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Analysis of Individually Ventilated Cage (IVC) Microenvironments During 21-d Cage Change Frequency for Mice Using Two Different Bedding Types

Jessica L Felgenhauer, DVM, MS, DACLAM,[1,*] Joanna N Copio, DVM,[1] Afra M Suri,[2] Rebecca Turcios, DVM,[1] Allison M Ostdiek, DVM, PhD, DACLAM,[1] George P Langan, DVM, DACLAM,[1] and Kerith R Luchins, DVM, DACLAM[1]

The *Guide for the Care and Use of Laboratory Animals* provides recommendations on sanitation frequencies for rodent caging equipment; however, it allows for performance standards to be used when extending this frequency for individually ventilated cage (IVC) caging. Our institution wanted to reexamine our current standards of care for mouse IVC caging, which includes a 14-d cage bottom and bedding change as well as the use of corncob bedding. This was driven by desire to reduce the stress to mice associated with cage change, and by recent literature showing a potential improved absorbency and multiple health and welfare benefits of paper pulp cellulose bedding products. Therefore, this study sought to compare the impact of different rodent bedding types (paper pulp cellulose and corncob) on mouse IVC microenvironmental parameters over a 14-d compared with a 21-d cage change frequency. Ammonia levels, temperature, humidity, urine latrine size, and the overall animal condition were assessed throughout the 21-d period. Our data indicate that IVC cage bottom and bedding change can be extended to 21 d for either paper pulp cellulose or corncob bedding based on ammonia levels, temperature, humidity, and the animal's overall condition. However, based on early cage change criteria, more frequent cage changes may be warranted before 21 d in cages with corncob, as there was a significantly increased urine latrine size in cages with corncob bedding compared with paper pulp cellulose bedding.

Abbreviations and Acronyms: CC, corncob; ECC, early cage change; PPC, paper pulp cellulose

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Introduction

The *Guide for the Care and Use of Laboratory Animals* provides recommendations on sanitation frequencies for rodent caging equipment. It states that "Solid-bottom caging … usually require[s] sanitation at least once a week"; however, it is noted that the use of IVCs "has led to investigations of the maintenance of a suitable microenvironment with extended cage sanitation intervals."[10] Ultimately, the "decreased sanitation frequency may be justified if the microenvironment in the cages … is not compromised."[10] To ensure appropriate microenvironmental parameters various methods can be used "includ[ing] measurement of pollutants such as ammonia and CO_2, microbiologic load, observation of the animals' behavior and appearance, and the condition of bedding and cage surfaces."[10]

The rodent cage sanitation frequency needed to maintain these microenvironmental parameters has been studied extensively. The frequency may be different depending on the type of cage component, however, as they serve distinct purposes. At our institution, we recently validated extending the use between sanitation of wire bar lids and filter top lids to 3 mo and automatic watering valves to 6 mo for mouse IVCs.[15] The sanitation frequency for other cage components has been evaluated by others. For example, for group-housed mice in IVCs, the change of cage bottoms and bedding has been recommended to occur every 14 d[21,22] while others have validated that the sanitation interval for these components can be extended to 21 d.[7,20] Others have described use of a performance-based approach to mouse cage change frequency using urine latrine characteristics versus a specific interval.[11,29]

Rodent bedding type can influence the microenvironmental parameters, and therefore can result in differing recommended bedding change frequencies.[4,9,12,18,24,25] However, there are conflicting data on which bedding products allow for a longer extension in cage bottom change frequency. One study assessing aspen and cellulose/paper bedding found comparable ammonia levels among the 2 bedding options.[6] Other studies have established that corncob (CC) bedding provides for a longer bedding service life.[4,12] This contrasts to more recent studies that showed cellulose/paper bedding resulted in lower ammonia levels than CC bedding.[18,25] In addition to the potential improved absorbency, there are multiple health and welfare benefits of paper/cellulose bedding products described in the literature. These include improved breeding performance,[1] preference of mice for this product,[3] less sneezing and lung pathology in the animals,[4] and lower endotoxin levels.[30]

Cage change is a stressful procedure that can affect mouse behavior and aggression and increase pup cannibalism.[5,14,27] This is due to the fact that cage change disturbs scent marks, disrupts the social hierarchy of the animals, and decreases social

