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Comparison of Indoor Air Quality between 2 Ventilation Strategies in a Facility Housing Rhesus Macaques (*Macaca mulatta*)

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Adequate indoor-air quality (IAQ)—defined by the temperature, relative humidity, and the levels of carbon dioxide, small particles, and total volatile organic compounds (TVOC)—is crucial in laboratory animal facilities. The ventilation standards for controlling these parameters are not well defined. This study assessed the effect of 2 ventilation strategies on IAQ in 2 rooms housing rhesus macaques (*Macaca mulatta*). We hypothesized that using a demand-controlled ventilation (DCV) system with a baseline ventilation rate of less than 3 fresh-air changes per hour (ACH) would maintain IAQ comparable to or better than the traditional constant flow rate (CFR) system at 12 fresh ACH. During a 60-d study period, each of the 2 rooms operated 30 d on DCV and 30 d on CFR ventilation. In both rooms, temperatures remained more consistently within the established setpoint during the DCV phase than during the CFR phase. Relative humidity did not differ significantly between rooms or strategies. CO_2 was lower during the CFR phase than DCV phase. Small-particle and TVOC levels were lower during CFR in the larger (3060 ft^3) room but not the smaller (2340 ft^3) room. During the DCV phase, the larger room was at the baseline airflow rate over 99% of the time and the smaller room over 96% of the time. The DCV strategy resulted in a baseline airflow rate of less than 3 ACH, which in turn provided acceptable IAQ over 96% of the time; higher ventilation rates were warranted only during sanitation periods.

Abbreviations: ACH, air changes per hour; cfm, cubic feet per minute; CFR, constant flow rate; DCV, demand-controlled ventilation; IAQ, indoor air quality; pcf, particles per cubic foot; TVOC, total volatile organic compounds.

For many decades, the research community and regulatory agencies have recognized the importance of maintaining a stable and safe environment for both research animals and the personnel working with them. Studies have shown that variability in environmental quality can alter or even compromise the validity of research studies.[7,12,31] For example, high levels of ammonia can radically alter the cellular composition of the trachea in rats,[12] thus confounding the effects of a particular procedure or compound under investigation. In addition, inadequate indoor air quality (IAQ) can pose a significant occupational hazard to workers.[16,17]

The environmental quality of a particular space is defined by several indicators, including temperature, humidity, and levels of gaseous or particulate contaminants. Ventilation systems function to regulate the levels of these parameters, in addition to providing oxygen and eliminating heat.[19] Moreover, ventilation removes airborne contaminants, such as odors and pathogens. Ventilation is the process of replacing the air present in a particular space with either fresh air from the outside or recirculated air that has been treated. The rate of ventilation can be measured in cubic feet per minute (cfm) of air per occupant, volume of air per floor area or, more commonly in the laboratory field, as air changes per hour (ACH), which refers to the number of times the volume of the entire space is exchanged in an hour.[10,21]

Although the *Guide for the Care and Use of Laboratory Animals*[19] provides standards related to physical plant design and specifications, to date it has not provided specific recommendations regarding the environmental quality and ventilation within an animal facility, owing to a paucity of scientific evidence. Notably, the *Guide*'s currently recommended ventilation rate of 10 to 15 fresh ACH for an animal room was based on anecdotal evidence, empirical information, and a scientific paper that was published more than 75 y ago.[7] The study published in 1938[25] concluded that using a recirculating air ventilation system providing at least 20% fresh air per change and 11 ACH was necessary to reduce odors to a 'satisfactory' level in a rudimentary animal room that housed rats and guinea pigs. The *Guide* acknowledges that this ventilation rate range does not reflect the effect of other factors on IAQ, which may, in practice, result in the over- or under-ventilation of a space.[19] The American Society of Heating, Refrigerating and Air-Conditioning Engineers states that IAQ is represented by the thermal conditions as well as the indoor air concentrations of pollutants that are known to affect people's comfort or health in a particular space.[1] The maintenance of IAQ in a space is highly dependent on effective ventilation, which is determined by several factors other than ventilation rate. Among these are the location and type of exhaust and supply vents, which will determine how well the air in the room mixes.[21] In addition, the arrangement of the cages and equipment within a room can create dead zones or high-velocity drafts, which can result in the accumulation of airborne contaminants or affect an animal's ability to retain heat and moisture, respectively.[18,19]

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Furthermore, the most recent guidelines from the American Society of Heating, Refrigerating and Air-Conditioning Engineers echo the *Guide*'s statement regarding the recommended ventilation rate, recognizing that a variety of factors, including husbandry protocols, bedding types, animal density, animal species, and ventilation efficiency, can render this ventilation rate range inadequate.[2] However, neither document provides any guidance on how to determine whether a particular ventilation rate in a specific space is sufficient. In the end, both documents simply refer readers to the literature on ventilation of the general laboratory setting.

Ventilation rate recommendations established for general laboratories are vague and broad, ranging from 4 to 15 ACH. However, in contrast to the laboratory animal field and recommendations made by the *Guide*, the general laboratory industry has examined the subject of ventilation in greater depth. Studies have concluded that the ACH range can be narrowed according to factors peculiar to the specific environment. One study[36] performed in an unoccupied laboratory measured the rate of clearance and the airborne concentration of diethyl ether by using different ventilation rates, ranging from 4 to 16 ACH. The results demonstrated that the greatest decrease in both chemical concentration and clearance rate occurred between 6 and 8 ACH, with little benefit gained beyond 12 ACH.[36] On the basis of this information, the ACH in that laboratory building was decreased from 14 to 8 ACH, thus ensuring personnel safety and saving $240,000 per year.[21]

In the last decade, the general laboratory community has made a pronounced shift in focus toward producing energy-efficient laboratories without compromising personnel health.[6] In addition to the goal of increasing efficiency, there is interest in reducing costs, given that as much as 60% to 70% of energy costs in a laboratory building are associated with HVAC functions.[21,36] In this effort to reduce costs associated with ventilation, the general laboratory industry has implemented additional strategies, including the reduction of ACH in rooms during unoccupied hours or rooms containing fume hoods. More recently, new approaches, such as demand-controlled ventilation (DCV), have been adopted.[6,21,23,33]

The DCV strategy is based on the concept that the ventilation rate is determined by the 'cleanliness' of the space. This approach requires frequent monitoring of the air quality for the levels of the specific hazardous agents expected to be present in a particular space.[6,21,33] The DCV strategy allows for the maintenance of a markedly lower ventilation rate during the time when the air quality is considered 'clean' or sufficient, instead of maintaining a constant high airflow rate intended to satisfy the worst-case scenario. A survey of more than 300 laboratory and animal spaces that were using DCV systems revealed that the air quality within these spaces was within acceptable ranges greater than 98% of the time.[32,33] This result suggests the feasibility of implementing this ventilation strategy, with considerable potential for energy savings.

