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Evaluation and Refinement of a Spot-change–only Cage Management System for Mice

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Maximizing operational efficiency while maintaining appropriate animal housing conditions is a continuous focus of research animal care programs. Our institution's longstanding approach to cage-change management included scheduled cage changes every 2 wk, with spot changes if cages met established visual criteria during the intervening period. This 2-wk plus spot changing (2WS) practice for mice housed in IVC was problematic during the COVID-19 pandemic when the need arose to minimize workload to reduce on-site staffing out of concern for employee health and possible absenteeism. With the approval of the IACUC, a spot-change–only (SCO) process was adopted, with the requirement to evaluate microenvironmental parameters under both practices to confirm acceptable equivalence. These parameters (humidity, temperature, and ammonia) were evaluated in a controlled study that found no significant difference between the 2 groups. Ammonia levels did not exceed 10 ppm in any group throughout the study. To assess operational differences between these 2 approaches, we collected cage-change data and employee feedback from facilities operating under these schemes. The SCO method required fewer cage changes than did the 2WS method (10.3% per day with 2WS and 8.4% per day with SCO). Despite this benefit, through a Plan–Do–Check–Act process that has been regularly employed at our institution, employee feedback identified important operational challenges associated with the SCO practice. The SCO approach was thus refined into a scheduled spot change (SSC) practice that builds on the SCO model by incorporating a scheduled focused cage evaluation period. Based on subsequent feedback, the SSC was found to retain the efficiency benefits afforded by the SCO model and simultaneously alleviated staff and operational concerns. This result underscores the importance of integrating staff feedback with a performance standard-based approach when assessing cage-change management.

Abbreviations: 2WS, change every 2 wk plus spot change; ICC, intracluster correlation coefficient; SCO, spot-change-only; SSC, scheduled spot change

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Introduction

The Guide for the Care and Use of Laboratory Animals (*The Guide*) states that, in general, primary enclosures should be sanitized at least every 2 wk.[15] The Center for Comparative Medicine at Massachusetts General Hospital historically followed a 2-wk scheduled cage-change approach for managing IVC. Ten years ago, however, it became evident that investigators were changing cages themselves between the scheduled changes because they perceived cages to be dirty. This unpredictable use of cage-change stations and clean cage components by research staff prevented the facility from ensuring predictable time, space, and resources for staff and challenged our ability to provide adequate numbers of clean cage components in a timely fashion.

To address these challenges, facility management personnel surveyed both animal care team members and research staff to generate a visual definition of what constituted a 'dirty' cage. This collaborative effort brought forth a new approach to rodent cage management whereby IVC were changed on a scheduled 2-wk basis but also spot changed as needed by animal care staff when cages met the agreed-upon visual criteria for a dirty cage. These criteria were presented in a pictorial format, termed a visual control, that provided a guide for animal care team members. This 2-wk plus spot change (2WS) approach was designed to satisfy our customer base by alleviating concerns that 2 wk between changes might not be appropriate for all cages and to help standardize the spot-change criteria for front-line staff.

In 2020, the SARS-CoV-2 (COVID-19) pandemic presented significant and abrupt challenges to laboratory animal care programs throughout the world, and many institutions adopted rapid, large-scale programmatic changes as part of their disaster response plans. To address anticipated staff absenteeism and promote physical distancing by reducing on-site needs, our program imposed restrictions such as limiting facility access, ceasing animal orders, and halting various research projects. Furthermore, the adoption of a spot-change–only (SCO) model of managing mouse caging was proposed. This practice would consist of eliminating the scheduled 2-wk cage change and of changing mouse IVC only when they met the criteria of the dirty cage visual control in the course of daily assessments. This revised plan was considered to decrease the number of daily cage changes, thereby reducing workload and on-site staffing needs.

Although spot changing and extended intervals for the changing of cages and cage components have been advocated by other programs,[1,2,19,37,39,42] eliminating the scheduled cage change raised the question of whether animal housing conditions would be affected. *The Guide* states that "decreased sanitation frequency may be justified if the microenvironment

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in the cages, under the conditions of use (for example, cage type and manufacturer, bedding, species, strain, age, sex, density, and experimental considerations), is not compromised."[15] The IACUC, therefore, approved the SCO plan but stipulated that this approach be assessed to ensure that microenvironmental conditions were similar under these 2 cage-change practices. To this end, we elected to evaluate, under controlled study conditions, the microenvironments of the 2WS and SCO cage-change models. *The Guide* defines the microenvironment as the immediate physical environment surrounding an animal (that is, primary enclosure such as a cage) and the macroenvironment as the physical environment of the secondary enclosure (that is, the room in which the animal is housed). Although *The Guide* defines acceptable conditions (humidity and temperature) for the macroenvironment only, it nonetheless stresses that exposure to fluctuations and extremes in these parameters may result in behavioral, physiologic, and morphologic changes that may negatively affect wellbeing and research outcomes.[15] Since the publication of the 8th edition of *The Guide*, the use of IVC has continued to increase, which has made microenvironmental conditions, which can differ from those of the macroenvironment,[20,29,33,34] increasingly relevant.