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[1]Animal Resources Center, Department of Surgery, University of Chicago, Chicago, Illinois; and [2]College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota
**Corresponding author. Email: jfelgenhauer@bsd.uchicago.edu*

stability in the group.[14,27] Due to these reasons, our institution wanted to reexamine our current standards of care for mouse IVC caging, which include a 14-d cage bottom/bedding change and CC bedding. Therefore, we decided to assess a paper/cellulose product and an extension to a 21-d cage bottom/bedding change frequency. To our knowledge, no other published literature has described validation of a 21-d change frequency using a paper/cellulose bedding product. To measure the microenvironment, we assessed ammonia levels, temperature, humidity, urine latrine size, and the animal's overall condition. Our hypothesis was that there would be no significant difference in the microenvironmental parameters between the 2 bedding types; and that no significant difference in the microenvironmental parameters would be seen at day 14 compared with day 21 after cage change compared with day 0.

Materials and Methods

Ethics review. All animal care and use were conducted in accordance with federal polices and guidelines and were approved by the University of Chicago's IACUC. The University of Chicago has a Public Health Service assurance with the Office of Laboratory Animal Welfare and is AAALAC accredited.

Animals and husbandry. A total of $n = 38$ cages of mice were used for the study. Mice were housed in the University of Chicago Animal Resources Center facilities (RRID:SCR_021806). The cage was considered the experimental unit in this study. Cages of adult male and female mice with an age range of 8 wk to 1 y from the program's training colony were used for this study, including C57BL/6, Crl:CD1(ICR), CFW, Crl:NIHBL(S), FVB/N, and various transgenic strains donated by researchers. Cages with 4 to 5 mice, housed by sex, were included in the study ($n = 19$ cages contained only females, $n = 19$ cages contained only males), and the housing density was static throughout the study. Cage densities of 4 to 5 mice per cage were used to ensure that the highest caging densities were included while using the largest possible sample size in the training colony. Mice were housed in solid-bottom polysulfone IVC cages (19.69 × 30.48 × 16.51 cm; Jag 75 micro-barrier IVC, Allentown, Allentown, NJ) set at 60 air changes per hour. The IVC rack exhaust plenums were connected to the building heating, ventilation, and air conditioning exhaust system. All cages and cage components (wire bar lids, filter tops, automated watering valves, and tunnels) were sanitized using a tunnel washer (Basil 6000, STERIS, Mentor, OH) with detergent (Labsan 120, Sanitation Strategies, Holt, MI). To ensure that an appropriate sanitation temperature (180 °F [82.2 °C]) was achieved, a temperature-indicating strip (TempTape 180, Pharmacal Research Laboratories, Naugatuck, CT) was run through the tunnel washer at the start of each day. All cages, cage components, bedding, and enrichment were then autoclaved prior to use (autoclave job no 971290, Primus, Orlando, FL) with a sterilization time of 20 min at 252 °F (122 °C). Cages contained 1 of 2 types of bedding: CC bedding (¼ in., 200 g per cage, Teklad 7097, Envigo, Indianapolis, IN) or virgin paper pulp cellulose (PPC) bedding (ALPHA-Dri® PLUS [120 g per cage, Shepherd Specialty Paper, Watertown, TN]). For enrichment, each cage contained approximately 4 g of specialty shredded paper (Bed-r'Nest, Lab Supply, North Lake, TX). A small amount of this shredded paper enrichment was moved during cage changing to ensure scent transfer from the old cage to the new cage. For nonaversive handling, specifically tunnel handling, the cages also contained a clear circular tunnel (mouse tunnel no. K3487, Bio-Serv, Flemington, NJ).[32] All mice were fed irradiated standard rodent diet (Teklad 2918, Envigo, Indianapolis, IN) and received reverse osmosis–treated chlorinated water through an automatic watering system (Avidity Science, Waterford, WI). Drinking water was treated with chlorine at 2.0 ppm and tested weekly to verify chlorine levels. Cage change of all cage components (wire bar lids, filter tops, automated watering valves, and tunnels) was performed every 21 d in a class II type A2 biosafety cabinet (NuAire, Plymouth, MN). Mice were transferred to the fresh cage using the tunnel present in the cage. Animal rooms were maintained on a 12-h light/12-h dark cycle, with humidity ranging from 30% to 70% and temperatures ranging from 68 to 76 °F (20 to 24 °C) in compliance with the *Guide for the Care and Use of Laboratory Animals*.[10] Mice were checked daily by the animal care staff to assess their health status and the availability of appropriate food, water, and cage conditions.

