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Assessing Accumulation of Organic Material on Rodent Cage Accessories

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According to the 8th edition of the Guide for the Care and Use of Laboratory Animals (the Guide), rodent cage accessories, such as filter tops, should be sanitized at least once every 2 wk. We performed a study to test the hypothesis that organic contamination (measured by ATP content, expressed as relative light units (RLU)) of cage accessories (wire bar inserts and filter top lids) does not differ at 2 wk (14 d) as compared with 30, 60, and 90-d time points after cage change even when in constant use. An additional time point for filter top lids of 180 d after cage change was also evaluated. Eight groups were studied: the wire bar inserts and filter top lids used for mice and rats, in both static and individually ventilated cages (IVC). When analyzing data from both mouse and rat static and IVC caging, we found that the mean RLU values for mouse IVC and rat static and IVC cage components were below 100,000 RLU at the 14-d time point. The mean value for the mouse static group was slightly above 100,000 RLU at this time point. Based on this observation, we considered 100,000 RLU to be an appropriate actionable level. We concluded that changing wire bar inserts at least every 14 d, as recommended in the Guide for sanitizing these components in mouse and rat static cages, may be considered acceptable. This interval could be extended for mouse and rat IVC cages up to 90 d while remaining below this limit. Filter top lids for mouse static cages should be changed at least every 30 d, but static rat and IVC mouse/rat filter top lids could be changed up to every 180 d, while still staying below this actionable level of contamination.

Abbreviations: Guide, 8th edition of the Guide for the Care and Use of Laboratory Animals; RLU, relative light unit(s)

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Mice and rats are the most commonly used mammals in biomedical research. The use of rodents in research has increased in recent years due to recently developed gene editing tools that allow their precise genetic modification and the development of specific rodent models of human disease.[9,15,21,27,28,48,51] Institutions typically house rodents in either static micro isolation or individually ventilated caging (IVC) systems. Institutions receiving National Institutes of Health (NIH) funding for animal-based research must follow the housing guidelines described for rodents in the Guide for the Care and Use of Laboratory Animals, 8th edition (the Guide).[23] The Guide contains specific recommendations for the sanitization of both rodent cages and cage accessories. According to the Guide, "In general, enclosures and accessories, such as tops, should be sanitized at least once every 2 wk. Solid-bottom caging, water bottles, and sipper tubes usually require sanitation at least once a week."[23]

The changing and sanitization of cages and cage components is among the most labor-intensive activities in the research animal facility and, therefore, one of the costliest. In addition to the financial impact, husbandry practices like cage changing and frequent sanitization of cages and cage accessories can have direct effects on research animals and may create experimental confounds,[5,16,20,43-46] impacting the outcomes of studies.[32,36] To perform studies using rodents in a cost-effective manner while minimizing experimental variables introduced by husbandry procedures, husbandry practices must be based on both animal welfare and scientific merit.

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The Guide states that "The increased use of individually ventilated cages (IVC) for rodents has led to investigations of the maintenance of a suitable microenvironment with extended cage sanitation intervals and/or increased housing densities. By design, ventilated caging systems provide direct continuous exchange of air, compared to static caging systems that depend on passive ventilation from the macroenvironment. As noted above, decreased sanitation frequency may be justified if the microenvironment in the cages, under the conditions of use (e.g., cage type and manufacturer, bedding, species, strain, age, sex, density, and experimental considerations), is not compromised. Verification of microenvironmental conditions may include 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."[23] Studies have been published to provide evidence-based recommendations to support changes to the Guide regarding sanitization intervals of cages and cage accessories.[6,17,42] Accrediting and regulatory agencies indicate that institutions may use site-specific and data-driven approaches to determine the ideal institutional frequencies for the sanitization of cages and cage components.[6] Thus, individual institutions should follow the recommendations of the Guide, but exceptions to the Guide are acceptable if evidence-based methodology is used in making such decisions and they are approved by the local Institutional Animal Care and Use Committee (IACUC).[1,33]

