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Effects of Intracage Ammonia on Markers of Pulmonary Endothelial Integrity in Mice Housed in Static Microisolation Cages

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Time-weighted exposure limits to ammonia are established for humans; however similar guidelines have not been defined for laboratory rodents. The *Guide* recommends maintaining air pollutants at concentrations below levels irritating to mucous membranes but does not provide specific values. Numerous studies have examined ammonia and its effects on animal health, yet none have assessed the effects of naturally occurring intracage ammonia on the lower pulmonary tree and pulmonary endothelial and epithelial integrity in mice. We performed several assays commonly used in mouse acute lung-injury studies (bronchoalveolar lavage fluid [BAL] cell counts and protein concentration, excess lung water content [ELW], Evans blue permeability assay [EBA], lung tissue myeloperoxidase assay [MPO], and lung histopathology) to evaluate the effects of exposure to cyclical, naturally occurring ammonia levels on pulmonary integrity and inflammation. C57BL/6 mice were maintained in static microisolation or open-top cages. Cages were changed weekly, and ammonia levels were measured for 6 wk on days 0, 1, 3, 5, and 7 of each cage-change cycle. Ammonia levels in static microisolation cages began to increase on day 3 and peaked at a mean of 141.3 ppm on day 7. Ammonia levels in open-top cages never exceeded 5 ppm. Neither BAL cell counts, protein concentration, ELW, EBA, nor MPO differed significantly between groups. Lung histopathology showed minimal, incidental changes in all mice. Our findings indicate that the ammonia concentrations in the static microisolation cages we used did not alter the integrity of the lower pulmonary tract nor influence key indicators used to assess acute lung injury.

Abbreviations: ALI, acute lung injury; BAL, bronchoalveolar lavage fluid; EBA, Evans blue permeability assay; ELW, excess lung water content; MPO, lung tissue myeloperoxidase assay

Numerous studies have examined the relationship between ammonia and the cage microenvironment in the context of cage design,[16,22,24,25] bedding type,[7,19,19,22,26] animal wellbeing,[9,23,27] and health.[8,9,17,21] Although many of these studies evaluated the role of ammonia in terms of inflammation and damage to the upper respiratory tract, little quantitative information is available regarding the effects of naturally occurring intracage ammonia on the lower pulmonary tree and pulmonary endothelial and epithelial integrity in mice. Furthermore, guidelines for exposure limits to ammonia in rodent species have not been established. The 8th edition of the *Guide for the Care and Use of Laboratory Animals*[11] recommends changing cages sufficiently frequent to maintain the concentration of air pollutants "below levels irritating to mucus membranes." Despite offering several factors to consider in minimizing these pollutants, the *Guide* ultimately relies on professional judgment and consultation between the investigator and animal care personnel to mitigate the potential health effects of these pollutants on laboratory animal species.

At our institution, several researchers study acute lung injury (ALI). This spectrum of pathologies results in disturbances to the alveolar endothelium and epithelium and leads to a loss of pulmonary capillary integrity. Disruption to this barrier ultimately causes lung injury through mechanical damage and decreased alveolar immune response. The American Thoracic Society recognizes several common measurements that demonstrate various aspects of lung damage within the ALI condition[14] and has identified a subset of these measurements as "very relevant" in identifying inflammatory responses and alterations in alveolar endothelium. These subset measurements include bronchoalveolar lavage fluid (BAL) cell counts and protein concentrations, excess lung water content (ELW), Evans blue permeability assay (EBA), lung tissue myeloperoxidase assay (MPO), and lung histopathology.

In addition, the American Thoracic Society recognizes 4 main features in characterizing ALI in the animal model—disruption of the alveolar–capillary barrier, a pulmonary inflammatory response, histologic evidence of tissue injury, and evidence of physiologic dysfunction. The assays we examined in the current study measure the first 3 features that characterize ALI. Integrity of the alveolar–capillary barrier integrity is assessed by evaluating fluid cellularity and protein concentration, lung edema, and extravasation of small proteins into airspaces as determined by BAL, ELW, and EBA, respectively. The pulmonary inflammatory response is determined by quantifying MPO, which reflects neutrophil accumulation in the lung and migration into the airspaces. The third feature, tissue injury, is assessed directly through histopathologic evaluation.[14]

Mice are commonly used to analyze the pathophysiology and treatment of ALI, but whether episodic exposure of mice to elevated levels of naturally occurring ammonia in the cage microenvironment affects physiologic indicators used to assess ALI is currently unknown. The purpose of this study was to determine whether episodic exposure to naturally occurring ammonia within the static microisolation cage during the prestudy period is a confounding factor in the ALI mouse model. In

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this context, the influence this type of ammonia exposure has on the integrity of the pulmonary endothelium and epithelium might also be evaluated.

