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Evaluation of a 16-week Change Cycle for Ventilated Mouse Cages

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The methods and conditions for housing research mice have been the subject of many discussions and publications in recent years. At our institution, we began to explore these matters with the goal of reducing stress in the animals and yet maintaining an environment that more closely resembled their habitat in the wild and yet was acceptable to researchers and the technicians that cared for the animals. Through a series of small inhouse studies, we derived a method that allowed the animals to stay in their established environment for longer than the standard 1- or 2-wk period. After several empirical studies, we concluded that the mice could stay in the same cage for 16 wk or perhaps even longer. To achieve this outcome, we perfected a method of removing 75% of the existing cage bedding and replacing it with clean bedding every 2 wk. To substantiate the validity of the method, we conducted a major study that evaluated the conditions of the cage, cage environment and the animals for a 16-wk period. In the study, we compared all of these factors in the 16-wk cages to a set of cages that were completely replaced on a 2-wk cycle. The mice in our study appeared to experience decreased stress, and observation also revealed that the 16-wk method was associated with increased pup survival in several colonies. The revised 16-wk method appears to create mouse cage conditions that are no different than the current standard (that is, every 1 or 2 wk) methods of cage changing.

Abbreviation: RLU, relative light units

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During the past decade, much has been published or posted on the internet concerning the housing and care of research mice. In addition, seminal guidelines, such as the *Guide*,[12] have been updated, and required performance on many issues has been delegated to professional judgment. However, this judgment is expected to be based on relevant data (performance standards) that substantiate the opinion and guidance of the professional which is, in most cases, the institutional veterinarian.

Many years ago, the animal care staff at our institution began to question why the cage accessories (wire tops and filters) needed to be washed on a 2-wk cycle when they appeared to be clean. To determine whether these accessories were significantly more contaminated at 16 wk than they were at 2 wk, we conducted a comparative study using swabs and cultures. The data (unpublished) revealed no significant difference between the 2 time points, and we subsequently submitted the results to the IACUC, which approved the changing of accessories on a 16-wk cycle. A similar study was conducted at another institution, in which the authors concluded that cage accessories at 180 d, in most cases, had ATP levels that were no different than accessories at 14 d.[13] A recent study[3] analyzed cage accessories after 6 wk of housing and found bacterial loads to be no different than from cages after 2 wk of housing 4 or 5 mice. All of these findings led us to question other guidelines regarding the changing of cage bottoms on a 1- or 2-wk cycle.

In 2009, we began to experiment with methods that would allow the cage bottom to remain in place for as long as the accessories. One of the major motivations to proceed in this direction was the observation by our staff that the mice appeared to be stressed by the procedure of removing them from an established environment and placing them in a totally new one. For the overall wellbeing of the animals and to reduce possible negative effects on research,[1,2] we began efforts to identify better ways to manage the microenvironment of our mice.

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Materials and Methods

This study was conducted in an AAALAC-accredited facility under an IACUC-approved protocol.

Study design. In this study, 2 groups of ventilated cages were established. In the control (that is, cage replacement) group of 20 cages, the cage bottoms were replaced with new cages and bedding every 2 wk. In the test (that is, cage scoop) group of 20 cages, the cage bottoms were not changed for 16 wk. Instead, we used an innovative method that allowed 75% of the bedding to be scooped from each cage every 2 wk and replaced with fresh bedding. The process consists of using sterile technique in a hood to remove the filter and wire top. By using a paper scoop, the mice are gently pushed to the back of the cage. Then, in a single sweeping motion, the scoop is moved forward and bedding removed. The scoop and bedding are placed in a small paper sack, sealed, and then placed in a garbage can outside the hood. A sterile bag of bedding is then placed in the cage and the wire top (along with the filter) is returned to the cage; the following URL links to a video of the process (http://155.101.192.53/16week; click on Media2.avi). For both groups, the wire-bar lids and filter tops were changed after 16 wk. Food and water were replenished as needed. All bedding and supplies were autoclaved to maintain sterility during the cage replacement or scoop process. Within each group of 20 cages (control and test), 10 contained corncob bedding and 10 contained paper bedding.

