Comparison of Environment and Mice in Static and Mechanically Ventilated Isolator Cages with Different Air Velocities and Ventilation Designs

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The purpose of this study was to compare environmental conditions and mice in cages with four different mechanical ventilation designs and a static isolator cage. Environmental conditions (air velocity, temperature, relative humidity, bedding weight change, airborne dust, NH_3, and CO_2) were compared for each cage type (n = 5 per cage). Bedding type was chipped hardwood. Mouse response in each cage type was evaluated by body weight, feed consumption, water intake, location of specific behaviors, and building of bedding mounds. Commercial polycarbonate mouse caging units (29.2 × 19.1 × 12.7 cm shoebox style, stainless-steel round wire bar lids, and 7-cm-deep isolator cage filter tops) were modified to fit the mechanical ventilation cage types and were used for the static isolator cages. Mechanically ventilated cages were fitted with forced air inlets centered 5 cm above the cage floor on the 19.1 cm-side of the cage. Inlet air velocity was either 40 or 200 feet/min (n = 10 cages each), and the air volume exchange rate was 9.3 L/min. In half of the mechanically ventilated cages, the exhaust air was forced through a filter in the isolator cage top, whereas in the remaining mechanically ventilated cages, the air was forced through a single exhaust port fixed in the narrow side of the cage top directly above the air inlet. Inlet air velocity but not exhaust design affected intracage air velocity distribution. Other environmental conditions were similar between the four mechanical ventilation designs. Relative to the mechanically ventilated cages, the static isolator cages had lower air velocities, higher relative humidities, higher NH_3 levels, higher CO_2 levels, lower body weight gain, and lower water consumption; temperatures, particulate levels, and feed consumption rates did not differ significantly between cage types. Locations of bedding mounds and behaviors were similar in all cage treatments.

The goal to improve environmental conditions in animal hous- ing facilities and the microenvironment to which animals are exposed has given rise to an evolution of ventilation designs for rodent iso- lator caging systems (ICS). In a comprehensive review of static and mechanically ventilated ICS, Lipman (1) indicated that the lack of common features makes comparative operational evaluations diffi- cult. Physical and biological ICS data from the literature have been used to develop computational fluid dynamic (CFD) models that can be used to predict ICS ventilation response (2). Because CFD model verification experiments were conducted with static mouse cages, a main objective of our study was to evaluate the intracage environments of ICS with two different mechanical ventilation sup- ply air velocities and two different air exhaust systems. The four mechanical ventilation cage treatments were compared to static iso- lator cages. In order to facilitate comparative operational evaluations, all physical and animal variables were kept as uniform as possible. Another specific objective of our research was to define how live mice would affect the intracage environment and to evaluate how the environments from the various cage treatments would affect the mice.

Prior to the animal experiment, initial environmental measure- ments were taken with Simulated Mouse Objects (SMO) in the cages to ensure that environmental conditions in the cages were ac- ceptable. Data collected under SMO conditions can be integrated into previously developed computational fluid dynamic (CFD) models to ensure increased accuracy in predictions of ICS ventila- tion. The individually ventilated ICS used in this study were designed, fabricated, and their physical attributes measured in order to reduce the possible variables that would be associated with different com- mercially available ICS. We used our experimental ICS to better define and compare experimental parameters; they were not intended to replicate any commercially available ICS.

Materials and Methods

General protocol and macroenvironment. Approval of all facili- ties and procedures for the use of laboratory mice was obtained from the University of Illinois Institutional Animal Care and Use Com- mittee prior to the initiation of this research. Prior to the conduct of this study, facilities, recording equipment, and environmental con- ditions within all cages were evaluated using bedded, fully assembled cages containing an SMO. The SMO approximated the area dis- placement and thermal emissions (2.6 W) of a group of five mature mice (3). Individual cage and SMO dimensions were the same as defined by Memarzadeh (2).

After the SMO stage of the experiment, the SMO was replaced with five female mice (Mus musculus) per cage. The mice (Hsd:ICR, CD-1; mean body weight, 26 g) were transported in a dedicated vehicle operated by the vendor (Harlan Sprague Dawley, Indianapo- lis, Ind.). The mice were housed in polycarbonate shoebox-style cages (approximately 29.2 cm long × 19.1 cm wide × 12.7 cm deep) with stainless-steel round wire bar lids for a 1-week period prior to experimental data collection. The number of mice per cage was the maximum allowable for the mouse weight and cage area (4).