Materials and Methods

Facility. The Biologic Resources Laboratory is the centralized animal facility at the University of Illinois at Chicago, which has an AAALAC-accredited animal care and use program. The facility regularly tests for and excludes *Helicobacter* spp., *Mycoplasma pulmonis*, pinworms, fur mites, murine norovirus, mouse rotavirus, mouse hepatitis virus, mouse parvoviruses, minute virus of mice, pneumonia virus of mice, reovirus 3, murine adenovirus types 1 and 2, polyomavirus, and Theiler murine encephalomyelitis virus.

Mice and housing conditions. Female C57BL/6J mice ($n = 60$; age, 6 wk) were obtained from The Jackson Laboratory (Bar Harbor, ME) housed 5 per cage. Female mice were selected to decrease the potential for intracage aggression. Mice were housed in autoclaved cages (polysulfone cage, 75 in^3 floor space, model PC75JHT, Allentown Caging, Allentown, PA), where 6 of the 12 cages were maintained under our facility's standard conditions for static microisolation caging, which include a wire bar lid and a microfilter top. The other 6 cages were maintained under open-top conditions without a microfilter top to mimic a controlled, room-level macroenvironment.

Each cage was prepared with 150 g of autoclaved corncob bedding (Envigo, Indianapolis, IN), an autoclaved cotton square (Ancare, Bellmore, NY) for nesting enrichment, and a bottle containing autoclaved municipal water. All cages were changed once every 7 d. Mice were housed in a restricted-access suite, maintained in the same room, and were the only animals in the suite for the duration of the study. Mice were observed daily and were minimally disturbed between cage changes. Cage lids remained securely on the appropriate cages and were removed only at the time of weekly cage change. The room was maintained on a 14:10-h light:dark cycle and had 10 to 15 air changes per hour. All animal work was performed in accordance with a University of Illinois at Chicago IACUC-approved protocol.

Cage modifications and gas measurement. Each cage was outfitted with an access hole (diameter, 1 cm), which was capped with a sterile thermoplastic elastomer plug (TPE-K65, Rubber Dynamics, Miami, FL). The hole was placed in the middle of the long side of the cage 1.5 inches from the bottom, thus approximating the level of the mouse nose (Figure 1). The plugs remained in place throughout the study, except for the brief periods of sampling for ammonia gas. Cages were placed on a standard rack in the middle of the room and randomized for rack level and rack position once each week at cage change.

Ammonia gas levels inside the cages were measured by using a bellows pump (Accuro, Drager, Pittsburg, PA) and ammonia colorimetry gas detection tubes (range, 5 to 600 ppm; model 5/a, Drager). The pump and gas detection tubes were operated according to manufacturer instructions.

Experimental design. Ammonia was measured by removing the elastomer plug and inserting the ammonia detection tube through the access hole. Measurements were taken on days 0, 1, 3, 5, and 7, between 0730 and 0930. Day 7 corresponded with the day of cage change; ammonia measurements were taken just before cage change and again afterward to reset the weekly cycle back to day 0. Ammonia measurements were collected under this schedule for 6 consecutive weeks. One mouse from each cage was randomly selected and used for each assay (BAL, ELW, and MPO) in combination with lung tissue collection for histopathology; 2 mice from each cage were used for EBA.