In addition to humidity and temperature, ammonia is a parameter that has been used extensively as an evaluative measure of microenvironmental quality in IVC rodent housing.[22] A known respiratory and ocular irritant to many species,[26] ammonia is released in mouse cages when urease-positive bacteria present in the bedding or feces encounter urine.[6,16,25,41,42] As the concentration of ammonia increases, adverse health effects begin to occur.[4-6,21,41] For humans, OSHA sets permissible exposure limits of 50 ppm as an 8-h time-weighted average.[24] The National Institution for Occupational Health and Safety similarly indicates an 8-h time-weighted average of 25 ppm for humans.[10] Despite these established limits in humans, extrapolation to mice has been challenging, and specific accepted exposure limits are not currently available for this species. *The Guide* states that "soiled bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry and keep pollutants, such as ammonia, at a concentration below levels irritating to mucous membranes."[15] Factors that influence ammonia accumulation in mouse IVC include—but are not limited to—housing density, biomass, sex, age, strain, type of housing, type of bedding used, and air exchange rate.[19,20,29,33]

Therefore, the initial aim of our work was to conduct a study that compared the microenvironment of IVC mouse caging under 2 experimental conditions: the 2WS (pre-COVID) model and the disaster-response plan SCO model. Humidity, temperature, and ammonia conditions were recorded both on a scheduled basis and whenever a cage was changed. We hypothesized that microenvironmental conditions would not differ significantly between these 2 experimental groups and that microenvironmental conditions under both scenarios would be acceptable. Given the current lack of consensus regarding permissible ammonia exposure limits in mice, we fixed the acceptable limit at 50 ppm for the purposes of our investigation in light of prior research and established human exposure limits.[34,38,41,42] In addition, guided by previous studies,[6,34] we determined that humidity and temperature conditions in our cages must remain within the macroenvironmental limits established by *The Guide* (that is, humidity of 30% to 70% and temperature of 68 to 79 °F [20.0 to 26.1 °C]). After this comparison of the pre- and post-COVID regimens (2WS and SCO, respectively), we assessed a third group of cages for humidity, temperature, and ammonia daily over a 2-wk period without spot changing. The purpose of this assessment was to characterize the full pattern of how humidity, temperature, and ammonia change over a 2-wk period without spot changing.

While these microenvironmental studies were underway, an efficiency review was simultaneously undertaken in animal rooms in which the 2WS and SCO practices had been implemented on a larger scale. We calculated the number of cages changed daily under each cage management system and solicited staff feedback on the 2 changing practices, particularly regarding the effect of each system on the ability of staff to complete routine tasks. With this additional information, we sought to characterize the operational benefits and pitfalls of both the 2WS and SCO approaches. The SCO model resulted in fewer daily cage changes than did the pre-COVID 2WS model, yet despite this benefit in labor, staff feedback identified operational concerns around task and personnel scheduling under the SCO model. As a result, we ultimately developed a hybrid model—the scheduled spot change (SSC)—and evaluated its efficiency. The SSC remains a spot-change–only cage management approach but incorporates a scheduled focused evaluation of each rack every 2 wk. This approach achieves the benefits of a SCO system and alleviates the operational concerns identified by our team.

Materials and Methods

Microenvironmental study. *Animals and housing*. All animal use was reviewed and approved by the IACUC at the Massachusetts General Hospital, an AAALAC-accredited program. Male CD1 mice (*Mus musculus*; *n* = 240; age, 12 to 14 wk; weight, greater than 30 g; Charles River Laboratories, Raleigh, NC), were housed 4 per cage (maximum density for adult mice per IACUC policy) in autoclaved polysulfone microisolation caging (11.75 in. × 7.25 in. × 5 in. [29.8 cm × 18.5 cm × 12.7 cm], Allentown Caging, Allentown, NJ). Cages were placed on a single individually ventilated rack (model MS75JU80MVSPSHR-R, Allentown caging, Figure 1). The rack air-handling system was set to 60 air changes hourly and was sampled monthly at 9 different locations on the rack to evaluate actual operation (model CFD+0jV0AE-0004, Allentown Caging). During the current studies, the measured air change rate ranged from 65 to 78 per hour at the cage level. Throughout the study, between 80% and 100% of rack slots were occupied. Room lights were maintained

Figure 1. IVC system for mice used at our institution.

on a 12:12-h light:dark cycle (recessed fluorescents, 325-540 lux, lights on, 0700). Room temperature was maintained at 68 to 71 °F (20 to 23 °C), and room humidity remained between 32% and 58% for the duration of the study. Mice had ad libitum access to food (Prolab Isopro RMH 3000 irradiated diet, PMI Nutrition International, Lexington, MN) and acidified water (pH 2.6 to 2.9) via an automatic reverse-osmosis system. Cages contained approximately 40 g (350 mL) of Sani-Chip (PJ Murphy, Montville, NJ) bedding (a blend of aspen or beech coupled with birch and maple) approximately 25 ounces of nesting material (Comfort Bedding, Biofresh, Patterson, NY) and an igloo (Bio-Serv, Flemington, NJ).

Colony health was monitored quarterly by serologic evaluation of sentinel mice exposed to dirty bedding. For the duration of these studies, all sentinels tested were seronegative for mouse hepatitis virus, mouse parvovirus, minute virus of mice, epizootic diarrhea of infant mice, ectromelia virus, Sendai virus, pneumonia virus of mice, Theiler's murine encephalomyelitis virus, reovirus, lymphocytic choriomeningitis virus, Hanta virus, lactate dehydrogenase elevating virus, murine cytomegalovirus, mouse adenovirus, polyoma virus, *Mycoplasma* spp., and cilia-associated respiratory bacillus. In addition, PCR testing for fur mites and pinworms was negative.

Microenvironmental sampling methodology. Sampling entailed simultaneous measurement of the ammonia, humidity, and temperature values within a given cage. Only cages containing 4 mice were sampled; cages that had fewer than 4 mice for any reason were excluded. Humidity and temperature readings were obtained by using a psychrometer (Figure 2) fitted with an external probe (model EP8710, General Specialty Tools and Instruments, New York, NY). Ammonia levels were obtained by using a digital single-gas monitor (Figure 2) fitted with an ammonia sensor (Micro IV G223, GfG Instrumentation, Ann Arbor, MI). The gas analyzer was connected via a short length of flexible plastic tubing to a rigid 8- to 10-in. (20 to 25 mm) length of polycarbonate tubing approximately 4 mm in diameter.

