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Disparities in Ammonia, Temperature, Humidity, and Airborne Particulate Matter between the Micro-and Macroenvironments of Mice in Individually Ventilated Caging

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Animal room environmental parameters typically are monitored with the assumption that the environment within the cage closely mirrors the room environment. This study evaluated that premise by examining macro- (room) and microenvironmental (cage) parameters in individually ventilated cages housing mice with variable amounts of bedding over a period of 17 d without cage changes. Intracage ammonia levels remained within recommended human guidelines but were higher than room levels, confirming that microisolation caging is efficient at preventing ammonia generated from animal waste from escaping into the room. Humidity and temperature within cages were consistently higher than room levels. Particles in the room predominantly consisted of fine particles (diameter less than 2.5 μm), presumably from the ambient atmosphere; some of these particles were found in the cage microenvironment. In addition, mouse activity within cages produced larger particles, and these particles contributed to substantially higher aerosol mass concentrations within the cage. These findings demonstrate that, although cage and room environmental parameters differ, knowledge of room environmental conditions can be used to predict certain conditions within the cage. This association is relevant in that typical animal care standard operating procedures rely on room measurements, not intracage measurements, which arguably are more important for assessing animal welfare. Further, location and ambient climate can influence particle concentrations in the room, and consequently within the animal cage, suggesting local weather patterns and air quality may account for variability among studies conducted at sites that are geographically divergent.

The environments in which laboratory mice are housed have a profound effect on mouse health, welfare, and the validity and reproducibility of scientific data. The environment at the cage level (that is, the microenvironment or primary housing) varies depending on caging type, air changes per hour within the cage and room, animal density, bedding type, and ambient environment. The room environment (that is, the macroenvironment or secondary enclosure) can be affected by human activity, building ventilation and air conditioning, cage density, cage ventilation type, and season. Measurements of environmental parameters have shown an inherent link between the cage (microenvironment) and room (macroenvironment).[17]

During the last several decades, modernization of housing and husbandry techniques for rodents primarily has sought to ensure biosecurity and biocontainment for animals, pathogens, and personnel.[16] Animal husbandry and environmental standards for the cage-level environment are intended to provide optimal animal welfare.[18] Isolator or filter-top cages were developed more than 40 y ago and are effective at maintaining pathogen-free rodent colonies and reducing cage-to-cage and room-to-cage transmission of airborne pathogens. In addition, isolator and filter-top cages help to protect personnel from animal-derived allergens.[2] However, the use of such static microisolation caging typically results in high ammonia and humidity levels in the microenvironment.[27] Past studies using mircoisolation housing have demonstrated that the temperature of air can have a key role in ammonia production.[23] The goal of improving environmental conditions in animal housing facilities has given rise to an evolution of designs for rodent isolator caging systems.[22] Individually ventilated cages have an isolated air supply, which can allow for higher-density housing of mice.[5,13,22,24] These caging developments have provided an improvement in environmental parameters when assessed by engineering standards developed for human exposures. Housing and husbandry practices are important variables that may influence the outcome of a research study, either by having a direct effect on animal behavior or physiology or indirectly by altering animal susceptibility to disease or by controlling the pathogen's environment.

Because many animal models are used extensively in different geographic regions and maintained by using significantly different housing types, macroenvironmental parameters that are not controlled at the room level (that is humidity, temperature, and airborne particulates) may alter the cage microenvironment in a way that is geographically or regionally sensitive. Conversely, microenvironmental or cage-level parameters with the capacity to influence the macroenvironment (that is, ammonia, particulate matter formed from animal activity) may have pronounced effects on human and animal health. Cage-level parameters are difficult and costly to monitor, so laboratory facilities typically rely on room-level parameters to estimate the animal's local

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conditions. Therefore, this study was initiated with the intent of monitoring the effects of room level environmental parameters on cage microenvironment (and vice versa) and to determine whether room-level measurements reliably reflect the animal's environment.