Despite new approaches and advances in understanding, a great need for more specific and objective guidelines to define environmental quality in animal rooms remains.[7,10,20,28] Currently, ventilation rates in animal rooms of most facilities are maintained at a constant ventilation rate of as high as 20 ACH to control odors and allergens at their highest anticipated levels.[33]Recently, several animal facilities using DCV have maintained ventilation rates of as low as 6 ACH yet achieved adequate IAQ.[33] Nevertheless, little scientific evidence is available to support a shift in the laboratory animal community toward a more individualized or evidence-based approach to the determination of optimal room ventilation.

To improve IAQ and animal and personnel safety and minimize cost, we wanted to apply a DCV strategy to our new primate facility, which was equipped with a variable air volume system. To this end, we chose to reduce ACH to the minimum required to handle the heat load in the room and maintain the correct pressurization for biosecurity purposes. However, to date, the lower limits of ACH in an NHP housing room using a DCV strategy have not been reported.

Therefore, the current study was aimed at determining whether we could maintain an IAQ that supports animal welfare and a safe working environment by using a DCV strategy with a base ventilation rate of less than 3 fresh ACH, when compared with a CFR ventilation system set at 12 fresh ACH. The IAQ parameters monitored were temperature, humidity, small particles (which carry allergens), volatile organic compounds (which are correlated with ammonia, considered an undesirable odor), and CO_2. The facility tested housed rhesus macaques (*Macaca mulatta*).

Materials and Methods

Setting. *Animals and caging.* Rhesus macaques in this study were housed at the University of Houston vivarium, an AAALAC-accredited facility, and were enrolled in IACUC-approved protocols. All of the animals originated from The University of Texas MD Anderson Cancer Center Keeling Center for Comparative Medicine and Research, which maintains an SFP colony (free of *Macacine herpesvirus*1, simian retrovirus type D, SIV, and simian T-cell lymphotropic–leukemia virus). Animals at the University of Houston were housed in accordance with the standards established in the *Guide* as well as the Animal Welfare Act and Animal Welfare Regulations.[3,4,19] All animals were housed in a commercial caging system (Primate 6.2 Break-Apart Primary Housing Unit, Britz and Company, Wheatland, WY). Each compartment had 6.0 ft^2 of floor space. These units allow for the provision of vertical and horizontal access between compartments when joined together for group-housing purposes. All animals were pair- or group-housed, except those that were exempt due to scientific or veterinary justification.

Daily cleaning and sanitation practices. All large debris, such as biscuits and food waste, found on the room floor were swept and placed in a biohazard container. The cage floors and pans were cleaned with water. The floors of the animal rooms were sanitized (LpH SE Concentrated Germicidal Detergent, Steris, Mentor, OH) by using a foamer that dispensed the detergent at a concentration of 0.4%. The detergent remained in the room for 10 min to allow sufficient disinfection. Finally, the floor was rinsed with water and dried with a squeegee. All cages were changed and sent through a rack washer every 14 d.

Room volume and volume of space per animal. The 2 primate housing rooms that were monitored during this study housed the maximal number of animals that could be supported by each room. Room A housed 2 juvenile (age, less than 3y) animals and 15 adult animals, for a total of 17 animals, 8 of which were housed in pairs; the remaining 9 were single-housed. Room B housed 13 juvenile animals in groups of 3 or 4 animals. The volume of room A was 3060 ft^3, and the volume of space per animal was 180 ft^3 of air per animal. The volume of room B was 2340 ft^3, and the volume of space per animal was 180 ft^3 of air per animal.

Room ventilation design. Air was supplied through radial diffusers located on the ceiling in the center of each space. The exhaust vents were located low in the corners of each room. The rectangular shape of the rooms prevents the creation of dead

space for air to become stagnant. The rooms were negatively pressurized at all times.

Room ventilation control. The ventilation rate to each individual room was controlled by an airflow control valve (Accel II Venturi valve, Phoenix Controls, Acton, MA). These valves were designed to maintain a fixed airflow by almost instantaneously responding to changes in static pressure in the ventilation system. All of the valves in the facility were 12-in. valves and designed to function in a variable air volume application capable of providing a flow rate range of 90 to 1500 cubic feet per minute (cfm). Each individual valve was set for a specific range limit, with minimal and maximal flow rate limits based on the volume, heat load, and airflow dynamics required in that particular room. Each valve was equipped with a valve controller (Celeris Valve Controller, Phoenix Controls) that could regulate the airflow into the room. This valve controller adjusted airflow based on information collected by both a temperature sensor and a humidity sensor within the room, as well as other room-control elements that relayed information about other room parameters, such as levels of volatile organic compounds and small particles.

All of the rooms in the facility were set to be at negative pressure to the adjacent labs and corridors. This parameter was accomplished by programming a 200- to 300-cfm offset between the supply valve and the exhaust valve. Room A had an offset of 300 cfm, and room B had an offset of 200 cfm.