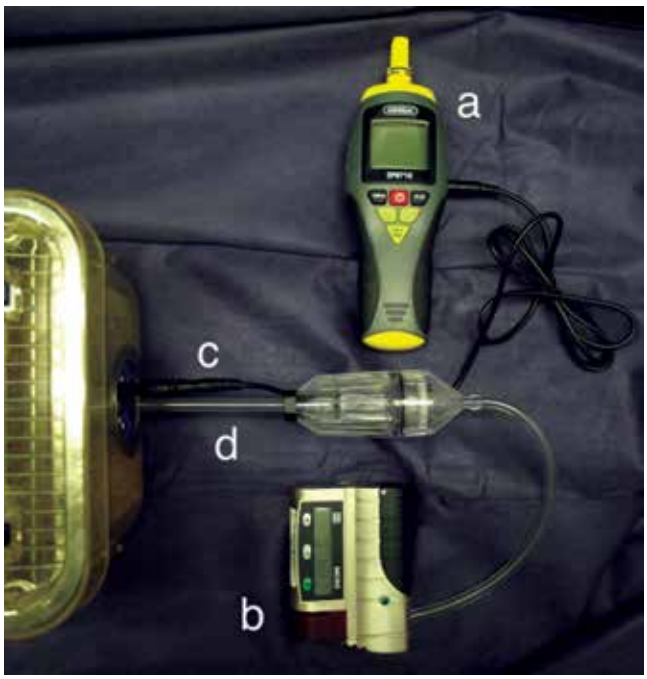

Figure 2. Positioning of sampling devices during collection of microenvironmental data. Psychrometer (a) and gas analyzer (b) positioned next to a cage. The psychrometer probe (c) and gas-sampling tubing (d) are inserted through the grommet for the automatic watering system.

To perform sampling, cages were removed from the rack, placed on a table, and sampled immediately, with special attention paid to ensuring the cage remained sealed until sampling was complete. The rigid length of polycarbonate tubing attached to the ammonia monitor was inserted into the cage through the automatic watering system grommet (Figures 2 and 3). Simultaneously, the psychrometer probe was inserted through the grommet (Figures 2 and 3). Both sampling instruments were inserted approximately 4 to 6 in. (10 to 15 cm) into the cage and rested roughly 1 in. (2.5 cm) above the cage bottom. The ammonia sensor had a built-in pump that, when activated, drew air from the cage through the tubing and into the analyzer. Based on manufacturer recommendations, the sample was allowed to stabilize for roughly 2 min before it was recorded. To improve the efficiency of the study, 2 identical gas analyzers were used. Approximately 30 s was required to obtain a stable reading from the psychrometer.

Both the ammonia gas analyzer and psychrometer were maintained according to manufacturer recommendations. For the ammonia sensor, maintenance included calibration every 6 mo, which was performed by the manufacturer just before the start of the study, as well as daily 'bump testing' immediately prior to use of the unit. Bump testing entailed exposing the monitor to a known ammonia source to ensure the unit could detect ammonia on the day of use. For bump testing in this study, we maintained a series of nonstudy mouse cages with visible urine spotting. Each day prior to use on study cages, these nonstudy cages were sampled to confirm that the analyzers could detect ammonia. Recalibration of the ammonia sensor was not required, given that the study was completed in less than 6 mo. In the case of the psychrometer, calibration was not required because the unit had been purchased new immediately before the start of the study.

Study design: 2WS and SCO microenvironmental comparison. Microenvironmental parameters were assessed under 2 experimental conditions: the pre-COVID 2WS system (n = 30 cages) and the disaster-response plan SCO system (n = 30 cages). To ensure sufficient statistical power (greater than 80%) for comparing the 2 experimental groups, sample-size calculations were performed under a noninferiority testing framework; additional details can be found in the online supplementary material (Figure S1). Cages in the 2WS group were changed entirely (cage bottom, wire bar, filter top, and igloo) every 14 d; these cages

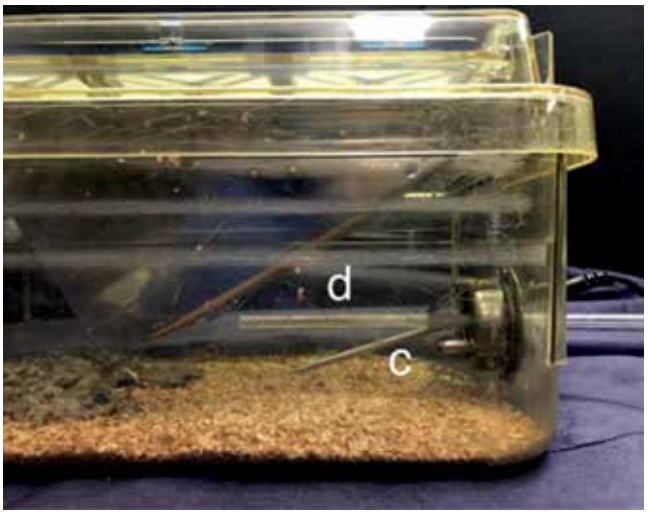

Figure 3. Side view of mouse cage showing the relative positions of the psychrometer probe (c) and gas-sampling tubing (d).

also were spot-changed between their scheduled cage changes if they met any of the criteria outlined in the spot-change visual control. Cages in the SCO group were changed only if they met the spot-change visual criteria. Regardless of experimental group, spot changing consisted of changing cage bottoms and igloos, with wire bars and filter tops changed only if they were visibly soiled or damaged, as stipulated in a previous IACUC-approved departure from *The Guide*.