Materials and Methods

Animals. Female ICR mice (n = 80; age, 9 wk) were obtained from a commercial vendor (Harlan Teklad, Madison, WI) and housed in a semibarrier animal room. According to health surveillance programs performed by the vendor and research institution, the mice were free from infections with mouse hepatitis virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, parvovirus, minute virus of mice, pneumonia virus of mice, epizootic diarrhea of infant mice, adenovirus, ectromelia, rotavirus, lymphocytic choriomeningitis virus, cytomegalovirus, polyoma virus, Sendai virus, and *Helicobacter* spp. The cages contained autoclaved aspen bedding chips (Harlan Teklad) at 3 different volumes as described previously.[34] Briefly, 5 cages each housing 5 mice per cage were provided with 250, 400, or 550 mL bedding. Internal control cages (n = 2) contained 400 mL bedding; one cage had 5 mice and was maintained on the typical 7-d cage change cycle during the experiment, whereas the other control cage was on the experimental 17-d cage-changing cycle but housed no mice. Mice were provided pelleted food (Harlan Teklad 8640) and access to water ad libitum by water bottles. The initial average weight of the mice was 28.07 g ± 1.97 g, and the average weight of the mice on the final day of the study was 29.89 g ± 2.16 g.

All research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to the principles stated in the *Guide for the Care and Use of Laboratory Animals*.[17] The protocol was approved by the Colorado State University Institutional Animal Care and Use Committee and was performed in an AAALAC-accredited facility.

Experimental design. Throughout the study, mice were housed in cages with dimensions of 19.56 cm × 30.92 cm × 14.93 cm (model 9, Thoren Caging, Hazelton, PA). This individually ventilated system recirculated room air. The rack unit had 2 separate HEPA filters. The supply module provided HEPA-filtered air to the system, and the exhaust module filters spent air from the unit. The rack was tested to calculate average air changes per hour prior to housing any mice in this study. Measurements ranged from 78.3 to 98.1 (average, 90.5 ± 4.1) air changes hourly for the 40 randomly sampled locations on the rack.[34] Modifications were made to all 17 cages so that cage air samples could be taken in situ to minimize disturbance to the mice. This modification consisted of inserting a small sampling port (diameter, 1.9 cm) into the front of the polycarbonate cage. The room and all the cages were monitored on a daily basis for humidity, temperature, ammonia, and particulate matter. A single sampling hose was used throughout the study. The hose was external to the cage and connected to the exterior of the sampling port, making an airtight seal. The cages were maintained under positive-pressure ventilation, therefore when the exclusion cap was removed, cage air would flow out the sampling port and into the tube. The tube was purged between samples, and any debris was removed.

Ammonia concentration was measured by using a photoionization detector, (MiniRae2000 Portable VOC Monitor, RAE Systems, San Jose, CA) that was calibrated to an isobutylene standard (Calibration Gas Mixture, NorLab, Boise, ID) according to the manufacturer's recommendation. The detector records the air concentration of specific gases in parts per million every 3 to 5 s. In addition to using the photoionization detector, ammonia in the room and all cages was also measured on day 17 by using a pump (Kwik-Draw Pump, Mine Safety Appliances, Pittsburgh, PA) and ammonia detector tubes (804405, Mine Safety Appliances). Both temperature and humidity were measured by using a hygrometer (Traceable Calibration Control Company, Fisher Scientific, Friendswood, TX) that was calibrated by the manufacturer before use.[34]

Particle measurements were made by using an aerodynamic particle sizer (model 3321, TSI, Shoreview, MN). The instrument counted and sized airborne particles within the range of 0.5 to 19.8 μm aerodynamic diameter. This device also estimated aerosol mass concentration within this range by assuming a standard particle density (density of water, 1.0 g/cm^3). A calibrated volume of air was introduced into the analyzer through a sampling hose (inner diameter, 1.3 cm) that was connected to the cage's sampling port. Each sample was collected for 120 s. Samples were taken between 0800 and 1000 every day.

The aerodynamic particle sizer had a flow rate of 5 L/min, whereas the photoionization detector had a flow rate of 400 mL/min. The cage volume was approximately 9 L, and ventilation was calculated to approximately 1.5 changes per minute.