Routine colony health monitoring was performed quarterly by exhaust dust testing via PCR. Excluded agents were Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse parvoviruses, reovirus, epizootic diarrhea of infant mice, mouse encephalomyelitis virus, ectromelia virus, lymphocytic choriomeningitis virus, murine adenovirus, murine cytomegalovirus, K virus, polyoma virus, mouse thymic virus, hantavirus, lactate dehydrogenase–elevating virus, *Filobacterium rodentium*, *Mycoplasma pulmonis*, *Salmonella* spp., *Citrobacter rodentium*, *Clostridium piliforme*, *Streptobacillus moniliformis*, *Corynebacterium kutscheri*, and endo- and ectoparasites, including *Hymenolepis* spp., *Giardia muris*, *Encephalitozoon cuniculi*, *Myobia musculi*, *Myocoptes musculinus*, *Radfordia affinis*, *Psorergates simplex*, *Syphacia* spp., and *Aspiculuris tetraptera*. Mouse norovirus, *Rodentibacter* spp., and *Helicobacter* spp. are endemic in this housing room.

Study design. We compared the 2 bedding types, CC ($n = 18$ cages, of which $n = 9$ cages contained only females and $n = 9$ cages contained only males) and PPC ($n = 20$ cages, of which $n = 10$ cages contained all females and $n = 10$ cages contained all males), over an extended cage change frequency of 21 d as compared with the standard 14-d cage change. No early cage changes (ECCs) were performed over the course of the study. Cages were excluded if they needed to be replaced for any reason (for example, cage flood) or if the total number of mice fell below 4 in the cage (for example, death of a mouse, separation due to fighting). There were 2 cages within the CC group removed from study due to these exclusion criteria: 1 cage was removed for fighting and 1 cage for loss of a mouse due to causes unrelated to the study, resulting in fewer than 4 mice in these cages. During the study period, the cages were monitored for ammonia, temperature, humidity, and urine latrine size.

Pain and distress scoring. In addition to the daily health checks by animal care staff, each mouse's overall condition was assessed weekly based on a published pain and distress scoring system previously used at the institution (Table 1).[17] A score of 1 to 4 was assigned to each animal with 1 representing no indications of pain/distress and 4 representing severe pain/distress.

Table 1. Animals were assessed weekly based on the pain and distress scoring system previously used at our institution[17]

Score	Observation
1, No indication of pain/distress	Normal; well-groomed, alert, active, good condition, asleep, or calm
2, Mild or anticipated pain/distress	Not well groomed, awkward gait, slightly hunched
3, Moderate pain/distress	Rough hair coat, squinted eyes, moves slowly, moderately hunched, depressed, lethargic
4, Severe pain/distress	Very rough hair coat, severely hunched, nonresponsive, dyspnea, dehydration

