The Effects of Cage Design on Airborne Allergens and Endotoxin in Animal Rooms: High-Volume Measurements with an Ion-Charging Device

JAMES A. PLATTS-MILLS,[1] NATALIE J. CUSTIS,[1] ALICE KENNEY,[2] AMY TSAY,[3] MARTIN D. CHAPMAN, PHD,[3] SANFORD H. FELDMAN, DVM, PHD,[2] AND THOMAS A. E. PLATTS-MILLS, MD, PHD[1,*]

Respiratory symptoms related to both endotoxins and animal allergens continue to be an important cause of occupational disease for animal technicians and scientists working with rodents. Better sampling methods for airborne allergens and endotoxin are needed to help standardize compliance with federal occupational health regulations. Using an ion-charging device, we sampled 20 mouse rooms and four rat rooms at the University of Virginia, along with 43 domestic living rooms in houses in the Charlottesville area with at least one cat or dog. The use of filter tops on cages corresponds to a 50-fold reduction in mean levels of both airborne allergens ($P < 0.001$) and endotoxin ($P < 0.001$). The use of vented cages with filtered exhaust ports was associated with additional reductions. However, the mean airborne endotoxin level in all rooms using filter tops without a filtered exhaust port on the cages was significantly lower ($P = 0.003$) than the level in domestic living rooms. Our results for maximum airborne allergens or endotoxin are comparable with previous reports. However, the sensitivity of the technique allows an accurate assessment of low-level exposure, which makes it possible to evaluate the effect of cage designs. In addition, this approach allows direct comparison with results for airborne allergen and endotoxin in domestic homes. The results could allow a more consistent approach to the application of occupational health guidelines.

Vivariums use a wide range of control strategies to protect both animals and technicians. Animals need to be protected from airborne pathogens, whereas for technicians, the primary recognized exposure risks are airborne animal allergens and bacterial products such as endotoxin. It has been suggested that the single most important control strategy for both airborne pathogens and allergens is the presence of filter tops on individual animal cages (1). Here we show that they are equally effective at reducing airborne endotoxin. Most barrier rodent housing uses filter tops on individual cages. Additional control strategies have been adopted with the intention of improving upon this simple method of isolation from airborne contaminants. Individually ventilated high-efficiency particulate air (HEPA)-filtered positively pressurized caging systems (vented cages) and HEPA-filtered changing stations have been recommended to further reduce the exposure of animals to airborne pathogens (2). A possible consequence of both of these adaptations is increased exposure of workers to allergens and endotoxin. In one commonly used vented caging system, the air leaving the cages blows along the edge between the filter top and cage bottom and into the room; similarly the use of laminar-flow clean workbenches blows air over the cages toward the worker. Further improvements have tried to eliminate these effects by redirecting the exhaust flow out of the room, by providing HEPA-filtered exhaust ports to individual cages, or, with regard to changing stations, the recirculation of as much as 70% of HEPA-filtered air within the booth (3).

Most air sampling has used air filters running at approximately 20 liters/min (and as low as 3 liters/min for endotoxin). Despite this approximate sampling standard, reported exposure estimates have varied considerably (1, 2, 4-6). In previous studies, reported differences in mean levels of airborne endotoxin exposure between low- and high-exposure environments varied by as little as 10-fold. We have published results on allergens in homes sampled by using an ion-charging device (ICD), which provides a higher sampling rate despite its passive (fanless) system (7). The consequently higher sensitivity for both allergen and endotoxin measurements allows a better estimate of the relative exposure levels in different environments. Further, the devices are both silent and safe and can be run for prolonged periods in domestic settings or animal houses. In animal rooms, using these devices offers an improved assessment of the effects of cage type on airborne allergens and endotoxin and allows comparison of the exposure data with domestic exposure data.