Statistical analysis. For the aerosol analyses, cages were grouped as a single set (microenvironment); resulting data were compared with room (macroenvironment) particle data. Particle concentration data were analyzed by using a mixed linear model for repeated measures, with day as a within-cage factor and bedding volume as a between-cages factor. Separate models were used for each particle size and total concentration. The dependent variable, particle number concentration, was transformed to natural log scale to normalize model residuals. If the particle count for a particular day and cage was zero, a value of 0.5 was substituted before obtaining the natural log. After goodness-of-fit indices for several covariance structures were compared, a compound symmetric covariance structure was chosen to model the covariance over time within cage. Analysis of ammonia, temperature, and humidity data was performed according to bedding volume groups by using ANOVA with a significance level of 0.05. Differences between the environmental parameters were analyzed by using a 2-tailed t test with unequal variance. In subsequent sections, cages grouped by bedding volume are labeled as 'micro' whereas the room is labeled as 'macro.' All statistical analyses were performed by using SAS version 9.1 for Windows (SAS Institute, Cary, NC), with significance defined for P values less than 0.05.

Results

Measurable quantities of ammonia were not identified in the mouse room over the course of the study. The mouse cages also had undetectable levels of ammonia for the first 11 d; however intracage ammonia levels were detectable starting on day 12 and tended to increased over time (Figure 1). Over the 17-d period, no microenvironmental value of ammonia exceeded 5.0 ppm, and the 15 microenvironments containing mice averaged less than 0.2 ppm overall. These levels are well within recommended human guidelines;[1] no guidelines exist specifically for mice.

Temperature was always lower in the room than in any cage during the 17 d of the study (Figure 2). All of the readings from both the room and cages were within the referenced guidelines of 64 to 79 °F on all days.[17] The difference in temperature between the microenvironments and macroenvironment was statistically significant ($P < 0.00001$) and ranged from 0.5 to 5.3 °F, with an average difference of 1.4 °F over the course of the study.

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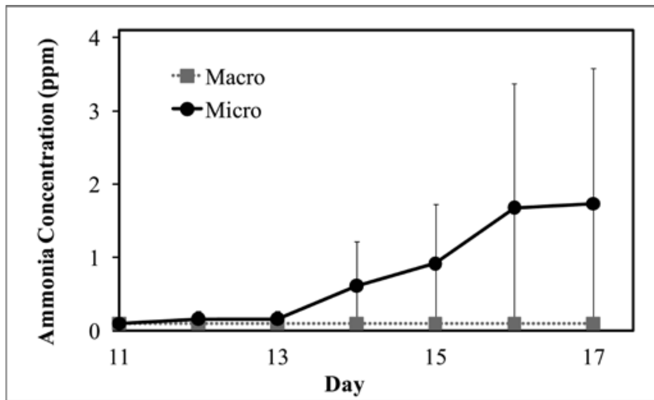

Figure 1. Average ammonia levels across all cages and the room during days 11 to 17 of the study. Error bars represent 1 SD of all cages averaged. Ammonia levels began to rise on day 12 and averaged 1.6 ppm for all the cages on day 17. The room ammonia levels remained below the limit of detection throughout the study.

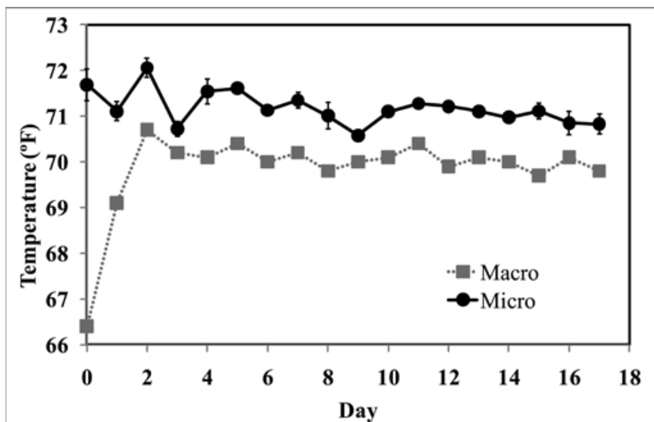

Figure 2. Average daily temperatures in cages and the room during the study. Error bars represent 1 SD for all cages together. Temperature was always lower in the room than in any of the cages during the 17 d of the study.