Outcome measures. The IAQ parameters measured in each animal room were temperature (°F), calculated relative humidity (%), CO_2 (ppm), small particles ranging in size from 0.3 to 2.5 µm (particles per cubic foot; pcf), and total volatile organic compounds (TVOC; ppm of isobutylene). Each room was equipped with a duct probe, which measured temperature and collected an air sample. This sample was sent through a common air-sampling backbone at a high flow rate of 20 L/min to an air data router (ADR500, Aircuity, Newton, MA) and then to a centralized sensor suite (SST700, Aircuity) for measurement. Sequential air samples from different rooms were tested every 30 to 40 s in the sensor suite. To prevent contamination of one sample by the previous sample, the sample conduits were made of a mixture of inert Kynar (Arkema, King of Prussia, PA) and micron-long strands of carbon atoms. This mixture created an electrically conductive matrix that prevented the buildup of particles and aerosols in the common conduit. Air samples from each room were tested for these parameters every 18 min, which corresponded to the amount of time needed to process an individual sample from each of the rooms measured by that sensor suite. The common sensor suite was composed of the following set of sensors (Aircuity): SEN-PAR-1, which measured particles sized 0.3 to 2.5 µm, SEN-TVC-1 and -3, which measured isobutylene (which is directly correlated with ammonia), and SEN-C2D-3, which measured CO_2 and moisture. The calibration of the sensor suite was accomplished through periodic (that is, every 6 mo) replacement of the sensors with a new set of sensors from the manufacturer. The data collected from these sensors were sent to data management software (Knowledge Center, Aircuity) and stored on a secure server. Using the data for levels of TVOC, CO_2, and small particles, the software had the capability of interfacing with the airflow control valves at the room level to alter the flow rate in the room.

Each room contained a second set of sensors that measured temperature and relative humidity (Phoenix Controls). The data collected by these sensors were relayed to a different secure server (Automated Logic Corporation, Kennesaw, GA) every minute. Using the measurements, this system had the capability of communicating with the air handler units to increase or decrease the humidity of the air supplied to the room.

Experimental design. Data were collected from each room for a period of 60 d (1 April through 30 May 2014). Each room operated 30 d on constant flow rate (CFR) ventilation and 30 d on DCV. The flow rate in each room during the CFR phase was set at 12 fresh ACH (calculated on supply) or 18 room ACH (calculated on exhaust). The flow rate during the DCV phase was set at a baseline rate of less than 3 fresh ACH (2 fresh ACH for room A and 2.7 fresh ACH for room B, calculated on supply) or 7.8 room ACH (calculated on exhaust). The maximal flushing rate during the DCV phase was 20 fresh ACH (calculated on supply). During 1 through 30 April 2014, room A was set at CFR, and room B was set at DCV, and during 1 through 30 May 2014, room A was set at DCV, and room B was set at CFR. Temperature and relative humidity were measured at 1-min sampling intervals, and TVOC, small particles, and CO_2 were measured at 18-min sampling intervals.

CFR ventilation. CFR ventilation typically is used by animal facilities to maintain ventilation rates in animal rooms at a constant ventilation rate that does not change, based on the highest anticipated odor and allergen levels.[21] Therefore, rooms that were set at CFR ventilation had both the supply and exhaust valves set at an unchanging flow rate. However, the humidity control system at the level of the air handler and the heating coils at the room level were controlled according to prevailing temperature and humidity levels sensed in the rooms.

DCV. DCV is based on the concept that IAQ parameters are not constant but fluctuate within a room. In contrast to the constant airflow rate used in the CFR strategy, DCV uses variable airflow rates according to real-time demands within a room. Essentially, rooms were maintained at a predetermined minimal flow rate necessary to support balanced air dynamics within the room. But to accommodate for fluctuations involving elevated parameters, the supply and exhaust flow rates were altered until acceptable ranges were restored.

Flushing thresholds for each room were calculated specifically for each room on the basis of the room volume, the flow-rate range of the valves, and the desired minimal and maximal ventilation rates (ACH) of room. The flushing thresholds of each individual room were programmed into the data-management software. When a divergence from these acceptable ranges was detected by the TVOC, CO_2, or small-particle sensors, a voltage signal was sent to the airflow control valve operating in that room that triggered an increase in the supply and exhaust rates in that room. As a result, the ventilation system flushed the air in the room by increasing the flow rate until the parameters returned to an acceptable level, at which point the flow rate returned to the predetermined minimal rate. Any time this process occurred, the room was considered to have undergone a DCV event.

The signaling process occurred by correlating the TVOC measurement in the room to a voltage. The voltage signal ranged from 0 to 5V. A voltage signal of 5V correlated with a clean room, which meant the room should remain at the minimal airflow. However, a voltage signal below 5V correlated with a room that required flushing. Lower voltage signals correlated with higher flow rates, with 0V representing the maximal airflow rates. Room-flush signals were correlated with TVOC levels only; however, the data-management software translated readings received from the CO_2 and particle sensors into TVOC measurements according to the following correlation: 1 ppm TVOC = 5,000,000 pcf small particles = 3000 ppm CO_2. The flush thresholds for room A were 0.39 ppm TVOC, 1,950,000

pcf small particles, and 1170 ppm CO_2. The flush thresholds for room B were 0.30 ppm TVOC, 1,500,000 pcf small particles, and 900 ppm CO_2.

Statistical analysis. The air-quality sensor data were compiled for each room and ventilation setting by using Excel (Microsoft, Redmond, WA) for data processing and SPSS (IBM, Armonk, NY) for statistical analyses. Means and SD were calculated for all air-quality parameters (temperature, relative humidity, TVOC, small particles, and CO_2) according to room and ventilation setting. Repeated-measures ANOVA was performed to evaluate main effects of changes in air-quality parameters between ventilation settings and rooms. Air-quality measures also were evaluated against OSHA or industry standards. The percentage of time that the measures were within the acceptable range was calculated and analyzed by using χ^2 tests. A P value of less than 0.05 was regarded as statistically significant.

Results

Overall air-quality values. Air quality was monitored continuously during the study period, and the measurements (mean± 1 SD) for temperature, relative humidity, TVOC, small particles, and CO_2 by room and ventilation setting are shown in Table 1. Repeated-measures ANOVA results indicated differences in air quality by rooms and ventilation settings for many of these variables, although the overall mean differences were small and may not be operationally relevant. Additional analyses focused on the air-quality values as compared with the OSHA or industry standard.

Temperature. The temperature of the rooms was programmed for 72 °F, and the acceptable range was defined as ±2 °F or between 70 and 74 °F. At CFR, the temperature in room A exceeded the acceptable range 91% of the time, whereas room B was outside the acceptable range 18% of the time. However, at DCV, the temperatures of both rooms were beyond the acceptable range less than 4% of the time. For both rooms, the difference between the CFR and DCV results was statistically significant ($P< 0.001$).