Department of Internal Medicine[1] and Center for Comparative Medicine,[2] University of Virginia, Charlottesville, Virginia 22908; Indoor Biotechnologies, Inc., Charlottesville, Virginia 22903[3]

[*]*Corresponding author*

Materials and Methods

Air sampling. Sampling of each room used two ICDs (Ionic Breeze Quadra, The Sharper Image, San Francisco, Calif.) in parallel running for 24 h and placed at least 1.8 m apart and at least 1.2 m from the wall. The machines cycle between two distinct flow rates. For each of the 14 devices used in this study, we timed the periods P_1 and P_2 of the two rates. By using a vanometer, the wind speed was measured at the center of 114 squares, each with an area of 9 cm^2, on a two-dimensional grid placed orthogonal to and directly in front of the machine. These speeds were summed to determine the total flow rate at each speed, V_1 and V_2. The total average flow rate was calculated as $(P_1V_1 + P_2V_2)/(P_1 + P_2)$ m^3/min. After sampling, the stainless-steel plates of the ICD were removed and cleaned with a series of three filters (Millipore prefilters, model AP20, 35 mm diameter, Millipore Corporation, Bedford, Mass.) dampened with sterile water. Each filter was placed in a 3-ml syringe and extracted overnight in 2 ml of sterile phosphate-buffered saline (PBS) at 4°C. The extracts were transferred into 3-ml tubes and stored at –40°C.

Animal room sampling. We sampled 20 mouse rooms and four rat rooms in four different animal facilities (vivariums), in addition to nonvivarium research laboratories with rodent experimentation at the University of Virginia. The mouse rooms varied in size from 15 to 70 m^3 and in occupancy from 96 to 1887 mice. The rat rooms varied in size from 20 to 90 m^3 and in occupancy from 86 to 476 rats. Each mouse room was sampled at least twice, and each rat room was sampled at least four times. The mouse and rat rooms used a

Table 1. Quantities of airborne endotoxin and allergen collected from animal rooms in 24 h

Cage type[a]	n[b]	Mus m 1 (μg/24 h)	Rat n 1 (μg/24 h)	Endotoxin (EU/24 h)
Mouse rooms				
Open	8	2.98 [1.69–5.25]	< 0.002	1930 [752–4940]
Static	20	0.024 [0.013–0.045]	< 0.002	47.8 [31.9–71.6]
Vented	10	0.081 [0.022–0.304]	< 0.002	63.6 [23.6–171]
Vented with exhaust	14	0.010 [0.005–0.019]	< 0.002	2.9 [1.9–4.6]
Rat rooms				
Open	12	0.009 [0.005–0.016]	3.53 [2.15–5.78]	10400 [6550–16500]
Vented	6	< 0.002	0.009 [0.004-0.017]	64.3 [21.2–195]

Data are presented as the geometric mean followed by the 95% confidence interval (in brackets).
EU, endotoxin units.
[a]Open cages had significantly higher airborne levels in each case ($P < .005$).
[b]"n" represents the total number of samples. Each mouse rooms was sampled at least twice, and each rat room was sampled at least four times.

variety of cage types, which were separated into the functional categories of open conventional, static filter-topped, positively pressurized vented, and positively pressurized vented with filtered exhaust. Open cages have only a metal grill to prevent the animals from exiting the cage. Static cages have a fitted filter top, whereas vented cages incorporate a filtered air input valve, which maintains a positive pressure in the cage relative to the room (all of the above cages were one of two models, Micrflo or Jag-75, from Allentown Caging and Equipment, Allentown, Pa.). Finally, vented cages with exhaust add a filtered exhaust valve (Thoren Maxi-Miser, Thorn Caging Systems, Inc., Hazleton, Pa.), so that air from the cage does not leak into the room. Each of the rooms studied used only one cage type, and there was no relationship between the size of the room and the cage type used. All of the rooms are equipped with ventilation systems designed to provide 10 complete air changes per hour. All animals were housed on crushed corn-cob bedding (Harlan Teklad, Indianapolis, Ind.), fed ad libitum with diets appropriate for the species (Harlan Teklad), and were under light:dark cycles of either 12 h:12 h or 14 h:10 h. All animals were used for research studies that had been approved by the University of Virginia Institutional Animal Care and Use Committee.

Domestic sampling. We sampled 43 living rooms (volume, 50 and 200 m^3) in houses in the Charlottesville area with at least one cat or dog for airborne endotoxin and the cat allergen Fel d 1. These samples were extracted and stored as described above.

Endotoxin assays. Because freeze–thaw cycles have been shown to significantly reduce the amount of endotoxin measurable in a sample, the extracts were assayed for endotoxin prior to storage by using the Limulus Amoebocyte Lysate test QCL 1000 (Bio-Whittaker/Cambrex, Walkersville, Md.), sensitive to 0.3 endotoxin units (EU)/ml (equivalent to 30 pg endotoxin/ml) (8). The sterile PBS used for extraction was used in each assay as a negative control and returned a value below the limit of detection in all cases.