During both SMO and live animal data collection periods, cages were ran- domly assigned to one of two levels of a stainless-steel cage rack that was located inside a 4.88 $\times$ 3.14 $\times$ 2.44 m controlled environment chamber (Hotpack, Philadelphia, Pa.). Air ventilation into the con- trolled environment chamber supplied approximately 18 air changes per hour, was HEPA-filtered, and served as the inlet air source for all cages. Air from the environmental chamber was exhausted di- rectly outside the animal facility. The environmental chamber had a

12:12-h photoperiod (lights on at 1:00 pm). Photophase illumina- tion was from four clear (60-W) incandescent light sources (General Electric Co., Cleveland, Ohio) that were equally spaced around the stainless-steel cage rack (intensity, 20 to 40 foot-candles, as mea- sured beside the cages at rack level). Because the least amount of photoactive responses appear to occur in the blue light spectra (5), scotoperiod illumination was from four blue (25-W) incandescent light sources (General Electric Co., Cleveland, Ohio; intensity, 0.2 to 0.4 foot-candles) that were located adjacent to the clear lights. The temperature and relative humidity were continuously moni- tored with hygrothermographs (model WD-37250-00, Oakton Instruments, Vernon Hills, Ill.). Temperature in the environmental chamber was 23.7 $\pm$ 0.1°C, and relative humidity was 43% $\pm$ 1.2%. Animal care records and mouse conditions were checked daily by Office of Laboratory Animal Resources personnel.

Cage ventilation design. Fifteen new commercial polycarbonate mouse isolation caging units (approximately 29.2 cm long $\times$ 19.1 cm wide $\times$ 12.7 cm deep shoebox-style cages with stainless steel round wire bar lids and 7-cm-deep isolator cage filter tops; N10 series, catalog no. N10MBTC, Ancare, Bellmore, N.Y.) were used for all cages in this study. There were five air ventilation treatments-four of the ventilation treatments used mechanical ventilation systems that provided 60 air changes per hour (ACH), and one treatment was a static isolator cage with no mechanical ventilation. The five ventilation treatments were designated as: high-velocity air supply with the air exhausted through a single-point exhaust (HS), high-velocity air supply with the air exhausted through a porous cage top (HP), low-velocity air supply with the air exhausted through a single-point exhaust (LS), low-velocity air supply with the air exhausted through a porous cage top (LP), and static isolator cage with a po- rous filter top but no mechanical ventilation (SM). Refer to Fig. 1 for a schematic diagram of the mechanically ventilated cages.

The configurations of the mechanical ventilation systems con- sisted of combinations of two types of air inlets and two types of air exhausts. High-velocity air inlet nozzles were cylinders (diameter, 3.8 cm) with six openings (inner diameter, 0.56 cm) on each. Air supply through the high-velocity nozzle openings results in veloci- ties of approximately 200 ft/min at the nozzle. The inlet air nozzle for the low-velocity configuration consisted of a cylinder (diameter, 7.0 cm) with 162 (inner diameter, 0.28 cm) openings on each cylin- der. The low-velocity air supply was designed to provide velocities of approximately 40 ft/min at the nozzle. Air-supply nozzles were installed centrally on one of the short (19.1-cm) sides of the cage-bottom, and the center of the air supply cylinder was approximately 5 cm above the cage floor. Supply airflow rate in all mechanically ventilated cages was 9.3 L/min, which provided 60 ACH. The single-point air exhaust was manufactured from a standard copper pipe cap (diameter, 6.3 cm) with a hose connector (outer diameter, 1.0 cm) attached through the solid flat side, and the open side was covered with filter media (no. 2024, Reemay, Old Hickory, Tenn.). The open (filtered) end was inside the cage-top, directly above the air supply, and the hose connector extended through the cage wall. The porous (diffuse) type of exhaust was through filter media (no. 2024, Reemay) sandwiched between the porous plastic assembly "screens" in the top of the cage top (the same setup and material as for standard isolator cage filter tops). A solid plexiglas sheet was sealed over the porous plastic assembly in the tops of the single-point exhaust
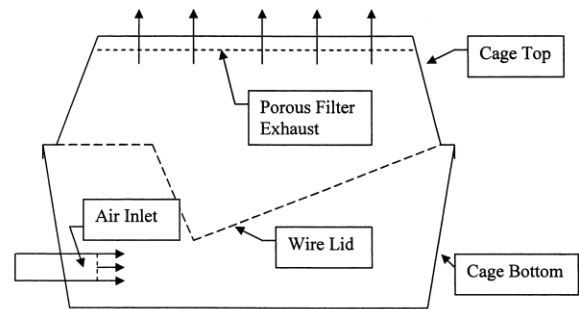
Mechanically ventilated cage with porous exhaust top (HP and LP)
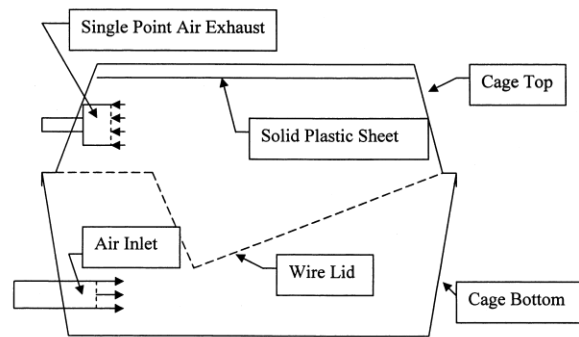
Mechanically ventilated cage with single point exhaust top (HS and LS)

Figure 1. Schematic diagram of mechanically ventilated mouse cages, with porous and single point exhaust tops—longitudinal cross-sections are shown. High- and low-velocity mechanical ventilation cages had air inlets that were centered in the cage bottom. Static isolator cages had porous exhaust cage tops but no mechanical ventilation air inlets.

cages. Cage tops of the mechanical ventilation cages were sealed to the cage bottom section for the entire period of each experiment so that all inlet air was exhausted from the ventilated cages through either the filtered single-point outlet or the porous tops under posi- tive pressure. The sealing material was replaceable weather-stripping (M-D Building Products, Inc., Oklahoma City, Okla.). SM cage filtered cage tops were not sealed to the cage bottoms, and the tops remained in place for the entire period of each experiment.

