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Nasal Histopathology and Intracage Ammonia Levels in Female Groups and Breeding Mice Housed in Static Isolation Cages

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Many factors influence ammonia levels in rodent cages, and high intracage ammonia has been associated with specific types of abnormal nasal pathology in mice. The use of autoclaved corncob bedding and the maintenance of low room humidity reduce the accumulation of ammonia in mouse cages. However, there are no engineering standards that define the limits of ammonia exposure for mice housed in static isolation cages. Regulatory guidance indicates that solid bottom cages must be sanitized at least weekly and that cage components in direct contact with animals must be sanitized at least every 2 wk. Common practice is to replace the bottoms and bedding of static isolation cages once weekly. To determine whether changing static isolation cages once weekly is an appropriate performance standard for mice, we prospectively evaluated the relationship between ammonia levels, nasal histopathology, and housing densities in various grouping strategies of mice housed in static isolation cages. Here, we report that the average nasal pathology score per cage and intracage ammonia levels were correlated, but nasal pathology scores did not differ among mice housed in breeding pairs, breeding trios, or female groups. In light of ammonia levels and histopathology scores as performance standards, these results suggest that a weekly cage-change frequency for static isolation cages does not result in adverse effects. Our results provide evidence to support current practices in the use of static isolation cages for housing laboratory mice in modern vivaria.

The 8th edition of the *Guide for the Care and Use of Laboratory Animals*[16] (the *Guide*) emphasizes the use of performance standards to evaluate housing and husbandry parameters for laboratory rodents. In terms of cage-change frequency, the *Guide* specifies, "Soiled bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry and to keep pollutants, such as ammonia, at a concentration below levels irritating to mucous membranes." However, the levels at which ammonia becomes irritating to the mucous membranes of mice have not been well defined, the minimal frequency of bedding changes needed to achieve such levels in static isolation cages is currently unknown, and quantitative guidelines have not been established. In the absence of numerical engineering standards to define these parameters, additional studies are needed to evaluate individual husbandry practices to establish acceptable performance-based standards that maintain the health and wellbeing of mice used in research.

Along with many other factors (including room ventilation, animal density, macro-environmental temperature and relative humidity, bedding type, bedding treatment and the amount of bedding used), cage-change frequency influences ammonia levels in the microenvironment of mice housed in static isolation cages.[4,7,11,28,30,33,34] In general, the frequency of cage changes must strike a balance between minimally disturbing the mice in their environment and providing clean bedding in accordance with the *Guide*. On one hand, increasing the frequency of cage changes to minimize ammonia levels may result in increased stress due to handling, more frequent disruption of pheromones and olfactory cues, undesirable effects on behavioral and sleep research, altered results of immunologic testing, and reduced reproductive success.[1,2,4,5,28] On the other hand, cage changes must be sufficiently frequent to maintain animals in a dry and clean environment and free of the negative effects of high levels of ammonia, carbon dioxide, and particulates,[16] because high ammonia levels have been associated with abnormal histopathology in the nasal cavities of mice[9,37] and rats.[3] Epithelial damage (attenuation, atrophy, degeneration, and necrosis) and suppurative or necrotizing rhinitis have been attributed to high ammonia levels, but the ammonia concentrations, duration of exposure, and other factors associated with these changes vary widely among studies.

As the use of IVC has become common, studies have focused more on describing the microenvironmental qualities, advantages, and disadvantages of these systems on rodent health and wellbeing, yielding a paucity of recent data on static isolation cages. IVC have been associated with lower intracage ammonia levels when compared with static isolation cages,[23] but recent studies suggest the use of ventilated cage systems may not totally avoid abnormal nasal histopathology[9] and may alter the expression of genes and studies of those genes.[21] Furthermore, the use of IVC has been associated with other behavioral deleterious effects, including aversion of the mice to high airflow rates within the cages,[19,28] altered behavioral test results,[20,38] temperatures that are below the thermoneutral zone for mice,[6,8] reduced production of pups,[36] and increased pup mortality.[28] In contrast, static isolation cages offer several advantages regarding safety and comfort for the mice, simplicity, ease of daily monitoring and ease of transport for the users, as well as low initial and maintenance costs. For example, in the event of environmental disasters resulting in catastrophic electrical failure, static isolation cages do not rely on electrical current for water

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supply or ventilation, thus providing a wider margin of safety compared with that of IVC.[15,18,24] Similarly, cages designed for use in ventilated systems may cause low-grade hypoxia[38] and the oxygen supply to mice may be depleted rapidly once a cage is removed from the ventilating rack,[15] complicating cage transport. In addition, direct observation of the mice during daily health checks is easier and more convenient for animal care staff when mice are housed in static isolation cages compared with cages in some ventilated racks, resulting in more effective health monitoring. Finally, observation during the weekly cage change helps to ensure that the animal care staff has the opportunity to better visualize individual mice, in contrast to the less-frequent cage changes that may occur when using ventilated cages.

For these reasons, we and others continue to prefer the use of static isolation cages for housing laboratory mice, and we have had many years of satisfactory use with weekly bedding and cage equipment changes. As the use of ventilated cages has increased, some laboratory animal professionals may have come to assume that static isolation cages changed once each week provide inferior housing conditions when compared with ventilated cages. To this end, we conducted the current study to evaluate our current husbandry practices.

We prospectively evaluated the relationship between ammonia levels and nasal pathology in mice housed in static isolation cages at different housing densities that mimic the housing densities for mice approved by our IACUC. We hypothesized that ammonia levels and nasal pathology would increase with increasing mouse housing density in static isolation cages. We characterized and quantified the nasal lesions under measured ammonia levels in mice housed in static isolation cages. Although ammonia levels were higher in cages housing groups and trios with offspring than in cages housing pairs with offspring, they did not differ between cages housing groups and cages housing trios with offspring. Furthermore the average pathology score did not differ among adult mice housed in breeding trios, breeding pairs, or cages housing 5 adult female mice. These results provide evidence to support the continued use of static isolation cages and trio breeding strategies for laboratory mice and suggest a once-weekly cage-change frequency for mice housed in these environments does not adversely affect mouse health.