Cages were checked daily by husbandry staff according to departmental standard operating procedures and entailed ensuring the presence of sufficient food, verifying appropriate operation of automatic watering systems, assessing cages for health concerns, and determining whether a cage required spot changing based on the visual control. On initial hire, staff received training on how to identify dirty cages using the visual control, and additional training of all staff occurs regularly to reinforce the document and the definition of a dirty cage. Animal care staff at our institution rotate tasks throughout facilities on a given day, therefore the same caregiver was not always responsible for cage checks of the same room on consecutive days. In total, 9 animal care staff who were blind to the purpose of the study and the experimental groups participated in this study.

During their daily inspection of cages, animal care staff flagged cages with a card if they appear to meet any of the criteria outlined in the spot-change visual control (Figure 4). Later that same day, research staff measured the microenvironmental parameters of cages requiring spot changing, after which cage components were changed. The date of every cage change was recorded. In addition to measuring microenvironmental conditions when spot changes were required, all cages were sampled on a once-weekly schedule. Cages that were part of the 2WS model were also sampled whenever scheduled cage changes occurred (Figure 5). For the duration of the study, research staff played no role in identifying which cages required spot changing.

Study design: microenvironment of 2-wk period without spot changing. Clean cages (n = 35) and components of a configuration identical to those in the 2WS and SCO microenvironmental comparison study were established on the same rack after completion of the first microenvironmental study. Mice from the previous study were used, with all cages starting the 2-wk period with 4 male CD1 mice. Microenvironmental conditions (humidity, temperature, and ammonia) were recorded daily for all cages.

Statistical analyses. To compare average microenvironmental conditions of 2WS and SCO groups over time, we modeled humidity, temperature, and ammonia parameters individually over time by using linear mixed effects models. We adjusted for regimen, time elapsed since the beginning of the study, and their interaction. A significant interaction term indicated that the trajectory of each microenvironmental parameter differed by regimen. Random intercepts and random slopes were included for the effect of time elapsed, accounting for clustering by cage. To evaluate whether the variability of the microenvironmental parameters were different between groups, we fit linear mixed-effects models for each of the 3 microenvironmental parameters as outcomes in each group separately, adjusting for time elapsed since the beginning of the study and using random intercepts

Figure 4. Visual control describing the various criteria resulting in spot-changing of a cage. Red circles highlight specific visual examples for staff. When a cage met any one of these criteria, the cage bottom and igloo were changed.

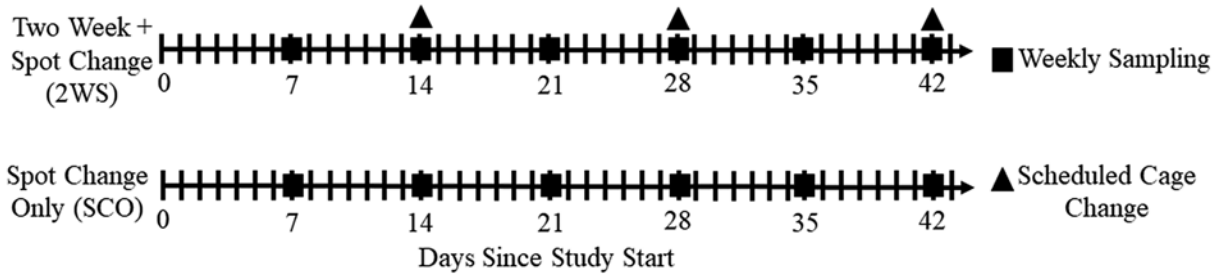

Figure 5. Schematic representation of microenvironmental sampling and cage-change time points for 2 cage management methods. All cages were sampled on a scheduled weekly basis (squares). 2WS cages were changed on a scheduled 2-wk basis (triangles). Under both regimens, cages would also be spot-changed and sampled on any day when they met any of the criteria outlined in the spot-change visual control.

to account for clustering by cage. The intracluster correlation coefficient (ICC) was then estimated from these models. The ICC is the proportion of variability explained in the model by between-cluster (that is, between-cage) differences; a higher ICC indicates that the outcome exhibits less variability over time within that group.[3] We compared the estimated ICC for each outcome variable between groups. The δ method was used to calculate 95% confidence intervals for ICC.[36]

The average cage change interval for the 2WS and SCO microenvironmental experimental groups was calculated by dividing the total number of days of cage residence by the number of cage changes, or equivalently, by measuring the average of the number of days between cage changes. The average number of days between cage changes was modeled by using a linear mixed-effects model, adjusting for regimen and accounting for clustering by cage by using random intercepts. All statistical analyses were conducted by using R, version 4.1.1.[27] Linear mixed-effects models were fit by using the *nlme* package in R. A *P* value of less than 0.05 was considered statistically significant.

Efficiency evaluation and review. *Staff feedback*. Our institution practices a culture of continuous improvement, specifically through the use of the Plan–Do–Check–Act process.[9] This management technique is predicated on the scientific method and encompasses a systematic approach to evaluating management practices, with particular emphasis on inclusivity through the collection of feedback from stakeholders at all levels of an organization. As part of the Plan–Do–Check–Act process, our department holds daily facility-based staff meetings during which staff are prompted to provide feedback regarding any difficulties faced during the workday. These meetings provide the opportunity for staff to share with their team leads and facility managers their experiences regarding the 3 cage-change systems. Staff also had opportunities to speak directly to the research team and share the challenges and benefits they noted between the cage-change practices.