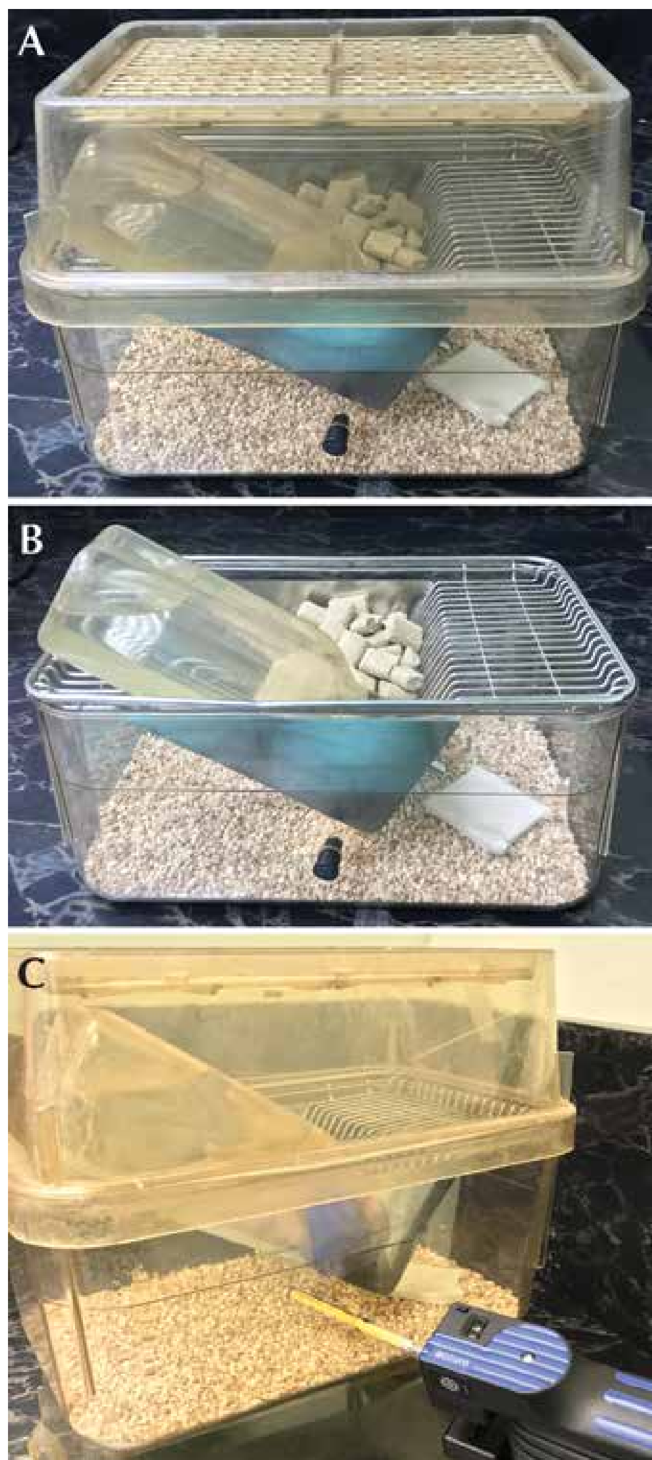

Figure 1. Cage modifications made to facilitate measurement of ammonia gas. (A) Static microisolation cage. (B) Open-top cage. (C) Ammonia gas sampling through the access hole.

BAL. The BAL procedure was performed as previously described.[4] Briefly, mice were anesthetized by using intraperitoneal ketamine (100 mg/kg; Henry Schein, Dublin, OH) and xylazine (5 mg/kg; Henry Schein). Lungs were instilled with 1 mL of cold HBSS (Invitrogen, Grand Island, NY) by means of tracheal cannulation. After centrifugation, the supernatant was collected for cell counts by using a standard hemocytometer technique and for protein concentration measurement by a commercial assay (Pierce BCA Protein Assay, Thermo Scientific,

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Rockford, IL). Cell counts were grouped according to housing condition.

ELW. ELW was determined as previously described.[20] Briefly, mice were anesthetized by using intraperitoneal ketamine (100 mg/kg; Henry Schein, Dublin, OH) and xylazine (5 mg/kg; Henry Schein). A blood sample from the inferior vena cava was collected to measure Hct. The whole lung was excised en bloc by means of thoracotomy and dissected free of connective tissues before being placed into preweighed tubes containing 1 mL of deionized water. The lung sample was homogenized, and aliquots of lung homogenate were separated and centrifuged. The supernatant of lung homogenates and whole blood from the collection tubes were measured for Hgb concentration (model Hb 201, HemoCue, Cypress, CA). Aliquots of lung homogenate, lung supernatant, and whole blood were weighed before and after being placed in a drying oven for at least 24 h to determine differences in water loss.

EBA. The EBA was performed as previously described.[6] Briefly, a 1% solution of Evans blue (Sigma-Aldrich, St Louis, MO) was prepared in PBS; bovine serum albumin was added to a final concentration of 4%. Mice were anesthetized using intraperitoneal ketamine (100 mg/kg; Henry Schein, Dublin, OH) and xylazine (5 mg/kg; Henry Schein). The right jugular vein was isolated and injected with 200 µL of the Evans blue solution, and the mouse was returned to its cage for 30 min. After appropriate anesthetic depth was achieved, the thoracic cavity was accessed and a small incision was made in the right ventricle to allow for perfusion of the lungs with PBS solution. Evans blue albumin was extracted from the lungs. The optical density of the supernatant was determined spectrophotometrically at 620 and 740 nm, and the amount of bovine serum albumin (in micrograms) per mass of wet lung (in grams) was calculated and compared with an Evans blue standard curve.