Materials and Methods

Mice and housing conditions. Male and female BALB/cJ and C57Bl/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 8 wk of age. These are the most common inbred strains used in our institution. We euthanized 3 male and 3 female mice of each strain immediately on arrival from the vendor to serve as controls for nasal histology. The remaining mice were housed in static isolation cages that had been modified (see later section) to facilitate gas-level measurements by the introduction of colorimetry tubes. The mice were acclimated for 2 wk prior to the study.

Mice were maintained in accordance with recommendations in the *Guide*[16] and housed in an AAALAC-accredited vivarium. The study was reviewed and approved by the IACUC of the University of Pennsylvania. Mice were housed in static isolation cages (polycarbonate mouse cages [7.5 in. × 11 in. × 5 in.; Max 75] topped with wire-lid feeder and water bottle holders [N10 Series Wire-bar Lid], closed with a microfilter top containing spun-bonded polyester filters, [reduced height, polysulfone lids])from Ancare (Bellmore, NY). Cages were provided 16 ounces (2 measuring cups) of autoclaved bedding (1/8-in. Bed-O-Cobs, The Andersons, Maumee, OH) by using an automatic bedding dispenser (Girton, Millville, PA); this amount of bedding provided a measured depth of 5/8 in. in a 75-in.^{2} cage. Wire-lid hoppers were filled at each cage-change day with autoclaved rodent chow (LabDiet 5010, LabDiet, St Louis, MO). Water bottles were filled with acidified water and autoclaved. Throughout the study, water and food were replaced when levels were thought to be below acceptable levels, to ensure mice had free access to food and water at all times. In accordance with parameters described in the *Guide*[16] our current husbandry practices include a minimum of once-weekly cage changes, regardless of whether mice are housed in static isolation or ventilated cages. In addition, cages deemed to be excessively soiled during daily health checks are changed more frequently, according to an inhouse photographic guide, at the discretion of the animal care staff. At our facilities, ventilated cages are also changed once weekly, because we value the opportunity to have our husbandry staff directly observe the mice at this frequency. We chose autoclaved corn-cob bedding as our substrate for all cages because published studies have shown that it maintains lower levels of ammonia than other bedding choices,[4,26,35] and autoclaving inactivates any heat-labile urease present in the bedding.[12]

Temperature and humidity were maintained in the room within the levels recommended in the *Guide*.[16] An Aircuity system (Newton, MA) automatically increases the number of air changes provided in the housing room above the minimum of 10 air changes hourly when the measured ammonia, temperature, particulates, or humidity level exceeds a set point. Ammonia levels remained undetectable in the room throughout the course of the study. In addition, minimal and maximal temperature and humidity levels are recorded by animal care staff daily as part of a general quality-control program. For this study, specific temperature and humidity values were monitored by using an in-room monitoring device at the time of sampling of intracage gases in the animal housing room. Mice were kept under a 12:12-h light:dark cycle, and the light level never exceeded 155 lx at the cage level. More than 10 air exchanges hourly of nonrecycled air was supplied to the room continuously, in accordance with the *Guide*.[16] Each cage of mice was provided one square of compressed cotton fibers for nest building (Nestlets, Ancare) at the time of cage change, according to our standard operating procedures for environmental enrichment.

Cage modifications and gas measurements. For each cage, a hole (diameter, 1 cm) was drilled in the center of the long side at 1.5 in. from the bottom (approximately at mouse nose level and well above the bedding surface). Each hole was tightly fitted with a cut-off Capiject blood collection tube that had a twist-off, replaceable cap (Terumo Medical Corporation, Elkton, MD). During intracage gas measurements, the cap was removed, and a measuring tube fitted with a rubber O-ring (model A140DV, O-Ring Silicone, Edstrom, Waterford, WI) was inserted into the tube and cage with a tight fit to avoid gas leaks (Figure 1). After acclimation, mice of each strain were housed in triplicates of groups (5 adult female mice), breeding trios (one male and 2 female mice), and breeding pairs (one male and one female mouse) for measuring and comparing intracage ammonia and carbon dioxide. Once they were randomized by housing group, mice remained in the colony for an additional 3 to 6 wk during ammonia measurements or until their offspring were weaned at 21 d of age. All cages were changed completely once weekly but minimally disturbed at all other times. Mice were observed daily through the closed cage, unless a problem was noted. Modified cages were washed, autoclaved, and filled

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Figure 1. Cage modifications were made to accommodate gas level measurements. Representative pictures depict a closed cage from the outside (upper left), an open cage from the inside (upper right), and closed cages during gas measurements (lower left and right).

with bedding in the same manner as all other cages in our facilities. Mice in trios and pairs were allowed to mate naturally, and offspring were maintained in each cage (with the adults) for 21 d. All mice were euthanized by CO_2 inhalation in accordance with recommendations outlined by the 2013 AVMA Panel on Euthanasia (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf).

Ammonia and carbon dioxide levels inside cages were measured by using a bellows air pump with a stroke volume of 100 mL (± 5%; Drager Tube Pump Accuro, Pittsburgh, PA) and ammonia (model 5/a tubes; range, 5 to 600 ppm) or carbon dioxide (model 100/a; range, 100 to 3000 ppm) colorimetry gas detection tubes (Fischer Scientific). Measurements were taken once daily from each cage between 0700 and 0900 on days 0, 3, 4, 5, 6, and 7 after cage change. Each cage was only opened during cage change (at day 7) or occasionally when food or water levels dropped below acceptable limits (only 2 cages, once each throughout the study). Measurements were collected for 3 consecutive weeks for each cage (without breeding) at which time animals in group cages were euthanized and processed for microscopic examination. In breeding cages measurements continued through an additional 3 wk while pups matured to weaning age at 21 d of age. One trio that did not produce offspring provided nonbreeding controls for these conditions. All animals were euthanized when the pups were 21 d of age in each cage, or in the case of the cage with no offspring, once 3 additional weeks of data had been collected. Male mice were allowed to remain in the cage with female mice during lactation and until weaning.