Fresh air was supplied equally into each forced ventilated cage at 9.3 L/min. An air pump (4F7 4EA, Gast Manufacturing, Inc., Benton Harbor, Mich.) was used to supply air to a common (multiport) static pressure tank, which distributed the air among the cages through flexible hoses. The airflow rate supplied by the pump to the static pressure tank was adjusted using a bypass valve. Airflow from the static pressure tank to each cage was measured with flow meters (model U-32458-52, Cole-Parmer Instrument Co., Vernon Hills, Ill.) and controlled using flow meter valves. Flow meters were cali- brated using a Mini-Buck Calibrator (model M-30, A.P. Buck, Inc., Orlando, Fla.). Correction factors were formulated by regression of cage flow meter values to the Mini-Buck Calibrator.

Experimental design and analysis. Seventy-five mice were allo- cated randomly into 15 designated cage groups that remained together throughout this study as five-mouse experimental units (MU). Three MU then were randomly assigned to each cage unit (A through E), and each cage unit (CU) was initially randomly as- signed to a particular cage ventilation treatment. Cage units were reassigned (sequentially rotated) to a different cage ventilation treat- ment on a weekly basis over the 5-week study until all CU (three MU each) had experienced each of the five cage ventilation treatments. This procedure allowed us to evaluate whether measurement of animal

responses by a particular CU (replication unit) was independent of cage ventilation treatment.

During each week, the following measurements were recorded: temperature, relative humidity, dust (mass and particle count), NH_3, and CO_2 (cage environment parameters); body weight and food and water utilization (animal response parameters); and activity and mound building at mid-photophase and mid-scotophase (animal behavior parameters). Environmental conditions were sampled from one cage in each of the cage ventilation treatments by fitting it with an instrumented cage top which remained in place for the entire week of each experiment. Animal responses were recorded weekly for all 15 cages and 75 mice.

Significant differences between variables were assessed using analy- sis of variance and Fischer's least significant difference tests at an inference level of $P < 0.05$ (SAS Institute, Inc., Cary, N.C.).

Data collection protocol and methods. The following data col- lection protocol was followed on a weekly basis:

Friday-Mice, feed, water, and bedding were weighed-out of one cage, and the mice were placed in a clean experimental cage of a different cage ventilation treatment.

Saturday and Sunday-Animal status was visually checked, but no measurements were collected.

Monday-Temperature, relative humidity, and dust were recorded from the five cages fitted with instrumented cage-tops (one cage for each cage ventilation treatment).

Tuesday-NH_3 and CO_2 gas levels were sampled from the five cages with instrumented tops. NH_3 measurements were taken 5 days after the mice were placed on new bedding, because it is a common
practice in many laboratories to change bedding at least every 5 days.
Wednesday-Location and activity of all 75 mice and the loca- tions of bedding mounds were recorded for all 15 cages at mid-photophase and mid-scotophase.

After the weigh-back measurements were obtained on Friday, the mice were maintained in their separate mouse experimental units (MU) and housed in individual open top rodent cages for approxi- mately 2 to 4 h. During this time period, all of the experimental cages (15) were cleaned, sanitized, and dried. All cages were given a fresh (weighed) supply of feed (Teklad 22I5 Rodent Diet [W] 8640; Harlan Teklad, Indianapolis, Ind.), bottled tap water, and an ap- proximately 1.3-cm depth of evenly spread bedding (Course Grade Beta Chips, 100% Hardwood; Northeastern Products Corp., Warrensburg, N.Y.). After this cleaning period, the mice were again weighed as a MU and assigned as a CU to a different cage ventila- tion treatment for the next week.

Weekly changes in body weight, feed and water use, and bedding weight were based on the weigh-in values from the previous Friday. This procedure was repeated five times so that all mice had been recorded in each of the experimental cage ventilation treatments (HS, HP, LS, LP, and SM). The procedure also allowed the evaluation of responses for the week after the change to a different experimental cage ventilation treatment (HS to HP, HP to LS, LS to LP, LP to SM, and SM to HS; this sequence was selected at random). This sequence of change in cage ventilation treatment was arbitrarily as- signed; however, time and facilities would not allow for all possible combinations.