Allergen assays. The samples from the rodent rooms were assayed using a two-site monoclonal assay for Rat n 1 and Fel d 1 and a monoclonal primary–polyclonal secondary assay for Mus m 1 (9). For each assay, 96-well plates were coated overnight with primary antibody diluted 1:1000 in carbonate–bicarbonate buffer. The plates then were washed and blocked with 1% bovine serum albumin in PBS containing 0.05% Tween-20. Samples were loaded, along with a standard curve in duplicate. After an hour, the plates were washed again and the secondary, biotinylated antibody was added. After the second incubation, streptavidin was added, and the plate was read in 0.1% H_2O_2–2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid). These allergen assays all were sensitive to 1 ng/ml.

Particle collection efficiency. A 20-liter/min air-sampling pump and an ICD were run in parallel in a room with artificially disturbed dust (using a vacuum cleaner without a filter) for 10-min periods (n = 8) to determine the collection efficiency of the ICDs. The 20-liter/min pump used the same type of prefilter for collection as was used to clean the ICDs. All samples were extracted overnight in 1 ml PBS and assayed for endotoxin and Fel d 1. The calculated flow rate of the ICDs was used to determine a sampling rate that takes into account the less-than-100% collection efficiency of the ICD.

Statistics. All mean levels are reported as geometric means with 95% confidence intervals (CI). For independent samples, t tests on log values were used to assess the significance of differences in mean levels of airborne allergens and endotoxin. All statistical tests were performed using SPSS 11.0, SPSS Inc., Chicago, Ill.

Results

Estimation of sampling rate. The flow rate of the 14 devices used in this study was 1.48 ± 0.09 m^3/min. The mean rate was used to determine the collection efficiency for ICDs sampling in parallel with a 20-liter/min air pump. The mean collection efficiency was 40.6% ± 9.0% for endotoxin and 51.7% ± 12.0% for Fel d 1. These were not significantly different. For the purposes of comparison to other studies, an estimated sampling rate of 670 liter/min was used, the product of the flow rate and a mean particle collection efficiency of 45%. We also have included the directly measured, absolute quantities of endotoxin or allergen collected over 24 h (Table 1). Using the estimated sampling rate, we have converted these quantities to airborne concentrations. For mouse and rat allergen, the concentrations ranged from < 0.01 to > 5 ng/m^3 and for endotoxin from < 1 to > 1000 pg/m^3.

Endotoxin. Figure 1 presents airborne endotoxin as a function of cage type and animal. Endotoxin levels from domestic living rooms are shown for comparison. The levels of endotoxin in all rooms with filter cage tops represent a 95% reduction from those from rooms with open cages, exceeding 99% for the cleanest rooms. The mean level of endotoxin in all animal rooms with filter tops without an exhaust port (5.64 pg/m^3; 95% CI, 3.71 to 8.31) was 100-fold lower than the mean level in rooms with open cages (567 pg/m^3; 95% CI, 385 to 835; $P < 0.001$). Meanwhile, rooms with vented cages with an exhaust port had significantly ($P < 0.001$) less endotoxin than did rooms with vented cages. The mean level of endotoxin in all animal rooms using filter tops without exhaust also was significantly ($P = 0.003$) lower than that in domestic homes with animals (15.1 pg/m^3; 95% CI, 9.09 to 25.3). Mean airborne endotoxin levels in nonvivarium research laboratories with rodent experimentation (4.28 pg/m^3; 95% CI, 2.60

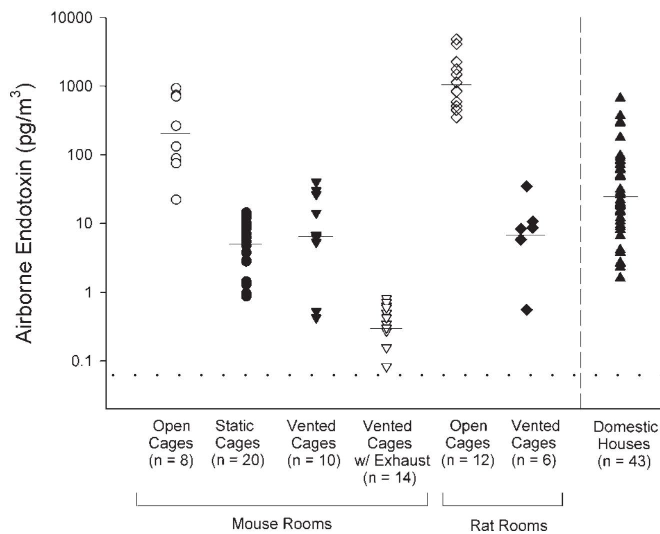

Figure 1. Quantities of airborne endotoxin in animal rooms, per cubic meter of air, by animal and cage type. Levels of endotoxin in domestic homes both with and without animals are shown for comparison. Dotted line represents assay threshold.