Animals. We selected animals that traditionally are considered to be aggressive because one goal was to determine whether our novel cage scoop method reduced their aggression. Both

groups (control and test) comprised 10-g SPF weanling male C57BL/6Cr mice (Charles River Laboratories, Wilmington, MA).

Housing. The animals were housed in no. 9 polysulfone cages with water grommets (Thoren Caging Systems, Hazelton, PA) that were placed in a ventilated rack with the grommets facing out. The cage air exchange rate in the ventilated racks was reduced from the normal 60 ACH to 30 ACH. According to *Guide*[12] recommendations regarding space, the maximal number of mice (that is, 5) was placed in each cage. Bottles were used to provide water to the animals; all mice received irradiated chow (Teklad 2920X, Envigo, Indianapolis, IN). Two common types of bedding material—corncob (Harlan Laboratories, Indianapolis, IN) and paper (Cell-Sorb Plus, Fangman Specialties, Cincinnati, OH)—were used within the ventilated cages. Both types of bedding were maintained at a cage depth of 1/4 in. The ventilated rack was housed in an environmentally controlled room with 15 changes of air hourly, and an average temperature of 22.8 °C. Average room humidity was 27.5%, and the average room CO_2 level was 490 ppm. During the study, no ammonia was detected in the room. Room ATP samples were taken from a specified location on a room wall, and the average room ATP was 1554 relative light units (RLU). All cages were manipulated in a Class II biosafety hood (type A/B3, Nuaire, Plymouth, MN).

Behavioral assessments. Three experienced animal technicians performed these assessments. They were blinded as to which cage-cleaning method was used. Using a score sheet (Figure 1), they independently evaluated the behavior of the mice just before cage change or scoop, immediately after cage replacement or scoop, and at 1 wk after cage replacement or scoop. During assessments, the cages were not removed from the ventilated rack. The technicians also evaluated the condition of the cages and bedding.

Environmental assessments. To compare the environment in cages that were replaced every 2 wk with that in cages in which only bedding was refreshed every 2 wk, we measured intracage ammonia, temperature, humidity, CO_2, and ATP. We used a handheld meter (HM70, Vaisala, Helsinki, Finland) to measure CO_2, temperature, and humidity. The instrument for measuring intracage ammonia levels (Micro IV, catalog no. G223, GfG Instrumentation, Ann Arbor, MI) was calibrated prior to each cage replacement or scoop session and is accurate from 0 to 200 ppm of ammonia. The sampling probes for both of these devices were introduced into the cages through the grommet and positioned at a standard midcage location about 1 in. above the bedding (Figure 2). Measurements were taken just before and just after each cage replacement or scoop. Swabs for ATP analysis (novaLUM II, Charm Sciences, Lawrence, MA) were collected from the right wall of each cage near the water holder.

Statistical analysis. The data comprise the environmental measurements that were taken every 2 wk immediately before cage replacement or scoop and so represent the maximal value across each 2-wk interval. That is, we assume that the value for the environmental variable will monotonically increase from the point of cage replacement or scoop until immediately prior to the subsequent cage replacement or scoop. Therefore, our data represent the highest possible environmental values, given that we had repeated measurements for each of our outcome variables (every 2 wk until 16 wk). Therefore, the data were analyzed by using mixed-effects linear regression (Stata, StataCorp, College Station, TX). In these models, we did not include a time variable, so the model basically used the mean of the measurements for each cage. The predictor variables were material (paper compared with corncob), method (replacement compared with scoop) and a material by method interaction term. As an added precaution against unmeasured confounders, the 2-wk cage change measurement that was the closest to the baseline value was included as a covariate to control for any baseline imbalance between groups. The mean outcomes for the 4 subgroups, which were defined according to cleaning method and bedding material, were obtained by using marginal estimation, which yields the predicted mean values from the model. We then compared the optimal combination for Utah (high altitude and low humidity) with each of the 3 other combinations by using a Wald posttest on the marginal mean estimates. The P values (significance, $P < 0.05$) from each of these 3 comparisons were adjusted for 3 multiple comparisons by using the Hommel procedure.[25]

