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Humidity and Cage and Bedding Temperatures in Unoccupied Static Mouse Caging after Steam Sterilization

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Contemporary rodent caging and equipment often are sterilized by steam autoclaves prior to use in facilities. This work assessed the microenvironment of unoccupied static mouse cages after steam sterilization to determine when internal temperatures had cooled to levels appropriate for rodent housing. Polycarbonate static cages containing food and corncob bedding were stacked (10 rows × 7 columns) in duplicate (front and back; *n* = 140 cages) on a storage truck and autoclaved to 249 °F (121 °C). Cages (*n* = 6) were assessed to represent top, middle, and bottom rows and edges of columns. After cage sterilization, hygrothermometers were placed in cages to measure internal cage temperature (IT), bedding temperature (BT), and cage humidity (CH) every 10 min for 150 min. At time 0, there were no significant differences in averaged temperatures or humidity across cage locations: IT, 95.9 °F; BT, 109.8 °F; and CH, 84.1%. Over time, significant positional effects occurred. Whereas IT and BT for cages in the center row cooled more slowly than those on the bottom row, CH in top row cages decreased more quickly compared with other cages. After 150 min, the average measures overall were IT, 75.8 °F; BT, 77.9 °F; and CH, 82.4%. Comparison of the overall measures at 150 min with those of cages cooled overnight (IT, 72.4 °F; BT, 71.0 °F; and CH, 49%) and cages housing mice (IT, 72.2 °F; BT, 70.7 °F; and CH, 82%) indicated that a poststerilization cooling period of greater than 2.5 h was necessary to achieve permissible rodent housing conditions at our institution, particularly with corncob bedding autoclaved within the cage.

Abbreviations: BT, bedding temperature; CH, cage humidity; IT, internal cage temperature.

Laboratory mice (*Mus musculus)* generally are maintained at macroenvironmental temperature and humidity ranges of 64 to 79 °F (17.8 to 26.1 °C) and 30% to 70%, respectively.[6] These parameters are monitored closely and controlled within rodent colony rooms. In contrast, little is done to routinely monitor the microenvironment within rodent cages, despite notable differences between the micro- and macroenvironments.[1,8] The specific microenvironment relevant to this study includes static microisolation caging for housing mice, complete with a layer of bedding on the cage bottom, fitted wire-lid with water bottle and food pellets within the indented hopper, and fitted microisolation top with filter. Within the cage itself, bedding substrates have been assessed for effects on ammonia levels,[10,11,13] ventilation effects[9,12] and temperature regulation and metabolism of mice.[3]

In our institution's strict barrier facilities, caging equipment and husbandry materials are sterilized prior to use for housing animals. The typical practice, after autoclaving, allows for a prolonged cooling period of as long as 16 h (overnight) of caging materials. However, unanticipated circumstances, such as equipment failures, shortage of husbandry personnel, and rodent disease outbreaks, may warrant an abbreviated poststerilization cooling period to facilitate adherence to service expectations of once-weekly change schedules. On occasion, as soon as poststerilization cages were cool enough to handle, they were put into use for housing animals. At a minimum, cage set-ups cooled on the storage truck for approximately 1 h before transport into the animal housing room for replacement of dirtied cages for housing mice.

Over the course of approximately 4 mo, independent reports of 2 occurrences from decentralized strict barrier facilities were received, wherein a total of 10 mice died unexpectedly without experimental or other recent manipulations. The only commonality between the 2 isolated circumstances was the report of recent changing into autoclaved cage set-ups. These animals were housed in rooms free from infectious rodent pathogens. Gross necropsies had revealed evidence of hypersalivation around the mouths and damp haircoats. The concern was raised that although the exterior of the cages were comfortable for animal care staff to handle, the interiors of the cages had not cooled to a range suitable for rodent housing.

Despite various adaptations such as highly vascularized ears and unhaired tails, which increase the surface area for heat loss, heat dissipation is limited in mice, resulting in a low tolerance for high temperatures. Studies have shown that acute heat stress can result in physiologic[5] and behavioral aberrations in mice.[4] Ultimately, if cages are not sufficiently cooled prior to housing mice, the animals potentially could succumb to heat stress and hyperthermia. To date, the evaluation of retentive heat within the microenvironment after autoclaving has not been assessed.