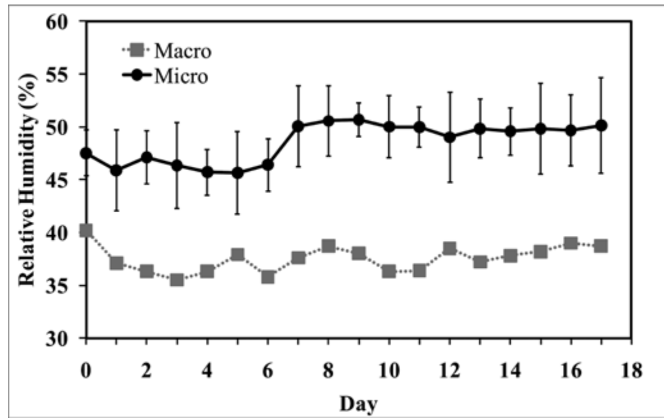

Figure 3. Average daily relative humidity in all cages and the room during the study. Error bars represent 1 SD of all cages averaged. At all time points, relative humidity was lower in the room than in any of the cages.

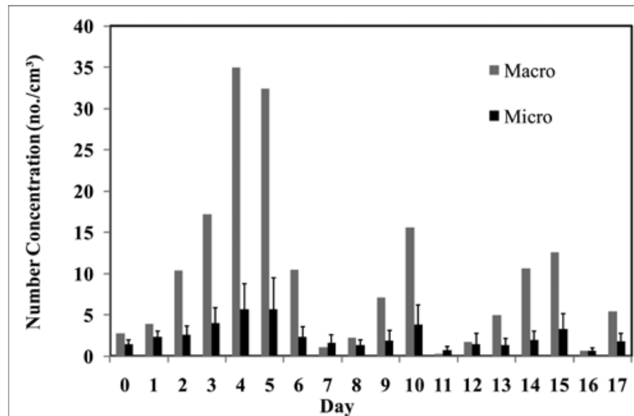

Figure 4. Average number of particles (diameter, 0.5 to 20 µm) per unit air volume within cages and the room during the 17-d study. Error bars represent 1 SD of all cages averaged. The mouse room had higher numbers of particles per unit air volume than did any of the cages on 14 of the 17 d during the study.

At all time points, relative humidity was lower in the room than in any of the cages (Figure 3), although all measurements were within referenced guidelines. The range of the difference between the 2 environments (cage and room) varied from 7.3% to 13.7%. Averaged across all days, cage relative humidity was 11% higher than that in the room, and this difference was highly significant ($P = 3.0 \times 10^{-19}$).

Because particle data were not normally distributed, values were log-transformed for statistical analysis. Mixed-model ANOVA was used to evaluate whether day, volume of bedding, or a day–bedding volume interaction was associated with particle number concentration within the cage. Small particles (less than 0.7 µm) were significantly influenced by day ($P < 0.0001$), bedding volume ($P = 0.011$), and the day–bedding volume interaction ($P = 0.002$). Medium-sized particles (1.3 to 4.4 µm) primarily were predicted by day of measurement. The primary predictor of particle counts for large particles (larger than 5.0 µm) was volume of bedding (Table 1).

On 14 of the 17 d during the study, the mouse room had higher particle numbers per unit air volume than any of the cages ($P < 0.0001$; Figure 4). There was no distinct pattern between this parameter and study day. However, day-to-day fluctuations in particle number per unit air volume occurred in both the room and cage, such that when room concentrations of particles were high, so were cage concentrations.

Linear regression analysis (Figure 5) of particulate matter concentrations in the room and cage revealed that the number of particles per unit air volume in the macroenvironment can be used as a predictor ($y = 6.49x - 6.18$; $R^2 = 0.93$) for that in the microenvironment. This relationship was dominated by the high concentration of small particles (that is, aerodynamic diameter less than 1 µm).