T-type thermocouples were used to measure and record tempera- tures outside and inside the cage. The thermocouples were calibrated using a water bath. Water bath temperatures were determined using a total immersion mercury thermometer (model 94-23403, vendor unknown, Taiwan). Correction factors were formulated by regres- sion of thermocouple readings on thermometer readings. Thermocouples were placed in the room, in supply air (inside the tube connected to the air inlet nozzle in cages with mechanical ventilation), and in each of the five instrumented cage tops. Six ther-

mocouples were inside the cages at heights of 12.5 cm and 19 cm from the cage bottom (three thermocouples at each height, spaced at - 6.5, 0, and 6.5 cm from the cage center along the median axis). Measurements at lower positions were not possible because the mice would interfere with the sensors. Thermocouples were connected to a data acquisition system (Model Personal DAQ 56 + PDQ2, Iotech, Inc., Cleveland, Ohio). In each cage location and for each cage ventilation treatment, temperature was determined from nine separate measurements of 20 samples each (a sample was measured every 10 sec).

Air velocities were measured using an omni-directional probe (model 8455, TSI, Inc., Shoreview, Minn.) calibrated in a benchtop wind tunnel (model 8390, TSI, Inc.). During velocity measurements, the cages contained bedding, water bottle, feed, and an SMO but no mice. Measurements were taken at three points 4 cm above the bottom of the cage (-6.5, 0, and 6.5 cm from the cage center along the median axis). In each cage location and for each cage ventilation treatment, velocity was determined from nine separate measurements of 20 samples each (a sample was measured every 10 sec). Air veloc- ity readings below 10 ftlmin are not as accurate as higher ones, but the readings were consistent across measurements.

Relative humidity was evaluated electronically using a thermohygrometer (model 900, General Electric, Woburn, Mass.). The relative humidity sensor probe was inserted through a port into the center of each instrumented cage top, and readings were taken after a 10-min stabilization period. The relative humidity sensors were calibrated with a psychrometer prior to recording the room and cage. The signal from the sensor was collected on a data acqui- sition system (Model Personal DAQ 56 + PDQ2, Iotech, Inc., Cleveland, Ohio) connected to an IBM compatible PC.

A laser particle counter (Aerodynamic Particle Sizer TSI 3320, TSI, Inc., Shoreview, Minn.) was used to evaluate the airborne par- ticle size distribution at 52 subranges within the overall range of 0.5 to 19.8 μm, and dust mass was determined assuming standard par- ticle density (density of water, 1.0 glcm^3). A calibrated volume of air was vacuumed into the analyzer through a sampling hose (inner di- ameter, 1.3 cm) that was connected to a plastic tube sealed into the middle of one side of the instrumented cage-top. The plastic tube had a removable cap, which remained closed except during air sam- pling, was located 2.5 cm down from the top horizontal surface, and extended 3.8 cm inside the cage.

Air samples for NH_3 and CO_2 analysis were obtained through the same cage-top port that was used for dust sampling. Air from the sampling port was pumped to an infrared CO_2 analyzer (model 880A, Rosemount, Inc., Chanhassen, Minn.) that was connected to a Kipp-Zonen strip chart recorder (Cole-Parmer). Values recorded for CO_2 were calibrated against four certified CO_2 standards (1.49%, 0.998%, 0.506%, and 0.248 %). NH_3 gas samples were taken with a Matheson-Kitagawa pump (model 8014-400A, certified model 42 CFR84, Montgomeryville, Pa.) connected with either model 105 SD (0.2 to 20 ppm) or model 105 SC (5 to 20 ppm) Kitagawa Precision Gas Detector Tubes (Matheson Safety Products, East Ru- therford, N.J.). Values recorded for NH_3 were calibrated against two certified NH_3 gas standards (52.5 and 74.8 ppm). All wire and tube connections that coupled the mouse cages to physical environment sensors attached through the polycarbonate isolator cage top and were sealed in place with pliable, replaceable weather-stripping ma- terial (M-D Building Products, Inc., Oklahoma City, Okla.). The total volume of intracage air extracted during each particulate, CO_2, and NH_3 sampling was 2.5, 3.4, and 0.2 L, respectively. The total internal volume of each cage was just under 11 L and time per sample was under 2 min, so there should have been little long-term disturbance of cage conditions from these samples
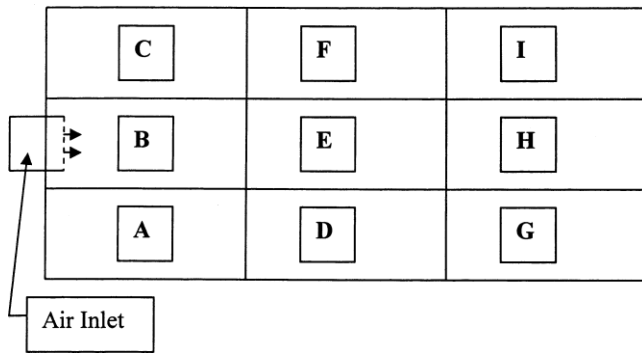
Figure 2. Map of mouse cage floor area showing the nine potential areas (A through I) used for the behavioral and mound-building recordings. Because of the location of feed and water in the wire lid above the mouse area, recorded eating behavior could only occur in areas A, B, D, H, and G, and drinking behavior could only occur in areas C and F.

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Cage locations of behaviors and bedding mounds were recorded on standardized charts assigned to each cage, by an observer that quietly entered and remained in the mouse room until a cage-floor area and one of six designated behaviors were recorded for each mouse. Designated categories of behavior were sleep, groom, move, drink, dig, eat, and climb. The number of mice involved in a desig- nated behavior and their locations during the time of observation were recorded. The recording charts schematically mapped the floor of the mouse cage into nine separate equally sized areas in relation to the sides and centers of the long and short axes of the cage (Fig. 2). Each area was designated by an alphabetic character; areas A, B, and C represented the one-third of the cage at the air inlet end (front); D, E, and F represented the transverse third (mid); and G, H, and I represented the remaining third (rear). Areas B, E, and H were cen- ter (median) along the narrow axis. The locations of the feed and

water supplies dictated that the eat behavior could only occur in areas A, B, D, H, and G; likewise, the drink behavior could only occur in areas C and F. Bedding mounds were recorded in areas that appeared to be elevated 2 or more cm above the surrounding level of bedding and were drawn directly on the recording charts. Video recordings were not used in this study, because only general activity and locations can be observed. Because records were obtained during the middle of the 12-h photophase and 12-h scotophase, clear video records were not accurate for precise counts of mice or mound locations.

Results

Physical results. (i) Air velocities. Air velocities (ftlmin) measured at 4 cm above the cage floor, along the median axis of empty cages that were fully assembled and bedded, at three evenly spaced cage locations (front, center, and rear) for all cage ventilation treatments are shown in Table 1. Mean air velocities for the high- and low-velocity cages were 65.3 and 42.3 ftlmin ($P < 0.05$), respectively. Exhaust design did not affect air velocity, but intracage pressures were 0.083 ± 0.004 and 0.003 ± 0.001 cm of water column ($P < 0.05$) for the single and porous exhaust designs, respectively. (ii) Air temperatures. Air temperatures (°C) measured at 12.5 and 19 cm above the cage floor, along the median axis of cages containing five mice each, at three evenly spaced cage locations (front, center, and rear) for the five different ventilation treatments are shown in Table 2. Points of measurement within the cages showed significantly different air temperatures, and there was no main effect of cage ventilation treatment or interaction. The top and middle measurement areas of the cages differed ($P < 0.05$) across all ventilation designs, and overall means for each were 24.33 ± 0.05 and 25.34 ± 0.06°C, respectively.

(iii) Relative humidity. Mean relative humidity (%) inside the different cage ventilation treatments is shown in Table 3. When the

Table 1. Effect of cage ventilation treatment on cage air velocity

Points of measurement[a]			Air velocity (ftlmin, mean ± standard error of the mean) according to cage ventilation treatment				
Location	h (cm)	L (cm)	HS	HP	LS	LP	SM
Bottom front	4	-6.5	98.83 ± 2.8	104.10 ± 1.2	52.90 ± 0.60	45.94 ± 0.41	2.93 ± 0.78
Bottom center	4	0	63.72 ± 1.6	71.74 ± 4.3	51.39 ± 4.5	52.18 ± 4.4	7.05 ± 0.25
Bottom rear	4	6.5	24.02 ± 1.6	29.59 ± 2.3	23.62 ± 2.8	27.83 ± 3.2	0.00 ± 0.0

h, height above bottom of cage; HP, high-velocity air supply with air exhaust through filter top; HS, high-velocity air supply with single-point air exhaust through filter; L, distance from cage center along median axis; LP, low-velocity air supply with air exhaust through filter top; LS, low-velocity air supply with single-point air exhaust through filter; SM, no mechanical air supply.
Analysis of variance for significance of main effects: Ventilation Design = $P < 0.001$, Points of Measurement = $P < 0.001$, Interaction = $P < 0.001$.
[a]Each value obtained from nine separate measurements of 20 samples each with no mice in the cages.

Table 2. Effect of cage ventilation treatment on air temperature (°C) and distribution in cage[6]