We hypothesized that static microisolation cages containing bedding were able to retain heat for prolonged periods of time after removal from the autoclave, thereby precluding their appropriateness for use for animal housing within 1 h of sterilization. The expectation for the study outcome was to

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establish guidance for improved rodent husbandry practices related to poststerilization cage changes throughout our animal facilities.

Materials and Methods

Polycarbonate mouse cages (7.25 in. × 11.5 in. × 5 in.; Max 75, Alternative Design, Siloam Springs, AR) were used in the studies. Cages were filled to an approximate depth of 0.25 in. with bedding (diameter, 0.12-in.; Bed-O-Cobs, Animal Specialties and Provisions, Quakertown, PA) by using an automatic bedding dispenser (Girton, Millville, PA). Wire-lid hoppers then were placed in the cages and the indented food hoppers filled to capacity with autoclavable rodent chow (LabDiet 5010, Animal Specialties and Provisions) and covered with a low-profile microisolation lid filtered by spun-bonded polyester filter media paper. Water bottles were autoclaved separately and not placed on autoclaved cage set-ups. Cages were stacked in 2 rows, 1 in front of the other, 10 high and 7 across, for a total of 140 cages on a rectangular stainless-steel storage truck (60 in. × 24 in. × 68.5 in.; Alternative Design) with 3 walled sides and 1 side open for loading purposes. The storage truck loaded with cages was draped with an autoclavable truck cover, leaving only the underside of the truck platform and support wheels exposed (Figure 1 A). The truck was placed into a sterilizer (GE Sterilizer, Getinge USA, Rochester, NY), and the sterilization process included 16 min at greater than 249 °F (121 °C) followed by a 5-min drying cycle. During each sterilization cycle, reports were printed continuously at 60-s increments to ensure that appropriate temperatures were reached and that runs completed all cycle steps. There were no errors in sterilization cycles for each testing period. An additional measure of autoclave function included placement of biologic indicators (Attest Biological Monitoring System 1262, 3M, St Paul, MN) with loads to be autoclaved, run in duplicate each month.

Once the drying cycle was completed, the storage truck was removed from the sterilizer and placed in a cooling room (450 ft^2; 15 air changes per hour) immediately adjacent to the cage-wash area. Cage assessments occurred during July and August; conditions in the cooling room during testing were 72.8 ± 2.3 °F with 48 ± 4.1% humidity, as measured by the facility monitoring system (Phoenix Controls, Newtown Square, PA, and Comdale Systems, Toronto, Ontario, Canada).

To determine internal cage temperature (IT), bedding temperature (BT), and caging humidity (CH), portable hygrothermometers (Timex TX5170 Indoor–Outdoor Thermometer with Indoor Hygrometer, Maverick Industries, Edison, NJ) with triple window views were placed inside the cages with an attached sensor tip buried into the corncob bedding. The measureable upper limit for humidity was 91%, according to the manufacturer's description.

Experiment 1. After sterilization, the storage truck immediately was removed from the autoclave and placed in the cooling room. To facilitate the placement of hygrothermometers in multiple cages, the cover was removed and 6 specific test cages were marked with coded tape to ensure they returned to their predetermined positions that represented the top, middle, and bottom of the rows and column edges, both vertically and horizontally (Figure 1 B). Cages were then temporarily unstacked and relocated to a second holding truck. Hygrothermometers were placed in cages in the center column designated as top center, middle center, and bottom center and in those on the columns at the end of the truck to represent the top side, middle side, and bottom side (Figure 2). Cages were restacked onto the original storage truck with the cages maintained as a closed unit. For testing purposes, the cover was left off the storage truck so the hygrothermometers could be seen. The time required to place the hygrothermometers and return cages to their original position was recorded, and hygrothermometers were left to acclimate for 10 min prior to beginning trials. The first measurement (T_0) was taken once the acclimation period concluded. After T_0, measurements were taken at 10-min intervals until 150 min. There were a total of 6 trials, 3 in the morning and 3 in the afternoon, including 3 trials for the front set of cages and 3 for the back set. To collect baseline values for comparison, hygrothermometer measurements were taken from a sterilized cage cooled overnight at the beginning of each trial.

