Determination of Air Distribution, Exchange, Velocity, and Leakage in Three Individually Ventilated Rodent Caging Systems

HUIPING TU, MS1,2, LOUIS J. DIBERADINIS, MS, CIH, CSP1, AND NEIL S. LIPMAN, VMD3,5

Abstract | Methods for characterizing the design and operation of ventilated caging systems were investigated to define and quantify differences in air distribution, exchange, velocity, and leakage in commercially available systems—units 1, 2, and 3. Intracage air distribution patterns were determined by visually observing smoke dispersion patterns after release at 3 intracage locations from a TiCl₄ smoke stick in 3 cages/system. Smoke distribution was rapid and complete in unit 1; visible leakage of smoke from the cage was observed. Smoke distribution in unit 2 was the slowest of all systems tested, and mixing was not complete; no visible leakage was observed. The smoke distribution in unit 3 was rapid and complete, but not as fast as in unit 1; no smoke leakage was observed. Intracage air exchange rates per h (ACH) were calculated by determining sulfur hexafluoride (SF₆) decay curves at 8 predetermined detection points after introduction of SF₆ into the supply air stream of 1 cage/system. Mean ± SEM ventilation rate were 65 ± 19, 42 ± 32, and 79 ± 21 ACH for units 1, 2, and 3, respectively. Intracage air velocity was measured at predetermined points within the cage, using a thermoanemometer. Mean ± SD air velocity was 36 ± 18 and 37 ± 12 linear feet/min for units 1 and 3. Air velocity was below the detection limit (< 10 linear feet/min) of the thermoanemometer for unit 2. Air leakage was determined qualitatively by measuring the concentration of SF₆ escaping from select cages for each system. Leakage of SF₆ was detectable from units 1 and 3, but not detectable from unit 2. Standardized testing methods were used in this study so that other users can adequately compare systems and understand more fully the advantages and limitations of particular systems.

Use of rodent caging systems that provide individually ventilated isolator cages is rapidly increasing. These systems have been shown to considerably improve the microenvironmental conditions to which rodents are exposed (1–5). Ventilated caging systems have also been shown to enhance containment capability at the cage level, reducing the opportunity for cross contamination (6, 7).

Individually ventilated caging systems are available from several manufacturers in a variety of configurations. In general, these systems provide filtered air directly into the cage, thereby pressurizing it. Caging systems may be purchased with exhaust systems that scavenging may also limit the potential of cross-contamination.

Optimal ventilation rates for individually ventilated cages have not been determined. Rates differ between systems, and manufacturers have frequently altered rates over time with various designs. In general, manufacturers have increased rates with newer designs. Although increased ventilation rates may have the advantage of decreasing microenvironmental ammonia, carbon dioxide, and humidity values, they will likely increase the velocity of air to which animals are exposed. Chilling, especially of hairless strains and neonates, and suspension of particulates in the air may be potentially detrimental effects. In addition, the shape of the wire bar lid, and the presence of feed and a water bottle may markedly impact ventilation patterns within cages and may contribute to differences reported between systems with respect to accumulation of microenvironmental contaminants.

It is unclear whether ventilated caging systems equipped with exhaust scavenging are suitable for use with hazardous agents. Although definitely unsuitable for high risk agents, they may be useful for housing rodents exposed to moderately pathogenic agents if equipped with effective scavenging systems. If air is exited directly into the building’s exhaust system, they also may be suitable for use with some volatile chemicals. Unfortunately, the effectiveness of exhaust scavenging from these systems has never been critically evaluated.

The study reported here was undertaken to investigate methods for characterizing the design and operation of ventilated caging systems to better understand and quantify differences in air distribution, exchange, velocity, and leakage in commercially available individually ventilated caging systems. These investigations begin to explore the possibility of developing standardized testing methods so users can adequately compare systems and understand more fully the advantages and limitations of particular systems.

Materials and Methods

Overall study design: Air distribution, exchange, velocity, and leakage rates were used to characterize three commercially manufactured individually ventilated caging systems. Air distribution within individually ventilated cages was determined by smoke visualization. Air exchange rates were extrapolated from decay curves after introduction of a known quantity of tracer gas into the cages. Air velocity was determined at predetermined points within individual cages, using a thermoanemometer. Air leakage from cages was qualitatively determined by measuring tracer gas concentrations surrounding cages into which tracer gas was released, as well as following smoke visualization.