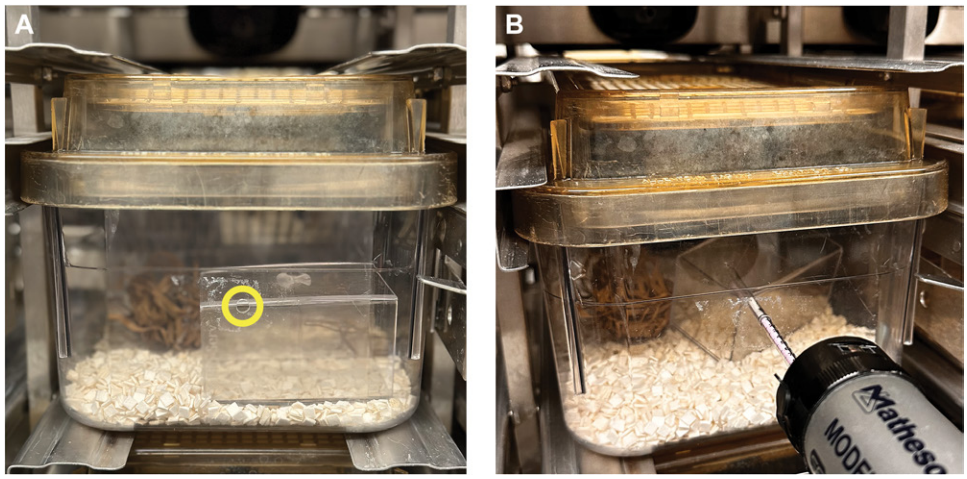

Figure 1. Ammonia was measured in each cage using an ammonia gas detection tube (while the cage remained on the ventilated rack) by the use of a predrilled hole in the front of the cage. (A) The hole was positioned on the midline approximately 6 cm from the cage bottom as shown by the yellow outline. (B) During measurements, reagent tubes were inserted approximately 6 cm into the cage while attached to a sampling pump.

Ammonia measurements. On days 0, 7, 14, and 21 between 0800 and 1100, ammonia was measured in each cage using an ammonia gas detection tube while the cage remained on the ventilated rack by the use of a predrilled hole in the front of the cage. The hole was positioned on the midline approximately 6 cm from the cage bottom as shown in Figure 1A. To minimize introduction of outside air during measurement, the holes were sized to closely approximate the diameter of the gas detection tube. The holes were covered with masking tape when ammonia measurements were not occurring. During measurement, reagent tubes (Ammonia Detector Tube, 10 to 200 ppm/5 to 100 ppm, no. 105SE, Kitagawa, Pompton Lakes, NJ) were inserted approximately 6 cm into the cage while attached to a sampling pump (toxic gas detector system model no. 8104-400A, Kitagawa, Pompton Lakes, NJ) as shown in Figure 1B. The sampling pump and tube were used according to the manufacturer's recommendations. Measurement outputs were given in parts per million.

Temperature and humidity measurements. For approximately half of the cages (n = 10 CC bedding, n = 10 PPC bedding), intracage monitors were used to measure the daily minimum, maximum, and average humidity and temperature (Wi-Com InSight™, Allentown, Allentown, NJ). The monitors were divided equally between cages containing males and females. These monitors were used per the manufacturer's recommendations. In brief, the monitors were placed inside the cage attached to the filter top lid with fabric hook-and-loop fasteners. Data were collected every 3 min and reported on an iPad that was placed in the animal housing room. Daily averages for humidity and temperature were calculated and recorded.

Urine latrine size. On days 7, 14, and 21 between 0800 and 1000 h, the urine latrine size was measured to assess the soiling/wetness of the bedding. The area of each urine latrine was measured by multiplying the largest length by the largest width and then added together to get a total urine latrine size per cage.

Statistical analysis. Data were considered significant if $P \leq 0.05$. Power calculations for group size were made using data from previous literature looking at ammonia levels up to 14 d after cage change, as no studies have looked at up to 21 d after cage change. Power analysis suggested groups of n = 8 to 10 to reject the null hypothesis with 95% probability and a power of 80%. Data were recorded into spreadsheets for recordkeeping (Excel, Microsoft, Redmond, WA). Data were analyzed using the statistical program R (R version 4.3.2 [R Core Team (2023)]). Analyses of ammonia levels, urine latrine size, temperature, and humidity were performed using linear regression with a random intercept to account for repeated measures.[2,13,19,23,31] Analysis of sex differences within each bedding type was performed using a t test.

Results

Pain and distress scoring. All mice received the lowest numerical score of 1 during daily health checks, with the exception of one mouse in the PPC group at day 14. This mouse was euthanized due to abnormal clinical signs observed, and the cage with the remining 4 mice remained in the study. Histopathology of this mouse determined that the clinical signs were unrelated to the study, as the diagnosis was disseminated lymphoma.

Temperature and humidity measurements. Both temperature and humidity in the cage remained stable, with no unforeseen fluctuations observed during the entire duration of the study. Temperature ranged from 73 to 76 °F (23 to 24 °C), and humidity ranged from 50% to 63%. It was seen that the temperature was significantly higher by 0.23 °F in cages housed with PPC compared with CC (P = 0.0001). It was also found that humidity was significantly increased in cages with PPC compared with CC with a 1% difference observed between bedding types (P = 0.0001).