Results

Intracage environmental parameters before cage replacement or scoop. The intracage ammonia measurement (mean ± SEM) immediately preceding a cage replacement or scoop was 5.8 ± 1.6 ppm for the cob–replacement combination of bedding and cleaning method, 9.7 ± 1.6 ppm for cob–scoop, 1.9 ± 1.5 ppm for paper–replacement, and 2.8 ± 1.5 ppm for paper–scoop. The paper–replacement combination gave the lowest ammonia level, which was significantly lower than that for cob–change ($P = 0.001$) but did not differ from cob–scoop ($P = 0.16$) or paper–scoop ($P = 0.65$). Intracage temperature (80.2 ± 0.1 °F [26.8 ± 0.1 °C]) did not differ significantly between methods and bedding types. Intracage levels of ammonia are shown in Figure 3.

Relative humidity within the cage was 30.0% ± 1.1% for the paper–replacement combination, 30.2% ± 1.0% for paper–scoop, 30.82% ± 1.0% for cob–scoop, and 32.0% ± 1.1% for cob–replacement cages. However, none of these values differed significantly from another.

CO_2 measurements were 1267.2 ± 212.1 for cob–replacement cages, 1670.2 ± 182.4 for cob–scoop, 1314.7 ± 186.5 for paper–replacement, and 1373.5 ± 181.4 for paper–scoop. These values did not differ significantly. ATP levels were 22447 ± 2515 RLU for paper–scoop cages, 26465 ± 2268 RLU for cob–scoop, 26483 ± 2285 RLU for paper–change, and 30700 ± 2415 RLU for the cob–replacement combination. None of these values differed significantly.

Animal behavior. Statistical analysis of the observation data collected immediately before cage replacement or scoop revealed no significant difference between methods in regard to the behavior of the mice. In addition, none of the other measured parameters (animal appearance, bedding condition, cage condition, cage accessories, and location preference) differed when compared between the scoop method and the 2-wk cage replacement method. In addition, the scores after 1 wk of habituation were essentially the same among all method–bedding combinations.

In particular, the appearance of mice on paper bedding was identical between the 2 cage-cleaning methods. Throughout the study, the mice were overall clean and healthy, with only an occasional slightly dirty appearance. Mice on corncob bedding in both scoop cages and replacement cages were normal in appearance at most of the 2-wk time points, and there were only 2 time points at which an animal's appearance scored greater than 0 (normal).

Regarding bedding condition prior to cage cleaning or scooping, paper bedding in the scoop cages was consistently only slightly soiled or wet. In the replacement cages, the bedding was clean and dry. At every 2-wk time point, cob bedding in the scoop cages was given a score of at least 2 or greater, with a single score of 3.1. In addition, the scoop cages had mostly clean walls and bottoms, with only a few cages being very slightly soiled. The replacement cages were consistently clean. In the paper–scoop cages, the accessories (wire top, filter) typically

Data Sheet Name:

Cage type Regular **ACH** 30 **Bedding** _____ **Water** Bottle **Diet** 2920X

Cage no. _____ **Date** _____ **Week** _____

1. Animal Behavior—evaluated immediately before bedding change, immediately after bedding change, and at 1 wk after bedding change (resting cage)
   0 = peaceful, resting, sleeping, mild to moderate activity
   1 = marked activity, marking
   2 = marked agitation
   3 = marked aggression and/or fighting
   4 = stereotypic behavior (bar chewing, jumping, pacing, circling, tail carrying, flipping)
   Before _____ After _____ 1 Week _____

2. Animal Appearance
   0 = clean, no wounds or hair loss, healthy appearing
   1 = very slightly dirty, very slight hair loss
   2 = slightly dirty, slight hair loss, slight wounds, slight barbering
   3 = moderately dirty, moderate hair loss, moderate barbering, unhealthy appearance
   4 = markedly dirty, marked hair loss, marked barbering, marked skin wounds, markedly unhealthy appearance