Notably, particle mass per unit air volume in the cages was always orders of magnitude greater than that measured in the room during all 17 d of the study (Figure 6). Study day and particle mass concentration showed no association in either the room or cages. Unlike the situation with particle number concentration, linear regression analysis of particle mass concentration in the room and cages demonstrated no relationship between the 2 environments.

The distribution of the mass of particulate matter, as a function of particle size, for both the room and cages is shown in Figure 7. As in Figure 6, the particle mass per unit air volume in the room was substantially less than the cumulative cage average. The large particles (that is, diameter greater than 10.0 µm) found in the cages comprised the majority of the total particle mass measured.

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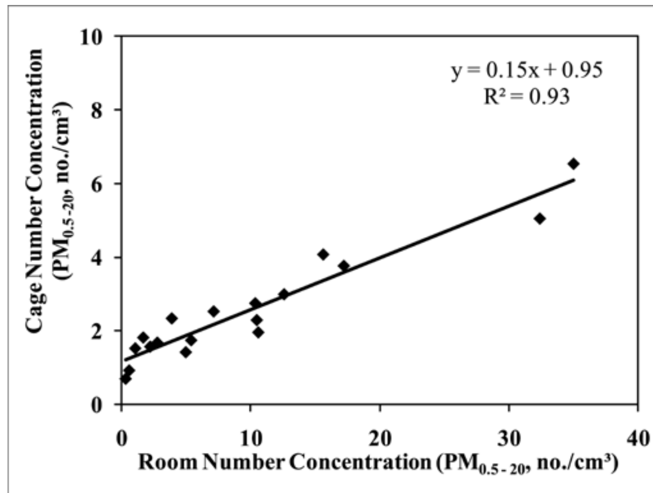

Figure 5. Linear regression graph of particle number per unit air volume (that is, particle number concentration) in the room and cages throughout the study. The number concentration of particles in the macroenvironment can be used as a predictor for that in the microenvironment; however, the particle mass concentrations in the two environments were not correlated.

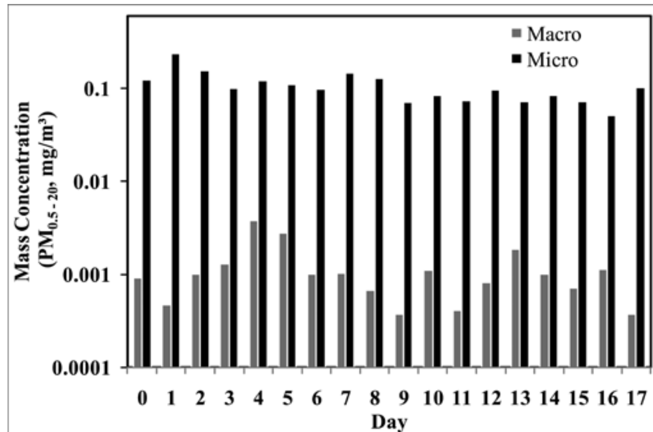

Figure 6. Daily particle mass per unit air volume for the cages and room during the 17-d study. Mass concentration of the particles measured in the cages was orders of magnitude greater than that in the room during the 17 d of the study. Note the *y* axis is log scale.

Discussion

This study evaluated the influence of the microenvironments of individually ventilated caging on the room macroenvironment and vice versa with respect to important environmental parameters. The microenvironment in an individually ventilated cage can affect both animal health and scientific study results and is influenced directly by the animal room macroenvironment. In addition, the environmental parameters within the room are influenced by variables such as weather, facility location, and engineering controls. Individually ventilated caging systems can be configured to have either positive or negative pressure related to the room, and filtration of both supply and exhaust air can occur through HEPA filters. Regardless of operating mode, the supply air mixes with animal dander, urinary proteins, and ammonia in the microenvironment of the cage.[20] The microenvironmental temperature, humidity, and gaseous and particulate composition can differ significantly from those of the macroenvironment, depending on the cage ventilation scheme and even the volume of bedding in the cage.[22,34]