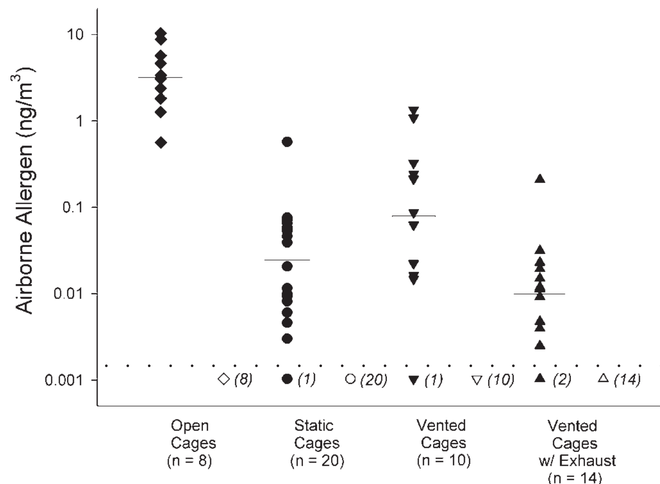

Figure 2. Quantities of the major mouse (Mus m 1; closed symbols) and rat (Rat n 1; open symbols) allergens per cubic meter of air collected in mouse rooms by cage type. Dotted line represents assay threshold.

to 7.06]) were not significantly less than that for animal rooms using filter tops without an exhaust port.

Mouse and rat allergen. Airborne samples from mouse rooms varied widely in allergen content, and the variation was mostly dependent on cage type (Fig. 2). The level of mouse allergen in all rooms with cage tops represents a 90% reduction from that in rooms with open cages, exceeding 98% for the cleanest rooms. The mean level of mouse allergen in all mouse rooms with filter tops without exhaust (0.037 ng/m^3; 95% CI, 0.020 to 0.070) was significantly ($P < 0.001$) lower than that of mouse rooms with open cages. Animal rooms with vented cages with exhaust had less airborne mouse allergen than did rooms with vented cages ($P = 0.013$). Fewer rooms and fewer cage types were available to sample for rat allergen (Fig. 3). Filter top cages were associated with a 99% reduction compared to open cages ($P < 0.001$). The mean level of airborne rodent allergen in all rooms using filter tops without an exhaust port in this study (0.024 ng/m^3; 95% CI, 0.013 to 0.044]) was significantly ($P < 0.001$) lower than that of the major cat allergen, Fel d 1, in 24 domestic homes with cats (0.73 ng/m^3; 95% CI, 0.36 to 1.47). All airborne samples from nonvivarium research laboratories were below the limit of detection for both Mus m 1 and Rat n 1.

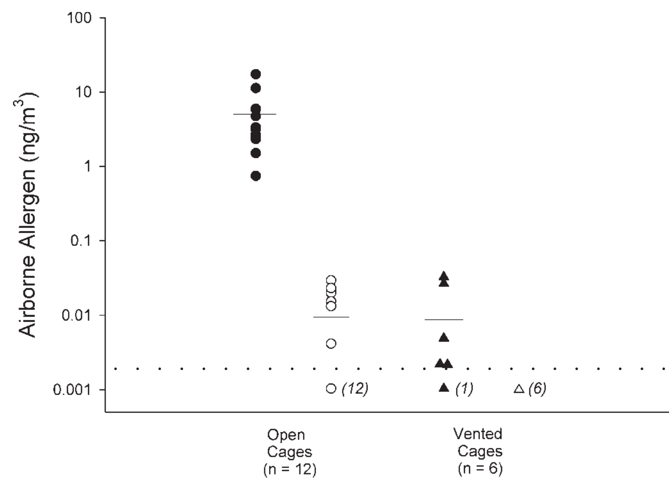

Figure 3. Airborne levels of the major rat (Rat n 1; closed symbols) and mouse (Mus m 1; open symbols) allergens in rat rooms by cage type. The detection of airborne mouse allergen in rat rooms is attributable either to passive transfer or some crossreactivity in the assay. Dotted line represents assay threshold.