Caging systems: Three commercially available double-sided ventilated caging racks were evaluated. Ventilated systems were equipped with HEPA-filtered supply and exhaust systems. All systems were evaluated as supplied by the manufacturer unless altered as described. All systems were operated independently of the

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1Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, 716 Princeton Blvd. No. 2, Lowell, MA 01851, 2Environmental Medical Service, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, 3Committee on Comparative Medicine and Pathology, University of Chicago, 5841 S. Maryland Avenue (MC 1030), Chicago, IL 60637. Send reprint requests to: Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021
facility's ventilation system. Systems evaluated included: unit 1 (Micro-VENT; 126 individually ventilated cages; Allentown Caging Equipment Co., Allentown, NJ), unit 2 (Maxi-Miser® IV; 96-cage rack; Thoren Caging Systems, Inc., Hazleton, PA), and unit 3 (Micro-Isolator A/V-WC1; 98-cage rack; Lab Products, Inc., Maywood, NJ). Unit 2, which has adjustable dampers regulating supply and exhaust flows, was operated with equal static pressure (0.3 in water column) in the supply and exhaust plenums.

The isolator cages, wire-bar lids, filters, water bottles, and automatic watering systems were standard for each rack and were supplied by the manufacturer. Polycarbonate shoebox cages had dimensions of 7.25 X 11.5 X 5.0 in. Each cage was equipped with a stainless steel wire-bar lid that held a 16-ounce water bottle and pelleted feed. Although no animals were housed within cages during the study, water bottles and feed hoppers were maintained filled with water and feed (Prolab 3000, Agway, Inc., Syracuse, NY). Each cage contained 850 cm³ of autoclaved pine shavings (Pine shaving laboratory grade, Northeastern Products Corp., Warrensburg, NY). Cages were modified for measurement purposes. Each cage was provided with four 1/4-in-diameter ports. The right side of the cage was divided into 4 equal size quadrants, each of which had a port placed in the center (Figure 1). Ports permitted air velocity measurements and tracer gas injection and sampling at one of 8 predetermined points within the cage. Two sampling points were accessed from each port. Sampling points were located 1/3 and 2/3 of the distance across the cage width at the same height off the cage floor as the port used for sampling. Ports were closed with tape when not in use.

Macroenvironment: The caging systems were evaluated within a holding room measuring 13.50 X 13.50 X 7.92 feet in an AAALAC-approved animal facility incorporating a clean-dirty corridor system. The HVAC system was a constant volume, terminal reheat type with direct clean steam humidification. Only caging under study was housed in the holding room. Ventilation provided seventeen 100% fresh air changes/h through two supply diffusers located in the ceiling. Air exited into two exhaust registers located approximately 6 in from the floor in the center of two opposing walls. Differential pressure provided directional airflow so that air flowed from the clean corridor into the holding room and out to the dirty corridor. The daily temperature (70.4 ± 0.1°F) and relative humidity (44 ± 0.4%) were determined by continuously operating hygrothermographs (Hygro-Thermograph, Belfort Instrument Co., Baltimore, MD).

Air distribution: Intracage air distribution patterns were determined by visually observing smoke dispersion after intracage release in 3 cages from each of the 3 systems. Smoke was released from a titanium tetrachloride (TiCl₄) smoke stick (Model 15-049, Liberty Industries, Inc., East Berlin, CT) inside the cage at a point, on a centerline between the cage front and back, intermediate between the cage floor and the wire bar lid at 3 predetermined locations: approximately 3 cm away from the front of the cage; in the middle of the cage; and approximately 3 cm away from the back of the cage. Smoke was observed until it was no longer visible. Each cage was evaluated in triplicate. The smoke pattern from each caging system was qualitatively described and depicted in line drawings.

Intracage air exchange rate determination: Intracage air exchange rates were established in each of the 3 cage systems by determining decay curves for the tracer gas sulfur hexafluoride (SF₆) after SF₆ input directly into each cage at the introduction point of the supply air stream (10). Decay curves were established in 1 cage/system. The air exchange rate (ACH) was determined at 8 predetermined detection points through one of the four ports, using a calibrated leakmeter (ITI model 61, Ion Track Instruments, Wilmington, MA). The leakmeter was calibrated, using a dynamic dilution calibrator (Dynamcalibrator Model 340-56-XV, VCI Medtronic, Santa Clara, CA). The air exchange rate for each cage evaluated per system was determined by calculating the mean of the decay curves generated for each of the 8 intracage detection points.

Air velocity determination: Intracage air velocity was measured at 8 predetermined points within the cage, using a thermonanometer (Velocicalc Plus-TSI velometer Model 8350, TSI Inc., St. Paul, MN) introduced through each of the four measurement ports (Figure 1) in each of 20 random cages evaluated. Intracage air velocity per cage was calculated as the mean of the 8 measurements taken in each cage.

Leakage rate determination: Air leakage from ventilated cages was determined qualitatively by measuring the concentration of SF₆ escaping from randomly selected cages for each system. Leakage was measured in 2.5 in below and 1 in away from the junction of the filter top and cage bottom, along the front of each cage after intracage SF₆ release. Leakage was expressed qualitatively as present or absent. The magnitude of leakage reflected the concentration of SF₆ detected when leakage was compared between systems. In addition, leakage was visualized after intracage smoke release from a TiCl₄ smoke stick.