Relative humidity. The relative humidity varied markedly throughout the day, according to the activities in the room (primarily cleaning with hot water), as supported by the high SD values (Table 1). No significant difference in relative humidity by ventilation setting was seen. However, for both ventilation settings, the average relative humidity was slightly higher during the month of April than during May.

CO_2. CO_2 concentrations in both rooms were significantly ($P < 0.05$) higher during the DCV period when compared with the CFR period; however, they were significantly lower than the current regulatory limit of 5000 ppm during a weighted 8-h period (Figure 1).

TVOC. TVOC levels were significantly ($P = 0.001$) lower in room A with CFR ventilation than in room A at DCV or in Room B at CFR or DCV. Nevertheless, TVOC levels were below the flushing threshold (0.3 ppm for room B and 0.39 ppm for room A) for 99% of the time in both rooms (Figure 2).

Small particles. The small-particle levels varied significantly ($P < 0.05$) throughout the day depending on the activities within the rooms. The small-particle levels for CFR in room A were significantly ($P < 0.05$)lower than levels for DCV in room A and lower than the levels for both CFR and DCV in room B. Although these levels differed, small-particle levels were below the 1.5 million pcf flushing threshold for 95% of the time in room B at DCV and 99% of the time in room A at both ventilation conditions and in room B at CFR (Figure 3). There were no statistically significant differences between ventilation strategies or between the rooms in the percentages of the time that small-particle levels were below the flushing threshold.

DCV events. In both rooms, 96.1% (49 of 51) of DCV flushing events occurred during staff-occupied hours (0630 to 1630), and the majority (94%) of these were associated with sanitation, which typically occurred between 0730 and 1200. DCV events were triggered by a rise in either TVOC, small particles, or both.TVOC levels rose quickly as the sanitation process began and returned to baseline once the sanitation process concluded (Figures 4 and 5). Although small-particle levels rose during the sanitation process, the amount of time for them to return to baseline was variable (not as predictable as the rise and drop in TVOC).

The patterns of rise and drop of TVOC and small-particle counts in room A were almost identical in magnitude and duration during CFR and DCV conditions (Figure 4). In contrast, in room B (Figure 5), the magnitude and duration of the TVOC and small-particle spikes were higher during DCV than during the CFR ventilation. Nonetheless, small-particle and TVOC levels exceeded the threshold for initiation of a DCV event less than 1% of the time.

Total supply airflow. Room A was set at 652.61 cfm for the CFR ventilation mode. During the DCV mode, room A had a baseline of 101.71 cfm and averaged 102.38 cfm over the month of May, with a peak of 379.28 cfm during a 13-min event. There were 11 DCV flushing events during May in Room A, with 0 to 2 events per day (Figure 6). There were 20 d with no DCV events. The average time per DCV event was 16.3 ± 7.2 min (range, 3 to 32 min). Room A was above baseline supply airflow for 179 min, or 0.4% of the time.

Room B was set at 468.27 cfm for the CFR ventilation mode. During DCV mode in April, room B had a baseline of 103.83 cfm and averaged 111.82 cfm over the month, with a peak of 781.87 cfm during a 42-min event. For the month of April, there were 40 DCV flushing events, with 0 to 3 events per day (Figure 6). There were only 2 d without events. The average time per event was 38.9 ± 37 min (range, 6 to 163 min). Room B was above baseline supply airflow for 1556 min, or 3.6% of the time.

Discussion

The IAQ in the rooms during the DCV portion of this study confirms the hypothesis that an HVAC system using a DCV strategy with a base ventilation rate of less than 3 ACH can support animal welfare and provide a safe working environment. Indeed, while on the DCV setting, the 2 rooms maintained all parameters below the flush threshold at least 96% of the time. The larger room was unexpectedly unable to provide enough heat to maintain the temperature within the programmed setpoint during the CFR phase. TVOC levels detected in both rooms greater than 99% of the time corresponded to an ammonia level of less than 2 ppm, which is below the odor threshold for humans.[35] CO_2 and small-particle levels were below the occupational exposure thresholds[27] in both rooms throughout the entire study. Relative humidity did not differ significantly between the 2 ventilation settings.

Acceptable dry-bulb macroenvironmental temperature ranges are based on the thermoneutral zone of each species. The thermoneutral zone is considered to be the range in which an animal can adjust its core temperature without having to alter its metabolism.[13,14] Several studies have been performed on thermoregulation, particularly in rodents, that have contributed to the development of the recommended ranges in the *Guide*. In addition to maintaining the temperature within a particular

Table 1. Air-quality parameters (mean ± 1 SD)

	Room A (3060 ft^3)		Room B (2340 ft^3)	
	CFR	DCV	CFR	DCV
Temperature (°F)	69.3 ± 0.9	72.0 ± 1.2	71.1 ± 1.6	71.3 ± 0.7
Relative humidity (%)	51.7 ± 6.0	49.3 ± 8.0	49.6 ± 5.9	53.2 ± 8.0
CO_2 (ppm)	487.7 ± 27.7	546.3 ± 46.8	482.1 ± 23.2	542.9 ± 45.7
Small particles (pcf)	374,473 ± 355,801	445,257 ± 282,141	464,146 ± 290,563	441,764 ± 482,701
TVOC (ppm)	0.091 ± 0.058	0.098 ± 0.037	0.098 ± 0.020	0.106 ± 0.095

range, the *Guide* also emphasizes the importance of preventing fluctuations in temperature.[19] Moreover, studies in the field of toxicology have demonstrated that the pattern of absorption of certain toxicants can be altered by ambient temperature.[22] In addition, certain toxicants can have a direct effect on the thermoregulatory system; thus, temperature fluctuations can become a confounding research variable.[15,22]

In the current study, the data demonstrated that the HVAC system tightly regulated the temperature in the rooms to within 2° F during both the CFR and DCV phases. Minimal temperature fluctuation was observed as indicated by standard deviations that did not exceed 1.6° F. The only situation in which temperature fluctuation was detected occurred during the daily sanitation process, which involved the use of hot water for approximately 1 h. Even though the temperature in both rooms during the CFR period was within the acceptable range established by the *Guide*,[19] the temperature exceeded the setpoint programmed into the system for significantly more time than when the rooms were set at DCV. Temperature readings in the larger of the 2 rooms were outside (typically below) the programmed setpoint more than 90% of the time. These low temperatures were a direct result of the inability of the reheating coil to handle the volume of air necessary to provide 12 ACH. In addition, this situation resulted in the use of a large amount of energy to generate enough cold air for the 12 ACH but also to run the supply and exhaust fans at a continuously increased load. Additional energy consumption can be related to producing and continuously supplying hot water to the heating coils. In the case of this building, which was built with a state-of-the-art variable air volume system designed for energy conservation, maintaining a constant airflow high enough to provide 12 ACH was beyond the capacity of the HVAC design.