Cage change calculations and statistics. Cage-change frequencies under 3 cage-change systems—2WS, SCO, and SSC—were ultimately assessed. SSC is an outcome of the controlled 2WS–SCO study and is discussed further in the Results section. In brief, it is a hybrid of the 2WS and SCO cage-change practices and consists of daily spot changes based on the visual control, with an additional scheduled 'focused' evaluation of each rack once every other week. Evaluations were performed in 2 animal facilities. One facility used the SCO model followed by the SSC model, whereas the 2WS model data came from a second facility. Animal health status, IVC housing systems, and husbandry were as described above for the microenvironmental study, except that cages contained mice involved in IACUC-approved studies and comprised various strains, sexes, and housing densities.

The number of daily cage changes and total cage census were recorded from specific rooms in each facility as they operated under each cage-change practice. From this information, the daily cage-change proportion, determined by dividing the number of daily cage changes by the total room census, was calculated. On weekends and holidays, cage change data were reported, but room and census data were not available. For these days, the census used in calculating the cage-change proportion was determined to be the average census of the days immediately before and after the weekend or holiday for which data were available. Using the calculated daily cage-change proportion, a Poisson regression model treating the number of cage changes each day as the outcome was generated, including an offset term for the census on a given day and group as a covariate. All statistical analyses were conducted by using R, version 4.1.1.[27] Linear mixed-effects models were fit by using the *nlme* package in R. A *P* value of less than 0.05 was considered statistically significant.

Results

Microenvironmental study. *2WS and SCO microenvironmental comparison*. Over the course of the study, 7 of 30 cages in the 2WS and 6 of 30 cages in the SCO groups were removed at various points because the cage population dropped below 4. In the 2WS experimental group, 409 total sampling events were recorded and included scheduled weekly sampling events, scheduled cage changes, and spot changes. For the SCO experimental group, 443 events were recorded. The 2WS group involved 132 scheduled cage changes and 170 spot changes, totaling 302 cage changes. By comparison, a total of 227 spot changes were recorded for the SCO group over the same period. The average cage-change interval was 6.3 d for the 2WS group and 7.7 d for the SCO group (between-group difference of 1.4; $P < 0.001$). For the SCO model, the cage change interval range was 2 to 17 d, whereas the range for the 2WS group was 3 to 14 d.

A total of 9 animal care technicians participated in husbandry of experimental cages; 6 were responsible for the majority of the study days (60 of 66 d). Among these 6 employees, the number of days on which each person conducted room checks was similar and generally randomly distributed.

Humidity and temperature for both experimental groups remained within acceptable target parameters throughout the 10-wk study. Measurements followed similar trajectories and varied to the same extent in both experimental groups (Figure 6). The interaction term of the linear mixed-effects model adjusting for regimen, time, and their interaction was used to evaluate whether the temperature trajectory differed between regimens; the effect was insignificant for both temperature ($P = 0.786$) and humidity ($P = 0.785$), thus providing no evidence that the temperature or humidity trajectory differed between

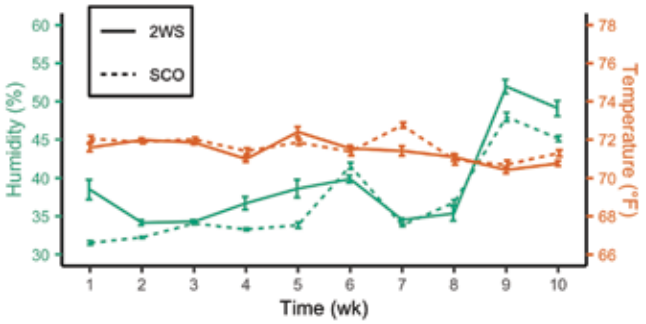

Figure 6. Weekly average temperature (right *y*-axis) and humidity (left *y*-axis) measurements for each group during the 10-wk study. Error bars, 95% confidence intervals.

experimental groups. The 95% confidence intervals for the ICC for temperature obtained from the linear mixed-effects model with only random intercepts adjusting for time, calculated after stratifying by group, were 0 to 0.082 °F for the 2WS group and 0 to 0.036 °F for the SCO group. These highly overlapping confidence intervals indicate that the variability in the temperature did not differ significantly over time between the 2 groups. Similar overlapping confidence intervals were observed for the humidity measures (0% to 0.077% for the 2WS group and 0% to 0.067% for the SCO group), thus providing no evidence that variability in humidity differed significantly over time between the 2 groups.

Although the ammonia sensors were reliably able to detect the presence or absence of ammonia, the 2 sensors differed slightly in their final readings in a few cases. These discrepancies were small and, where noted, did not change the determination of whether a cage was within or outside of acceptable ammonia levels. In these cases, the value reported was the average of the 2 readings. In total, 1 of 409 sampling events (0.25%) under the 2WS regimen detected ammonia whereas 6 of 443 sampling events (1.4%) under the SCO regimen detected ammonia. Among the cases in which ammonia was detected, the highest concentration recorded was 10 ppm (well below our study maximum of 50 ppm). Thus, ammonia levels did not differ significantly between groups, and modeling approaches were not needed to compare ammonia levels between experimental groups.

Microenvironment of 2-wk period without spot changing. Overall 33 of 35 cages reached the desired 2-wk endpoint with 4 adult mice present. At no time did the humidity or temperature of any cage fall outside the acceptable range. Ammonia was first detected on day 7 after cage change. At the conclusion of the 2-wk period, ammonia was detected in 10 of the 33 cages (30%). Among these 10 cages, 4 (12%) had levels below 25 ppm, 4 (12%) had levels that were between 25 and 50 ppm, and 2 cages (6%) had levels that were 50 ppm or greater. Of these 2 cages, the highest recorded concentration was 85 ppm in one and 165 ppm in the other (Figure 7).