Health monitoring and rodent quality assurance. The institutional rodent quality assurance program includes a soiled-bedding sentinel program to monitor and detect infectious diseases in rodent colony rooms. Female Swiss Webster (Tac:SW) mice are acquired at 5 to 6 wk of age and housed in pairs, in a cage at the bottom of each rack of colony mice (one cage per side of the rack). A predetermined amount of soiled bedding from each of the colony cages is added to the sentinel cage weekly, and sentinel mice are monitored daily for clinical signs of disease. Sentinel mice are tested inhouse for fur mites, pinworms, mouse hepatitis virus, epizootic diarrhea of infant mice virus, Theiler virus, mouse minute virus, and mouse parvovirus, during each of 3 quarters of each year. Screening serology tests for virus infections are performed by ELISA on antigen-coated plates purchased from Charles River Laboratories (Wilmington, MA). When weakly positive or positive results are obtained, confirmatory tests are performed by indirect immunofluorescence using antigens prepared internally or provided by Dr Susan Compton (Yale University, New Haven, CT). Once each year, live sentinel mice from each rack are shipped to Charles River Laboratories for euthanasia

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and comprehensive monitoring, which includes testing for 23 viral and bacterial agents by serology, upper respiratory and gastrointestinal tract cultures, tests for endo- and ectoparasites, and gross necropsy examination. In addition, mesenteric lymph nodes from barrier-housed mice are tested by PCR analysis for mouse parvovirus DNA. The University of Pennsylvania does not exclude murine norovirus, *Pasteurella* spp., or *Helicobacter* spp. from mouse housing facilities.

In addition to the use of our established sentinel program, serum samples were collected and submitted for infectious disease testing from mice used for the ammonia measurement studies. Randomly selected samples from 10% of the mice (10 samples) were submitted to Charles River Laboratories Research Animal Diagnostic Services (http://www.criver.com/files/pdfs/research-models/rm_ld_c_rads_us_a.aspx) for serology (MFIA Mouse Assessment Plus). All results were negative, indicating no evidence of infectious diseases that might have confounded study results.

Nasal pathology. After euthanasia, mouse heads were skinned and fixed in 10% buffered formalin for at least 72 h prior to decalcification. Once decalcified, 4 transverse sections were made by using anatomic landmarks as follows. One section was taken immediately behind the whiskers, another section taken in front of the eyes, a 3rd section immediately behind the eyes, and the last section was taken at the level of the external ear canal. Paraffin-embedded microtome sections (5 µm) were mounted on slides and stained with hematoxylin and eosin for evaluation. Sections were examined blindly by a board-certified veterinary pathologist (AKB) with extensive rodent pathology experience. Three sections (rostral, middle, and caudal) of the nasal cavity were examined from each mouse. According to the presence or absence of epithelial cell attenuation, degeneration and necrosis, and mucosal and submucosal inflammation, lesions were scored indicating minimal or no pathology (score, 0), mild changes (1), moderate disease (2), or severe lesions (3).

Statistical analysis. Average ammonia levels and nasal pathology scores were calculated by group, and standard deviations are depicted in each corresponding figure. Nasal histopathology scores, ammonia, and carbon dioxide levels were compared between groups by using one-way ANOVA. In cases where ANOVA showed a difference between groups, posthoc analysis (Tukey Highly Significant Difference) was used to determine groups that differed. Results from different strains were compared by using Student *t* tests for 2-tailed distributions assuming equal variances. In all cases, P values less than 0.05 were considered indicative of a significant difference. There were no significant differences between strains.

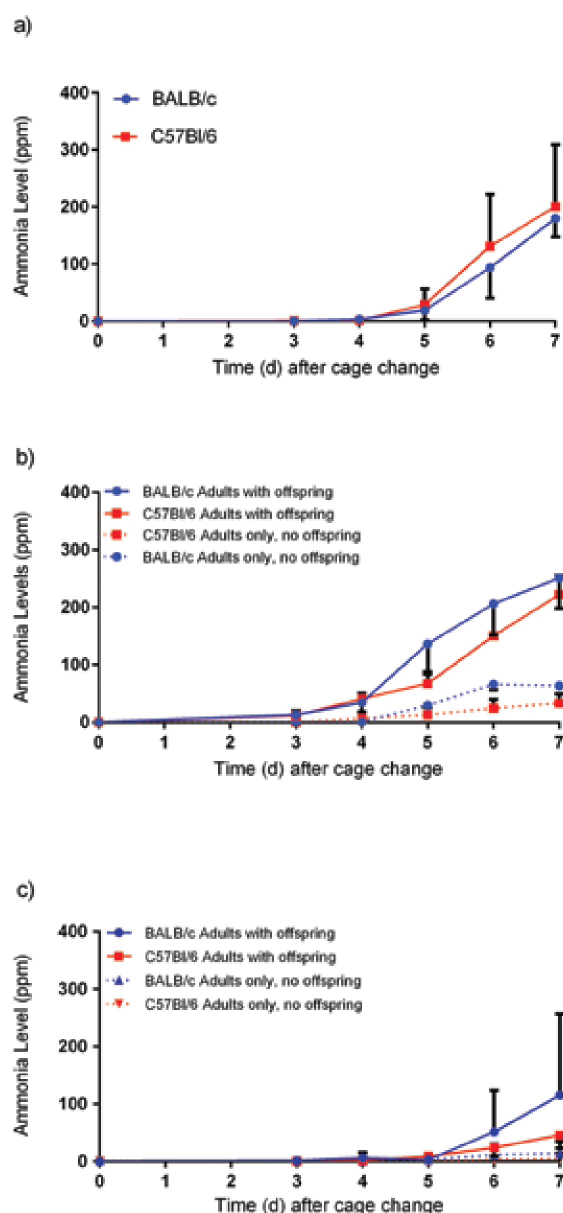

Figure 2. Intracage ammonia levels (mean ± 1 SD) were measured daily in cages of mice housing (A) groups, (B) trios before and after offspring, and (C) pairs before and after offspring for 3 wk. The average of 3 wk measured for 3 cages per group is depicted, except where only 2 of 3 C57Bl/6 trios produced offspring (*).