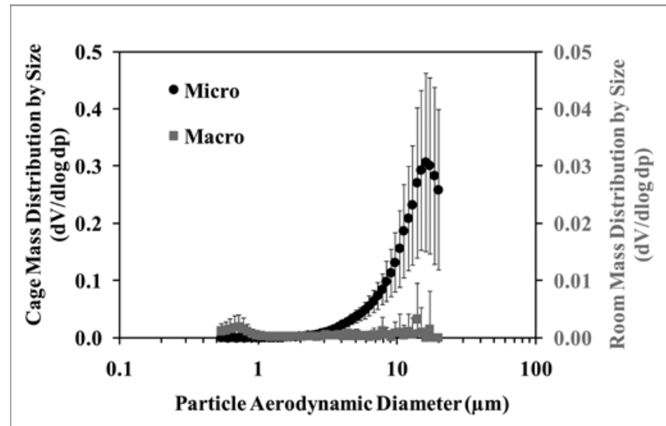

Figure 7. Daily mass distribution by size of particles within the room and cages during the 17-d study. Error bars represent 1 SD. The room's particle mass was substantially smaller than the cumulative average of the cage's particle mass, and the large particles (diameter, greater than 10.0 µm) found in the cages comprised the majority of the total particle mass measured.

Benefits of individually ventilated caging include reduction in the spread of infectious diseases within rodent colony mice[7,13] and reduction in ambient rodent allergen concentrations and odors in the room if animals are housed under negative pressure.[20] Disadvantages include high costs associated with purchase, operation, and upkeep.[32] Previous studies have demonstrated that the microenvironment of static caging is influenced heavily by the room macroenvironment.[21] Our current study demonstrates that a significant relationship between the room and cage environments also exists.

Ammonia was not detected in the room during the 17-d study. None of the cages had detectable ammonia until day 12, and throughout the study, no individual cage level exceeded 4.8 ppm (Figure 1). Ammonia levels detected using the PID and the Kwik-Draw Pump/detector tubes showed no statistical differences between the 2 devices.[34] These observations indicate that caging airflow and filtration prevented microenvironmental ammonia from contaminating the room, thereby preventing human exposure. The high number of air changes per hour in the caging and the relatively low humidity levels in the room likely were responsible for this finding. Other reports indicate extensive variations in ammonia levels depending on the type of housing, time of year, current climate during experimentation, and the methodology of detection;[27,31-33] therefore, extrapolation of our current findings to other caging systems and facilities should consider these additional factors. Because we tested only 1 type of mouse housing and because room humidity in the current study (35.8% to 40.2%) was at the low end of recommended ranges, future studies should evaluate ammonia production over time by using other types or brands of caging at different humidity levels. However, the current study clearly demonstrates that microenvironmental cage ammonia levels are likely to exceed room ammonia levels when individually ventilated caging is used.

All temperatures measured in this study were within the ranges (18 to 26 °C; 64 to 79 °F) recommended by the *Guide for the Care and Use of Laboratory Animals*.[17] No temperature differences were noted between bedding volume groups, and no temporal relationship between temperature and day after cage change was noted. However, room temperature was consistently lower (1.4 °F on average over the course of the study, with a range of 0.5 to 5.3 °F) than the temperatures of the cages. These results suggest that room temperature is likely to underestimate

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cage (microenvironmental) temperatures for mice housed in individually ventilated caging. This bias particularly should be considered when room ambient temperatures are at the low or high end of recommended or required ranges.