MPO assay. The procedures for this assay followed previously published protocols.[10] Briefly, mice were anesthetized using intraperitoneal ketamine (100 mg/kg; Henry Schein, Dublin, OH) and xylazine (5 mg/kg; Henry Schein) and then underwent cervical dislocation. The lungs were removed en bloc by means of thoracotomy. The left lung lobe was preserved for histopathology, and the right lobe was weighed and homogenized in a standard MPO homogenate medium. The homogenate was sonicated 3 times on ice and centrifuged for 30 min. A 10-µL sample of the supernatant was loaded onto a cuvette plate with phosphate buffer. Change in absorbance was measured at 460 nm for 3 min in triplicate. MPO activity was expressed as the change in absorbance per minute per gram of tissue.

Lung histopathology. In conjunction with the MPO assay, the lungs were removed en bloc from the thorax. The left lung lobe was collected and fixed in 10% neutral buffered formalin for histopathologic evaluation. Samples were embedded in paraffin and sliced into 5-µm sections before staining with hematoxylin and eosin. Histopathologic evaluation was performed by a board-certified veterinary pathologist who was blinded to the housing condition of each mouse.

Statistical analysis. All assays were performed by technicians blinded to experimental groups. Ammonia levels in static microisolation cages and for open-top cages were averaged at each time point for the duration of the study. Statistical analysis was performed by using a Student *t* test for 2-tailed distribution with an assumed equal variance (Prism version 7.2, GraphPad Software, San Diego, CA). A *P* value of less than 0.05 was considered significant. Data are presented as mean ± SEM.

Results

BAL. BAL total cell count (mean ± SEM) was $1.59 \pm 0.21 \times 10^5$ cells/mL for mice in static microisolation cages and $1.64 \pm 0.16 \times 10^5$ cells/mL for those in open-top cages. Protein concentration in BAL fluid was 215.90 ± 15.39 µg/mL for the static microisolation group and 244.2 ± 18.10 µg/mL for the open-top group. Neither total BAL cell count ($P = 0.8602$) nor protein concentration ($P = 0.2614$) differed significantly between mice housed in static microisolation compared with open-top cages.

ELW content. ELW (mean ± SEM) of the mice in the static microisolation cages was 14.58 ± 0.72 µL and in the open-top cages was 18.23 ± 2.04 µL. These values did not differ ($P = 0.1226$) between the 2 housing conditions (Figure 2).

EBA. EBA in the static microisolation cage group was 16.05 ± 1.72 µL/g compared with 13.94 ± 1.04 µL/g for the open-top cage group. The values showed no significant difference ($P = 0.3064$) between the 2 housing conditions (Figure 2). One mouse each was excluded from the static microisolation and open-top conditions due to technical difficulty; data were not collected from these 2 animals.

MPO assay. MPO was 8.11 ± 0.82 units/min/g in the static microisolation group and 8.80 ± 0.09 units/min/g in the open-top group ($P = 0.5879$; Figure 2).

Lung histopathology. No noteworthy histopathologic changes were present in any of the 12 lung samples submitted (Figure 3). Alveolar walls were thin and did not contain excessive numbers of cells. Bronchi and bronchiolar spaces were free of cellular infiltrate. An occasional macrophage was observed within an alveolar space. Epithelial cells were occasionally hypertrophic and, in rare cases, dissociated and free in the lumen. Collectively, these changes were minor, rare, and unassociated with any particular animal or group. The pathologist considered these changes to be incidental findings.

Ammonia measurements. Ammonia concentrations in the room remained below the level of detection (that is, less than 5 ppm) throughout the study period. Ammonia measurements in all open-top cages were below the detection threshold at all time points, except for one cage that had a level of 5 ppm just before cage change. Static microisolation cages had a detectable increase in ammonia on day 3, and ammonia concentrations steadily increased thereafter until the time of cage change on day 7. Mean ammonia concentrations in the 6 static microisolation cages on day 7 ranged from 22.4 to 141.3 ppm throughout the study (Figure 4).

Discussion

Ammonia is a naturally occurring air pollutant in the microenvironment of static microisolation cages. In this study, relevant markers of pulmonary endothelial and epithelial integrity and inflammation commonly assessed during ALI research were investigated within the context of the microisolation caging system. The purposes of this study were to evaluate the influence of naturally occurring intracage ammonia on the health of the lower pulmonary tract in mice and to evaluate whether static microisolation caging is appropriate for the maintenance of mice before the initiation of ALI studies.