Adenosine triphosphate (ATP) is a molecule found in all living and recently deceased organisms. Thus, its presence can be used as an indicator of the presence of biologic residues or contamination on surfaces. To evaluate surface contamination using ATP testing, a surface is swabbed, and the sample is exposed to an ATP releasing agent that lyses cells. The sample is

then exposed to an ATP-activated light producing substrate/cofactor (luciferin), an enzyme (luciferase) and oxygen. The amount of light produced during the enzymatic reaction is directly associated with the amount of ATP present and the light emitted can be quantified in relative light units (RLU). ATP-based microbiologic monitoring has been used in many industries in which evaluation of sanitization practices is essential for maintaining health and safety. These industries include food and beverage, health laboratories, hospitals, the pharmaceutical industry, and others.[10,13,19,26,55] Similarly, this methodology has become the standard for measuring surface contamination in animal research facilities. Therefore, this approach can be considered the gold-standard methodology to use for evaluation of sanitization practices.[2,3,6,16,42,53]

Currently, we house mice and rats in static cages that are changed at 1-wk intervals, and individually ventilated cages that are changed at 2-wk intervals. Cages are spot-checked on days not scheduled for a full cage change, and a cage is changed if it meets established cage change criteria. Historically, wire bar inserts were changed at 90-d intervals and filter top lids were changed at 180 d. These intervals were based on data from a previously published manuscript.[42] However, the brand of caging and cage accessories employed at our institution differs from what was used in that study, and literature results are inconclusive regarding optimal sanitization intervals for cage accessories. To support objective decisions regarding sanitization intervals of the cage accessories used at our institution, we performed a study so that we could make evidence-based decisions on our current cage changing practices and determine the best interval(s) for sanitization of cage accessories (wire bar inserts and filter top lids). We chose ATP evaluation as the best methodology to evaluate surface contamination and efficacy of cage and cage accessory sanitization in this study.

Materials and Methods

Animals. Use of animals in this study was approved by the Medical College of Wisconsin (MCW) Institutional Animal Care and use Committee (IACUC) as part of the institution animal health surveillance program. Sentinel animals were used. Collection of the samples did not require specific IACUC approval, because no animal contact occurred during collection. Sample collection took place when routine animal husbandry procedures were being performed. The MCW animal care and use program is fully accredited by AAALAC International. We used CD-1 IGS mice (Charles River Laboratories, Wilmington, MA) and CD (Sprague–Dawley) IGS rats (Charles River Laboratories, Wilmington, MA) for this study. Sentinel animals are ordered at 3 wk of age and placed in cages for sentinel use when they are approximately 4 wk of age. Sentinel animals are euthanized every 4 mo to collect blood and tissues as required for diagnostic testing. Sentinel animals were used in this study because they were housed under consistent cage conditions, and because long-term housing of cages on individual racks was noted in a similar study.[17] In addition, use of sentinel animals allowed us to use animals for multiple purposes, and thus helps to minimize the number of animals used for research purposes.[41] Mice and rats were exposed to dirty bedding from the cages of animals used for research in their respective rooms. Sentinel mice were negative for mouse hepatitis virus, minute virus of mice, generic parvovirus, murine parvoviruses 1 to 5, Theiler murine encephalomyelitis virus, mouse rotavirus, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, REO3 virus, lymphocytic choriomeningitis virus, Ectromelia virus, mouse adenoviruses 1 to 2, murine polyomavirus, *Encephalitozoon cuniculi*, *Cilia-associated respiratory bacillus*, *Clostridium piliforme*, mouse cytomegalovirus, pinworms, and fur mites. Sentinel rats were negative for rat coronavirus/sialodacryoadenitis virus, generic parvovirus, rat parvovirus, rat minute virus, Kilham rat virus, Toolan H-1 virus, rat theilovirus, *Pneumocystis carinii*, Sendai virus, pneumonia virus of mice, *Mycoplasma pulmonis*, REO3 virus, lymphocytic choriomeningitis virus, *Cilia-associated respiratory bacillus*, Hantaan virus, *Clostridium piliforme*, mouse adenoviruses 1 to 2, pinworms, and fur mites.