Results

Relationship among ammonia levels, nasal histopathology, and housing density of mice housed in static isolation cages. We obtained healthy, genetically identical mice of 2 strains from a commercial vendor and examined their nasal histology sections before and after housing in static isolation cages in a single room for as long as 8 wk. Accordingly, 6 BALB/cJ (3 male and 3 female) and 6 C57Bl/6J (3 male and 3 female) mice obtained at 8 wk of age showed no microscopic nasal lesions on arrival (controls). The remaining mice were then allocated into triplicate cages that housed groups, trios, or pairs, as described in the Materials and Methods. Average ammonia levels measured over time in cages housing groups, trios with offspring, and pairs with offspring showed a direct relation between animal density and increasing ammonia levels overall, but most cages maintained ammonia levels below 50 ppm until day 5 after cage change (Figure 2). Ammonia levels were below the limits of detection (5 ppm) on all days measured in cages housing only pairs of mice with no pups. Cages housing adult mouse trios with no offspring similarly maintained low levels of ammonia (<25 ppm) on all days measured. However, ammonia levels in cages housing groups or trios and pairs with their offspring were on average higher than 50 ppm on days 6 and 7 after cage change. Ammonia levels were compared at day 7 (for all weeks measured) between housing strategies (Table 1, Figure 3). The average ammonia levels were significantly ($P < 0.05$) higher in cages housing trios with offspring than in cages housing pairs with offspring when average ammonia levels at day 7 were compared for both strains and when individual strains were compared separately. Similarly, the average ammonia levels were significantly ($P < 0.05$) higher in cages housing groups than in cages housing pairs with offspring when average am-

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Table 1. Average ammonia levels and nasal pathology scores in mice housed in static cages

	Cage no.	Average ammonia level (ppm) on day 7	Average nasal pathology score	Individual nasal pathology scores	No. of pups in cage	No. of litters
Groups						
BALB/c	1	126	1	1, 1, 1, 1, 1	NA	NA
	2	176	1	1, 1, 1, 1, 1	NA	NA
	3	238	1.4	1, 1, 1, 2, 2	NA	NA
C57Bl/6	4	27	0.6	0, 0, 1, 1, 1	NA	NA
	5	176	1.6	1, 1, 1, 2, 3	NA	NA
	6	400	2	1, 1, 2, 3, 3	NA	NA
Trios						
BALB/c	7	168	1.3	0, 1, 3	6	2
	8	235	1.3	1, 1, 2	10	2
	9	350	2.7	2, 3, 3	4	1
C57Bl/6	10	23	0.3	0, 0, 1	0	0
	11	188	2	0, 3, 3	4	1
	12	256	2.5	2, 2.5, 3	10	2
Pairs						
BALB/c	13	2	0.5	0, 1	1	1
	14	69	0.5	0, 1	5	1
	15	184	2.5	2, 3	6	1
C57Bl/6	16	26	1	0, 2	5	1
	17	40	1	0, 2	4	1
	18	70	1	1, 1	5	1

NA, not applicable.

monia levels at day 7 were compared for both strains and when individual strains were compared separately. Importantly, the average levels of ammonia in cages that housed trios with offspring did not differ from those in cages housing groups of 5 adult female mice.

Nasal lesions of variable severity were recorded in mice after 3 wk (groups) or 6 wk (trios and pairs) of housing in static, filter-top cages with corncob bedding (Table 1). Regardless of housing strategy, the average nasal pathology scores did not differ between BALB/c (P = 0.86) and C57Bl/6 (P = 0.11) strains of mice. For mice housed in groups (Table 1), one of the cages (no. 4) maintained average levels of ammonia below 50 ppm at day 7 after cage change for 3 consecutive weeks, and the mice in this cage had the lowest nasal pathology scores in this cohort. Another cage (no. 6) reached the highest average level of ammonia (400 ppm on day 7) and had the highest pathology scores. These results suggested an association between ammonia levels and nasal pathology. Similar associations between ammonia levels and pathology scores were noted in mice housed in trios and pairs (Table 1). When the average ammonia level at day 7 was compared with the average pathology scores per cage, increasing ammonia levels were positively correlated with histopathology score in all groups (Figure 4). However, individual pathology scores varied widely between mice in individual cages, all of which had been exposed to similar levels of ammonia. In addition, nasal pathology scores did not differ (P = 0.18) when comparing groups, trios with offspring, and pairs with offspring. Our results suggest an association between maximal ammonia levels in individual cages and the incidence and severity of nasal mucosal changes that are associated with damage due to high ammonia. However at commonly used housing densities, we detected no significant differences in the severity of nasal lesions, regardless of housing scheme.

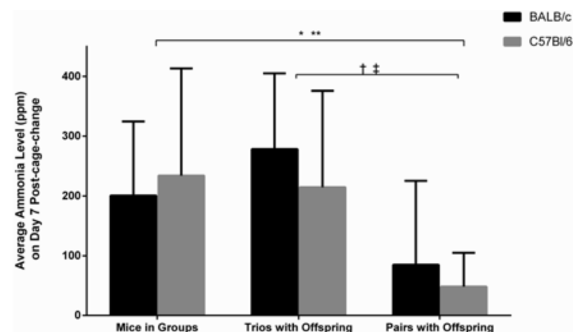

Figure 3. Intracage ammonia levels (mean ± 1 SD) measured on day 7 after cage-change were measured in triplicate cages from each group for 3 wk. The average of 3 wk measured for 2 or 3 cages per group is depicted. Cages of trios and pairs before offspring were born are omitted from this analysis. Cages of mice in groups contained 5 adult female mice. The numbers of mice per cage ranged from 7 to 13 (average, 11) for mice in trios and from 3 to 8 (average, 6) for mice in pairs. The average ammonia levels on day 7 were higher in groups than in pairs with offspring for C57Bl/c and BALB/c mice (†, $P < 0.05$ for each) and for both strains combined (‡, $P < 0.01$). The average ammonia levels on day 7 were higher in trios with offspring than in pairs with offspring for C57Bl/6 and BALB/c mice (*, $P < 0.01$ and $P < 0.05$, respectively) and for both strains combined (**, $P < 0.01$). There were no significant differences between groups and trios with offspring ($P > 0.05$).