Ammonia measurements. Mean ammonia levels in cages with PPC were 0.2 ppm (SEM = 0.2 ppm) at day 0, 2.2 ppm (SEM = 1.3 ppm) at day 7, 9.8 ppm (SEM = 6.0 ppm) at day 14, and 10.3 ppm (SEM = 5.0 ppm) at day 21. Mean ammonia levels in cages with CC were 0.3 ppm (SEM = 0.2 ppm) at day 0, 2.6 ppm (SEM = 1.4 ppm) at day 7, 14.7 ppm (SEM = 4.1 ppm) at day 14, and 16.5 ppm (SEM = 5.0 ppm) at day 21. During the course of study, the maximum ammonia level found was 89 ppm in a cage housed with PPC and 80 ppm in a cage housed with CC.

When both bedding types were compared with themselves as baseline day 0, there were no significant differences in ammonia levels for PPC bedding at day 7, 14, or 21 when compared with baseline day 0. Compared with baseline day 0, there was no significant difference in ammonia levels for cages housed with CC at day 7; there was a significant difference found on days 14 and 21 (Table 2). When the bedding types were compared with each other, there were no significant differences at any day between PPC and CC: days 0 (P = 1.0), 7 (P = 7.1), 14 (P = 0.2), or 21 (P = 0.2) (Figure 2A). In assessment of day 21 after cage change

Table 2. Data values for each bedding type at each progressive weekly time point to original baseline values (day 0), for both ammonia and urine latrine size, over the total study time

	Ammonia		Urine latrine size	
	Day	*P* value	Day	*P* value
PPC	7	0.77	7	0.72
	14	0.12	14	0.17
	21	0.19	21	0.25
CC	7	0.42	7	0.03*
	14	0.002†	14	0.002†
	21	0.009†	21	0.008†

A statistically significant increase in ammonia levels was evident at days 14 and 21 compared with day 0 in cages containing CC bedding. A statistically significant increase in urine latrine size was found at each subsequent week for cages containing CC bedding, compared with baseline values at day 0. *, $P \leq 0.05$; †, $P \leq 0.01$.

Table 3. Comparison of sex differences between male and female mice in each bedding type

	Ammonia		Urine latrine size		ECC	
	Day	*P* value	Day	*P* value	Day	*P* value
PPC	7	0.77	7	0.72	7	1
	14	0.49	14	0.47	14	0.47
	21	0.65	21	0.97	21	0.64
CC	7	0.67	7	0.75	7	0.65
	14	0.99	14	0.38	14	0.65
	21	0.94	21	0.56	21	0.28

There were no statistical differences between sexes in ammonia, urine latrine size, or number of cages reaching ECC.

compared with day 14, there were no statistically significance differences in intracage ammonia levels at day 21 compared with day 14 in cages housed with PPC ($P = 0.98$) or CC ($P = 0.53$) when the beddings were compared with themselves (Figure 2B).

Sex differences between male and female mice were assessed for each bedding type. There was no significant difference between the sexes found in ammonia levels in either bedding type at any time point (Table 3).

Urine latrine size. Mean urine latrine size measurements in cages with PPC were 0.0 cm^2 (SEM = 0.0 cm^2) at day 0, 2.2 cm^2 (SEM = 1.3 cm^2) at day 7, 10.8 cm^2 (SEM = 6.1 cm^2) at day 14, and 8.9 cm^2 (SEM = 3.6 cm^2) at day 21. Mean urine latrine size measurements in cages with CC were 0.0 cm^2 (SEM = 0.0 cm^2) at day 0, 15.3 cm^2 (SEM = 4.4 cm^2) at day 7, 43.3 cm^2 (SEM = 18.5 cm^2) at day 14, and 51.4 cm^2 (SEM = 17.4 cm^2) at day 21.

When bedding types were compared with themselves at baseline day 0, there was no significant difference in urine latrine size for PPC bedding at any day; however, for CC bedding there was a significant difference in urine latrine size at each time point (Table 2). When cages housed on PPC were compared with cages housed on CC, there was no significant difference at day 0 ($P = 1.0$) for urine latrine size. There was a significantly increased urine latrine size in CC cages compared with PPC cages at days 7 ($P = 0.04$), 14 ($P = 0.02$), and 21 ($P = 0.01$) (Figure 3A). When compared with themselves, there was no statistically significance difference in urine latrine sizes at day 21 compared with day 14 in cages housed with PPC ($P = 0.82$) or CC ($P = 0.22$) (Figure 3B).