3. Bedding Condition
   0 = clean and dry, very small amount of feces, no odor
   1 = clean and dry, some feces, very slight odor
   2 = slightly soiled and slightly wet, slight feces, slight odor
   3 = moderately soiled and moderately wet, moderate feces, moderate odor
   4 = markedly soiled and moderately wet, marked feces, marked odor

4. Cage Condition (walls and bottom)
   0 = clean walls and bottom
   1 = very slightly soiled
   2 = slightly soiled
   3 = moderately soiled
   4 = markedly soiled

5. Cage Accessories (wiretop, filter) Condition
   0 = clean wiretop and filter
   1 = very slightly soiled
   2 = slightly soiled
   3 = moderately soiled
   4 = markedly soiled

6. Location Preference (F, front; R, rear; NP, no preference)
   Before _____ After _____ 1 wk _____

Figure 1. This observation data sheet was used to assess animal condition and behavior as well as cage and accessory condition just prior to and just after cage replacement or scoop. An additional assessment was made 1 wk after cage replacement or scoop.

were clean with a few very slightly soiled ones. Accessories in the paper–replacement cages were consistently clean. In the cob–scoop cages, only a few accessories were slightly soiled, with most of them appearing clean. The cob–replacement cages likewise had mostly clean accessories.

Discussion

We initiated this study out of a desire to reduce the apparent stress of our mice at cage changing. Our animal care staff consistently observed stress-associated activity (running, jumping, aggression and fighting) in the animals when they were moved from their established cage environment to a new cage with fresh bedding. The optimal frequency of cage changing for mice has been the subject of numerous recent studies.[4,8,11,14,15,17,18,24] The handling and the loss of territorial cues associated with cage changing apparently are stressful to mice.[1,2,4,9,10,15,17,22] Mice are territorial animals and they mark their areas with urine pheromones.[1,2,4,9,10,22] Smell is the most important sense in mice, and fecal, plantar and salivary odor cues are used.[9,22,23] When mice are moved from an established environment to a new one, they become stressed because nothing is familiar to them. Reestablishing their territory often results in fighting among the animals in the cage.[10,22,23] Others[1,2,4,5,22] have made similar observations and have recommended the transfer of a handful of dirty bedding to the new cage. At the beginning of our study, we tried this method but did not observe any difference in the behavior of the animals. This result led us to conclude that we should leave the mice in their current cage and remove some of the soiled bedding. Through a series of small studies, we concluded that removing and replacing about 75% of the bed-

Figure 2. The intracage environment was assayed by using 2 sensitive instruments whose sampling probes were inserted into the cage through the grommet opening. Assays were made just prior to and just after cage change or scoop.

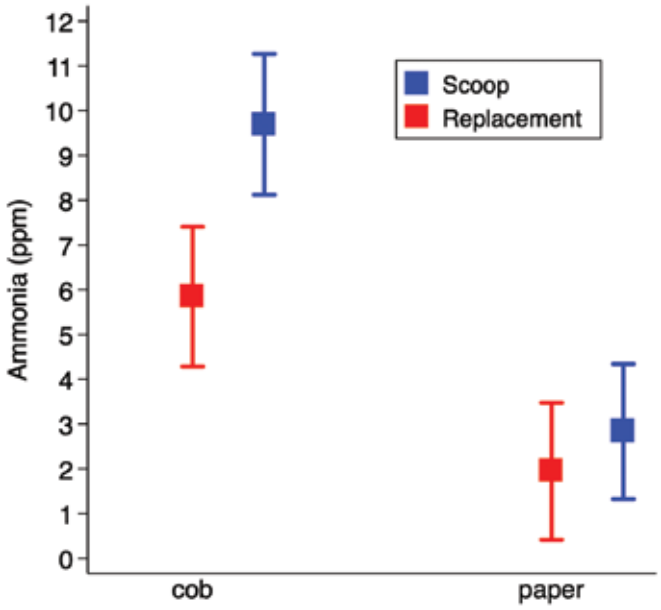

Figure 3. Intracage ammonia levels (mean ± SEM) just prior to cage replacement or scoop depending on bedding type and change method.

ding left sufficient scent markings in the bedding and nesting material and on the cage that the stress level of the animals was decreased. These studies also allowed us to precisely define how we removed the used bedding.