Results

Air distribution: Figure 2 depicts the air distribution patterns observed after smoke release from 3 points in each of the 3 caging systems. In unit 1, smoke moved quickly, and rapidly diffused throughout the cage after release from all 3 points. Smoke distribution and diffusion throughout the cage was most rapid after smoke release from the back of the cage, the point closest to the supply air nozzle. Smoke distribution was turbulent when it was released from the point in the center of the cage. Smoke dispersion was rapid when it was released at the most forward point in the cage, however smoke initially concentrated at the front of the cage. Smoke was observed to escape from the junction of the cage bottom and lid at the front of the cage after smoke release at all 3 intracage points. The leakage of smoke was greatest when it was introduced directly into the supply air stream at the back of the cage. Compared with units 2 and 3, unit 1 provided the most rapid and complete smoke distribution.

![FIG. 1. Schematic representation of cage ports (4) located on the right side of a test cage.](image-url)
In unit 2, smoke distribution was poor after release from all 3 intracage points. Smoke moved slowly and slightly forward after release from the smoke stick at the back of the cage; it moved slowly upward and slightly forward after release in the center of the cage, and it moved slowly upward and backward after release from the front of the cage. No smoke was observed to exit the cage after smoke dispersion from any of the 3 intracage release points. In comparison with units 1 and 3, unit 2 had the least rapid air distribution.

In unit 3, smoke distribution was similar to that observed in unit 1, except that air moved slightly more slowly and leakage of smoke from the cage was not observed.

**Intracage air exchange rates**: Intracage air exchange rates per h (ACH) were determined from SF$_6$ decay curves at 8 predetermined intracage locations; mean ± SD values were 65 ± 19, 42 ± 32, and 79 ± 21 ACH for units 1, 2, and 3, respectively. Select SF$_6$ decay curves are provided in Figure 3 (a-c). In general, SF$_6$ decay was linear from all 8 points evaluated in units 1 and 3. Curves generated from several points in unit 2 indicated increasing concentrations of SF$_6$ before ultimately decreasing, an indication of poor air mixing in particular areas of the cage. Increasing concentration of SF$_6$ was observed at one evaluation point during the measurement period. An ACH value of zero was assigned to this determination.

**Air velocity determination**: Intracage air velocities were determined from 8 intracage points in 20 cages from each system. Mean ± SD linear air velocity was 36 ± 18 and 38 ± 12 linear feet/min (fpm), with a range of 20 to 100 and 20 to 72 linear fpm for individual detection points in units 1 and 3, respectively. Air velocity was below the detection limit of the thermoanemometer (< 10 linear fpm) for unit 2.

**Air leakage determination**: Air leakage was determined qualitatively by measuring SF$_6$ escaping from the front of cages of each unit. The SF$_6$ was detectable escaping at the front of the cages of units 1 and 3, but was not detectable from cages of unit 2. The concentration of SF$_6$ escaping from unit 1 was approximately 2 times that escaping from unit 3.

**Discussion**

There are a multitude of reasons for the burgeoning popularity of ventilated caging systems. Results of several studies have indicated substantial alteration of microenvironmental conditions to which rodents are exposed when housed in static isolator caging systems (11-14). Modern static isolator caging systems cause greater microenvironmental alteration, compared with previously used systems (11, 12). They enhance protection from contamination with adventitious microbial agents (6, 7). The development of transgenic and "knock-out" technology has led to the creation of extremely valuable and frequently irreplaceable lines of animals that need to be provided with the highest volume and quality...
quality environment and protected from cross contamination. These systems also permit greater numbers of cages to be maintained in the same floor space. If integrated into the building’s HVAC appropriately, ventilation rates can be reduced, thereby reducing facility construction and operational expenses (15). However, ventilated caging systems are purchased at a substantial cost premium, compared with static systems.

Because provision of ventilation to individual cages is the principal reason for which these systems are purchased, buyers should understand the advantages and disadvantages of particular systems and have access to critical information so they can compare systems on the basis of accurate and standardized test data. Unfortunately, this information is frequently unavailable for particular systems, or if available, may not permit comparison between systems because different testing methods and performance criteria have been used in the evaluation.

It is extremely difficult to test and obtain accurate information on ventilation characteristics for these systems. The technology available to determine ventilation is designed for rooms or buildings; it is not designed to accurately evaluate enclosures the size of rodent cages, which have a volume of less than 1 ft³, or whose air supply or exhaust rates may be less than 0.5 cubic feet per minute (CFM).