ALI is an inflammatory condition that induces alterations in lung epithelial and endothelial barriers. This condition is characterized by microvascular endothelial injury, which leads to increased capillary permeability, transepithelial neutrophil migration, and a shift of protein-rich fluid into the interstitium.[23] ALI in humans is clinically well defined;[2] however, the condition in animal models is difficult to extrapolate to the human disease state, due to the lack of a single physiologic marker

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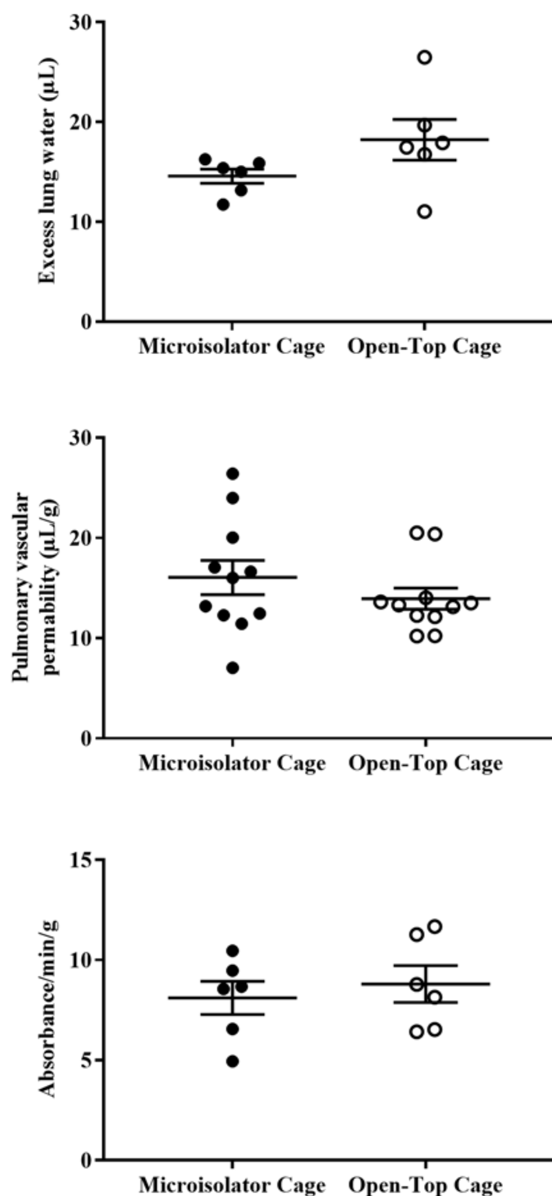

Figure 2. Effects of housing condition on ELW, top; EBA, middle; and MPO, bottom. Values are expressed as mean ± SEM. Neither excess lung water (P = 0.1226), Evans blue assay (P = 0.3064), nor myeloperoxidase (P = 0.5879) differed significantly between housing conditions. Closed circles represent static microisolation cages; open circles represent open-top cages.

with sufficient sensitivity and specificity to assess the various etiologies and gradations of ALI. For these reasons, the American Thoracic Society recommends using multiple assays and histopathology, which we assessed here, to serve as correlates of the main features of ALI to define and characterize the ALI experimental animal model.[15]

Some of the main features of experimental ALI are determined by measuring disruption to the pulmonary endothelial and epithelial barriers. Evidence for altered endothelial barrier integrity and permeability is supported by the presence of protein-rich fluid recovered after BAL and the accumulation of fluid within the lung, as determined by ELW. Normally, the pulmonary endothelium is impermeable to proteins found in the vascular system, such as albumin, but during inflammatory states, pulmonary permeability is altered, and small proteins leak from the vascular space. Because albumin can be traced by using Evans blue dye, EBA can be performed to evaluate pulmonary endothelial damage by measuring albumin leakage consequent to inflammation.

An acute inflammatory response and tissue injury are other characteristics of ALI, which manifest as neutrophil accumulation and activation within the lung and present histologically as diffuse alveolar damage. BAL can demonstrate inflammation within lung airspaces by quantifying cellular influx, which primarily includes macrophages, lymphocytes, and neutrophils. When neutrophils become activated within airspaces, they release myeloperoxidase and accordingly, MPO can be used to assess activated neutrophil shift into alveolar spaces. