Journal of the American Association for Laboratory Animal Science
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Vol 45, No 6
November 2006
Pages 44-48

Particulate Matter in Animal Rooms Housing Mice in Microisolation Caging

Gregory L Langham,[1] Robert F Hoyt,[2] and Thomas E Johnson[3,*]

Reactions to allergens created by laboratory animals are among the most frequently encountered occupational illnesses associated with research animals. Personnel are exposed to these allergens through airborne particulate matter. Although the use of microisolation caging systems can reduce particulate matter concentrations in rooms housing mice, the operating parameters of ventilated caging systems vary extensively. We compared room air in mouse rooms containing 5 different types of caging: 1) individually ventilated caging under positive pressure with filtered intake air and exhaust air returned to the room (VCR+), 2) individually ventilated caging under negative pressure with exhaust air returned to the room (VCR–), 3) individually ventilated caging under positive pressure with exhaust air returned to the heating, ventilation, and air-conditioning (HVAC) system, 4) individually ventilated caging under negative pressure with exhaust air returned to the HVAC system, and 5) static microisolation cages. We found that rooms under VCR conditions had fewer large particles than did those under other conditions, but the numbers of 0.3 μm particles did not differ significantly among systems. Static, positive or negative pressure applied to caging units as well as route of air exhaust were found to have little influence on the total number of particles in the atmosphere. Therefore, considering the heat load, odor, and overall particulate concentration in the room, placing individually ventilated caging under negative pressure with exhaust air returned to the HVAC system appears to be the optimal overall choice when using microisolation housing for rodents.

Abbreviations: HEPA, high-efficiency particulate air; HVAC, heating, ventilation, and cooling; IVC, individually ventilated caging; SC, static microisolation caging; VCO–, individually ventilated cages under negative pressure and with exhaust air returned to the HVAC system; VCO+, individually ventilated cages under positive pressure and with exhaust air returned to the HVAC system; VCR+, individually ventilated caging under positive pressure with filtered intake air and exhaust air returned to the room; VCR–, individually ventilated caging under negative pressure with exhaust air returned to the room

Human allergies to laboratory animals are among the most common health concerns of animal care personnel,[25] and working with laboratory animals correlates with a high occupational risk for the development of animal-related allergies.[2,3,6,12,22] Between 10% and 46% of the more than 90,000 laboratory animal workers in the United States have developed allergies to laboratory animals,[2,13,26] and approximately 10% of these likely will develop a postexposure persistent occupational asthma.[7] Although the United States government has issued publications outlining steps employers should take to prevent occupational asthma in employees,[22,27] occupational exposure limits to allergens and particulate matter in the workplace are not highly regulated.

For the majority of laboratory animal species, the allergens of concern are in hair, dander, urine, and saliva.[3,13,35] Particulate matter generated by laboratory animals can lead to the development of species-specific allergies in humans.[2] The human health effects of inhaled particulate matter depend on total deposition and the total mass inhaled. Both of these measures show apparent site selectivity within the lung.[30] Factors that can increase the risk of allergenic exposures to laboratory animal personnel include those as simple as the type of contact bedding used to house rodents,[8,30,34] but the most important are the level of exposure to animal allergens and controls in place to reduce the transfer of allergens into the breathing zone.[2,3,6]

Since the 1980s, individually ventilated caging systems (IVCs) have been incorporated to house rodents used in biomedical research. In the past, published literature focused predominantly on improving the microenvironment for the rodent species using this type of housing.[39] The use of IVCs can safeguard valuable research rodents from specific animal pathogens[5,18] while allowing increased housing densities of rodents.[16]

The ventilation rates of IVCs vary from 25 to 120 air changes per hour and can be maintained with either positive or negative intracage pressure. The microenvironment or primary enclosure that contains the rodents has its own temperature, humidity, and gaseous and particulate composition of air. Temperature and humidity can differ significantly between the microenvironment and macroenvironment depending on the cage ventilation scheme.[4,20,21] Although optimal operating conditions to minimize allergen exposure are unknown, recent studies report a moderate decrease in airborne mouse allergen when IVCs are operated under negative pressure.[7,9,31]

IVCs can be configured with either a positive or negative pressure differential and with filtration of both supply and exhaust air through a high-efficiency particulate air (HEPA) filter. Regardless of operating mode, the air mixes with animal dander, urinary proteins, and ammonia in the microenvironment. When an IVC is under positive pressure to the room, supply air inflow exceeds the exhaust flow, so particulate matter may spill into the working environment of personnel. The negative ventila-

Received: 17 Apr 2006. Revision requested: 5 June 2006. Accepted: 5 June 2006.
[1]Centers for Disease Control and Prevention, National Center for Infectious Diseases, Scientific Resources Program, Animal Resources Branch, Lawrenceville, Georgia; [2]National Institutes of Health, National Heart, Lung and Blood Institute, Bethesda, Maryland; [3]Colorado State University, Department of Environmental and Radiological Health Sciences, Fort Collins, Colorado.
[*]Corresponding author. Email: Thomas.Johnson@colostate.edu

tion mode provides less supply air than exhaust capacity, so the air mixture containing allergenic particulate matter is retained within the cage and exhausted through the HEPA filter. Filtered exhaust air from the cage is then either reintroduced into the room or exhausted into the building heating, ventilation, and cooling (HVAC) system. Although these types of rodent housing systems pass air through a HEPA filter prior to delivery to each cage, an industry standard does not currently exist to document that rodent IVCs are truly self-contained. Optimal operating conditions to minimize the exposure of laboratory personnel to particulate, organic, and nonorganic matter created by laboratory animals are unknown.

In this study, we compared the particulate concentrations in mouse rooms containing microisolation caging operated under 5 different scenarios. Although the concentration of particulate matter cannot be directly correlated with allergen concentration, we viewed it as a marker of the filtration efficiency of the caging systems.

Materials and Methods

The data collection for this study used current technological and laboratory practices consistent with approved animal protocols of laboratory animal facilities within the National Institutes of Health (Bethesda, MD). Several primary predictor variables were used to assess the working environment of lab rooms: 1) the operating mode of the IVC as positive or negative to the animal room, 2) the rack exhaust returned to the animal room or exhausted out of the room, and 3) static caging versus IVCs. This study was conducted in 36 laboratory animal rooms (16,795 cages total) containing mice housed in either IVCs (Thoren Caging Systems, Hazelton, PA) or static Micro-Isolator™ cages (Lab Products, Seaford, DE). All of the animal rooms were composed of masonry block covered with epoxy resin paint and received 100% fresh air from their facility HVAC systems. Each room had ceiling-mounted air-intake diffusers. The number and location of diffusers varied, as did the number and location of exhaust air vents within rooms. All animal rooms were negative in pressure with respect to the hallways, as validated by an automated monitoring system. Each room was certified to have between 10 to 15 or more air changes per hour, as recommended in the *Guide for the Care and Use of Laboratory Animals*.[24]

Five basic microisolation configurations were examined in this study: positive pressure with room exhaust; positive pressure with external exhaust; negative pressure with room exhaust; negative pressure with external exhaust; and static. Rooms having IVCs with HEPA-filtered exhaust air *returned* back into the animal room were designated as VCR+ if the cages were operated under *positive* airflow pressure and as VCR– if they were under *negative* airflow pressure. Rooms having IVCs operating under *positive* airflow with HEPA-filtered air *out of the room* (that is, into the HVAC system) were labeled as VCO+, or VCO– if they were operating under *negative* airflow. Rooms with only *static* microisolation *caging* were labeled as SC. We assumed that each IVC leaked air containing particulate matter into the laboratory room while operating under positive pressure.

All animal room conditions were maintained on a 12:12-h light:dark cycle at 22 to 24 °C and 40% to 60% relative humidity in accordance with the *Guide for the Care and Use of Laboratory Animals*.[24] Temperature and humidity were recorded at each sample location by the use of a temperature and humidity probe (Royco, Hach Ultra Analytics, Grants Pass, OR) attached to the particle counter. In addition, 26 of the 36 animal rooms contained a Class II biological safety cabinet, but none of the

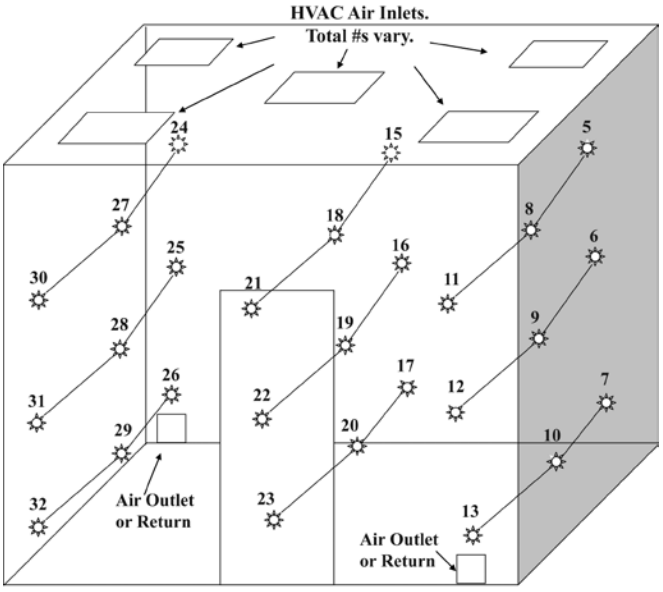

Figure 1. 3-dimensional room plan. Stations 1 through 4 were reserved for the HVAC inlets. Stations 14 and 33 to 35 were reserved for HVAC outlets. Stations 5 through 13 and 15 through 32 were sampled at 30.5, 152.4 (breathing zone), and 213.4 from the floor. The numbers of air inlets and outlets per room varied.

units were used before or during air sampling. No air samples were collected during the animal husbandry tasks of cage changing, room cleaning, or animal handling, because previous studies showed that these tasks affect the particulate counts in each room.[6,8,12,15,30]

Rooms under VCO–, VCR+, VCR– and SC conditions contained mice on hardwood bedding (BetaChip Hardwood Screened Lab Bedding, Northeastern Products, Warrensburg, NY). Rooms under VCO+ conditions housed mice on a similar bedding of woodpulp (catalog no. 001.10006, Tek-Fresh Laboratory Animal Bedding, Harlan Teklad, Madison, WI). The air in each room was sampled at predetermined locations in a 3-dimensional, vertical and horizontal design with numerically labeled stations (Figure 1); the exact location of each station varied according to room size. Rooms of similar size were located and used for sampling. All air samples were collected using pre-calibrated particle counters (model 243A, Hach Ultra Analytics, Grants Pass, OR). The particle counter used simultaneously measures aerosolized particulate matter at 0.3, 0.5, 5.0, and 10.0 µm in diameter as well as the volume of air sampled. The size range of air particles sampled accommodates the broad size range of particles in which laboratory allergens have been reported.[11] Results from this type of air sampling device are comparable to those from gravimetric sampling techniques,[19] and this device is particularly suited for comparative measurements.[37]

Air samples were collected with the air sampling probe for 65 s at each station at a rate of 1 L of air per minute (Figure 1); the sampling time includes the manufacturer's recommended 5-s purge at the initiation of each sample collection. Immediately prior to air sampling, the particle counter was automatically reset to 0. At the end of each collection time, particulate matter data were identified by station number and automatically stored in the particle counter memory. Air samples were taken at approximately the same location in each room relative to room dimensions without moving the IVCs, mobile shelving, or other equipment to maximize the accuracy of measurements

Table 1. *P* values of comparisons of particle number by size and cage type

Cage types compared		Particle size (µm)			
		0.3	0.5	5	10
Static	VCO–	0.8814	0.0475[a]	0.7687	0.6050
Static	VCO+	1.0000	0.2836	0.0763	0.5928
Static	VCR–	0.5890	0.0030[a]	0.0096[a]	0.0055[a]
Static	VCR+	0.6794	0.1112	0.6876	0.7243
VCO–	VCO+	0.9205	0.9394	0.0047[a]	0.0484[a]
VCO–	VCR–	0.9868	0.8699	0.0005[a]	0.0001[a]
VCO–	VCR+	0.9910	0.9952	1.0000	0.9991
VCO+	VCR–	0.6924	0.4444	0.9832	0.2974
VCO+	VCR+	0.7679	0.9947	0.0059[a]	0.1138
VCR–	VCR+	1.0000	0.6589	0.0005[a]	0.0003[a]

[a]Particle counts differ significantly ($P < 0.05$) between cage types.

of particulate counts within the working environment. The air at 30.5, 152.4, and 213.4 cm from the floor was sampled with the attached tripod probe. The 152.4-cm sampling point was equivalent to the breathing zone of laboratory animal personnel; at this level and within a 30.5-cm radius of the head, allergen levels can be as much as 15 times higher than those of ambient air.[15,28,36] Stations 15 to 23 sampled air from approximately the center of the room, whereas the outer stations (5 to 13 and 24 to 32) sampled air approximately 76.2 cm from the walls (Figure 1). Stations 1 to 4 were reserved for the HVAC inlets, and stations 14 and 33 to 35 were reserved for HVAC outlets.

At the completion of data collection for each room, data were downloaded by use of the software provided by the particle counter manufacturer (Hiac-Royco Logger, version 1.3, Hach Ultra Analytics). These files were then converted to Excel (Microsoft, Redmond, WA) file format as raw data. Variation between rooms was minimized by accounting for animal density (that is, number of cages) and room size (in m^3), by calibrating all particle counters, and by standardizing sample collection locations among rooms.

Data analysis. All data analysis was accomplished using SAS software (version 8.0, SAS Institute, Cary, NC). Data first were checked for normality by use of Shapiro-Wilkes and Kolmogorov-Smirnov tests as well as residual plots. Data then were log transformed, and variables were examined to determine whether grouping was statistically reasonable. Variables were grouped and parameters discarded according to results from analysis of variance at the 95% confidence level. Tukey-Kramer tests were performed to determine statistical differences among operating modes for the IVCs. Finally, models were produced to predict particle counts for a specific particle size and cage ventilation scheme. A P of <0.05 was considered statistically significant.

Results

The data were not normally distributed, and particle count data were log (base 10) transformed. The residual plots for the transformed data indicated near-normal distribution. Table 1 shows the statistical comparisons by cage type and particle count.

The number of cages did not have a significant effect on the number of particles in the atmosphere. Temperature had a minimal effect on the number of particles in the atmosphere. At most, a 10 °C increase in temperature increased the counts of 10- and 5-µm particles each by roughly 60% and 0.3-µm particle counts by 7%, with little change in the 0.5-µm particle count. The data did not indicate that any particular set of rooms had an unusually high number of particles in the inlet air.

Table 2. Highest and lowest particle concentrations as a function of cage type

	Particle size (µm)			
	0.3	0.5	5.0	10.0
Highest number of particles	VCO+	SC	VCR+	VCO–
Lowest number of particles	VCR+	VCR–	VCR–	VCR–
Difference	18%	70%	59%	58%

The number of 0.3-µm particles in the atmosphere did not differ significantly according to cage type. The number of particles in the atmosphere associated with the various caging systems is shown in Table 2. VCO+ cages reduced 5- and 10-µm particles, but the VCR– cages were superior. Table 3 shows the actual estimated particle counts for each caging system and particle size. Although the number of particles differs significantly between VCR– and VCO– operating conditions, the resulting effect is small and is unlikely to be relevant in a practical sense.

Discussion

This study quantified and compared the airborne particulate matter in laboratory animal rooms of similar size and animal population. Controlling for all known potential confounders in this study was impractical, but we documented as many numerical and non-numerical parameters as possible. Examples of potential confounders that could not be controlled, but may have affected the data, include: potential dead spaces in airflow at a specific sampling location (station number), particles produced or brought in by personnel entering the rooms during sampling, the levels of particulate matter brought in from the various HVAC systems, and leaking cages or HEPA filters. Another possible confounder was the type of bedding used (VCO+ caging did not have hardwood chip bedding; however, no particular trend was associated specifically with this cage type). A previous study examining the influence of bedding on the reduction of allergenic proteins found that the use of noncontact (absorbent) pads did indeed reduce allergens,[8] but further studies are needed to ascertain the influence of different types of bedding in combination with the various caging systems. We could not control for the age of each building, outdoor wind patterns, seasonal patterns affecting fresh air inlets, or possible local construction projects. Factors such as bedding changes and relative humidity, known to affect ammonia production rates in rooms housing mice in static microisolation cages,[21] were documented and controlled.

The number of small (0.3 and 0.5 µm) particles was not significantly influenced by cage operating mode because particles of this size are not effectively removed by HEPA filters.[40] Therefore, as expected, the sampling location, number of cages, time, and temperature all had greater influence on the number of 0.3-µm particles than did the type of cage. Although HEPA filters do not completely filter 0.5-µm particles, the operating mode of the cage does influence the number of these particles somewhat. At larger particle sizes, HEPA filtration is more effective and the significance of the number of cages is lost while the operating mode of the system becomes statistically significant, albeit irrelevant in a practical sense (Table 3).

Previous studies indicating that IVCs operating under negative-pressure mode reduce allergen levels in the macroenvironment[4,7,9] have led to a general assumption that the design of a room ventilation system will reduce room particulate counts,[17,20]

Table 3. Estimated number of particles present for a given operating mode and particle size

	Particle size (µm)			
Operating mode	0.3	0.5	5	10
Static	1.34×10^6	2.93×10^5	56	8
VCO-	1.16×10^6	7.85×10^4	79	12
VCO+	1.33×10^6	1.14×10^5	24	5
VCR-	1.08×10^6	5.08×10^4	21	3
VCR+	1.09×10^6	9.39×10^4	81	11

with the additional assumption that overall particle counts correlate with allergen concentrations.[29] However, our statistical modeling indicates that the operating mode had little practical effect on the overall level of particulate matter in the rooms.

Although no particular particle size is associated with endotoxin or mouse allergens, the total particle count appears to correlate with the amount of endotoxin and mouse allergens in the atmosphere.[28,29] Mouse care and research activities have the greatest influence on the amount of endotoxin and mouse allergens in the atmosphere.[29] Therefore, the operating mode of cages is only one factor in minimizing aerosolization of animal allergens. Current ventilated caging equipment incorporates allergenic controls regardless of the operating condition or setup of the animal room. Our study shows that counts of 0.3-µm particles and smaller are not influenced by cage operating mode. In addition, the IVC operating mode does not strongly influence counts of larger particulate matter. From a practical standpoint, the number of particles does not differ among operating modes (Table 3), because all of them have increased quantities of the smaller particles. Because particle size distributions are typically log-normal, we expect that small particles will comprise the majority of any sample.

Previous studies have shown aerosolized allergens range from 0.4 µm to larger than 6 µm in diameter.[8,12,28,30] Although particles of 0 to 10 µm are considered to be respirable,[10,38] the percentage deposition of these particles varies as a function of both particle size and anatomic location (that is, nose versus mouth). Total deposition increases with particles from 0.5 to 7 µm.[14] Particles larger than 10 µm tend to deposit in the upper respiratory tract, 4- to 10-µm particles in the thoracic region, 1- to 5-µm in the lower respiratory region, and particles smaller than 4 µm can reach the alveolar regions of the lung.[28,33] Although small particles (0.3 to 0.5 µm) are most likely to reach the deep respiratory tract and are more numerous than larger particles, larger particles are still inhaled and could cause deleterious effects within the upper respiratory tract. Reducing all particulate matter through caging, bedding, and other engineering methods should be a goal of the overall animal husbandry program.

Although our data show that the VCR– configuration yields the lowest particulate count, other factors, such as the heat load on the room from animals and odors from ammonia, are still present. According to our data, the IVC system configuration that best removes heat and animal cage-related smells is VCO–: ventilated cages under negative pressure with all air exhausted out of the room. This mode theoretically would have 3% higher particulate counts at 0.3 µm, 21% higher at 0.5 µm, and 58% higher at 5 and 10 µm than VCR–, which was statistically the best-performing system. However, as we stated earlier, considerations other than particle count influence caging decisions, especially where numerical differences between systems are slight. The VCO– configuration with the addition of a separate portable electrostatic precipitation unit may provide the best reduction of in-room particulate counts, heat, and odor.

To limit or control research and animal-related occupational exposures, three measures should be applied in a hierarchy. These should include engineering controls, work practices or administrative controls, and the use of personal protective equipment.[23,25] Practices that could reduce occupational exposures to allergens and improve air quality of the working environment include providing 100% HVAC fresh air into the animal rooms,[24] using IVCs that accommodate less frequent cage changing,[24,32] and increasing the room ventilation rate for rooms housing mice in static microisolation cages[21] according to heat load calculations by species.[1] In addition, the use of filter-top bonnets on static microisolation cages may reduce rodent room allergen concentrations.[8,12,31,34]

The importance of using personal protective equipment cannot be overemphasized in reducing exposure to particulate matter containing allergenic substances. The National Institutes of Health's Laboratory Animal Allergy Prevention Program has recommended the use of an N-95 disposable respirator for personnel with known allergies to laboratory species.[23] Because personnel may find that the prolonged use of respirators is hot and uncomfortable, the use of powered air-purifying respirators, which provide cool air to the face, may be an alternative that is more acceptable to workers and result in better compliance with the use of personal protective equipment.

In light of our data, we suggest that the use of VCO– cages with continuous filtration and ventilation to room exhaust yields the best overall conditions for workers when heat load, odor, and particle counts all are viewed as important. The implications and outcome of this study may assist facility directors and occupational health and safety specialists in designing programs to minimize risks to the health and safety of laboratory personnel while safeguarding of the animals housed in individually ventilated caging systems.

Acknowledgments

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the United States Government, Centers for Disease Control and Prevention, the National Institutes of Health, or Colorado State University. The authors would like to acknowledge the generous assistance from D Wilson and the staff of the Occupational Health and Safety Branch of the National Institutes of Health and thank D Smith for his consistent encouragement and Aimee Buelow for her assistance with this manuscript.

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