Examples of nasal pathology observed. Nasal histopathology ranged from none or minimal (score, 0) to severe (3). Upon arrival to our institution, the nasal sections examined from all 12 mice in the control group were within normal limits and therefore received histopathology scores of 0 (Figure 5 A). Similarly, several other mice maintained normal nasal histology while housed in static isolator cages under study (Table 1, Figure 5 B). Lesions observed in other mice included epithelial attenuation,

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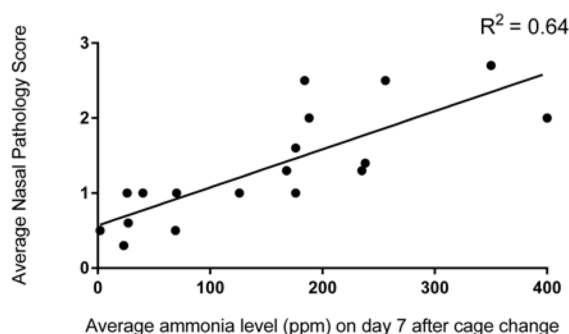

Figure 4. Intracage ammonia levels (mean ± 1 SD) were measured daily in triplicates of cages housing groups, trios before and after offspring, and pairs before and after offspring for 3 wk. The average measures on day 7 after cage-change for each individual cage were compared with the average nasal pathology scores of all mice in each cage. There is a positive correlation between the average nasal pathology score and average ammonia level at day 7 for each cage, but the average nasal pathology scores in all cages remained below 3, with the mean of all scores (1.3) indicating mild changes overall.

degeneration, and necrosis (Figure 5, C and D), submucosal lymphoplasmacytic inflammatory infiltrates (Figure 5, E and F), and mucosal and submucosal suppurative infiltrates (Figure 5, G and H) in some cases.

Room temperature, relative humidity, ammonia and intracage carbon dioxide levels. This prospective study was performed between March and May, and macroenvironmental temperatures in this room ranged from 67.2° to 69.4° with an average of 68.4 °F during the course of the study. Ammonia levels in the room were below the limits of detection (<5 ppm) throughout the course of study. The relative humidity in this housing room ranged from 27% to 36%, with an average of 30%, during the course of the study. Although the room humidity occasionally fell below the 30% humidity parameter in the *Guide*, the humidity within static isolation cages is, on average, 11% above room humidity,[27,31] and no problems associated with low humidity, such as ringtail in pups, were seen in this room or other rooms in our vivaria kept at similar ranges of humidity. Carbon dioxide levels were measured prospectively at the same times as ammonia. Figure 6 shows the average CO_2 levels measured in cages of different housing densities before and after breeding. Cages with female groups of 5 mice had the highest levels of carbon dioxide measured. Cages with trios had intermediate levels of carbon dioxide, and cages with pairs had the lowest levels of carbon dioxide measured before pups were born. In cages housing trios of mice, the appearance of offspring did not significantly influence carbon dioxide levels in each group. However, in cages housing pairs of mice, the levels of carbon dioxide increased when offspring were present (Figure 6). However, we did not detect a direct correlation between ammonia and carbon dioxide levels in this study (data not shown).

Discussion

Prior studies of open cages, static or ventilated isolation cages compared the effects of bedding type,[10,14,26,35] relative humidity,[7,23,31] cage location,[13] temperature,[31] housing density,[9,14,25,33,34] cage-change frequency,[14,29,30,37] and ventilation rates[32] in a variety of experimental settings. The majority of these studies were performed in IVC systems or open cages.[9,14,22,25,30,31,33,34,37] Mouse preference studies have shown that, when given a choice, mice choose a static isolation cage over a ventilated cage.[19] In contrast, mice exhibited no clear preference for, or aversion to, experimental ammonia levels up to 100 ppm in a cage-choice paradigm,[13] suggesting that mice may not find ammonia as aversive as do humans, who find ammonia aversive at 25 ppm or greater. Among the few prior studies describing nasal pathology as an indicator of ammonia toxicity, one found that animals had normal nasal histology irrespective of intracage ammonia levels,[35] another found microscopic changes only after experimental exposure for more than 13 d to high ammonia levels and that the mean ammonia levels that caused rhinitis were 181 ppm for 18 d of duration,[37] and yet another found microscopic respiratory changes in breeding pairs and trios of mice housed in IVC despite low intracage ammonia levels.[9]

We undertook the current study in static isolation cages to answer questions that may contribute to the establishment of engineering guidelines for intracage ammonia levels, which are absent from the *Guide*.[16] Published data have shown that chronic mucosal irritation and degeneration of respiratory epithelium can be due to high ammonia levels.[10,37] In the study reported here, we measured intracage ammonia and carbon dioxide levels and evaluated the degree of histologic changes in the respiratory tract of mice, to use respiratory histopathologic changes as a relevant performance standard under housing densities and conditions currently in use in our vivaria. For this purpose, we included groups of 5 adult female mice, breeding pairs, and breeding trios (2 female and one male) of mice. Carbon dioxide levels did not correlate with housing density or days since cage change, and the observed large variations in CO_2 levels may have been due to variations in the tightness of the fit between cages and lids or in biomass within the cage. Levels of ammonia exceeding 50 ppm were seen in cages housing groups of 5 female mice and mice with litters at days 6 and 7 after the cage bottom had been changed. Overall, we saw a correlation between animal density and maximal ammonia levels at the end of the week. Trio breeding cages with litters had a significantly higher maximal level of ammonia when compared with pair breeding cages. In addition, group-housed mice experienced higher maximal levels of ammonia than did breeding pairs. However, the average ammonia levels at day 7 after cage change did not differ between groups of mice and breeding trios. The *Guide*[16] states that "sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter." The lack of detrimental effects seen in the current study support the continued use of trio breeding, provided that animal numbers are managed carefully and that pups in trio breeding cages are weaned promptly at 21 d of age.