The *Guide* states that relative humidity in animal housing areas should be between 30% and 70%.[17] Humidity in the room and in all cages stayed within this recommended range during the current study. Relative humidity was consistently 7.3% to 13.7% (average, 11.0%) lower in the room than in any of the cages, a difference with high statistical significance. Although the *Guide* states the environment must be maintained within certain relative-humidity guidelines, our findings show that the animal room is not strictly representative of the microenvironmental conditions of individually ventilated caging. This lack of association is particularly relevant in facilities without humidity controls and that might experience ambient humidities below 30% or above 70% for part or all of the year. For example, our data suggest that intracage humidity would remain above the 30% range as long as room humidity exceeds approximately 20% at temperature ranges and air change rates similar to those in the current study. Conversely, our data imply that room relative humidity near the upper limit of the recommended range (70%) could push the relative humidity in cages above this limit. As noted previously, humidity levels play a pivotal role in ammonia production, as higher humidity enhances bacterial generation of ammonia.[11] High humidity levels prevent fecal and urinary desiccation and provide the optimal setting for bacterial propagation and subsequent ammonia production.[26] Humidity levels have also been shown to effect microenvironmental particle levels. For example, mouse allergens were shown to decrease from 3 ng/m^3 at 15% relative humidity to 0.5 ng/m^3 at 65%. This decrease in particles is presumed to reflect decreases in static charge as relative humidity increases and therefore increases in particle adhesion forces.[35] Low humidity has been associated with disease conditions such as ringtail and dermatitis. Consequently, humidity levels can affect experimental results and animal health and therefore should be considered when comparing experiments from different institutions and determining environmental standards for animal facilities in humid or arid environments.[10,25]

Particles generally are characterized by size,[15] which is a determining factor for deposition and site selectivity within the lung.[8,29] The health effects of inhaled particulate matter can depend on both the total mass inhaled and the particle number per unit air volume. The total number per volume is a gross count without regard to the size of the particles, whereas the mass concentration is based on the product of total particulate volume and average particle density. Mass concentration typically is dominated by large particles because particle volume is associated with the cube of particle diameter, assuming spherically shaped particles (*Mass* is proportional to *density* × *diameter*^{3}). For example, a 10-µm particle is 1000 times more massive than a 1.0-µm particle of equal density.

Particulate matter air pollution consists of both solid and liquid compounds suspended in air and usually represents a mixture of contaminants from various sources.[8] Fine-particle air pollution (defined as having diameters less than 2.5 µm in aerodynamic diameter) has been linked to increased human morbidity and mortality.[30] Exposure to particulate matter may result in inflammation and neoplasia.[3,14] Laboratory housed rodents may be exposed to variable concentrations of particulate matter. Particles can stimulate airway inflammation or elicit an immune response in humans and are likely to have similar effects on laboratory housed animals.[30] The effect of particles on humans and research subjects depends on particulate composition, which may include biologic materials such as waste, bacteria, dander, pollen, and viruses.[15] Although guidelines for mass and number concentration of particles in the environment have been established for humans, similar recommendations are not available for laboratory animal housing.

Exposure to airborne rodent allergens in housing areas is a function of both the number and size of particles in the environment.[28] Therefore, quantification of particulate matter concentrations can help identify specific activities that generate high levels of particles and airborne allergens.[19] Particle levels in animal rooms are highly variable throughout the day, depending on caretaker activity levels. Because changing cages accounts for most of the animal caretaker's day, and this action generates high particle counts, any practical reduction in exposure to particles is dependent on decreased particle generation during cage changes.[19] Factors that can increase the risk of allergenic exposures to laboratory animal personnel include those as simple as the type and presumably the volume of bedding used to house rodents.[12,29] However, the most important risk factors to personnel are the actual levels of particle exposure and the controls in place to reduce the transfer of particles (allergens) into the worker's breathing zone.[4,6,9]

Airborne particles are present both within the cage and the room as demonstrated in this study. No temporal relationship between particle number and mass concentration in the cages or room was determined over the 17 d period, suggesting fecal and urine content do not significantly alter the character of airborne particles within the room or cage. Room air contained many relatively small particles, whereas the cage contained a small number of large particles which comprised the majority of the total particle mass within the cage (Figure 8). These data suggest that these large particles in the cages most likely were generated from within and, despite high levels of filtration, a small percentage of ambient particles enter the cage from the room or ventilation ducts.