Even though relative humidity, as compared with temperature, requires less precise control of variation, given the broad acceptable range of 30% to 70% for most mammals,[19] extreme variations in humidity can have detrimental effects on animal health. Low (<25%) relative humidity has been associated with ringtail in rats, as well as with an increased incidence of environmental dust particles.[7,9,26] High relative humidity can have detrimental effects on the respiratory tract of animals housed in microisolation cages by increasing the rate at which ammonia is produced.[12,24]

In the current study, relative humidity was more variable than was temperature in both the CFR and DCV phases but consistent between both. Fluctuations in humidity occurred daily, coinciding with room sanitation practices requiring the heavy use of hot water. This use caused relative humidity to increase from a baseline of approximately 50% to as high as 80%. The return to baseline generally took approximately 3 h, thus contributing to the high standard deviations in relative humidity in both rooms during both the CFR and DCV phases. Relative humidity was not affected by the ventilation strategy. Notably, the mean relative humidity in the rooms was slightly lower during the month of May as compared with April. This difference is likely due to the fact that the average outside relative humidity during May was higher and thus the dehumidification cycle in the air handler was initiated more often than during April.

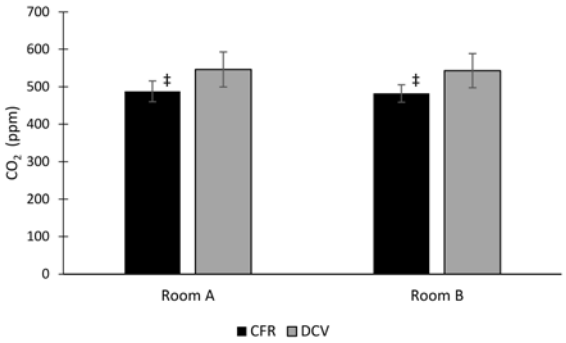

Figure 1. Average CO_2 concentrations during the study period for each room and ventilation strategy. Average CO_2 levels were significantly (‡, $P < 0.001$) lower during CFR conditions compared with DCV in the same room.

Currently, there are no safety guidelines for CO_2 levels for animals. Studies performed on rodents housed in individually ventilated cages have shown that average levels of 1250 ppm to 3000 ppm within the cages are not uncommon.[34]Because the cages used in the current study were open, control of CO_2 concentrations that would meet the more stringent standards for human occupants were applicable.

CO_2 levels were correlated with the airflow to the rooms. The average CO_2 levels found in the rooms in the present study were around 480 ppm for the CFR strategy and around 540 ppm for the DCV strategy. Therefore, CO_2 levels were significantly lower in both rooms during the CFR phase. Nonetheless, these concentrations were both within previously reported ambient room levels (440 to 530 ppm).[34] Even at the higher DCV average of 546 ppm, CO_2 levels were much lower than OSHA's permissible exposure level of a time-weighted average of 5000 ppm.[27] Therefore, both strategies met OSHA standards, although higher airflow rates like those used during the CFR phase are more effective at maintaining lower CO_2 levels.

Numerous gaseous organic and inorganic contaminants can be found within an animal facility. These can be released from the animal bedding, the animals' waste, sanitizing solutions, and the animals themselves.[20,33] Of these, ammonia is measured most often in animal facilities, because it is produced by bacteria found in the bedding and can have detrimental effects on the health of animals and humans alike. The odor threshold of ammonia in humans is around 2 ppm, and irritation levels are between 30 and 60 ppm, depending on the exposure method.[35]Some animal species are believed to be more tolerant of high ammonia levels; however, ammonia levels above 125 ppm can cause detrimental changes in the respiratory tract of rats.[12,31]

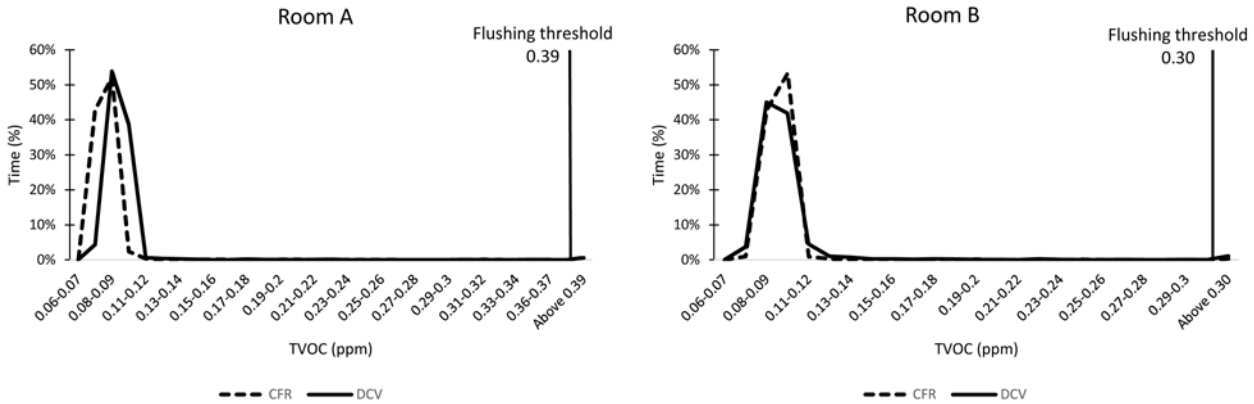

Figure 2. Measured TVOC levels as a percentage of time during the study period for each room and ventilation strategy.

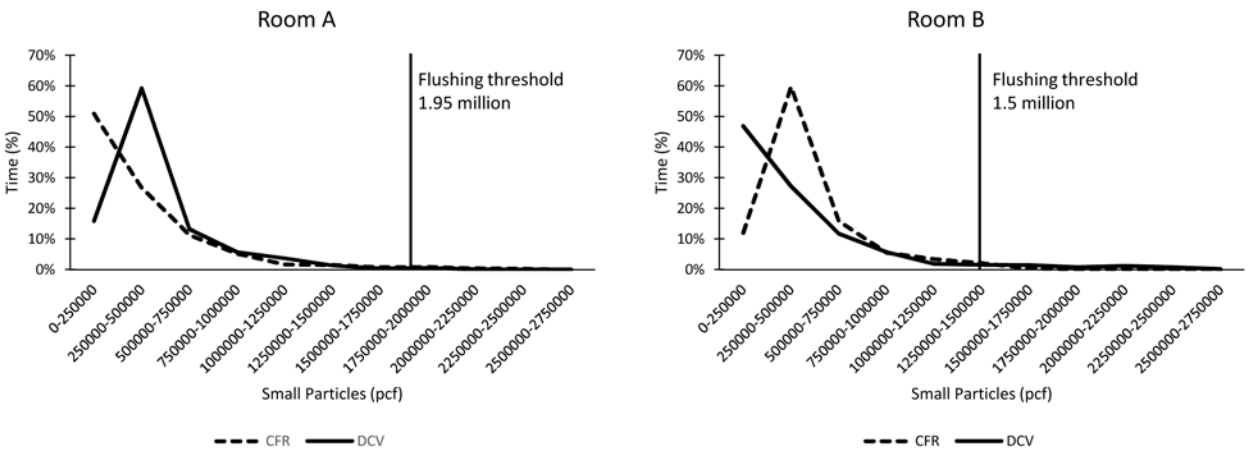

Figure 3. Measured small-particle counts as a percentage of time during the study period for each room and ventilation strategy.

The TVOC sensor used in the current study detected several organic and inorganic compounds, but it was calibrated to report in terms of ppm of isobutylene. The fact that elevations in TVOC were detected only during the cleaning process suggests that the compounds were aerosolized during cleaning. The sanitizing agent was ruled out as the compound detected by the sensors during the cleaning process by performing the same procedure in an unoccupied room (data not shown). Therefore, we assume that all TVOC detected during this process were likely ammonia particles. Under this assumption, a direct correlation between isobutylene and ammonia of 1:9.4, as reported by the manufacturer of the sensor, allowed for a simple calculation of ammonia level. Assuming all TVOCs detected were ammonia, the levels in all rooms were below 2 ppm 99% of the time for both the CFR and DCV strategies (Figure 2). The only time when the TVOC level corresponded to an ammonia level higher than 2 ppm was during a brief period shortly after the onset of a sanitation event in a room. Room A under CFR had lower levels of TVOC than did any of the other 3 experimental conditions (Table 1), likely because as the larger room, it had a higher airflow rate to achieve the 12 ACH required during this phase. This higher rate, in turn, resulted in a lower level of TVOC. However, in both rooms levels were at all times markedly below the current OSHA permissible exposure limit of 50 ppm during a weighted 8-h period or even the more stringent threshold limit value of 25 ppm during a weighted 8-h period advocated by the American Conference of Governmental Industrial Hygienists.[27]

With the recognition of laboratory animal allergies as an important occupational hazard, several studies have attempted to document the concentrations and distributions of the different aeroallergens within animal facilities.[8,17,30] A goal of such studies has been the development of a guideline for acceptable ranges of aeroallergens in an effort to minimize their negative effect on people who work in the laboratory animal field. Two studies correlated particular tasks within an animal facility with an increase in risk of developing allergies to laboratory animals.[11,20] Other studies correlated the concentration of various allergens with the size and number of particles present, suggesting that controlling allergen concentrations is highly dependent on controlling the levels of particles that carry the allergens.[28,29] However, to date, a direct correlation between the levels of particles and the incidence of allergies among laboratory animal personnel has not been established.

Most allergens are carried on particles ranging from 0.3 to 15 µm in aerodynamic diameter, with a significant portion found on particles smaller than 4 µm, a size considered to be a respirable particulate. Controlling respirable particulates is especially important because particles of this size are deposited directly in the alveolar region of the lungs and travel the furthest, because they remain airborne for the longest time.[17]The particulate sensor we used in the current study measured particles 0.3 to 2.5 µm in size. As seen in Figure 3, the small-particle numbers observed throughout the entire study were below the flushing threshold more than 99% of the time in room A and more than 95% of the time in room B.