Points of measurement[a]			Temperature (°C, mean ± standard error of the mean) according to cage ventilation treatment					Overall mean
Location	h (cm)	L (cm)	HS	HP	LS	LP	SM	
Room			23.7 ± 0.2	23.6 ± 0.1	23.6 ± 0.1	23.6 ± 0.1	23.8 ± 0.1	23.7 ± 0.1
Middle front	12.5	-6.5	25.6 ± 0.3	25.7 ± 0.1	25.4 ± 0.4	25.4 ± 0.2	25.8 ± 0.1	25.6 ± 0.1
Middle center	12.5	0	25.3 ± 0.3	25.2 ± 0.2	25.0 ± 0.3	25.1 ± 0.2	25.5 ± 0.1	25.2 ± 0.1
Middle rear	12.5	6.5	25.2 ± 0.3	25.1 ± 0.2	25.2 ± 0.2	25.2 ± 0.2	25.5 ± 0.1	25.2 ± 0.1
Top front	19	-6.5	24.1 ± 0.2	24.3 ± 0.2	24.2 ± 0.2	24.2 ± 0.2	24.3 ± 0.1	24.2 ± 0.1
Top center	19	0	24.6 ± 0.3	24.6 ± 0.2	24.7 ± 0.2	24.6 ± 0.2	24.6 ± 0.1	24.6 ± 0.1
Top rear	19	6.5	24.1 ± 0.2	24.0 ± 0.2	24.3 ± 0.2	24.1 ± 0.2	24.3 ± 0.1	24.2 ± 0.1
Overall mean	na	na	24.8 ± 0.1	24.8 ± 0.1	24.8 ± 0.1	24.8 ± 0.1	25.0 ± 0.1	na

h, height above bottom of cage; HP, high-velocity air supply with air exhaust through filter top; HS, high-velocity air supply with single-point air exhaust through filter; L, distance from cage center along median axis; LP, low-velocity air supply with air exhaust through filter top; LS, low-velocity air supply with single-point air exhaust through filter; na, not applicable; SM, no mechanical air supply.
Analysis of variance for significance of main effects: Ventilation Design = $P < 0.117$, Points of Measurement = $P < 0.001$, Interactions = $P < 0.998$.
[a]Each value obtained from nine separate measurements of 20 samples each, with five mice per page.

Table 3. Effect of cage ventilation treatment on air environment inside cage[a]

	HS	HP	LS	LP	SM
Relative humidity (%)	46.06 ± 3.96	48.30 ± 1.99	47.60 ± 3.93	47.64 ± 2.91	78.42 ± 2.39[b]
Particulates (mglm^3)	0.257 ± 0.058	0.350 ± 0.063	0.315 ± 0.128	0.236 ± 0.070	0.134 ± 0.021
NH_3 (ppm)	0.00	0.20 ± 0.1	0.20 ± 0.1	0.1 ± 0.1	47.8 ± 28.5[b]
CO_2 (%)	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.56 ± 0.07[b]

HP, high-velocity air supply with air exhaust through filter top; HS, high-velocity air supply with single-point air exhaust through filter; LP, low-velocity air supply with air exhaust through filter top; LS, low-velocity air supply with single-point air exhaust through filter; SM, no mechanical air supply.

[a]Each value (mean ± standard error of the mean) represents replicate measurements taken weekly over a 5-week period. There were five mice per cage and different mice each week.

[b]Significantly different (P < 0.05) from values for other ventilation designs.

Table 4. Effect of photoperiod on activity and floor area[2] use[3] for all cage ventilation treatments (HS, HP, LS, LP and SM)[1]

Percents of Overall Total for Observed Behavior and Location
Photoperiod = Light:

Location:	A-front	B-front	C-front	D-mid	E-mid	F-mid	G-rear	H-rear	I-rear	Total
Sleep	17.1	1.3	9.6	5.6	0.0	12.0	3.5	1.9	33.1	84.0
Groom	1.1	0.0	0.5	0.8	0.0	0.5	0.5	0.5	3.7	7.7
Move	0.0	1.3	0.0	0.0	0.0	0.3	0.3	0.5	0.8	3.2
Drink	0.0	0.0	0.5	0.0	0.0	0.3	0.0	0.0	0.0	0.8
Dig	0.3	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.8
Eat	0.8	0.3	0.0	1.1	0.0	0.0	0.0	0.3	0.0	2.4
Climb	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.5	0.0	1.1
Total	19.2	3.2	10.7	7.5	0.0	13.1	5.1	3.7	37.6	100

Percents of Overall Total for Observed Behavior and Location
Photoperiod = Dark:

Location:	A-front	B-front	C-front	D-mid	E-mid	F-mid	G-rear	H-rear	I-rear	Total
Sleep	11.2	0.5	4.8	2.9	0.0	4.0	3.5	5.1	33.6	65.6
Groom	0.8	0.5	0.8	0.3	0.0	0.5	0.8	0.5	4.3	8.5
Move	1.6	4.5	1.1	0.5	0.8	0.5	1.1	3.7	0.5	14.4
Drink	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.3
Dig	1.1	0.0	0.0	0.0	0.0	0.0	1.6	0.3	0.5	3.5
Eat	2.1	0.0	0.0	0.8	0.0	0.0	0.3	0.0	0.0	3.2
Climb	0.5	1.1	0.3	0.0	0.0	0.0	0.5	0.0	1.1	3.5
Total	17.3	6.7	8.3	4.5	0.8	5.1	7.7	9.6	40.0	100

[1](HS = High velocity air supply - single point air exhaust through a filter, HP = High velocity air supply - air exhaust through a porous filter top, LS = Low velocity air supply single point air exhaust through a filter, LP Low velocity air supply - air exhaust through a porous filter top and SM = Cage without mechanical air supply).