Particle concentration measurements as a function of size and their interactions with either bedding volume or day (Table 1) demonstrate that small particles are related to day of sampling, whereas large particles were related to bedding volume. The association of fine-particle count with day supports the conclusion that the small particles are introduced to the room by the HVAC system by means of the ambient atmosphere. Small particles derived from outdoor air most likely would vary by the time of day, local weather, air-quality patterns, and ambient air pollution levels. Conversely, because greater amounts of bedding were associated with higher numbers of large particles, large mass particles apparently are generated by the movement of animals within the cage. The lack of relationship between particle mass concentration and day suggests that the production of particulate matter within the cage is independent of the degree of cage 'cleanliness.' Therefore, the cage-change interval may not affect the particle mass concentrations within the cage. Similar studies did not demonstrate detrimental effects of particles on mouse health, behavior, or wellbeing during the 17 d with no cage changing,[34] and the natural habitat of mice would suggest they are well-adapted to long-term exposure to large particulates generated by movements within the nest.

In summary, we found no temporal relationship between particle number and mass concentration in either the cages or room, suggesting these parameters are independent of cage-change interval. The room (macroenvironment) contained many relatively small particles, which apparently penetrated into cages (Figure 5). Although the cage (microenvironment)

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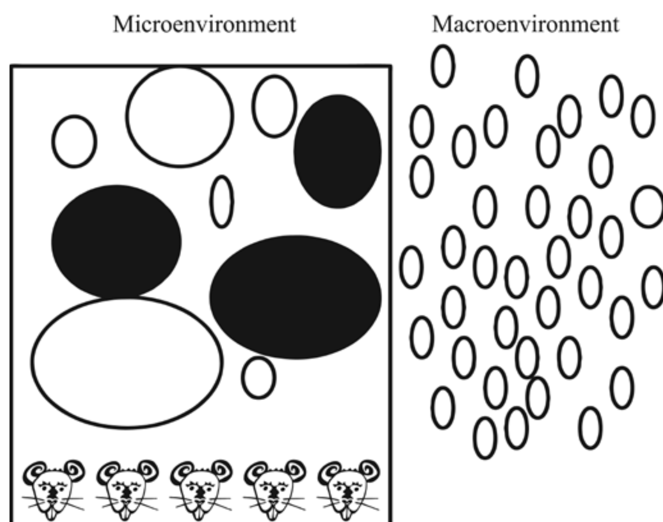

Figure 8. Schematic depiction of particles in cage and room showing fewer yet larger particles in cages but more yet smaller particles in room.

Table 1. *P* values for particle number per unit air volume in individually ventilated cages as a function of particle size and related to day, bedding volume, and their interaction

Particle size (µm)	Day	Volume	Day × volume
0.54	**<0.0001**	**0.012**	**0.029**
0.67	**<0.0001**	**0.011**	**0.002**
0.78	**<0.0001**	**0.031**	**0.001**
1.29	**0.003**	0.288	0.115
2.46	**0.006**	0.104	0.066
3.79	**0.021**	0.062	0.104
4.37	**0.006**	0.074	0.119
5.045	0.062	**0.044**	0.082
7.23	0.311	**0.018**	0.181
10.38	0.488	**0.016**	0.206
12.86	0.590	**0.013**	0.322
14.86	0.637	**0.009**	0.161
17.15	0.589	**0.010**	0.233
>19.81	0.627	**0.005**	0.124

Bold type indicates significant variables.

also contained large particles that contributed to the majority of within-cage mass concentrations, these large particles were not detected within the room macroenvironment. We hypothesize the variable spikes in particle number concentration in the room correlated with ambient weather patterns, suggesting the outdoor air quality has an important influence on room particle number concentration, which in turn affects the number concentration of particles within the cage. This finding may be highly relevant when comparing studies performed at urban versus rural locations or in areas strongly affected by fine and ultrafine particulate matter air pollution.

This study documents significant interaction between room and cage microenvironments for mice housed in individually ventilated caging and simultaneously demonstrates significant differences between measurements taken in the room versus at cage level. These findings are important for consideration of regulatory requirements and guidelines for environmental parameters at the cage level—particularly with respect to temperature and humidity. Finally, we have demonstrated that individually ventilated caging is highly efficient at preventing contamination of ambient room air with potentially allergenic particles and ammonia.

Acknowledgments

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