Sex differences between male and female mice were assessed for each bedding type. There was no significant difference between the sexes found in urine latrine sizes for either bedding type at any time point (Table 3).

In addition to urine latrine size measurements, we also retrospectively assessed the percentage of cages that would reach ECC criteria from size alone based on the University of Chicago's ECC criteria of greater than 19.4 cm^2 (greater than 3 $in.^2$) at each time point. This size criterion was based off the cage change criteria from a previous publication.[11] However, no cages were changed before the 21-d point in this study. Based on the urine latrine size measurements, the following percentages of cages would have met ECC criteria housed with PPC: 0% at day 7, 20% (SEM = 11%) at day 14, and 20% (SEM = 11%) at day 21. The following percentages of cages would have met ECC criteria housed with CC: 40% (SEM = 12%) at day 7, 61%

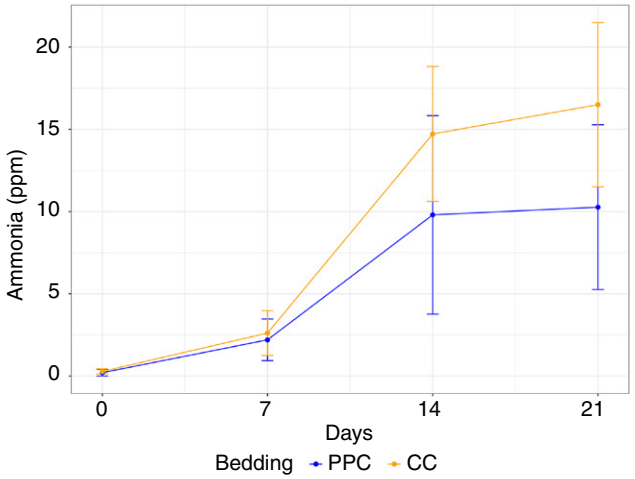

Figure 2. Ammonia levels (ppm) were evaluated in both PPC and CC bedding types. Weekly measurements of ammonia levels in PPC-bedded cages were compared with ammonia levels in CC-bedded cages. There were no significant differences in ammonia levels at any time point between the PPC- and CC-bedded cages. There was no significant difference in ammonia levels from day 14 to 21 in either bedding type when compared with themselves. Significance was determined for $P < 0.05$. Data are shown as mean ± SEM.

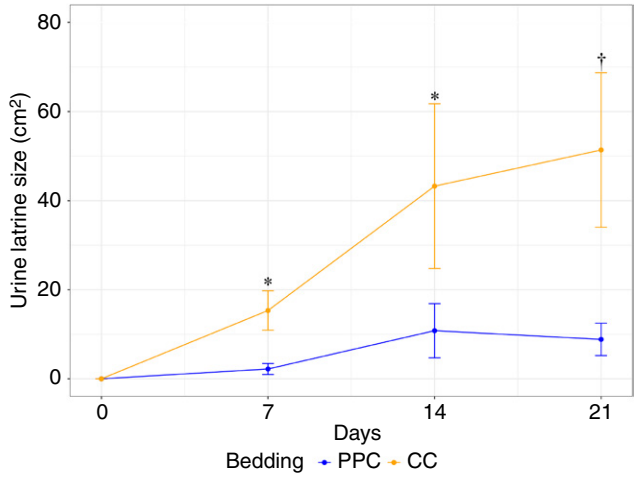

Figure 3. Urine latrine size (cm^2) was evaluated in both PPC and CC bedding types. Weekly measurements of urine latrine size in PPC-bedded cages were compared with urine latrine size in CC-bedded cages. There was a significant increase in urine latrine size in CC-bedded cages at days 7, 14, and 21 compared with PPC-bedded cages. Urine latrine size was evaluated for the different bedding types independent from each other at day 14 compared with day 21. There was no significant difference in urine latrine size from day 14 to 21 in PPC- or CC-bedded cages. *, $P \leq 0.05$; †, $P \leq 0.01$. Data are shown as mean ± SEM.