[2]Areas A, B, and C represent the one-third of the cage area at the air inlet end. Areas B, E, and H represent the median third of the cage. Feed and water are centered over areas D, E, and F. (see Fig. 2).

[3]Values represent percent of 375 observed behaviors recorded during five replicate evaluations of 15 cages each (recorded at mid-photophase and mid-scotophase).

relative humidity inside each cage was subtracted from the relative humidity of the room (inlet) air, the mean difference in percent relative humidity was 4.5%, 5.0%, 5.6%, 4.6%, and 34.6% in the HS, HP, LS, LP, and SM treatments, respectively. When the differ- ences in relative humidity between inside the cage and room were compared to values when no mice were present in the cages, the mean differences were -0.04% and 10.9% for cages with no mice and mice (five per cage), respectively. When mice were not present, relative humidity was the same across all cage ventilation designs, as expected.

(iv) Particulates. Airborne particle mass (mg/m^3), sampled on the same day as temperature and humidity, is shown in Table 3. Particle counts per cm^3 in the 0.5- to 19.8- m range were skewed to the smaller end of the size range in all cage ventilation treatments. Par- ticles in the 0.5-to-2- and 2-to-4- m ranges represented 65% and 24% of the total count, respectively. There were no statistical differ- ences in particle counts across the cage ventilation treatments.

(v) NH_3 and CO_2. NH_3 and CO_2 levels were consistently higher in the SM cages ($P < 0.05$) than any of the other cage treatments (Table 3).

Response of mice to cage environment. (i) Behavior and bedding mound locations. From the data summarized in Table 4, it is apparent that more mice were observed at both ends of the cages than in the center. The stainless-steel cage lid extends to within 4.5 cm of the cage floor in the cage center, in the mid-cage area (floor areas D, E, and F in Fig. 2). Sleeping in a group was the most frequent obser- vation at both mid-photophase and scotophase (84% and 66%, respectively).

The percentages of the total bedding mounds (196) recorded in the front, middle, and rear areas of the cages were 3.0%, 84.2%, and 12.8%, respectively. Ventilation treatment did not influence the number or distribution of bedding mounds. When bedding mounds were observed, they generally extended into two or three areas across the narrow axis of the cage. Mice generally slept in a hollowed area (nest), and this nest and rim was not recorded as a mound. Mounds in the middle of the cage would often extend up to the bottom of the feed and water areas of the stainless-steel cage lids.

(ii) Animal management conditions-mouse, feed, water, and bedding weights. Body weight gain, body weight gain after the sequential change in cage design, and water consumption of mice were lower ($P < 0.05$) for the week of SM cage assignment (Table 5). Weekly increase in bedding weight was 65 ± 2, 68 ± 4, 68 ± 2, 71 ± 4, and 106 ± 4 glcage in the HS, HP, LS, LP, and SM cage designs, respectively. All mechanically ventilated cage designs showed a lower bedding weight increase ($P < 0.05$) than did the static isola- tor (SM) cages.

When evaluated over all treatments and replications, body weight differences did not vary significantly ($P < 0.05$) between MU and CU, and they were independent of cage ventilation treatment. Mean MU (five mice) body weight increased ($P < 0.05$) from 117.31 ± 0.92 g to 140.6 ± 1.24 g at the beginning of the first and fifth week of the experimental period, respectively.

Table 5. Effect of cage ventilation treatment on mice[a]

	HS	HP	LS	LP	SM
Body weight gain	1.59 ± 0.31	1.98 ± 0.29	1.79 ± 0.27	1.49 ± 0.25	0.71 ± 0.32^b
Gain for week after change	1.57 ± 0.38 SM to HS	1.76 ± 0.32 HS to HP	1.50 ± 0.27 HP to LS	1.41 ± 0.29 LS to LP	0.54 ± 0.37^b LP to SM
Feed consumed	25.8 ± 0.40	26.9 ± 0.69	26.5 ± 0.49	26.5 ± 0.54	25.5 ± 0.61
Water consumed	41.37 ± 1.32	40.43 ± 1.13	40.30 ± 1.22	39.60 ± 1.38	34.48 ± 1.08^b

HP, high-velocity air supply with air exhaust through filter top; HS, high-velocity air supply with single-point air exhaust through filter; LP, low-velocity air supply with air exhaust through filter top; LS, low-velocity air supply with single-point air exhaust through filter; SM, no mechanical air supply
[a]All values (glmouselweek) represent the mean $\pm$ standard error (n = 15) of the mean of weekly measurements from three cages of five mice each during the week of cage design exposure. Measurements were replicated over a 5-week period.
[b]Significantly different ($P < 0.05$) from values for other ventilation designs

.Discussion

Cage ventilation designs, which caused different air velocities within the cage and different avenues of air exhaust, did not influence the mean cage air temperature, and our finding is similar to results previously reported for static and ventilated cages (6-8). Forced convection heat exchange may have varied between mice and their environment at different air velocity treatments; however, this study was not designed to evaluate different perceived temperatures at different air velocities. Particulate levels were not significantly different between the various ventilation configurations. In general, the particulate level of our HEPA-filtered room air was low (0.04 mglm^3), and the mean intracage level was 0.218 mglm^3 higher. In a previous study (9) in which room air had a higher mean particulate level (0.98 mglm^3) than in our study, the mean intracage level was 1.03 mglm^3, which is a smaller increase than we measured.