Husbandry. Mice were housed in static micro isolation cages (Allentown 75 JAG, Allentown Caging, Allentown, NJ) or individually ventilated caging (IVC) (model no. MS75JU70MVSPSHR-R, Allentown Caging, Allentown, NJ). Rats were housed in static micro isolation cages (Allentown 140, Allentown Caging, Allentown, NJ) or IVC (model no. RS10147U40MVSPSHR-R, Allentown Caging, Allentown, NJ). IVC air changes were approximately 60/h. Additional, age-matched, replacement animals were ordered so that we could maintain a consistent housing density in each cage in the event that an animal had to be removed from a cage or died during the study. No original study animals were replaced during the study. The environment in the rooms housing study animals was controlled as follows: temperature (68 to 72 °F [20.0 to 22.2 °C]; relative humidity, 30% to 70%; 1410-h light:dark cycle). Cage changing was performed in laminar-flow cage-changing stations (model 612, AllerGard Dual Access Small Animal Cage Changing and Transfer Stations, Nuaire, Plymouth, MN). Cages contained hardwood bedding (Sani-Chips, PJ Murphy, Montville, NJ). Naturalistic nesting material (Enviro-Dri, Waldschmidt and Sons, Madison, WI) was provided to all mouse cages, and a paper towel was provided to each rat cage. Cages were changed at least once every 14 d, and more often as necessary, according to established standard operating procedures. Criteria for a cage change were 10% or more of the cage floor space visibly wet, or 33% or more of the cage floor covered with fecal material. Even though mandatory cage changes were scheduled to occur every 14 d, cages were typically changed every week because they met one or both of the cage change criteria. Cages and caging supplies, including bedding, were autoclaved prior to use. Animals were fed an irradiated, commercial rodent diet (PicoLab Laboratory Rodent Diet, LabDiet, St Louis, MO). Animals received water that had undergone reverse osmosis filtration and subsequent hyperchlorination to 3 ppm (Edstrom Industries, Waterford, WI). Animal care staff wore dedicated footwear and personal protective equipment that consisted of a disposable gown and gloves when performing animal husbandry tasks. Animal rooms were swept and mopped daily using a detergent compound (GP100, Sanitation Strategies, Okemos, MI) except on weekends and holidays. Racks with IVC cages were washed every 6 mo, and racks that held IVC cages used in this study were cleaned prior to use to prevent any unwanted variables associated with air flow that could confound the experimental results.[12]

Experimental design. Organic debris accumulation on the wire bar inserts and filter top lids of mouse and rat static and individual ventilated caging (IVC) systems was evaluated. A total of 8 groups were included in the study and 15 cages were assigned to each group for a total of 120 cages ($n = 15$). Mice were housed at a density of 3 animals/cage and rats were housed at 2 animals/cage. This is close to the approximate average cage housing density employed at our institution (data collected previously).

ATP Testing. We used ATP testing because it is an effective method to detect cells and organic debris. This test has strong

linear predictability and has been used in previous studies involving the assessment of organic contamination of cage accessories.[2,3,6,16,42,53] We took samples from a 4 ×4 cm area on each wire bar insert and filter top lid of each cage and evaluated the swab for organic contamination in the form of ATP (expressed as relative light units (RLU)) by using luciferase test swabs (PocketSwab Plus, Charm Sciences, Lawrence, MA) and using the same methodology described in a similar study.[42] Swabs were taken at day 0 (prior to housing animals in each cage to verify no organic contamination) 7 d, 14 d, 30 d, 60 d, and 180 d (filter top lids only) after an initial cage change. Wire bar inserts and filter top lids were not replaced until the last time point was completed for each cage component accessory. The same individual collected all samples.

Statistical Analysis. We used statistical software (Prism version 8.4.2; GraphPad Software, San Diego, CA) to carry out our analyses. We compared the amount of ATP (measured as RLU) between day 14 and all other time points using one-way analysis of variance (ANOVA) with a Bonferroni Correction. Summary data are expressed as mean ± SEM. Differences were considered significant when $P < 0.05$.

