ANOVA, $P > .05$; $n = 35$ in room and $n = 80$ in cages).

with a high prevalence of symptoms, which lead us to hypothesize that a 10-fold reduction in allergen levels will be clinically meaningful. These experiences are in keeping with studies showing that allergic reactions to animal proteins can be dose dependent.^{26,29} A few individuals may have symptoms at even low doses of allergen, but a 10-fold reduction in ambient allergen should provide clinical improvement for other individuals.

Room ventilation

Our results indicate that, under the described conditions, increasing the room ventilation rate was not necessarily effective for reducing levels of ambient aeroallergen. Real-time particulate data support the trends observed with the allergen data; increasing the room ventilation rate did not reduce levels of real-time particulate matter. Although the ventilation system removed CO₂ efficiently at up to 15 ACH in a room with unventilated cages, little additional benefit was achieved by further increasing the rate to 20 ACH. The allergen results contrast an earlier study, in which an increase from 8 to 15 ACH did reduce aeroallergen levels in a rat room.⁹ The ventilation system in the rat room was much different than the mouse room described here, and this could explain the contrasting results. In the rat room, air was centrally and longitudinally supplied at the ceiling and exhausted at various vertical heights and horizontal intervals on the wall behind the animal racks.

The mixing ventilation system of the experimental room described here was designed to minimize thermal stratification within mouse racks by creating a turbulent airflow. Computational fluid dynamics modeling of this design type predicted that although thermal stratification should be minimized, this ventilation design may not provide efficient air distribution in rooms with a thermal load.³⁰ The thermal load produced by animals and equipment may drive an upward airflow that cannot be overcome by the downward flow of supply air, resulting in reentrainment and recirculation of contaminated air and dead air zones.

In general, the amount of aeroallergen in a room is a function of the rate of allergen production (animal density and population type) and the rate of allergen removal (ventilation dilution and removal of contaminant). Computer modeling has suggested that an increased air change rate in a conventional mixing ventilation system,

such as that described in our experimental room, will not accelerate the removal of airborne particulate trapped within dead spaces or recirculating currents.³⁰ This ventilation design apparently is less effective for removing particulate under these conditions. Thus an increase in fresh air change rate would not necessarily be expected to reduce the amount of aeroallergen in the mouse room. These findings are consistent with conclusions that appropriate ventilation design is important for a healthy animal room macroenvironment.

Three innovative room ventilation systems have recently been developed to improve the removal of airborne contaminants: the one-way airflow (OWAF) system, the central soffit system, and displacement ventilation. In the OWAF system, the animal racks are isolated within the room by rigid perforated polycarbonate curtains. Clean air is supplied centrally to the room, flows through the curtain and across the animal racks, and is exhausted at the ceiling behind the curtains. Operation of the OWAF system in rat rooms reduced the levels of airborne particulate by approximately 96% in one study³¹ and reduced the level of aeroallergen by 86% in a second study.³² The central soffit concept is to optimize the capture of contaminated air at the ceiling and reduce air recirculation. Air is supplied through a longitudinal air duct, or soffit, centrally located on the ceiling and equipped with radial diffusers along the center axis. Exhaust extracts are located along the length of the soffit. The centrally supplied air warms and flows upward, carrying contaminants, where it is retained by the soffit and drawn into the exhaust ducts.³⁰ Displacement ventilation provides cool air at low velocity near the floor, which rises as heat is gained from sources in the room (eg, personnel, animals, and equipment). The thermal lift carries contaminated warm air toward the ceiling, where the room exhausts are located.^{33,34} Displacement ventilation has been demonstrated to be effective in improving air quality (gaseous components) by a factor of 2 to 18 as compared with high supply/high exhaust mixing ventilation.³³

In summary, we have identified 3 effective means of aeroallergen control in mouse rooms. (1) Filter tops on unventilated cages significantly reduce the concentration of aeroallergen. The filter tops evaluated in this study were simple and inexpensive; filter tops can be an afford-

able step toward reducing allergen within an animal colony. (2) In the type of ventilated cage system evaluated in this study, negative cage pressure can reduce ambient aeroallergen levels compared with positive cage pressure. (3) Ventilated cage-changing tables can reduce aeroallergen in the workers' breathing zone. Such tables can also reduce the spread of contamination, measured as bacterial CFUs, throughout an animal room or into negatively pressurized cages.

Conventional mixing ventilation may not provide effective contaminant control in animal rooms. However, recent innovations in animal room design have produced several viable alternatives for improved ventilation and reduced exposure to aeroallergens.

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