Static isolator cages had higher mean levels of relative humidity and CO_2 and NH_3 concentrations than did mechanically ventilated cages, regardless of ventilation inlet velocity and exhaust design. Intracage humidity control appears more related to air exchange rate than to other ventilation parameters. The air exchange rate used in our mechanically ventilated cages was around 60 ACH, and the mean intracage relative humidity was $4.95 \pm 0.71\%$ higher than inlet (room) air. In studies in which vented cages received around 23 ACH, relative humidity was 18% higher than in the room (6), and a significant decrease in intracage humidity has been reported to occur between 40 and 60 ACH (8). When air exchange rate was in the range of 70 to 196 ACH, the cage bedding moisture content was only 2% while static cage bedding moisture content was 16.4% (10). In our study, the mean bedding weight gain was 1.9 glmousel day with 60 ACH and 3.0 glmouselday in the static isolator cage. The most likely explanation for this trend is that increased moisture evapo-ration from the bedding occurs in the mechanically ventilated cages.

Where comparisons were made, intracage NH_3 and CO_2 levels, like humidity, generally related more to air exchange rate than ventilation design or velocity (1, 6, 8, 9). In our study, with the exception of high NH_3 measurements from two SM cages (mean, 115 and 120 ppm), NH_3 and CO_2 levels both were consistent within a given ventilation design. Even if these two excessively high NH_3 samples were dropped from the data analysis, the NH_3 in the static isolator cages continued to be significantly higher than in other cage de-signs, and the means were 0.0, 0.18, 0.16, 0.12, and 1.3 ppm for the HS, HP, LS, LP, and SM cage ventilation treatments, respectively. In general we have noted in our research and as reported by others that when mean intracage NH_3 levels are high (> 25 to 50 ppm), there is low air exchange, high air relative humidity, and high bedding mois-ture content. There is also a large range of NH_3 levels. Another factor that should be associated with NH_3 levels in mouse cages is bedding pH. In research addressing the mass generation rate of ammonia from poultry manure, the pH of the manure is more closely related to gaseous NH_3 generation than to any other manure or bedding characteristic (11). In that study, very little NH_3 generation occurred at pHs lower than 6.5 to 7.0, and this association is related to the pKa (9.3) of NH$^+$. Another important factor that may have caused the higher variability in NH_3 measured in the SM treatment was the type of bedding used. Although chipped hardwood bedding is com-monly used in mouse cages, it is not as effective as ground corncob bedding for controlling NH_3 emissions (12).

The locations of certain designated behaviors and bedding mounds we reported were intended to be used for more realistic application of actual cage conditions in future CFD modeling of mouse cage ventilation. For example, in light of the behavior-location results, an estimated heat production from mice sleeping in a group likely would be representative about 75% of the time. However, because mice occupied both ends (especially the corners) of the cage, locat-ing the mouse group in the center of the rear area of the cage would not always be an accurate representation. The presence of bedding mounds across the center width of the cage that we noted in 30% of observations should also to be considered for ventilation modeling. This factor would appear to be especially important for modeling mechanically ventilated cages that force air along the long axis near the floor (bedding) level. Another consideration is that during the 150 recordings of mound observations, 61 cages had no distinct bedding mounds. During the light phase of the daily photoperiod, 55% of the cages had mounds and 45% had no mounds, and in the dark, 64% had mounds whereas 36% had no bedding mounds. These mound location data indicate that the mounds are rearranged or modified daily and may be a result of the digging behavior that was noted for 3.5% of the mice during mid-scotophase.

When compared with those of mice in mechanically ventilated cages, the water consumption and body weight gain of our mice were lower when they were housed in the static isolator cages. Mean body weight gain during the week that mice were in the SM cages was less than half that of the week before or after being housed in the SM cages. Body weight gain was consistently lower in SM cages during each week of the experiment. During Weeks 4 and 5, the mice that were transferred into and housed in the SM cages showed a mean loss in weight (-0.09 and -0.4 glmouselweek, respectively). Mean weekly feed consumed was not significantly different and was 132 and 127 glcage of five mice in the mechanically ventilated cages (HS, HP, LS, and LP) and SM cages, respectively. It is possible thatmore feed was spilled into and left in the wetter bedding of the SM cages than in the ventilated cages, but that cannot be confirmed from the data collected in this study.

The results of our study support the need for additional research that tests CFD models of ventilation patterns in mouse cages and that takes into account intracage thermal distribution, humidity, and bedding mound location. In addition, studies addressing the relationships between bedding moisture, age, and pH and the mass generation rate of gaseous NH_3 may be beneficial for explaining the wide range of intracage NH_3 levels (2).

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