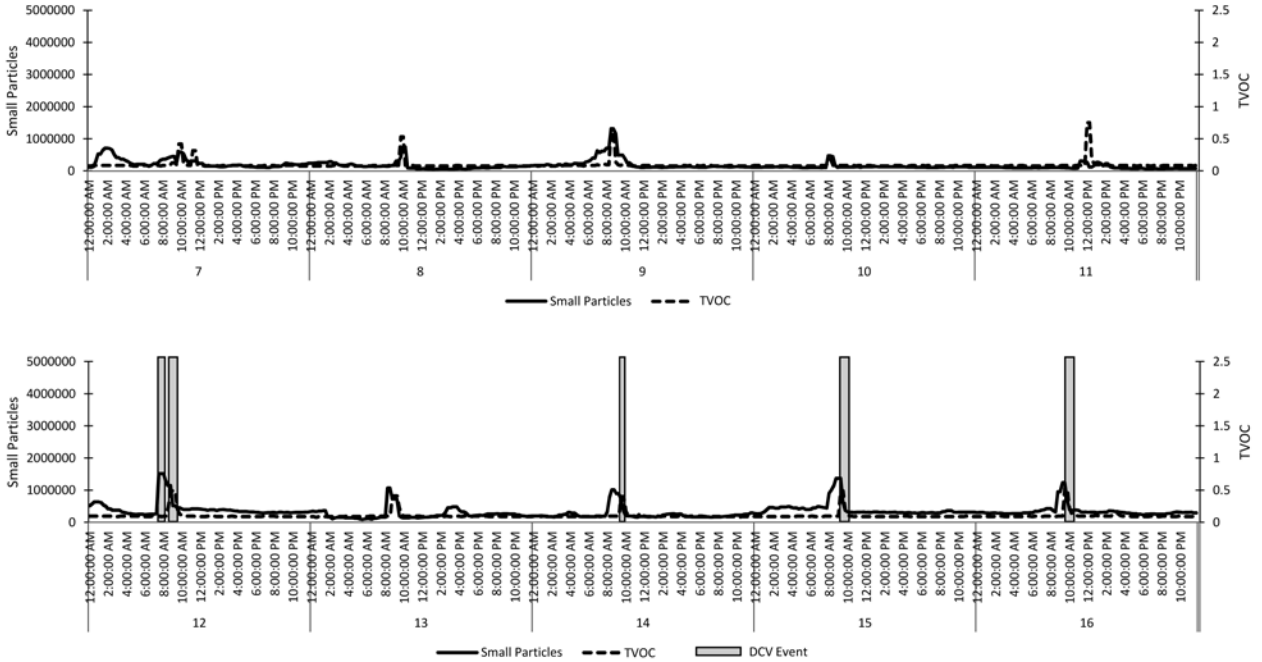

Figure 4. Patterns of small-particle counts, TVOC, and DCV flushing events for a typical work week in room A under CFR (top) and DCV (bottom) conditions.

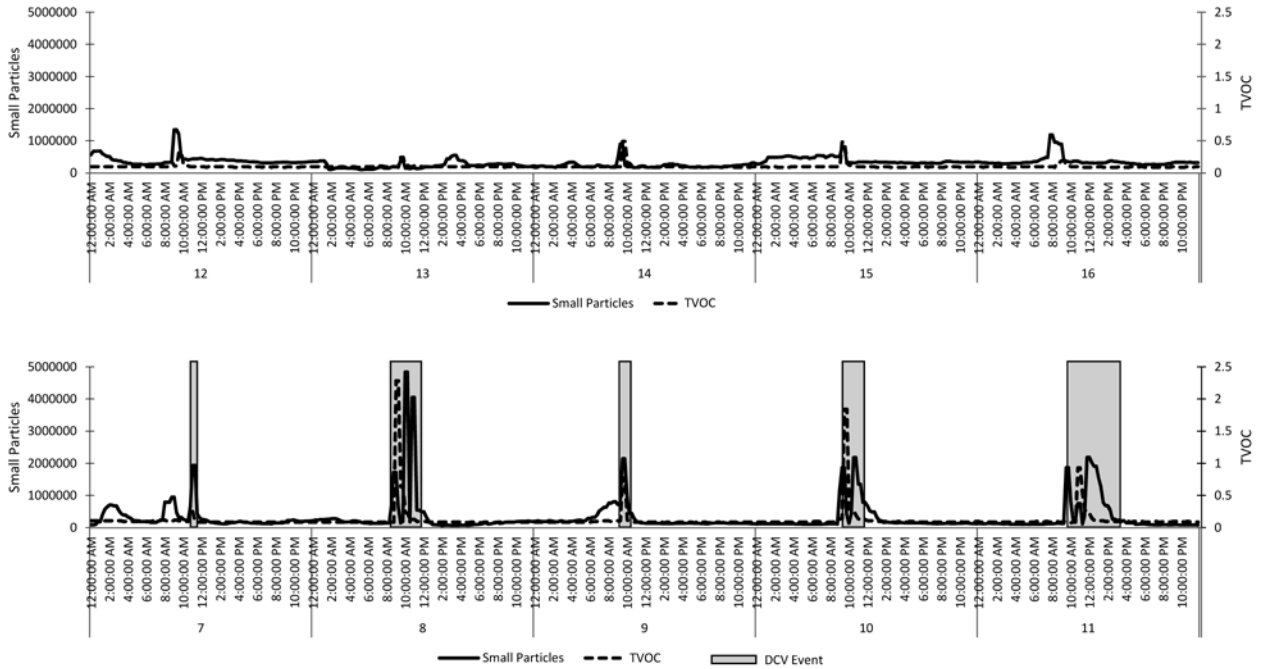

Figure 5. Patterns of small-particle counts, TVOC, and DCV flushing events for a typical work week in room B under CFR (top) and DCV (bottom) conditions.

In our experience, the respiratory protection requirements used within vivaria vary greatly from facility to facility. In the case of NHP rooms, a surgical mask is always required, but these masks provide no protection against aerosolized small particles. Even though most of the research on laboratory animal allergens has focused on rodents, a few cases of sensitivity to NHP have emerged.[37] In addition, in an epidemiologic study performed in Japan, 24% of people working with NHP reported having laboratory animal allergies.[5] These reports, together with the fact that 96% of DCV events detected during working hours occurred in association with sanitation, suggest that more stringent respiratory protection may be justified when this work is performed. Additional research and risk assessment are needed to evaluate this conclusion, but based on our preliminary data, the implementation of such a policy may benefit employee health.