Lesions within the nasal cavity were limited to the nasal epithelium in study mice. When histologic changes were present in the nasal cavity, most of the lesions seen were evaluated as mild in extent and severity under these housing conditions. There were no significant differences in the average nasal pathology score of mice housed in groups, trios, or pairs in static isolation cages. However, the maximal intracage ammonia level per cage was correlated ($R^2 = 0.65$) with the nasal histology scores of individual animals housed under the 3 paradigms, suggesting that higher ammonia levels can be expected to cause more severe lesions. Our conclusion from these data is that intracage ammonia levels in cages housing breeding trios with litters may result in detectable damage to the nasal mucosa. In response to this finding, we require that pups must be weaned promptly at 21 d of age in trio breeding cages. If mice are bred in harems with more than 2 adult female mice per cage, each pregnant female mouse must be removed from the harem to a separate cage for parturition and nursing.

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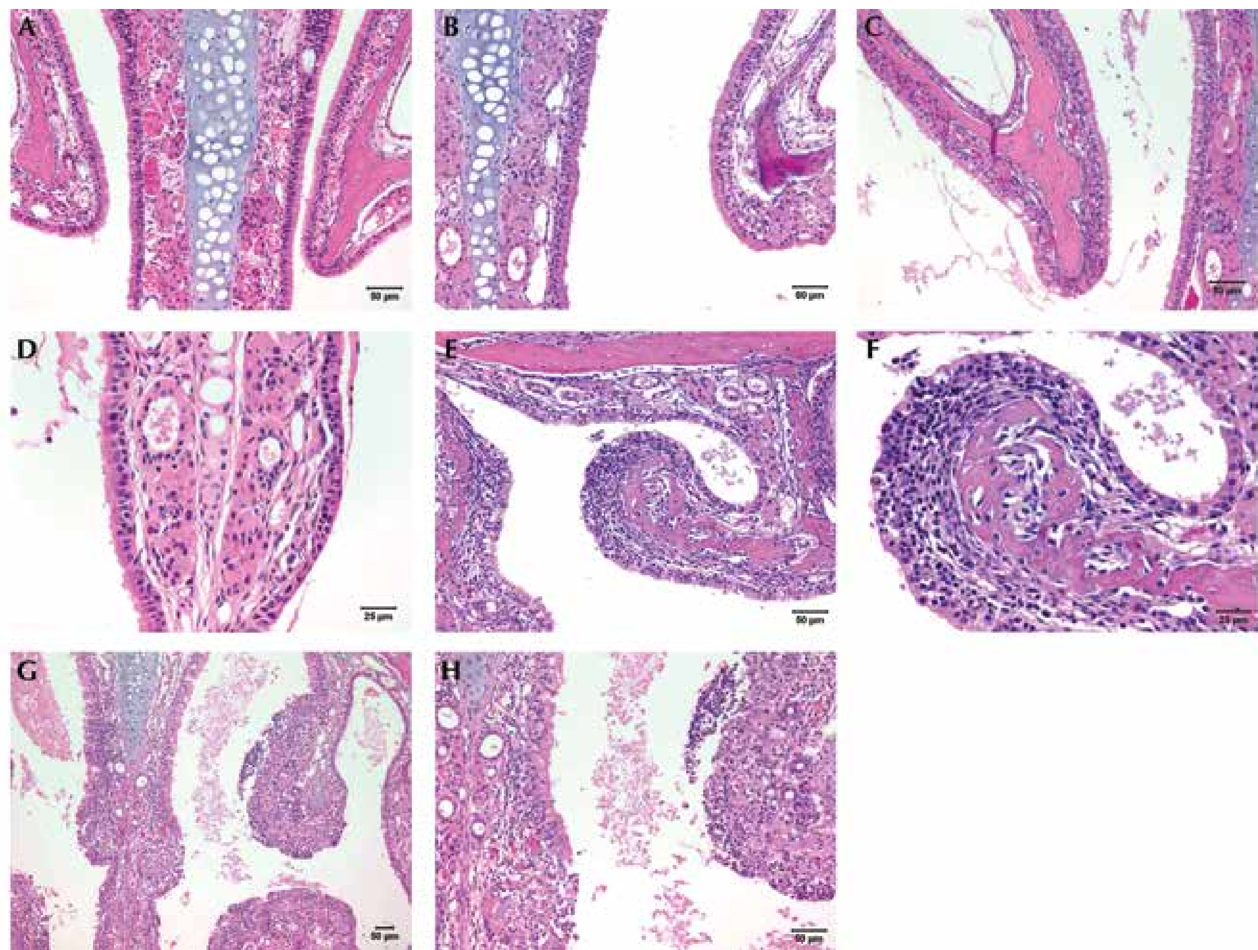

Figure 5. Representative examples of nasal histopathology from mice housed in static isolation cages. (A) Control group, no nasal pathology (score, 0); magnification, 20×. (B) Experimental group, no nasal pathology (score, 0); magnification, 20×. (C and D) Mild epithelial degeneration and ciliary loss (score, 1); magnification: 20× (C), 40× (D). (E and F) Moderate epithelial degeneration and necrosis with mild submucosal inflammatory infiltrates (score, 2); magnification: 20× (E), 40× (F). (G and H) severe suppurative rhinitis with marked epithelial degeneration, necrosis, and sloughing; also marked mucosal and submucosal suppurative inflammatory infiltrate (score, 3); magnification: 10× (G), 20× (H).