Control study. To assess the baseline parameters at which clinically normal mice typically are housed in our barrier facilities, we placed hygrothermometers in occupied mouse cages and recorded the measurable cage parameters. Mice from an inhouse breeding colony of coisogenic 129/SvJ were maintained on an approved Institutional Animal Care and Use Committee protocol and housed at a density of 5 mice per polycarbonate cage in a standard colony room (207 ft^2; 15 air changes hourly; 12:12 light:dark cycle). Occupied cages were located on a housing rack (Alternative Design) capable of supporting 112 cages; the overall room was at 60% cage capacity at the time of measurements. Additional features of the room included a class II biosafety cabinet (NuAire, Plymouth, MN). The macroenvironent was monitored by the computerized system described earlier for the cooling room. Room ventilation was balanced so that air flowed from the corridor into the colony room and exhausted through HEPA-filter iris exhaust dampers in the rodent room.

Hygrothermometers were placed in 6 occupied cages, 2 each on the first row (top), fifth row (middle), and eighth row (bottom); 3 test cages were in the middle column, and 3 test cages were on the end column. Four trials were conducted over the course of 2 d, 1 trial each in the morning and afternoon of both days. Measurements began after 10 min of hygrothermometer acclimation and were taken every 10 min for 60 min. Only IT and CH within the cage were measured because the mice interfered with the probes thereby preventing collection of BT readings. A modified experiment was performed to measure BT to determine any effects that the presence of mice in occupied cages might have conferred on the bedding. A sterilized cardboard barrier was fashioned and placed in the cage to prevent animal contact with the hygrothermometer probe during the course of the trial. For BT, measurements were collected every 10 min for 30 min from 3 occupied cages.

Statistical analyses. Cross-sectional and longitudinal data analyses were performed. A studentized *t* test was used to compare the mean values in outcome between observations measured in the front and back areas at the same time period and cage (or spot) on the loaded storage truck. The effect of spot outcome was examined at each time period by using analysis of variance. If front and back observations did not differ in outcome in the previous step, the observations were analyzed as repeats. Bonferroni adjustment was used if no significant effects of front and back area were noted. For the control studies evaluating occupied cages, a studentized *t* test was used to compare the mean values in outcome between all observations measured.

Mixed-effects models[7] were used to evaluate the longitudinal patterns of temperature inside the cage, temperature of the bedding, and humidity inside the cage over time. This statistical procedure accounts for the repeated time measurements at each spot location and adjusts for the within-spot correlation in the longitudinal data analysis. For each location and time

measurement, there were 6 measurements: top, middle, and bottom center and top, middle, and bottom side. A separate mixed-effects model was examined for each outcome. Changes in outcomes across time were not entirely linear; therefore, both linear and curvilinear effects of time were examined. Time was treated as both a fixed and random effect so that variation over time at both the population and individual location level could be evaluated. In addition, a random intercept was included in the model so that each location could vary from the average at baseline. T statistics were reported for examination of the effect of any polynomial function of time on outcome.

All results are reported as mean ± 1 SD. All analyses were performed by using SAS software with the Proc Mixed procedure (version 9.1.3, SAS Institute, Cary, NC). All statistical tests were 2-tailed, and statistical significance was defined as a *P* value of less than 0.05.

Results

Significant effects associated with cage location on the autoclaved storage truck became evident shortly after monitoring began. In assessing microenvironmental conditions across the 6 locations on the storage truck at 1 h (T_{60}) after sterilization (the time at which cages could be handled comfortably), the IT (mean ± 1 SD) was 84.3 ± 3.6 °F, BT was 89.7 ± 5.1 °F, and CH was 82.2% ± 11.3%.

Experiment 1. For comparison, unoccupied sterilized cages cooled overnight, measured values were: IT, 72.5 ± 1.2 °F; BT, 71 ± 1.2 °F; and CH, 49% ± 4.5%. For experimental sterilized cages, removing the storage truck from the sterilizer, placing the hygrothermometers, and reloading the cages onto the truck took 12.8 ± 4.4 min. Because hygrothermometers were left to acclimate for an additional 10 min before beginning the trials, time 0 (T_0) readings were not taken until approximately 25 min after removal from the autoclave. Measurements from sterilized cages were averaged across trials (n = 6), and the maximum (and minimum) individual cage measurements recorded were IT, 109.3 °F (71.8 °F); BT, 124.9 °F (71.6 °F); and CH, 91% (51%) across all time points to 150 min, independent of location. A Student *t* test showed no differences between the front and back cages; therefore for all trials, front and back measurements were collapsed, and values averaged depending on location.

The overall effect of location on the stacked truck with respect to cooling is shown in Table 1. For IT, the temperature did not differ significantly across locations until 60 min after monitoring began. At T_{60}, the IT of the top-side cage was significantly lower (81.8 °F, $P = 0.037$) and that of the middle-center cage was significantly higher (88.3 °F, $P = 0.0017$) from the mean temperature for all of the other cage locations at that time point. The middle-center cage had the highest temperatures throughout the monitoring period and was used as a reference point for the longitudinal analysis. Compared with the middle-center cage, the bottom-side cage cooled more quickly ($P = 0.0044$). The middle-side and bottom-center cages also had steeper declines in IT over time than did the middle-center cage, although the overall differences were not significant (Figure 3 A).

For BT, the effect of location on the truck had a significant effect on outcome at 50 min after the initiation of monitoring. Similar to IT, the BT of the top-side cage was lower (88.12 °F, $P = 0.025$) and that for the middle-center cage was higher (97.77 °F, $P = 0.0032$) compared with the average bedding temperature for the remaining cage locations at the same time point. Again, with the middle-center cage as the reference point, bedding in the bottom-side cage cooled more quickly ($P = 0.0002$). The middle-side and top- and bottom-center cages had steeper declines in bedding temperature (that is, they cooled more quickly) than did the middle-center cage, but the overall differences in temperature were not significant (Figure 3 B).