Results

Data were collected to evaluate organic debris accumulation, quantified as RLU. We did not directly compare static and IVC between groups. Because the *Guide* states "In general, enclosures and accessories, such as tops, should be sanitized at least once every 2 wk," we instead compared time points within groups against the 14-day time point to determine when significant increases occurred and thereby when cage components should be changed or cleaned as determined based on the 14-day point.

No significant differences were found in RLU between day 14 and other time points in the mouse static and IVC wire bar groups, except at day 90 (P = less than 0.0001 in both groups, Figure 1 A and B). No significant differences were detected in the rat static wire bar group, except at days 60 and 90 as compared with day 14 ($P = 0.0084$, P = less than 0.0001, Figure 1 C), with similar results in the rat IVC wire bar group ($P = 0.0367$, $P = 0.0011$, Figure 1 D). No significant differences were found in the mouse static and IVC filter top groups, except at day 90 as compared with day 14 ($P = 0.0364$, P = less than 0.0001, Figure 1 E and F). Differences were found in the rat static and IVC filter top groups at day 90 as compared with day 14 ($P = 0.0232$, $P = 0.0011$, Figure 1 G and H). Mean RLU values were similar when comparing wire bar group data between mice in static and IVC caging (Figure 1 A and B) and were comparable to the rat IVC wire bar group. Mean values were considerably higher in the rat static wire bar group as compared with the IVC wire bar group (Figure 1 C and D). For example, at day 14 in the rat static wire bar group, the mean RLU value was 81058 ± 23873) whereas mean value for the IVC wire bar group was 56121 ± 10295. At day 90, the rat static wire bar group mean was 544747 ± 116619), and the rat IVC wire bar mean was 89966 ± 14073.

With regard to filter tops, the static groups had higher mean RLU than did the IVC groups for both mice and rats. For example, the mean value for the mouse static filter tops at 14 d was 2239 ± 1220 whereas the mean value for the IVC mouse filter tops was 941 ± 274. At 90 d, the mean mouse static filter top value was 254441 ± 82705, and the mouse IVC filter top value was 15865 ± 5200). (Figure 1 E and F). For rats, at day 14 the mean value for static filter tops was 635 ± 278, whereas the mean for rat IVC filter tops was 565 ± 261. At 90 d, the mean rat static filter top value was 9712 ± 5093, and the rat IVC filter top group value was 4447 ± 1087 (Figure 1 G and H). Mean organic debris accumulation was lower in every group at the 180-d time point, as compared with the 90-d time point in the static cages (Figure 1 E, through H).

The mean RLU value for wire bars from the mouse static group was 110,530 at 14 d. The mean RLU value for the rat static wire bar group was below 100,000 RLU at 14 d, but was higher than this value after 14 d. The IVC wire bars for both mice and rats displayed mean values below 100,000 RLUs out to 90 d (Figures 2 and 3). For filter tops, only the mouse static filter top group showed mean RLU values above 100,000. This occurred at 60 d (Figure 4).

Discussion

A study of IVC housing of mice showed that the cage bottom and bedding becomes soiled within days after mice are placed in a cage.[6] However, the microbiologic and organic contamination of wire bar inserts and filter top lids was much slower.[6] That information, combined with air quality, as judged by ammonia concentrations in the cage, led those authors to recommend extending the intervals between cage accessory sanitization out to 6 wk.[6] In another study, an evaluation of cage accessory sanitization intervals was performed by measuring the microbiologic and organic load of cage accessories in both static and IV caging systems.[42] That study found no significant difference in microbiologic or organic debris accumulation at 90 d, as compared with 14 d after cage change.[42] Our current study used ATP quantification as a measure of organic debris accumulation on wire bar inserts and filter top lids of static and IVC housing either mice or rats. We performed the study to collect data for determining optimal sanitization intervals for these cage accessories as relevant to the recommendations of the Guide. Our caging systems are different than the caging systems used in the previously mentioned studies, and we sought to develop standards for cage accessory sanitization based on data collected at our institution.