This study was intentionally designed to closely mimic the housing density and cage conditions for mouse housing in static isolation cages that are used throughout our vivaria. However, our study design did result in several limitations to the interpretation of our results. First, individual cages with similar numbers of mice showed variable intracage ammonia levels. We hypothesize that some of this variation may be due to the fact that the cage bottoms selected for customization with a gas measurement port and the cage tops used in this study had previously been used for mouse housing and that no attempt was made to control for variations in the 'tightness' of various cage bottom–lid pairs. These variations in tightness in fit may have been present when the cages and lids were new or may have developed with repeated sanitization and autoclaving. Variations in the tightness of the fit of cage tops over individual cage bottoms may be more important than previously thought in the levels of intracage ammonia in mouse cages. Despite the presence of a filtered top, most of the ventilation for a static isolation cage has been shown to occur around the lid and not through the spun polyester filter.[17] Second, our breeding mice produced litters of various numbers of pups, and we chose not to cull pups to make the number of pups per cage uniform. This variation in litter size mimics conditions found in our vivarial breeding cages but is likely to have added variability to intracage ammonia levels in the breeding cages in this study. Third, although our barrier housing areas maintain clinically healthy mice, we do not exclude *Pasteurella pneumotropica*, *Helicobacter* spp., or other bacterial agents that cause sporadic respiratory inflammation. We saw no evidence of bacterial infection in the respiratory passages of the animals in this study, but it might play a subclinical role in some colonies. In addition, our group-housing paradigm included only groups of female mice—groups of male mice could have been expected to produce higher levels of ammonia.[37] Because male mice of many strains fight when cohoused beyond puberty, we did not include all-male groups in the experimental design because of a humane concern regarding unnecessary pain or distress and because fighting would necessitate disruption of groups under experimental study. Finally, the results presented in our current study pertain specifically to the timing and conditions described and evaluated only 2 healthy strains of commonly used mice. Our findings, however, may be directly relevant to mouse housing in static cages beyond our institution.

Static isolation cages have withstood the test of time as a highly useful housing system for laboratory mice. We conducted this study to test the hypothesis that ammonia levels and nasal pathology would increase with high mouse density in static isolation cages. We conclude that static isolation cages provide acceptable performance standards for modern vivaria. Further studies are needed to determine whether increasing the cage-change frequency in static isolation cages abrogates nasal pathology in mice.

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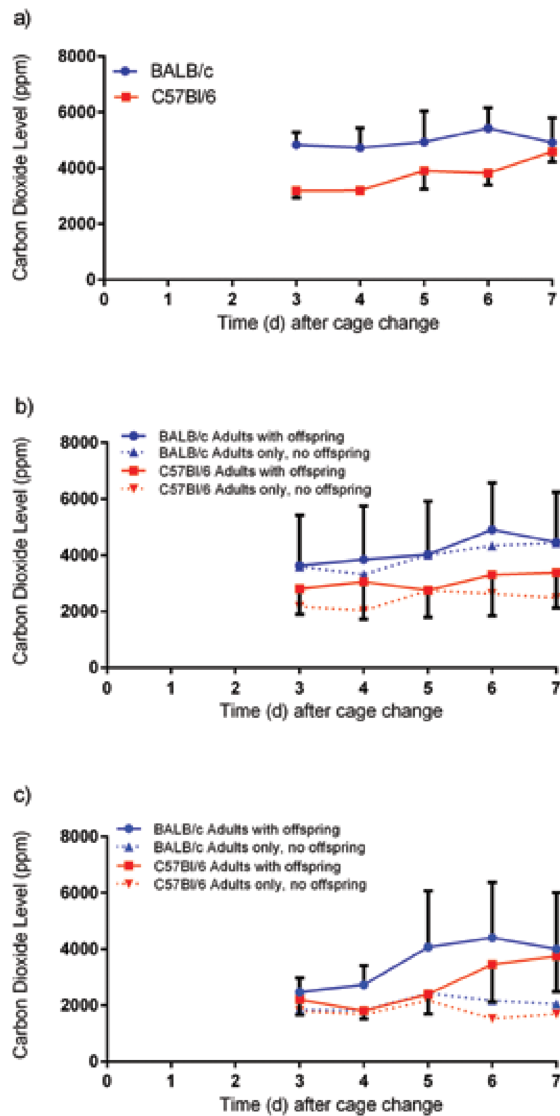

Figure 6. Intracage carbon dioxide levels (mean ± 1 SD) were measured daily in cages of mice housing (A) groups, (B) trios before and after offspring, and (C) pairs before and after offspring for 3 wk. Each line represents measures from 3 individual cages within each group, except when only 2 of the 3 cages of C57Bl/6 trios produced offspring (*).