Our initial approach to determine intracage ACH was based on determining the volume of supply air provided to each cage. We attempted to measure the air provided to each cage through the cage supply air outlet, and we estimated the air delivered by measuring the total volume of air being supplied by the air fan and dividing that value by the number of cages on the rack (data not shown). Both methods had pitfalls.

Accurate measurement of small air volumes is difficult and, even if measured accurately, the change in static pressure may affect the volume of air delivered because the cage is not in place when the determination is made. This is most critical for unit 2 because air must pass through the filter lid before entering the cage. The manufacturer of unit 1 has developed a system for supply air determination, using a modified cage that directs the supply air through a rigid tube to the front of the cage so that it can be measured. However, issues related to change in static pressure remain when measuring supply air by use of this equipment. The ACH within each cage is difficult to determine accurately on the basis of the total quantity of air supplied by the fan. The total quantity of air delivered by the fan can be determined, but it must then be assumed that this quantity is delivered equally to all cages. In addition, it must be assumed that none of this supply air leaks prior to entering the cages. Furthermore, if the cage is under “negative” pressure, additional air will flow into the cage from the room.

Use of a tracer gas, such as sulfur hexafluoride, can provide a more accurate reflection of the actual air flow conditions within the cages. This technique has been used largely for determining air exchange rates in rooms or buildings (10, 16). It has more recently been used to evaluate the cage environment (17).

The advantages of this technique are its quantitative nature and the fact the condition in an individual cage can be measured directly. Tracer gases used are stable and safe. The limitations of the technique are the initial costs of the equipment ($10,000) and the need to develop a standard study protocol. An additional limitation for tracer studies in small volumes (i.e., cages) is the specific sampling equipment chosen. Some analytical instruments sample at high air flow rates relative to the cage volume. This can result in the technique itself introducing 4 to 5 ACH. In our study, we used a sampling rate of 100 cc/min, which by itself would only result in 1 to 2 ACH. This represents <10% of the air changes being measured and probably has minimal effects on the relative results.

We evaluated several ventilation characteristics of these cages.
in the filter top beneath either the supply air orifice or the supply and exhaust air orifices, open when the cage is placed on the ventilated rack. The valves permit exhaust and/or supply air to exit and enter the cage directly, without having to penetrate the filter medium. Use of both valves would likely improve intracage air distribution and enhance air velocity and ACH values. Additional studies are needed to evaluate unit 2 equipped with air valves.

The ideal ACH value for individually ventilated cages remains unknown. The criteria used to determine an ideal ACH rate should be formulated from objective data on microenvironmental ammonia and carbon dioxide accumulation, the provision of stable temperature and humidity within suggested ranges, and adequate air distribution throughout the animal cage. A recent study examined microenvironmental conditions in these same 3 caging systems. Ammonia was detected several days earlier and accumulated in substantially greater amounts in cages from unit 2 than in cages from either unit 1 or 3. The practical importance of these findings remains unclear, as ammonia concentration detected in unit 2 was negligible before day 7, and unless cage changing is done less often than weekly, would be of little concern. Because of differences in design, the ideal ACH value will likely differ between caging systems. The air velocity to which animals are exposed should be minimized to prevent drafts that can cause chilling, which is of special concern for neonates and hairless strains, and also to limit the dispersion and generation of particulates within the cage.

Release of intracage air into the room is an important factor to consider. If isolator cages are used for primary containment, air must not escape without filtration into the room. Unit 2 permits adjustment of dampers in the supply and exhaust plenum, allowing cages to be maintained at positive or negative pressure with respect to the room. Units 1 and 3 maintain cages under positive pressure attempting to scavenge escaping air. Users must recognize the advantages and limitations of each system, and when alterable as with unit 2, must understand the mode in which the equipment is being operated.

Ideally, ventilated cage systems should provide product and personnel protection. Product protection refers to the protection of animals housed within the cage from exposure to microbial agents carried by other animals or people. Personnel protection refers to protection of people who handle or care for the animals from exposure to airborne materials, particulates and volatile gases, present and/or generated within the animals’ cage. Airborne materials may be generated by the animal or may be a component of the animal’s feed or bedding. Objective criteria must be developed to determine whether one or both goals are met. Class-II biological safety cabinets must also provide product and personnel protection. To ensure these criteria are achieved, objective testing methods were developed to evaluate class-II biological safety cabinet performance. These methods, published as the National Sanitation Foundation Standard 49, are standards of manufacture and testing that assure the buyer of compliance. The methods used in this study followed the general concepts of the published standard and are provided to provoke thought and discussion among designers and users of ventilated caging systems.

References

17. The H. L. Turner Group Inc. 1993. Final report to the Jackson Laboratory summarizing tracer decay PIV air exchange rate testing. Harrison, ME.