Nests and nesting material contain scent markings that are calming to the animals, more so than bedding contaminated with urine and feces.[1,9,22,23] Therefore our method is to avoid removing nests and nesting material and remove only bedding laden with feces and urine. Several studies[22,23] suggest that bedding laden with urine and feces may increase aggression. Nests can keep the mice warm and keep the cage cool enough to prevent fights.[9] In addition, nests conserve energy, reduce food use, and improve breeding performance.[9] Given our findings, we designed a large controlled study to evaluate the behavior of the mice and the conditions within the cage when we removed bedding on a regular schedule but left the mice in the same cage.

Throughout our industry, mouse cages are routinely changed on either a 1-wk or 2-wk cycle.[4,7,11,15] At the time of our study, we were using a 2-wk cycle for cage bottoms with a 16-wk change cycle for filter tops and wire-top lids. On the basis of these schedules, we designed a study that left the mice in the same cage for 16 wk, with a partial (75%) bedding change every 2 wk. We reduced the number of air changes hourly in the cages to 30 rather than the typical 60.[9,15] We also used male C57BL/6Cr mice exclusively and placed the maximal number (5) in each cage. Because our facility traditionally has used paper and corncob bedding materials, we used both types in our study.

Furthermore we wanted to compare the condition of the scoop cages with that of cages in which all components were replacement after housing mice for 2 wk. On the basis of previous studies,[13,14,15,16] we selected the intracage parameters to monitor. In the animal research field, there are no established regulations—only recommendations—concerning the acceptable intracage environment for rodents. The gas eliciting the greatest concern regarding intracage levels is ammonia,[5,7,8,11,13,15-17,19-21,24] and most facilities have arbitrarily adopted a cage limit of 25 ppm.[7,11,15,17,24] This limit is based on the National Institute for Occupational Safety and Health standard for human exposure during an 8-h period.[7,13] Because there are no established limits for other chemicals or conditions within the mouse cage, we concluded that if the conditions within the scoop cages were equal to or better than those of the replacement cages at 2 wk (control cages), then the scoop method of cage changing was acceptable.

Ammonia levels within all cages were meticulously measured at the end of each 2-wk period after cage replacement or scoop. This parameter, along with animal behavior, was considered the most critical for determining whether the scoop method was acceptable. In our facility, cages with paper bedding had lower ammonia levels than cages with corncob bedding. Rapid ammonia production occurs under conditions of high humidity, and the type of bedding and the moisture in it markedly influence the production of this gas.[5,13] The greatest ammonia level in scoop cages with paper bedding was 4.2 ppm which occurred at the 16-wk time point. In replacement cages with paper bedding, the highest level was 1.4 ppm, which occurred at the end of the 2nd week. In cages with corncob bedding, the highest level of ammonia in replacement cages was 24 ppm which was measured at 2 wk. The highest average level of ammonia in scoop cages with corncob bedding was 29 ppm, which likewise occurred at 2 wk. Considering that both of these cage types contained corncob bedding and both had high ammonia levels at 2 wk but that later dropped considerably suggests an equipment malfunction. Importantly, in the cages with paper bedding, the average level of ammonia over the entire study was 2.8 ± 1.5 ppm in the scoop cages and 1.9 ± 1.5 ppm in cages that were changed completely every 2 wk. The results were slightly different for cages that contained corncob bedding. The scoop cages consistently had higher ammonia levels than the replacement cages. However, except for week 2, the levels in both scoop and replacement cages dropped and were well within the acceptable range (less than 25 ppm). The average levels for the entire study revealed that the scoop cages with corncob bedding contained 9.7 ± 1.6 ppm of ammonia and the replacement cages contained 5.8 ± 1.6 ppm. The measurements before changing or scooping were the most critical because they revealed the conditions in the cages after housing mice for 2 wk. To establish a new starting point for every 2-wk period, we also measured ammonia in the cages after they had been replaced or scooped. Regardless of the bedding type or cleaning procedure, the ammonia levels in all cages were close to 0 ppm. This result