Discussion

Our results suggest that the use of filter tops on individual cages is associated with large reductions in both airborne allergen and endotoxin, each of which have been shown to cause symptoms in animal room technicians (10, 11). Given the remarkable efficacy of cage tops, the question is whether further improvements are relevant. A recent study reported a moderate decrease in airborne mouse allergen levels (from 1.62 ± 0.56 ng/m^3 to 0.31 ± .08 ng/m^3) in an animal room by switching vented cages with exhaust valves from positive to negative pressure (2). This change was reported to be beneficial for animal handlers and appeared to be without negative consequence to the animals, because there was no increase in pathogen transmission (12). However, most animal housing, even where equipped with vented cages with filtered exhaust ports, is not capable of making this transition, which is unique to cages with positive individual ventilation (PIV). Further, that study only examined one type of PIV cage comparing positive and negative setups and did not give a complete context for the achieved reduction. In the results we present here, exposure to both animal protein and endotoxin was higher in many domestic homes when compared with rodent facilities using filter-topped caging of any configuration. This finding suggests that the use of filter tops can achieve a high level of control for both allergens and endotoxin.

The absolute exposure estimates for airborne allergen and endotoxin obtained by using pump-based sampling systems have varied greatly (Table 2). It is our belief that the use of low-rate (~20 liters/min) sampling methods sacrifices both range and sensitivity. The consequent airborne data are often confusing, and differences in exposure levels rarely are assigned statistical significance. In the case of endotoxin, the quantities collected are so low that measurement is only possible with a "low-level" assay (e.g., a cutoff of 0.006 EU/ml). This magnifies the Limulus Amoebocyte Lysate assay's existing difficulties with inter-assay variability and the risk of contamination (13, 8).

High-rate sampling is susceptible to two problems: 1) an artificially low estimate of allergen exposure per m^3 because the air was sampled repeatedly, and 2) disturbance of the room that increases the flux of allergen into the air. The second problem (disturbance of particles) impairs the ability of high-rate HEPA filters to clean the air of allergens (14-18). We argue that the device used here represents a middle ground. The flow rate at the outlet of the device is much lower than with most fan-driven devices, thus they are less

Table 2. Comparison of exposure estimates in published studies

Year	Group	Sampling rate (liters/min)	Sampling time (h)	Mus m 1 (ng/m^3)	Rat n 1 (ng/m^3)	Endotoxin (pg/m^3)
1999	Reeb-Whitaker et al. (16)[a]	20	12	Low: 1.1 ± 1.0 High: 5.1 ± 0.4	not applicable	not applicable
2001	Lieuter-Colas et al. (8)[b]	20	1	not applicable	Low: .43 ± 1.00 High: 27.36 ± .73	Low: 30 ± 790 High: 570 ± 730
2003	Schweitzer et al. (18)[c]	20	8	Low: 0.30 ± 0.06 High: 1.01 ± 0.18	not applicable	not applicable
2003	Pacheco et al. (14)[d]	15 (Mus m 1) 3 (endotoxin)	< 1	Low: 38 (0–92) High: 75 (14–179)	not applicable	Low: 219 (24–414) High: 566 (12–1463)
2004	Current report	670[e]	24	Low: 0.010 [0.005–0.020] High: 3.11 [1.75–5.44]	Low: 0.009 [0.003-0.016] High: 3.66 [2.23–5.99]	Low: 0.301 [0.197–0.477] High: 1080 [679–1710]

Data are presented as mean ± 1 standard deviation for the first three studies, mean (range) for the fourth study, and geometric mean followed by the 95% confidence interval (in brackets) for the fifth study.
[a]"Low" is the level in rooms with vented cages with exhaust; "high" is the level in rooms with open cages.
[b]"Low" and "high" indicate lowest and highest mean values among 12 rat rooms with identical cages of an unspecified type.
[c]"Low" represents level in rooms with vented cages with exhaust, "high" represents level in rooms with static cages.
[d]"Low" and "high" are the lowest and highest mean values from a variety of animal facility environments.
[e]This rate is estimated from the flow rate and collection efficiency of the ion-charging device.

likely to contribute significantly to the flux of particles into the air. Still, the higher sampling rate offers improved sensitivity and thus a better measurement of relative airborne levels between environments. We have recorded absolute differences between rooms of 1000-fold for both allergen and endotoxin.