As observed for the TVOC levels, room A in the CFR phase maintained a significantly lower mean small-particle level than in the other 3 experimental conditions (Table 1). This finding likely was due to the fact that this room had the highest airflow during the CFR phase of all 4 experimental conditions. The amount of time that small-particle levels exceeded the flush threshold in room B was slightly above the

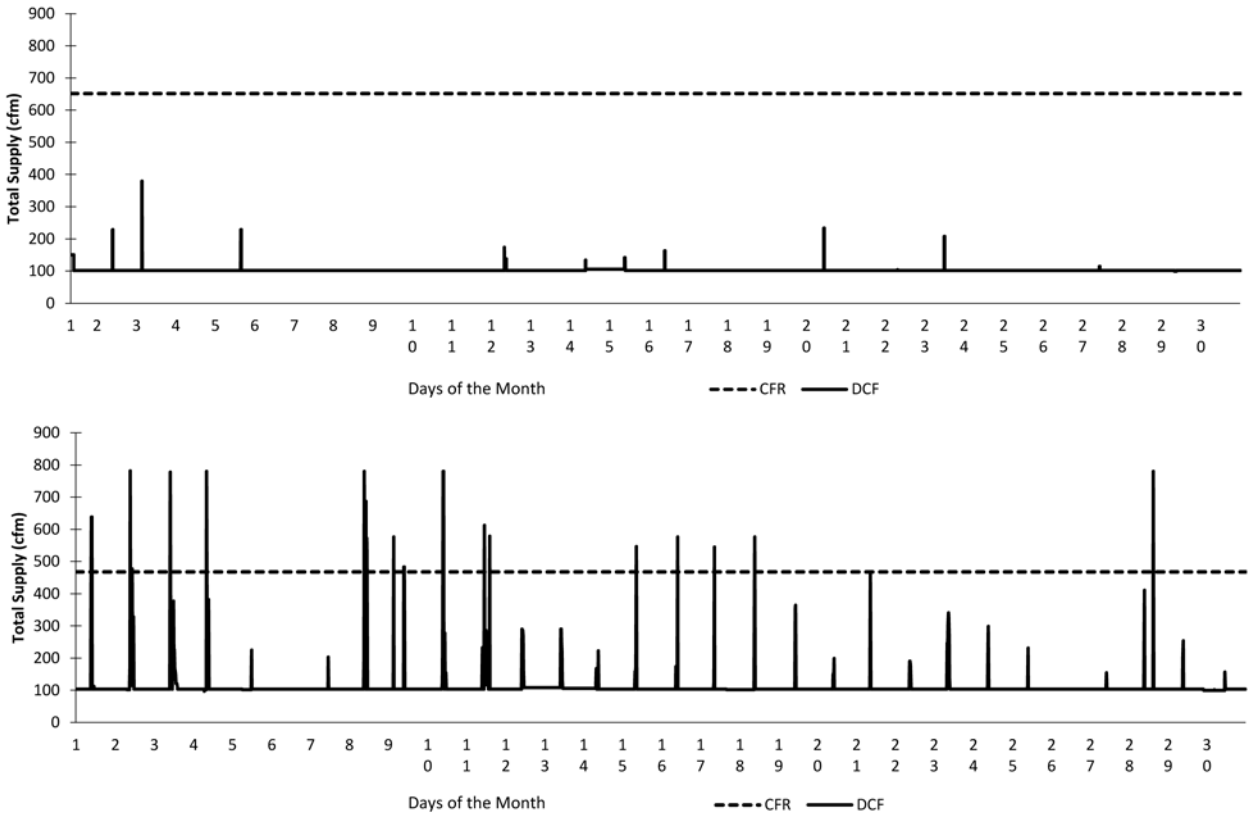

Figure 6. Total air supply (in cfm) by day during the entire study period for rooms A (top) and B (bottom).

1.2% reported elsewhere for NHP open cages.[33] However, this difference can be attributed to a number of factors, such as housing density, housing conditions (single- compared with group-housed), and activity level, which is highly dependent on the age of the animals. Even though there is no accepted range regarding the number of respirable particles present in animal facilities, the numbers we obtained for both strategies were much lower than the governmental occupational exposure threshold for not-otherwise-specified inert dust particles of 15 million pcf.[27]

The numbers of DCV events in each of the rooms differed markedly (Figure 6), with room B having 3 times as many events as room A. In addition, the average duration of DCV events in room B was twice as long as that in Room A. This difference, although unexpected, might be attributable to the population demographics in the rooms. The average age of animals housed in room A was 5.9 y, and only 2 of the 17 animals in that room were juveniles (younger than 3 y). In contrast, the average age of the animals in room B was 2.3 y, and all 13 animals were juveniles. In addition, all of the animals housed in room B were group-housed (3 or 4 animals per group), whereas only 8 of the 17 animals in room A were pair-housed. Juvenile NHP, particularly when housed in groups, typically are far more active than are adult animals, which often results in a much increased spread of fecal material throughout the cages. This situation, in turn, requires a more robust, thus longer, daily sanitization of the cages, perhaps explaining the prolonged duration of DCV events in room B. At this time, a difference in the number of DCV events due to a difference in the demographic of the population housed in the room has not been reported by others in the field. This generally higher activity level might also be responsible for the additional temporary increases in the levels of small particles in room B.

Even in the room with the highest number of DCV events, the air quality within the room was considered to be adequate 96% of the time. This result suggests that the higher air supply during the CFR phase constituted a waste of energy and money, because it was unnecessary more than 96% of the time. Based on the current average yearly cost per cfm at our facility, the constant flushing represents an additional $1500 per year in energy costs for this particular room. If this concept is applied to the remaining 80 rooms in our facility, many of which are considerably larger than this room, the cost savings could be close to $200,000 annually, not to mention the associated decrease in the 'carbon footprint.'

Given current legislative and public pressures to develop more 'eco-friendly' or sustainable ways of conducting research, DCV may represent the future of ventilation systems in the laboratory animal field. However, to adopt DCV globally, considerable efforts are still needed to develop acceptable ranges for some of the parameters that characterize IAQ. For example, despite strong correlations between particle size and allergen load,[28,37] a clear correlation between the numbers of particles and the risk of developing clinical allergies to laboratory animals has not been developed. In addition, this relationship may vary by species and therefore should be evaluated among the species typically used as laboratory animals. Such data would facilitate the development and adoption by the regulatory agencies of a standard acceptable range for small particles in a vivarium. In addition, in contrast to the defined acceptable occupational exposure threshold for ammonia, there is no defined acceptable range for ammonia exposure in animals. Although some support the idea that different species have different tolerance ranges for ammonia, no studies have been attempted to develop an acceptable range of ammonia concentration for the various

laboratory species. This information would not only aid in standardizing ventilation requirements but would also eliminate varying levels of ammonia as a possible confounding factor in research studies.

Even though more work is necessary to define acceptable IAQ in laboratory animal facilities, our study confirms that advances in HVAC technology, such as DCV, will be key in future efforts to conserve resources.

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