also substantiates the fact that the scoop method removed a significant quantity of ammonia-laden bedding at each 2-wk interval. Our data thus show that scoop cages, regardless of the type of bedding, had slightly higher levels of ammonia than did the cages that were replaced every 2 wk. Importantly, however, the average levels of ammonia never reached the arbitrary limit of 25 ppm, except at the 2-wk time point in cages containing corncob bedding. As previously mentioned, this result may represent an unrecognized equipment malfunction. At every other time point, the levels in both types of cages and bedding were well below the 25-ppm level. Therefore, according to the evaluation of the most important parameter, intracage ammonia, the scoop method is acceptable for use with either paper or corncob bedding.

Throughout the 16-wk study, intracage temperature remained very stable regardless of the type of bedding or the cage procedure. For all cages, the average temperature remained at either 26.7 or 27.2 °C. Therefore, in regard to temperature, scooped cages were identical to cages that were replaced every 2 wk.

Intracage humidity in both types of cages and bedding varied during the study. However, measurements made before the change point resulted in the same overall study value of about 30% for cages with paper bedding that were scooped or changed out. A very similar result was obtained in cages with corncob bedding, where regardless of the cage procedure, the humidity level was about 31%. In addition, humidity levels in the scoop cages were no different than those of cages that were replaced.

Measurements of intracage CO_2 likewise demonstrated that the scoop method was acceptable for mouse husbandry. In cages with paper bedding before the change point, the study value for scoop cages was about 1373 ppm, whereas in the replaced cages, the value was about 1314 ppm. The measurements after cleaning revealed study values that were almost identical among scoop cages and replacement cages. In cages with corncob bedding, the value before scooping was numerically lower (but only slightly) than that of the replacement cages. Measurements from corncob cages after cleaning revealed values that were almost identical between the scoop and replacement methods. Like the other parameters measured in this study, CO_2 levels revealed the 2 cage management methods to be almost identical.

During the study, we wanted to measure the quantity of cellular material, including bacteria, on the inner surfaces of the cages. Rather than relying on cultures for bacteria, we elected to measure the total cellular content by ATP levels. ATP is a nucleotide found in the mitochondria of all plant and animal cells, including bacteria; by using a luminometer, the test gives immediate results and is extremely accurate.[8,11] This method of assessment has been applied to evaluate facility sanitation in another study.[6] To reduce variability in our measurements, we always sampled the same area in each cage. Because we primarily were interested in the cellular load at the end of each 2-wk period, we measured this parameter just before the cage was scooped or replaced. However ATP levels did not differ significantly between the types of cages. This finding is important because it reveals that the scoop cages are not any 'dirtier' than the cages that are cleaned frequently.

Animal behavior was observed by the same 3 animal technicians throughout the study, and they did not know whether the cages had been scooped or replaced. All cages were observed immediately before manipulation and after the cages had been scooped or replaced and returned to the rack. Both observations were made while the cages were docked in the rack. Prior to manipulation, as expected, the behavior of animals in all cages was identical. The cage scoop and replacement processes each took about 15 min once the cages had been removed from the rack. This elapsed time probably allowed for a decrease in animal activity by the time the cages were replaced in the rack. Statistical analysis of the behavior data revealed no significant difference between the cage cleaning methods. In addition, although not quantitatively measured, the technicians manipulating the cages in the hood noticed greater activity in mice moved into new cages as compared with those experiencing bedding removal, regardless of the bedding type. Similar observations are also reported currently by other technicians in our facilities where we have established this method of cage cleaning. In addition, researchers using the scoop method have reported that several mouse colonies with breeding difficulties and low pup survival have become more normal.

Animal appearance was observed prior to cage manipulation only. The scores recorded by the observers were identical for both methods of cage management. Throughout the 16 wk study, all mice appeared to be clean and healthy.