A better understanding of the dynamics of excess sampling is critical for comparing our data to low-rate estimates. Animal rooms aim for a ventilation rate of 10 air changes per hour, whereas comparable values for domestic houses with the windows closed are often less than 0.5 air changes per hour. The implication is that flux of allergen or endotoxin from surfaces including cages in an animal house must be rapid (19). Although the factors that influence this flux are not well defined, it is generally considered that activity of animals, predominantly at night, is an important source. In addition, cage changing and cleaning add further variables, as do the type of bedding and the sex of the animal (20). Our previous results demonstrated that the quantity of particles carrying rat allergen becoming airborne from rat litter was critically dependant on the humidity of the litter (21). This same factor may well play a leading role in relation to dust on the floor of animal houses and in domestic houses.

The National Research Council (NRC) report "Occupational Health and Safety in the Care and Use of Research Animals" (11) recommends the use of personal protective equipment for animal workers who are "at risk of exposure to animal allergens." That recommendation is the basis for the occupational health criteria used by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Clearly, it would be valuable to have a standardized method of quantifying this risk. It is generally acknowledged that workers find respirators to be "hot and uncomfortable during prolonged use" (2). In our units, this is a leading source of animal technician complaints. For this reason, engineering controls are a more sensible method of reducing exposure. However, the claim that changing the pressure in vented cages from positive to negative represents a practical and available method of decreasing exposure ignores the prohibitive cost of equipping animal houses which do not have such technology, especially those where pathogen transmission is not a major factor and static cage tops are generally employed. In all of the animal rooms studied here, cage changes are performed in the animal room. It would be interesting to see the effect of isolating these cage changes in a separate room, as cage changes and animal transfers may have the largest effect on ambient levels in rooms with cage tops. It is likely that the effects of disturbance are variable for different allergens and for endotoxin. Because all allergen proteins, because of their molecular weights, have a saturated vapor pressure close to zero, the amount of airborne allergen is a function of the number and size of the particles that carry them. For example, cat allergen (particle size, 2 to 20 μm) is found airborne in undisturbed rooms, whereas mite allergen (particle size, 14 to 30 μm) is not (7, 16).

Ideally we would have a standard for measurement of airborne allergen and endotoxin, however there are many problems with defining one. Firstly, air-sampling techniques are still too variable. Secondly, the technique for assaying endotoxin is best carried out shortly after extraction and is inherently difficult to transfer to another site. Thirdly, the values for airborne allergen that are relevant to allergic subjects are so low that maintaining levels this low is generally considered impractical. Our measurements suggest that cages with filter tops are sufficient for diminishing the risk of personnel exposure to both endotoxin and allergen. In addition, there does not appear to be sufficient objective basis for the regulation that animal workers should wear respirators in rooms with cage tops. The implication is that the NRC guidelines and AAALAC requirements should be interpreted as applicable for technicians working in rooms with open cages or while they are changing cages.

Acknowledgments

These studies were supported by NIH Grant No. AI-20565, and we are grateful to The Sharper Image for an educational grant and for supplying the air-sampling device used in these studies.

References

1. **Reeb-Whitaker, C. K., D. J. Harrison, R. B. Jones, J. B. Kacergis, D. D. Myers, and B. Paigen.** 1999. Control strategies for aeroallergens in an animal facility. J. Allergy Clin. Immunol. **103:**139-146.
2. **Schweitzer, I. B., E. Smith, D. J. Harrison, D. D. Myers, P. A. Eggleston, J. D. Stockwell, B. Paigen, and A. L. Smith.** 2003. Reducing exposure to laboratory animal allergens. Comp. Med. **53:**487-492.