Efficiency evaluation. *Staff feedback.* The 9 animal care technicians that performed husbandry during the course of this study provided feedback on the 2WS and SCO practices. Feedback was consistent among staff regarding the key benefits and pitfalls of each approach. Staff agreed that the 2WS model provided essential predictability and patterns that were absent under the SCO model. For example, in a room with 6 racks, under the 2WS model, 3 would be changed over the course of one week (Monday, Wednesday, Friday) and the other 3 during the second week. This schedule of full-rack changes allowed staff to estimate fairly accurately the number of cage changes required on a given day. This system also helped staff to predict where those cages requiring spot changes based on the visual control were more likely to be found (that is, on racks furthest from their last scheduled change).

Under the SCO approach, staff consistently remarked that the elimination of scheduled rack changes resulted in the loss of the key predictability provided by the 2WS model. They felt they could no longer accurately estimate the number of daily cage changes needed or the distribution of dirty cages throughout the room. As a result, they felt their time in rooms was spent less efficiently.

The SCO approach revealed staff concerns regarding the use of a model that was based solely on a visual control. Although the visual control outlines specific criteria for what defines a dirty cage, it was the 'borderline' cages—those that were close, but did not clearly fit, the definition of a dirty cage (and thus require cage changing)—that presented the most concerns for staff. Husbandry staff at our institution rotate room assignments on a daily basis; thus multiple employees may provide daily room checks for a given animal room over the course of a week. As a result, some staff working under the SCO model felt compelled to change borderline cages. Two reasons were generally given for this approach to such borderline cages. First, they were concerned that a second staff member identifying dirty cages in the next day or 2 might inaccurately conclude that the cages were similar on the preceding days and thus judge them as having previously been missed by staff. Second, staff felt compelled to change borderline cages because they were concerned about leaving more work for their colleagues the next day.

The SCO model raised several critical operational challenges. For one, the 2WS model required scheduled cage changes during the week, thus decreasing the work required on weekends. When scheduled changes were removed under the SCO model, each day's workload was effectively the same. As a result, whereas staffing requirements previously had been lower on weekends because scheduled rack changes occurred on weekdays, the SCO approach required equal staffing throughout the 7-d week. This demand for a relative increase in weekend staffing was difficult to sustain.

The erratic number of daily cage changes under SCO led to additional challenges. The inability to reliably anticipate the amount of time required for staff to perform room checks and cage changes made it difficult to accurately allot staff time to other tasks, such as cage washing, autoclaving, and cage setup. Furthermore, although the predictability of 2WS had helped keep the availability of daily clean cages in step with demand, the SCO model resulted in processing and storing of more clean cages than were generally needed to ensure a sufficient supply in case a large number was required.

The SSC model. Despite the consensus from staff that SCO presented various challenges, employees agreed that the method offered potential for labor savings because, unlike 2WS, SCO allowed them to avoid changing cages that were obviously clean. Countermeasures to address staff concerns regarding SCO were discussed, and the proposal was made to trial a third model, a hybrid of the 2WS and SCO models termed the scheduled spot-change (SSC) method. This approach employed daily spot-change–only but added a focused evaluation of each rack in a room every 2 wk. Cages on racks that were not due for a focused evaluation would be changed if they met the visual control criteria on that day. However, on racks scheduled for a focused evaluation, the technician was expected to change not only those cages that met the criteria but also all borderline cages, which had previously caused staff concern in the SCO

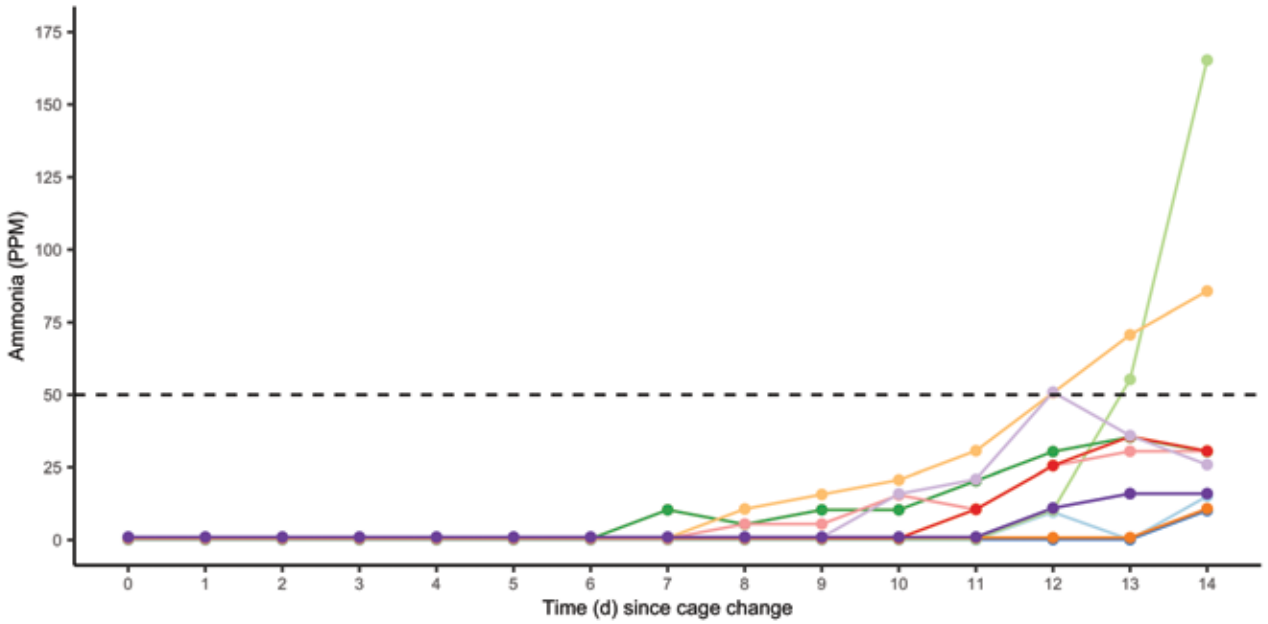

Figure 7. Ammonia levels recorded from 10 cages that had detectable ammonia levels over a 2-wk period without spot changing. Colored lines represent each of the 10 individual cages; the dashed line indicates 50 ppm ammonia. The remaining 23 cages in this study did not produce detectable ammonia.

model. Borderline cages were those that staff anticipated would meet the visual definition of a dirty cage in the next 2 or 3 d.