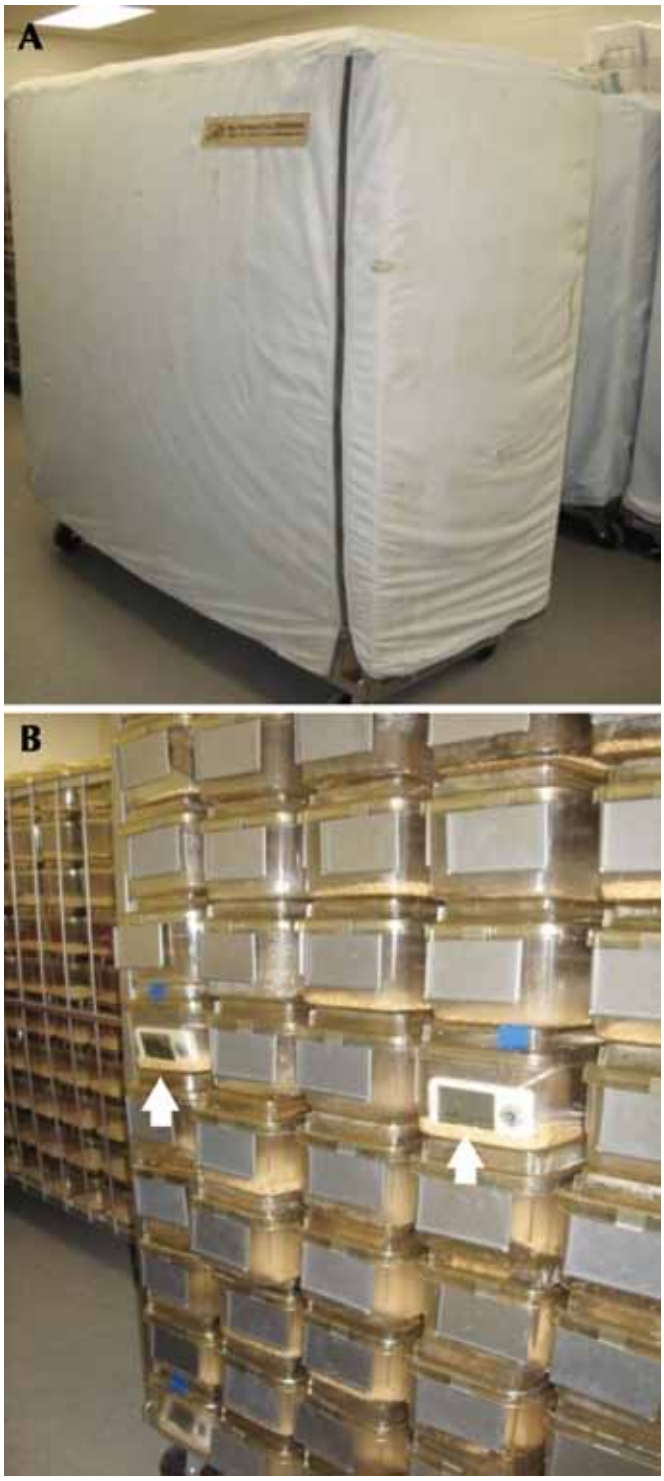

Figure 1. (A) Autoclavable storage truck (that is, cart for cages) as it appears after removal from the autoclave. The cover is zipped into place and typically remains over the cages until they have cooled and been moved to an animal housing room for routine weekly cage changes. (B) Hygrothermometer placement (see white arrowheads) in unoccupied cages. The LCD hygrothermometer screen was placed at the front of the cage for visualization of temperature and humidity readings during the monitored cooling period.

The humidity inside the cage differed significantly across locations within the first 10 min of monitoring. At T_{10}, the top-

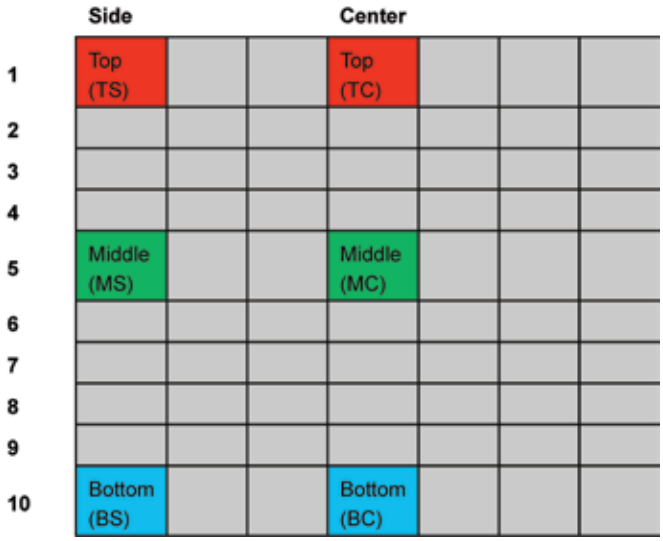

Figure 2. Schematic of hygrothermometer placement in the 6 unoccupied cage locations on the storage truck, after removal from the autoclave. TS, top side; MS, middle side; BS, bottom side; TC, top center; MC, middle center; BC, bottom center

side cage had a significantly lower humidity (76.5%, $P = 0.032$) than the average humidity for the rest of the cage locations. Over time, the bottom-center cage had the highest humidity and was used as a reference point for longitudinal analysis. Compared with the bottom-center cage, CH at the top-side ($P < 0.0001$) and top-center ($P = 0.0015$) locations dropped significantly more rapidly than others (Figure 3 C).

Control study. Measurements taken from occupied cages showed no differences in values between cage locations and repeated trials, therefore all data were averaged. At the time of testing, the average temperature of the colony housing room was 68.7 ± 0.8 °F, and average room humidity levels were 54% ± 5.8%. Measurements from cages housing adult mice were: IT, 72.2 ± 2.6 °F; BT, 70.7 ± 0.38 °F; and CH, 82% ± 4.5%.

Discussion

This study was designed to assess the microenvironment of unoccupied static mouse cages after steam sterilization to determine when internal temperatures had cooled to levels deemed appropriate for rodent housing. The cage set-ups were preloaded with food and bedding prior to autoclaving so that all environmental materials that came into close contact with the mice, including water bottles (autoclaved separately), were sterile. We hypothesized that although staff could handle the exterior of the polycarbonate cages, cage humidity and temperatures of the microenvironment and bedding were not appropriate for routine rodent housing.