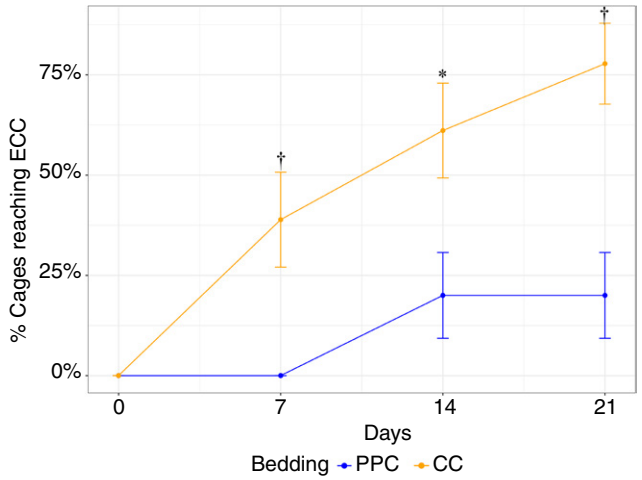

Figure 4. Assessment of cages that reached the University of Chicago's early cage change criteria based on urine latrine size greater than 19.4 cm^2 (3 $in.^2$). CC-bedded cages had a significantly higher number of cages needing the early cage changed compared with PPC-bedded cages on days 7, 14, and 21. *, $P \leq 0.05$; †, $P \leq 0.01$. Data are shown as mean ± SEM.

(SEM = 12%) at day 14, and 78% (SEM = 10%) at day 21. Cages housed on CC would have received significantly more ECC compared with cages housed on PPC at days 7 ($P = 0.009$), 14 ($P = 0.03$), and 21 ($P = 0.002$) (Figure 4). There was no significant difference observed between the sexes in ECC levels (Table 3).

Discussion

Due to the stress placed on mice associated with cage change, a primary focus of this study was to assess refinement of the cage change process through extension from 14 to 21 d for mice housed in IVC caging systems. Our data suggest that a cage change extension to 21 d from 14 d is acceptable in mice with no differences in intracage ammonia levels or urine latrine sizes found at day 21 compared with day 14 between either bedding type evaluated in our study (Figures 2B and 3B). Other studies showed increases in ammonia levels over time; however, these ammonia levels were taken after cages were removed from the IVC rack, whereas in our study ammonia levels were assessed on the rack to better mimic what the mice experience within the cage for most of their time.[4,11,18,25,26]

High ammonia levels are known to lead to health concerns in mice, including damage to nasal turbinates.[16] Within this study, mice showed no visible health effects on either bedding type throughout the 21-d period, indicating no observed animal welfare concerns with the extended cage change schedule. While scoring of pain and distress was performed as part of our study, we did not look at other animal welfare parameters such as negative stress responses to the extended cage change. Future studies looking at behavioral changes, stress parameters (for example, corticosterone levels, lymphocyte/neutrophil ratio), and breeding performance would be valuable. The maximum ammonia level found within an individual cage over the study period was 89 ppm in a PPC cage and 80 ppm in a CC cage. A previous study by others did not find more than mild nasal turbinate changes on histopathology until approximately 200 ppm of ammonia;[16] another study found changes at approximately 50 ppm,[28] and another study did not find any observable differences in mice with ammonia levels of 100 ppm and lower,[20] all supporting the assumption that the ammonia levels found in our study likely did not cause more than mild changes within the nasal structures of the mice. While histopathology was not performed as part of the present study, such evaluations would be helpful in future studies to ensure no observable differences between day 14 and 21 in internal structures of mice seconday to exposure to elevated ammonia levels.

We noted for multiple cages of mice, housed with either CC or PPC, that ammonia levels were higher at day 14 and then lower at day 21. We theorized that this could be due to absorption of the urine followed by drying or removal of ammonia via the air changes that are part of the normal IVC function. In addition, while ammonia measurements were taken at the same time period each day, the timing of when mice last urinated could play a role in ammonia levels in the cage at the time of measurement. While it has been shown that increased bedding volume does not lower ammonia levels, it is thought that there is an optimal amount of bedding that allows ammonia to be filtered out of a cage, and the absorption level/pattern of beddings play a role in decreasing ammonia build up in cages.[8] As PPC and CC bedding have been shown to be highly absorptive,[4,6,12,18,25] it is possible that the absorption process resulted in lower ammonia levels in the cages.