This study provided many assessments. Mean organic debris accumulation on wire bar inserts, demonstrated by RLU values, was higher in static cages than in IVC (Figures 2 and 3). The same relationship occurred when comparing static and IVC filter tops, and a large difference was detected between the mouse static filter top group as compared with the other filter top groups (Figure 4). The reason(s) for these differences may be multifactorial. The Guide indicates that nesting material should be provided to rodents and states; "If provided in sufficient quantity to allow nest building or burrowing, bedding also facilitates thermoregulation."[23] IVC at our institution provide approximately 60 air changes per hour, which is 4 times higher than the room air exchange rate, whereas static cages provide air changes that are the same as the room air exchange rate. We suspect that higher airflow in the IVC resulted in animals spending more time in, or on, the nesting material provided in each cage for reasons associated with thermoregulation. Reduced animal activity may have reduced aerosolization of particulate matter and, subsequently, reduced organic debris accumulation on cage accessories in the IVC. A second factor to consider is the impact of airflow on aerosolization of particulate matter inside cages. The significance of air flow inside cages and its impact on the accumulation of organic debris on cage accessories is unknown. The high air flow in IVC rack systems causes aerosolization of debris inside cages, and this debris is subsequently exhausted and accumulates in IVC rack plenums over time. This debris accumulation forms the substrate for exhaust air duct testing of IVC rack systems to detect the presence of excluded agent nucleic acid.[25] Higher airflow in the IVC

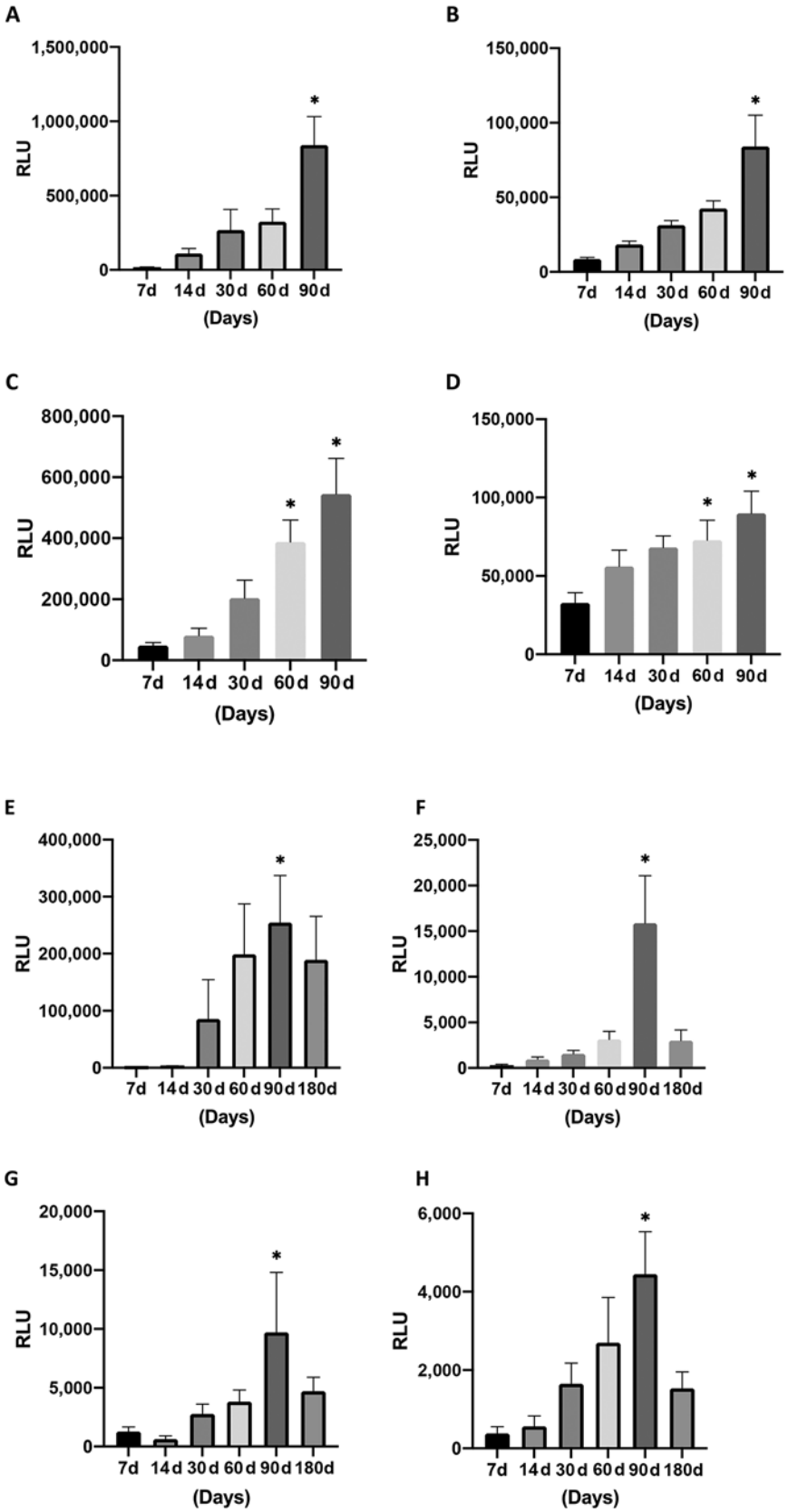

Figure 1. Graphs depict the number of relative light units (RLU) in the 8 groups; (A) mouse static cage wire bar lids, (B) mouse IVC cage wire bar lids, (C) rat static cage wire bar lids, (D) rat IVC cage wire bar lids, (E) mouse static cage filter top lids, (F) mouse IVC cage filter top lids, (G) rat static cage filter top lids, (H) rat IVC cage filter top lids. Significant difference(s) ($P < 0.05$) between 14 d and other time points are marked with an asterisk *. Data is represented as the mean at each time point with standard error of the mean (SEM).

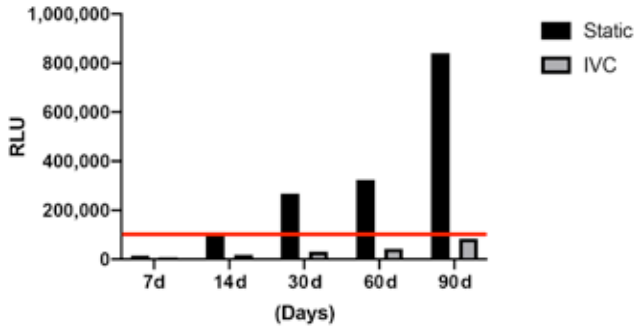

Figure 2. Number of relative light units (RLU); mouse wire bar lids (static and IVC cages). Data is represented as the mean at each time point. The red line is the actionable level which is designated at 100,000 RLU.

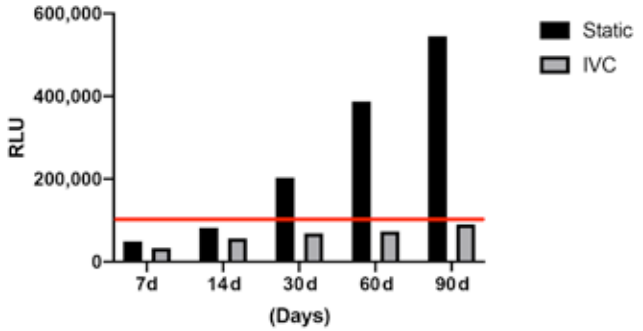

Figure 3. Number of relative light units (RLU); rat wire bar lids (static and IVC cages). Data is represented as the mean at each time point. The red line is the actionable level which is designated at 100,000 RLU.

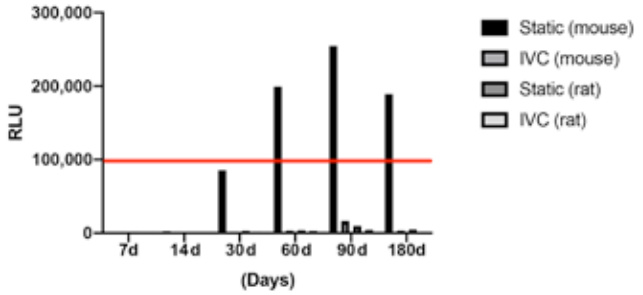

Figure 4. Number of relative light units (RLU); mouse and rat filter top lids (static and IVC cages). Data is represented as the mean at each time point. The red line is the actionable level which is designated at 100,000 RLU.