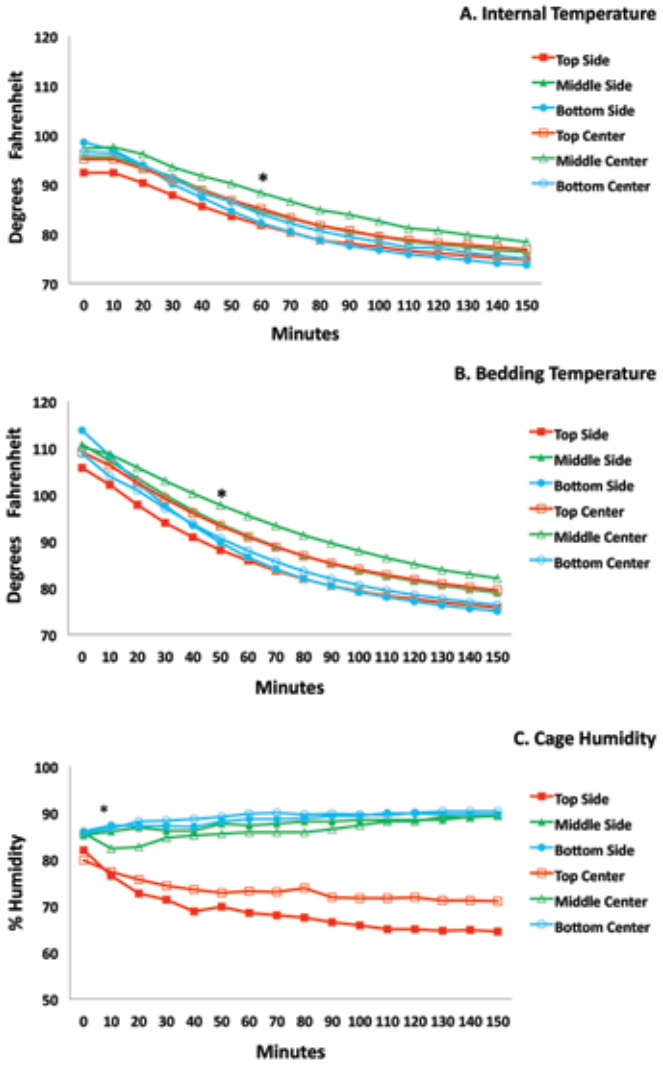

Figure 3. Time course (min) of cooling and dehumidification of sterilized unoccupied caging. (A) Internal cage temperature. (B) Temperature of corncob bedding. (C) Cage humidity. Repeated trials were averaged and plotted for each of the 6 positions on the storage truck. Red lines depict cages from the top row, green lines depict cages from the middle row, and blue lines depict cages from the bottom row. The asterisks indicate the 10-min timeframes during which cage location significantly affected measured parameters during the cooling period.

Table 1. Time to significant effect of cage location on internal temperature, bedding temperature, and cage humidity during the cooling period

Time (min)	Internal cage temperature	Bedding temperature	Cage humidity
0	0.67	0.67	0.46
10	0.74	0.60	***0.049***
20	0.56	0.40	0.0041
30	0.44	0.18	0.0011
40	0.20	0.067	0.0004
50	0.065	***0.017***	0.0003
60	***0.014***	0.0037	0.0001

Values in the columns under headings of IT, BT, and CH represent the P values when the main effect of cage location was evaluated statistically; significant differences are those yielding a P value of less than 0.05. Italicized type indicates the first time point at which differences were significant.

At 1 h after initiation of monitoring, the time at which our practice occasionally had warranted cage-changing procedures, the mean internal cage temperature was at least 80 °F, corncob bedding temperatures were approaching 90 °F, and cage humidity was approximately 80%; all values were higher than acceptable macroenvironmental parameters of the *Guide*.[6] Significant effects of temperature, relative to cage location on the autoclaved storage truck, were noticeable within the first 60 min of cooling. Cages with tops exposed to room air and the bottom cages resting on the metal truck cooled more rapidly than cages in the middle of the truck, particularly the middle-center cage. The significant differences in microenvironmental parameters were important to note, because they represented thermal pockets of particularly hot cages, mainly at the center of those columns stacked on the storage truck.