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References

1. **Bind RH, Minney SM, Rosenfeld S, Hallock RM.** 2013. The role of pheromonal responses in rodent behavior: future directions for the development of laboratory protocols. J Am Assoc Lab Anim Sci **52:**124–129.
2. **Bittman EL, Kilduff TS, Kriegsfeld LJ, Szymusiak R, Toth LA, Turek FW; Joint Task Force of the Society for Research on Biological Rythms and the Sleep Research Society.** 2013. Animal care practices in experiments on biological rhythms and sleep: report of the joint task force of the Society for Research on Biological Rhythms and the Sleep Research Society. J Am Assoc Lab Anim Sci **52:**437–443.
3. **Broderson JR, Lindsey JR, Crawford JE.** 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am J Pathol **85:**115–130.
4. **Burn CC, Mason GJ.** 2005. Absorbencies of 6 different rodent beddings: commercially advertised absorbencies are potentially misleading. Lab Anim **39:**68–74.
5. **Burn CC, Mason GJ.** 2008. Effects of cage-cleaning frequency on laboratory rat reproduction, cannibalism, and welfare. Appl Anim Behav Sci **114:**235–247.
6. **Clough G, Wallace J, Gamble MR, Merryweather ER, Bailey E.** 1995. A positive, individually ventilated caging system: a local barrier system to protect both animals and personnel. Lab Anim **29:**139–151.
7. **Corning BF, Lipman NS.** 1991. A comparison of rodent caging systems based on microenvironmental parameters. Lab Anim Sci **41:**498–503.
8. **David JM,** Knowles S**,** Lamkin DM**,** Stout DB**.** 2013. Individually ventilated cages impose cold stress on laboratory mice: a source of systemic experimental variability. J Am Assoc Lab Anim Sci **52:**738–744.
9. **DiVincenti L Jr, Moorman-White D, Bavlov N, Garner M, Wyatt J.** 2012. Effects of housing density on nasal pathology of breeding mice housed in individually ventilated cages. Lab Anim (NY) **41:**68–76.
10. **Domer DA, Erickson RL, Petty JM, Bergdall VK, Hickman-Davis JM.** 2012. Processing and treatment of corncob bedding affects cage-change frequency for C57BL/6 mice. J Am Assoc Lab Anim Sci **51:**162–169.
11. **Ferrecchia CE, Jensen K, Van Andel R.** 2014. Intracage ammonia levels in static and individually ventilated cages housing C57BL/6 mice on 4 bedding substrates. J Am Assoc Lab Anim Sci **53:**146–151.
12. **Gale GR, Smith AB.** 1981. Ureolytic and urease-activating properties of commercial laboratory animal bedding. Lab Anim Sci **31:**56–59.
13. **Green AR, Wathes CM, Demmers TG, Clark JM, Xin H.** 2008. Development and application of a novel environmental preference chamber for assessing responses of laboratory mice to atmospheric ammonia. J Am Assoc Lab Anim Sci **47:**49–56.
14. **Horn MJ, Hudson SV, Bostrom LA, Cooper DM.** 2012. Effects of cage density, sanitation frequency, and bedding type on animal wellbeing and health and cage environment in mice and rats. J Am Assoc Lab Anim Sci **51:**781–788.
15. **Huerkamp MJ, Thompson WD, Lehner ND**. 2003. Failed air supply to individually ventilated caging system causes acute hypoxia and mortality of rats. Contemp Top Lab Anim Sci **42:**44–45.
16. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
17. **Keller LS, White WJ, Snider MT, Lang CM.** 1989. An evaluation of intracage ventilation in 3 animal caging systems. Lab Anim Sci **39:**237–242.
18. **Krohn TC, Hansen AK.** 2002. Carbon dioxide concentrations in unventilated IVC cages. Lab Anim **36:**209–212.
19. **Krohn TC, Hansen AK.** 2010. Mice prefer draught-free housing. Lab Anim **44:**370–372.
20. **Logge W, Kingham J, Karl T.** 2013. Behavioural consequences of IVC cages on male and female C57BL/6J mice. Neuroscience **237:**285–293.
21. **Logge W, Kingham J, Karl T**. 2014. Do individually ventilated cage systems generate a problem for genetic mouse model research? Genes Brain Behav **13:**713–720.
22. **Martinewski A, Correia CS, de Souza NL, Merusse JL.** 2012. Mouse housing system using pressurized cages intraventilated by direct-current microfans. J Am Assoc Lab Anim Sci **51:**177–180.
23. **Memarzadeh F, Harrison PC, Riskowski GL, Henze T**. 2004. Comparison of environment and mice in static and mechanically ventilated isolator cages with different air velocities and ventilation designs. Contemp Top Lab Anim Sci **43:**14–20.
24. **Nagamine CM, Long CT, McKeon GP, Felt SA.** 2012. Carbon dioxide and oxygen levels in disposable individually ventilated

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cages after removal from mechanical ventilation. J Am Assoc Lab Anim Sci **51:**155–161.
25. **Paigen B, Svenson KL, Von Smith R, Marion MA, Stearns T, Peters LL, Smith AL.** 2012. Physiological effects of housing density on C57BL/6J mice over a 9-month period. J Anim Sci **90:**5182–5192.
26. **Perkins SE, Lipman NS**. 1995. Characterization and quantification of microenvironmental contaminants in isolator cages with a variety of contact beddings. Contemp Top Lab Anim Sci **34:** 93–98.
27. **Perkins SE, Lipman NS**. 1996. Evaluation of microenvironmental conditions and noise generation in three individually ventilated rodent caging systems and static isolator cages. Contemp Top Lab Anim Sci **35:**61–65.
28. **Reeb-Whitaker CK, Paigen B, Beamer WG, Bronson RT, Churchill GA, Schweitzer IB, Myers DD.** 2001. The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. Lab Anim **35:**58–73.
29. **Reed B, Hawkins P, Latham N, Westwood K, van Driel K, Battram C, Golledge H, Farmer AM, Osborne N, Jennings M, Hubrecht R.** 2008. Report of the 2006 RSPCA/UFAW Rodent Welfare Group meeting. Lab Anim (NY) **37:**216–222.
30. **Rosenbaum MD, VandeWoude S, Johnson TE.** 2009. Effects of cage-change frequency and bedding volume on mice and their microenvironment. J Am Assoc Lab Anim Sci **48:**763–773.
31. **Rosenbaum MD, VandeWoude S, Volckens J, Johnson T.** 2010. Disparities in ammonia, temperature, humidity, and airborne particulate matter between the micro- and macroenvironments of mice in individually ventilated caging. J Am Assoc Lab Anim Sci **49:**177–183.
32. **Silverman J, Bays DW, Baker SP.** 2009. Ammonia and carbon dioxide concentrations in disposable and reusable static mouse cages. Lab Anim (NY) **38:**16–23.
33. **Smith AL, Mabus SL, Muir C, Woo Y.** 2005. Effects of housing density and cage floor space on 3 strains of young adult inbred mice. Comp Med **55:**368–376.
34. **Smith AL, Mabus SL, Stockwell JD, Muir C.** 2004. Effects of housing density and cage floor space on C57BL/6J mice. Comp Med **54:**656–663.
35. **Smith E, Stockwell JD, Schweitzer I, Langley SH, Smith AL**. 2004. Evaluation of cage microenvironment of mice housed on various types of bedding materials. Contemp Top Lab Anim Sci **43:**12–17.
36. **Tsai PP, Oppermann D, Stelzer HD, Mahler M, Hackbarth H.** 2003. The effects of different rack systems on the breeding performance of DBA/2 mice. Lab Anim **37:**44–53.
37. **Vogelweid CM, Zapien KA, Honigford MJ, Li L, Li H, Marshall H.** 2011. Effects of a 28-day cage-change interval on intracage ammonia levels, nasal histology, and perceived welfare of CD1 mice. J Am Assoc Lab Anim Sci **50:**868–878.
38. **York JM, McDaniel AW, Blevins NA, Guillet RR, Allison SO, Cengel KA, Freund GG.** 2012. Individually ventilated cages cause chronic low-grade hypoxia impacting mice hematologically and behaviorally. Brain Behav Immun **26:**951–958.

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