The bedding in all cages was assessed at the end of 2 wk of habitation and just before the bedding was either scooped or the entire cage was changed out. The paper bedding in scoop cages was consistently only slightly soiled and slightly wet. In contrast, the paper bedding in the cages that were replaced was clean and dry. Similar results were seen in cages with corncob bedding that were scooped or replaced. The slight soiling and wetness in the scoop cages may seem to be a drawback to this method. However, further consideration of the findings makes it apparent that the presence of feces, urine, and nesting material is key to maintaining the scent markings in the cages and ultimately reducing the stress level of the animals.[1,4] In addition, it is important to recognize that the ammonia level in the scoop cages with paper or corncob bedding never exceeded the universally accepted limit of 25 ppm.

In cages with paper bedding, the walls and bottoms of the scoop cages typically were clean, with only some cages very slightly soiled. The cages with paper bedding that were replaced were always clean. Cages containing corncob bedding were similar to paper bedding cages. Specifically, the scoop cages with paper bedding were very clean, and the corncob cages were slightly soiled. But, again, the condition of the scoop cages contributed to the calmness exhibited by the animals in these cages. Any soiling of the cage walls and bottom in the scoop cages, regardless of the bedding, contained scent markings that calmed the mice.[1]

The condition of the cage accessories was similar to that of the cages themselves. The scoop cages, regardless of bedding type, had slightly higher scores (indicating increased soiling) than the replacement cages. But, in general, they were clean throughout the study. However, the slight soiling probably contained scent markings because the mice often climbed on the wire lid. The markings identified territories and individual animals, which resulted in a calm environment.[1]

In this study, the mice did not show any preference for location within the cages, regardless of the bedding or the management of the cages. In some studies,[15,19] the animals congregated in certain areas of the cages presumably to avoid air currents. This huddling probably did not occur in our study, because we reduced the airflow in each cage to 30 air changes hourly, which is half of the normal rate. In addition, the cages we used diffuse the entering air through a filter on top of the cage, thus eliminating streams of forced air.[9,15]

We recognize several important operational and environmental benefits from our novel cage-change method. Under the scoop method, an individual plastic mouse cage is washed

3 times in a year as compared with current common schedules of 26 to 52 times. This difference will result in a significant reduction in power expenses, and the life of the cage and accessories will be extended by years. This cage-change method will dramatically decrease the labor needed to wash cages. Environmentally, the scoop method is markedly 'greener' than the procedures used by most of the industry. Untold millions of gallons of water can be saved and huge quantities of chemicals will not be flushed into the sewer, again representing another marked reduction in operational expenses.

The current study was conducted in ventilated racks. Following the success of this investigation, we questioned whether the same method could be used with cages sitting on a shelf and having no positive intracage ventilation (static caging). We have initiated a study to evaluate those scenarios, the results of which will be presented in a future report.

In conclusion, the results from this study successfully prove that cage conditions in which the bedding is partially removed every 2 wk over a 16-wk cycle are, indeed, at least comparable to those of a standard cage that has housed mice for 2 wk and subsequently is completely replaced. In addition, our initial notion was that the bedding removal procedure resulted in calmer and less active mice. The study data, along with our observations and use of the method since 2009, also support these conclusions. The scoop method has been used with both sexes of numerous diverse mouse strains, and we have recognized no differences in either the health or behavior of any of the mice compared with traditional cage-changing methods. In addition, the researchers are very pleased with the condition of their animals and the research data collected from them. Since the original study, which we report here, we have made refinements in the scooping process. We conducted a second 16-wk study to evaluate another paper bedding (Paperchip, Shepherd Specialty Papers) and found the results to be better than those from the paper bedding that we used in our initial study. To simplify the procedure of adding bedding to the cage after it has been scooped, we arranged for the packaging of 4.48 oz. of bedding in a thin paper bag (WF Fisher and Son). The autoclaved bag is dropped into the cage, and the mice chew through the paper and scatter the bedding. In addition, this modification allowed us to stop placing a sheet of enrichment paper in the cage, because the bedding bag fulfills that purpose. A recent AAALAC site visitor called the scoop method "revolutionary," and we believe that it is. The method establishes an environment for the mice that is more compatible with their behavior characteristics rather than what we humans think is best for them according to our standards.

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