The differences in humidity, dependent on location on the truck, in unoccupied cages were as expected. Cages in the top row with unobstructed filter tops adjacent to the room macroenvironment had the lowest initial humidity and the fastest decrease in CH over time. The cages located at the middle and bottom of the truck maintained a high humidity throughout the monitored cooling period of 2.5 h. In previous studies, cage-top filters substantially impeded air from flowing freely; therefore air-change rates in animal cages differed depending on their position on the housing racks.[8,12] Compared with cooled unoccupied cages, occupied cages showed a 30% increase in CH, exceeding room humidity limits recommended in the *Guide*.[6] Comparison of CH for unoccupied and occupied cages has been reported to differ by approximately 10%,[9] and CH typically is 10% to 20% higher than the relative humidity of the housing rooms.[1,12] The heat load generated by animals is a principal factor driving intracage ventilation,[12] which further increases the importance of ensuring cages are thoroughly cooled before housing animals. Given that the humidity of the housing room was within the recommended range,[6] other routine aspects of housing mice, including the amount of soiled bedding, caging density, body mass of animals, and presence of a water bottle likely all contributed to the increased CH in occupied cages. Mice remained active, in good body condition and clinically normal when housed in the described microenvironmental conditions.

Available guidance on bedding choices is limited,[2,6] and choices depend on cost and husbandry practices, in addition to whether ventilated or static caging is preferred. In the present study, the degree and chronicity of increased BT after sterilization were of great interest and indicated that corncob materials were heat-retentive, despite cooling of other aspects of the caging materials (plastic polymers). We demonstrated that those cages that had the hottest bedding, especially those stacked in the middle of the storage truck, also had the highest internal cage temperatures. This pattern was in contrast to the cages located on the top rows and side columns of the storage truck, all of which had aspects of their cage sides exposed to ambient room temperatures during the cooling period. Heated bedding likely would be a major contributing factor to heat stress and potential hyperthermia in mice placed on these substrates, potentially causing pain and distress and further impairing thermoregulation in already hot and humid cages. When the IT, BT, and CH of unoccupied cages that had cooled for 150 min after sterilization were compared with those of cages housing mice, the parameter with the greatest difference (approximately 7 °F) was that of BT. Throughout our facilities, corncob bedding is used almost exclusively due to its ability to minimize ammonia levels and maximize waste absorption in rodent cages; therefore, we did not evaluate the potential effects that other bedding substrates might have imparted on the poststerilization microenvironment.

One component of the caging set-up that we were unable to evaluate simultaneously, due to the 3-feature limitation of the hygrothermometer, was the temperature of the autoclaved rodent chow pellets that were placed in the wire-bar hopper. We presumed that this organic material could be another source of retained heat within the cage that could contribute to increases in IT and CH. Animals placed in an overly warm cage would not have direct contact with the food materials; therefore, food that cooled slowly over time would not be the foremost concern when housing animals. We were unable to control various aspects of the cooling process, including the time to unload and repack the storage truck and the allowance for hygrothermometer acclimation. In addition, we presume that heat escape occurred when cage lids were lifted briefly for hygrothermometer placement. Regarding determination of an appropriate cooling period for caging, the experimental manipulation and acclimation of cages on the storage truck effectively lengthened the overall poststerilization cooling period prior to recording T_0 in our study. We did remove the protective truck cover for ease of recording measurements, although typically our practice is to leave the sterilized truck draped until such time that the truck is transported in situ into the animal room for changing. We anticipate that leaving the truck cover over the stacks of cages might prolong increased cage temperatures and humidity levels due to restricted air flow under the cover. Once cages are removed from the truck and the cage lids removed within the change station or flow hood, we predict an immediate lowering of IT and relative CH due to introduction of ambient air; however, this study did not address these aspects of cage dehumidification.

Although overnight cooling is recommended and preferred, our occasional practice in response to unanticipated caging needs had been to allow sterilized cages to cool for approximately 1 h prior to use for animal housing. As a result of our study, particularly the finding that corncob bedding retains heat after sterilization, we changed this practice. Although we continue to drape the truck of sterile cages until change-out occurs, the adjustment to a prolonged cooling period of at least 3 h has been sufficient to prevent animal welfare concerns related to hyperthermia from autoclaved caging within our high-barrier facilities.

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