This new practice was trialed in the same facility in which 2WS and SCO had been used, thereby offering an opportunity to receive feedback from staff who had worked under all 3 models. Staff agreed that the addition of the focused evaluations helped to address the management of borderline cages and that SSC returned the predictability of room cage-changing patterns. In turn these features helped planning and scheduling and saved on labor and supplies by keeping to the general premise of not changing a clean cage. In addition, with focused evaluations during the week, the bulk of cage changes could then be performed on weekdays, allowing resumption of a 5-d work week schedule, with more staff able to have weekend time off.

Cage-change calculations and statistics. The 2WS method review included 31 d of data, during which a total of 1114 cage changes were performed, including both scheduled full-rack changes and spot changes, with a census population for the same period of 10,798 cages. These data yielded an average daily cage-change proportion of 10.3%. For the SCO method review, a period of 30 d identified 15,269 cage changes with a period census of 180,853 cages, yielding an average daily cage-change proportion of 8.4%. Finally, for the SSC review, a period of 47 d identified 13,312 cage changes with a period census of 164,621 cages, yielding an average daily cage-change proportion of 8.1%. The estimated ratio of daily cage-change proportions obtained from the Poisson regression model comparing SCO with SSC was 1.044; that is, the proportion of cage changes on any given day was significantly ($P < 0.001$) higher (4.4%) for SCO than for SSC. Comparing the 2WS ratio with the SSC ratio indicated that significantly ($P < 0.001$) more (27.6%) daily cage changes occurred under the 2WS model. In terms of absolute differences in cage-change proportion, the average was 2.23 percentage points higher for 2WS than SSC, 1.88 percentage points higher for 2WS compared with SCO, and 0.35 percentage points higher for SCO than SSC.

Discussion

Although research animal facilities use spot-changing of mouse cages as either a primary management approach or an adjunct to a scheduled cage change, a widely accepted consensus is not available regarding the visual criteria that should trigger a cage change. Given this uncertainty, institutions should verify that new processes do not negatively affect housing conditions before such changes are implemented on a broad scale. With the support of the IACUC, our program implemented an SCO approach to rodent cage management in response to the COVID-19 pandemic. However, our department was charged with verifying that the microenvironment under any new management system did not differ from that associated with our historical practice. By evaluating the microenvironment of mouse cages, as represented by ammonia, humidity, and temperature, we verified our hypothesis by identifying no significant differences in microenvironmental conditions using the 2WS and SSO approaches to mouse cage management.

A secondary aim of this study was to evaluate the operational performance of our cage-change practices during actual use in our facilities. This goal was accomplished by collecting cage-change data while simultaneously soliciting feedback from husbandry staff. Review of operations under the SCO and 2WS cage-change practices revealed that the SCO system reduced daily cage changes by 18% compared with the 2WS system. Despite this finding, and the fact that the microenvironment data supported the use of an SCO model, staff feedback consistently identified numerous pitfalls associated with this approach. To address these unanticipated challenges, we developed the hybrid SSC system, which follows SCO practices but adds a scheduled 2-wk focused evaluation of each rack in a room.

This hybrid approach helped to alleviate operational and staff concerns associated with the original SCO model and simultaneously retained the efficiency benefits gained from SCO compared with the pre-COVID-19 2WS model. Cage change data showed that the SSC model reduced daily cage changes from 10.3% during 2WS to 8.1% during SSC. This 2.2% difference

equates to a relative reduction of 21% fewer daily cage changes under SSC compared with 2WS. Extrapolating this figure over 1 y at an institution with 35,000 cages (such as our own) translates to upward of 770 fewer cage changes daily, or 280,000 annually. At our institution, we calculated that the average cage change requires approximately 80 s; cutting daily cage changes by 2.2% could save as much as 17 h of labor per day at our institution. When programs are faced with absenteeism or other staffing challenges, the reduction in labor afforded by this management approach could be important for addressing such concerns. Furthermore, this reduction could also provide vital savings in consumables (for example, bedding, food) and equipment (for example, reduced use of tunnel washers, autoclaves).

Although previous studies have described performance standards associated with mouse cage management practices,[2,14,35,41] few have addressed the operational implications of such practices, especially with emphasis on feedback from staff.[17,25,39,42] The cage-change data alone allowed us to conclude that the SCO approach was superior to the 2WS model and to make the decision to adopt SCO practices on a large scale. However, the critical feedback from our husbandry staff led to the refinement and adoption of a better system. This outcome underscores the importance of integrating performance standards and operational assessments, with particular attention to staff feedback.

Despite the clear benefits of the SSC system compared with the 2WS system, 18% of the cages that underwent daily microenvironmental sampling for 2 wk without spot changing had ammonia levels of 25 ppm or greater at the end of the 2-wk period, with only 2 cages attaining 50 ppm or more. The finding that the average cage-change interval for the SCO group was 7.7 d and the first ammonia level that met or exceeded 25 ppm occurred on day 11 leads to the conclusion that the visual accumulation of urine—and therefore ammonia—was not necessarily our primary basis for cage changes under an SCO system. Discussions with members of the husbandry team revealed that feces accumulation was the predominant trigger for spot changes under both the 2WS and SCO systems. The spot-change visual control indicates that cages should be changed when the ratio of feces to bedding is greater than 50:50. These criteria are based on the perception of cleanliness by the observer and not on empirical data or actual assessments of animal welfare. With the knowledge that cage changes typically occur before significant levels of ammonia accumulate and that cages are changed based on these subjective criteria, the cage change interval might safely be prolonged by refinement of the visual guide. An extended cage-change frequency could not only benefit programs by sparing resources but also reduce staff exposure to allergens[11,30] and improve animal welfare, given that negative effects of cage changes on animal wellbeing have been documented.[18,28,31,40] A thorough assessment of how accumulated feces might influence welfare is a critical component to achieving this refinement.

To establish appropriate cage-change intervals in mouse caging, questions should focus on establishing the exposure limits for ammonia in mice. We set our acceptable limit at 50 ppm because previous work has shown that, although overt clinical signs may not develop in mice at such concentrations, histopathologic effects have occurred at similar levels.[21,41] Despite these findings, some argue that mice naturally inhabit small burrows where such waste products would concentrate, but confirmatory studies have not yet been undertaken. However, in one study, mice of several strains displayed no significant differences in preference for inhabiting spaces of very low to very high (for example, 110 ppm) levels of ammonia.[12]

We used CD1 male mice in this study for several reasons. This strain is one of the top 5 strains ordered at our institution. In addition, these mice are comparatively large, with a 12-wk average weight of 35 to 45 g, compared with 25 to 30 g for a C57BL/6J. This characteristic, coupled with the fact that males may produce more urine than females,[5,41] led us to use male CD1 mice because we believed they would represent an extreme in terms of urine production and thereby rigorously challenge the microenvironment and the spot-change process. Prior studies have used CD1 mice for similar reasons.[6,41] A recent study housed 4 male CD1 mice on 4 distinct bedding types and measured ammonia accumulation over 2 wk.[38] For 3 of the bedding types, approximately 10% to 30% of cages reached or surpassed the threshold of 50 ppm by the end of the 2-wk period. In our study, by comparison, only 2 of 33 (6%) of cages attained 50 ppm ammonia or more by the end of the same period. To our knowledge, our study represents the first such evaluation using a hardwood blend bedding consisting of mostly aspen or beech, birch, and maple. Another study found that cages with trio breeding CD1 mice housed on corncob bedding did not exceed 50 ppm ammonia over the course of 2 wk, whereas pairs generally did not exceed 25 ppm over the same time period.[6] A third study evaluated ammonia accumulation by using 5 male CD1 mice per cage housed in IVC with corncob bedding.[41] In that case, ammonia generally exceeded 50 ppm by 9 or 10 d after cage change, whereas cages with 3 mice did not accumulate excessive levels of ammonia for at least 2 wk after cage change.

The current study was designed to model high-density housing of a common mouse strain and gain insight into effects on the microenvironment. However, our results are limited to the type of rack, cage type, housing density, bedding, and air flow conditions that are used in our institution. The policy for mouse housing density at our institution is more conservative than that of *The Guide,* and a different institution might house CD1 mice at higher densities than we do. Therefore, other programs should be cautious when generalizing these current results to their operational conditions and should ensure that new cage-change regimens are evaluated before they are adopted on a widespread basis. We used high-density housing of large male mice as representative of an extreme microenvironment, but other housing configurations, particularly breeding pairs with large litters, could represent another extreme that could challenge the assumptions that can be drawn from our study. A further limitation is the temporal nature of our study, given that the bulk of this study was conducted in the spring. During this period of the year, the humidity of our facilities is relatively low and consistent, but we have experienced significant fluctuations, with comparatively low humidity in the winter and high humidity in the summer. This feature is noteworthy because ammonia levels can be influenced by humidity levels, with higher humidity linked more closely to elevated ammonia in mouse cages.[32] Although our mice are housed in IVC and are thus insulated from the macroenvironment to some extent, higher or lower relative humidity of the macroenvironment may have nonetheless produced different results had we conducted this study in midwinter or the height of summer. Finally, our study focused on comparing microenvironmental conditions as our primary evaluative measure for modifying our cage-change regimen. Microenvironmental parameters are only one factor in animal wellbeing; other studies assessing cage change management have evaluated other metrics, including breeding performance, fecal cortisol levels, and animal behavior.[6,23,39] We are considering future investigations in these areas.

The use of a spot-change visual control raises the question of how intertechnician variability might affect results, such as the interval between cage changes. In reviewing the data from the 2WS compared with SCO microenvironment study, we found that 6 technicians performed the majority (approximately 90%) of the cage changes and that this responsibility was relatively evenly distributed across these employees. In addition, all technicians received spot-change training, and cage change intervals were normally distributed, with very few outliers. As a result, we are reasonably confident that intertechnician variability did not play a significant role in the study.

In conclusion, our microenvironmental comparison showed no significant difference between scheduled 2-wk cage changes and as-needed spot changing and a spot-change–only approach to mouse cage management. This outcome gave confidence to both the IACUC and the Center for Comparative Medicine that a spot-change–only approach could be adopted as part of our disaster-response plan to the COVID-19 pandemic. Furthermore, through a problem-solving approach that used both employee feedback and cage-change data, we were able to refine the SCO process and adopt a superior method—the scheduled spot-change process. This refinement allowed us to embrace a more efficient means of cage management that addressed staff and operational concerns.

Supplementary Materials

Figure S1. Power calculations.

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