could have resulted in increased aerosolization of debris and increased accumulation on cage accessories as compared with static cages. Alternatively, the higher airflow could have had a cleansing effect, resulting in reduced adherence of organic debris to cage accessory surfaces. A third factor is the number of animals in cages. The number of mice housed per cage was higher than the number of rats housed per cage. Having more mice in individual cages is equivalent to having more animals whose activity could contribute to aerosolization of particulate matter. This could promote increased accumulation of organic debris on mouse cage accessories. Animals in this study were housed at the standard cage density for mice and rats at our institution. If animals were housed at a higher density, cage accessories could have become soiled more quickly and result in higher mean RLU values for the different groups studied. Future studies could evaluate the effects of air flow on animal activity and debris accumulation on cage accessories in cages with differing housing densities in both static and IVC systems. This would allow objective determination of the effect of these factors on organic debris accumulation on cage accessories. In the filter top groups, organic debris accumulation was somewhat lower in every group at the 180-d time point as compared with the 90-d time point. We have no explanation for this finding.

One of the goals of this study was to determine a method that could be used to determine appropriate minimal sanitization intervals for the wire bar inserts and filter top lids in either static or IVC caging systems for mice and rats. Similar studies have evaluated factors such as air quality, microbiologic load, and organic debris accumulation as metrics to determine necessary sanitization intervals for cage accessories.[6,17,42] One study evaluated intra cage air quality, animal welfare assessments, and microbial load on cage top surfaces.[6] Mice were housed at a density of 4 or 5 in an IVC, and data were collected at selected time points. During 6 wk of continuous housing, ammonia levels were below a defined upper limit of 25 ppm for all time points. After 8 wk of cage occupation, no significant welfare issues were noted and microbial load decreased over time. The authors concluded that the lack of significant differences in these factors between time points justified an extended sanitization frequency up to 6 wk for cage top components.[6] A second study evaluated microbiologic loads from micro isolation cage tops (MCT) in mouse and rat IVC and static cages over a 90-d period.[17] Animals were pair-housed. Bacterial contamination performance standards and statistical evaluation between time points was used to determine appropriate sanitization intervals. The authors concluded that 14 d is an appropriate sanitization interval for rat MCT, but this interval can be extended to 90 d for mouse MCT.[17] Finally, a third study evaluated bacterial load and organic debris accumulation on filter top and wire-bar lids in mouse and rat IVC and static cages over a period of 180 d.[42] Mice were housed at 5 animals per cage and rats were pair-housed. Selected time points were compared with a 14-d time point for all groups studied. Statistical analysis was performed to determine differences between groups, and ATP levels did not differ with cage type between 14 and 90 d. The number of bacterial colonies were also not significantly different between 14 and 120 d. In most cases, significant differences did not occur between 14 and 180 d.[42]

ATP is present in all living cells. It is typically measured from living cells, but it may also be detected from dead cells. It can be used as a proxy to evaluate surface contamination by organic debris. Detection of ATP may be affected by the type of debris on a surface being evaluated, disinfectant use, and environmental conditions. All that said, we did not clean the surfaces that were being evaluated and all other extrinsic factors were consistent for each group being studied and compared. We assume that the majority of ATP measured was from living cells, and that the primary variable affecting the quantity of organic debris on surfaces was time.[6,10,13,16,19,26,42,53,55]

In our study, we considered comparing all time points to the 14-d time point for each group being studied, with statistical comparison to the 14-d time point being the primary factor for establishing an appropriate sanitization interval. However, when evaluating the wire bar insert data for mice and rats in both static and IVC caging, we noted that the mean RLU value for the mouse static wire bar group was slightly above 100,000 at 14 d, whereas the mean RLU values for mouse IVC and rat static and IVC cage components were below 100,000 at 14 d (Figures 2 and 3). The static cages housing mice had mean RLU filter top values that exceeded 100,000 at 60, 90, and 180 d. The values in

all of the other filter top groups never exceeded 100,000 RLU (Figure 4). Based on these observations, we determined that 100,000 RLU could be considered an actionable value, because all groups studied had mean RLU values slightly above or below 100,000 at 14 d. To our knowledge, our study is the first to define an actionable RLU level for organic debris accumulation on wire bar inserts and filter top lids.