3. **Gordon, S., S. W. Fisher, and R. H. Raymond.** 2001. Elimination of mouse allergens in the working environment: assessment of individually ventilated cage systems and ventilated cabinets in the containment of mouse allergens. J. Allergy Clin. Immunol. **108:**288-294.
4. **Lieuter-Colas, F., P. Meyer, P. Larsson, P. Malmberg, N. Frossard, G. Pauli, and F. De Blay.** 2001. Difference in exposure to airborne major rat allergen (Rat n 1) and to endotoxin in rat quarters according to tasks. Clin. Exp. Allergy **31:**1449-1456.
5. **Menzies, D., J. Popa, J. A. Hanley, T. Rand, and D. K. Milton.** 2003. Effect of ultraviolet germicidal lights installed in office ventilation system on worker's health and wellbeing: double-blind multiple crossover trial. Lancet **362:**1785-1791.
6. **Pacheco, K. A., C. McCammon, A. H. Liu, P. S. Thorne, M. O'Neill, J. Martyny, L. S. Newman, R. F. Hamman, and C. S. Rose.** 2003. Airborne endotoxin predicts symptoms in non-mouse-sensitized technicians and research scientists exposed to laboratory mice. Am. J. Respir. Crit. Care Med. **167:**983-990.
7. **Custis, N. J., J. A. Woodfolk, J. W. Vaughn, and T. A. Platts-Mills.** 2003. Quantitative measurement of airborne allergens from dust mites, dogs, and cats using an ion-charging device. Clin. Exp. Allergy **33:**986-991.
8. **Milton, D. K., D. K. Johnson, and J. H. Park. 1997.** Environmental endotoxin measurement: interference and sources of variation in the Limulus assay of house dust. Am Ind Hyg Assoc J. **58:**861-867.
9. **Ferrari, E., A. Tsay, and P. A. Eggleston, A. Spisni, M. D. Chapman.** Environmental detection of mouse allergen by immunoassay for recombinant Mus m 1. J. Allergy Clin. Immunol. **108:**288-294.
10. **Bush, R. K., R. A. Wood, and P. A. Eggleston.** 1998. Laboratory animal allergy. J. Allergy Clin. Immunol. **102:**99-112.
11. **National Research Council.** 1997. Occupational health and safety in the care and use of research animals. National Academy Press, Washington, D.C.
12. **Myers, D. D., E. Smith, I. Schweitzer, J. D. Stockwell, B. J. Paigen, R. Bates, J. Palmer, and A. L. Smith.** 2003. Assessing the risk of transmission of three infectious agents among mice housed in a negatively pressurized caging system. Contemp. Top. Lab. Anim. Sci. **42(6):**16-21.
13. **Douwes, J., P. Versloot, A. Hollander, D. Heederik, and G. Doekes.** 1995. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl. Environ. Microbiol. **61:**1763-1769.
14. **De Blay, F., M. D. Chapman, and T. A. Platts-Mills.** 1991. Airborne cat allergen (Fel d 1). Environmental control with the cat *in situ*. Am. Rev. Respir. Dis. **143:**1334-1339.
15. **Gore, R. B., S. Bishop, B. Durrell, L. Curbishley, A. Woodcock, and A. Custovic.** 2003. Air filtration units in homes with cats: can they reduce personal exposure to cat allergen? Clin. Exp. Allergy **33:**765-769.
16. **Luczynska, C. M., Y. Li, M. D. Chapman, and T. A. Platts-Mills.** 1990. Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). Measurements using cascade impactor, liquid impinger, and a two-site monoclonal antibody assay for Fel d I. Am. Rev. Respir. Dis. **141:**361-367.
17. **Swanson, M. C., A. R. Campbell, M. J. Klauck, and C. E. Reed.** 1989. Correlations between levels of mite and cat allergens in settled and airborne dust. J. Allergy Clin. Immunol. **83:**776-783.
18. **Wood, R. A., E. F. Johnson, M. L. Van Natta, P. H. Chen, P. A. Eggleston.** 1998. A placebo-controlled trial of a HEPA air cleaner in the treatment of cat allergy. Am. J. Respir. Crit. Care Med. **158:**115-120.
19. **Swanson, M. C., A. R. Campbell, M. T. O'Hollaren, and C. E. Reed.** 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. Dis. **141:**1578-1581.
20. **Sakaguchi, M., S. Inouye, H. Miyazawa, H. Kamimura, M. Kimura, and S. Yamazaki.** 1990. Evaluation of countermeasures for reduction of mouse airborne allergens. Lab. Anim. Sci. **40:**613-615.
21. **Platts-Mills, T. A., P. W. Heymann, J. L. Longbottom, and S. R. Wilkins.** 1986. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. J. Allergy Clin. Immunol. **77:**850-857.