While there was no statistical difference between the bedding types at any time point, when compared with themselves at day 0, the cages housed with PPC bedding had no statistically significant increases in ammonia levels at any time point. However, compared with day 0, cages housed on CC bedding showed statistically significant increased ammonia levels at days 14 and 21. This could indicate that PPC bedding types may result in lower ammonia levels, and further investigation may be warranted to determine the cause. It is possible that this is due to the absorption process of PPC bedding. Upon visual assessment of urine latrines in the CC compared with PPC bedding, there was a distinct difference between the beddings. Urine latrines in CC bedding had more distinct demarcations, with clumping of bedding and pooling of urine occurring when soiled and wet. However, clumping does not appear to happen with the PPC bedding, as we observed a more diffuse distribution with wicking and absorption of urine. This resulted in smaller urine latrine sizes in the PPC group. It is possible that this absorption process could explain, in part, the steady ammonia levels found in PPC cages over the 21-d study period (Table 2). As husbandry staff performed ECCs based on appearance, the use of PPC beddings would have resulted in fewer ECCs compared with cages on CC bedding, which could also result in decreased stress on mice from more frequent cage changes.

At the University of Chicago, our staff members are trained to use visual indicators including a urine latrine size that is greater than 19.4 cm^2 (3 $in.^2$) to help determine when an ECC is needed, based on a previous study.[11] When comparing the bedding types, there was a clear difference in urine latrine sizes noted between the bedding types (Figure 4). Urine latrine sizes were significantly larger in CC cages than in PPC cages at days 7, 14, and 21. In addition, there was no significant difference in urine latrine sizes at any time point in PPC cages compared with week 0; however, urine latrine sizes were significantly larger starting at the first week after cage change and continued to increase in CC cages. When evaluating the urine latrine sizes in this study using our visual indicator for ECC, it was found that starting at day 7, 40% of CC cages reached this cage change criterion, and continued to increase up to 78% of cages reaching this criterion by day 21. Cages housed on PPC had a significantly lower percentage of cages requiring ECC, with 20% of cages meeting criteria at both days 14 and 21. Based on the practices at the University of Chicago, these data show that, based on visual indicators of urine latrine size, IVC cages

housed on PPC require fewer ECCs compared with those on CC. In addition, because we found as no adverse health effects related to this study, and because we found no ammonia levels to exceed 100 ppm (mean at 21 d in PPC = 10.3 ppm and CC = 16.5 ppm), extension of the visual indicators of ECC currently used might be the subject of future studies.

In addition to the limitations discussed above, this study was performed on nonexperimental, nonbreeding mice from the University of Chicago's training colony. While mice were from various strains, they were healthy mice with no known phenotypic health conditions. Future studies looking at ammonia and urine latrine sizes in mice with increased urination (for example, diabetic strains, aged mice) would be beneficial to help provide a more global view of animals that are typically housed within research institutions. In addition, while scoring of pain and distress was performed as part of our study, it was not within the scope of our study to assess other animal welfare parameters such as negative stress responses to the extended cage change interval. Future studies examining behavioral changes, stress parameters (for example, corticosterone levels, lymphocyte/neutrophil ratio) and breeding performance would be valuable. In conclusion, our data support the extension of cage change interval from day 14 to day 21 for mice housed on both PPC and CC bedding, with no significant differences in health score, ammonia levels, or urine latrine sizes in either bedding type at day 21 compared with day 14 being found. While there was a statistically significant increase in both temperature and humidity seen in the PPC bedding type compared with CC bedding, it was negligible and not of a practical concern. In addition, although there was no significant difference between PPC and CC bedding in ammonia levels, PPC bedding may be preferred due to significantly smaller urine latrine size and the percent of cages reaching ECC criteria. These reduced parameters would result in fewer ECCs, which would likely decrease stress to the animals and the amount of time husbandry staff spend performing cage changes.

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Conflict of Interest

The authors have no conflicts of interest to declare

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