A potential weakness of this study is the fact that we did not test air quality. This assessment is typically performed by evaluating intra cage ammonia concentrations, as others have done.[6] Air quality has also been measured in studies that evaluated rodent cage change frequency.[7,22,29,37,38,40,47,50,56,57] We did not feel that evaluation of air quality would provide a significant benefit to this study because the majority of cage air quality contamination is associated with the bedding and animal waste on the cage floor. In our study, cage bottoms were changed at the intervals recommended by the Guide. That said, air quality is a parameter that may be investigated in future studies.

Another potential weakness in this study is that we did not evaluate microbiologic load as a measure of contamination of wire bar and filter top lids. This parameter has been evaluated in other similar studies,[6,17,42] and as such we felt that evaluation of microbiologic contamination of cage accessories would not provide a significant benefit to this investigation. Cages, cage accessories, bedding, and nesting material used in this study were all autoclaved prior to use. The food offered to animals was irradiated, and the water was treated by reverse osmosis followed by hyperchlorination. Because all materials coming into contact with the animals used in the study were decontaminated, microbiologic contamination of cage accessories would likely be associated with the natural flora of the animals housed in each of the cages studied. We felt that evaluation of ATP alone would provide a better measure of cage accessory contamination because ATP evaluation can be used to evaluate the presence of any organic material containing ATP, which includes microorganisms, cells, and other organic debris.[53] However, a recent study found a poor correlation between ATP levels and total bacterial colony counts for mouse and rat micro isolation tops over time.[17] As a result of those findings, the authors of that study decided to use bacterial counts expressed in colony forming units (CFU) as the primary measure of bacterial load. The caging systems used in that study were similar to those used in our study. In the future, additional studies may be performed to more accurately determine the correlation between ATP measurements associated with organic debris accumulation and bacterial contamination of cage accessories over time. Such information would be useful for determining the best method to evaluate cage accessory contamination in future studies.

Another parameter that could have been evaluated in this study is cage bedding type. Different types of bedding may affect animal wellbeing, the conditions of the cage environment.[8,18,22,24,31,35] and experimental outcomes in rodent studies.[30,49] Different types of bedding may affect the accumulation of debris on cage accessory surfaces because bedding types differ in their composition and size. Evaluation of the effects of different types of bedding on the accumulation of organic debris on the surface of cage accessories may be evaluated in the future.

Finally, factors that affect the wellbeing and welfare of animals should be a primary consideration when evaluating the sanitation of cage accessories. Modification of the cage accessory sanitation intervals may affect the wellbeing of animals housed in the affected cages. Indicators of animal wellbeing such as behavior, immunologic and physiologic responses, and the reproductive performance of individual animals should be considered. Evaluating these parameters was not feasible for this study, but the effects of cage accessory sanitation on the aforementioned indicators could be evaluated in future studies using methods already described in the literature.[4,5,7,8,11,14,17,22,32,34,36,39,52,54,56]

The *Guide* recommends that cage accessories, such as tops, should be sanitized at least once every 2 wk.[23] In this study we evaluated both wire bar lid inserts and cage tops. Based on the *Guide's* recommendations, we used the measurement of organic debris at a 14-d timepoint as the standard point of comparison for all other time points in the studied groups. This comparison indicated that an actionable level of 100,000 RLU could be used as a standard for all groups studied (Figures 2, 3, and 4). Based on this actionable level, we adopted, with IACUC approval, a protocol of changing wire bar inserts every 14 d in static mouse and rat cages. This interval could be extended to up to 90 d for wire bar inserts in mouse and rat IVC. Likewise, mouse static cage filter top lids should be changed every 30 d, but static rat and IVC mouse/rat filter top lids can be changed up as infrequently as every 180 d. Other institutions that perform animal-based research using rats and mice may consider adopting similar sanitization intervals for mouse and rat cage accessories.

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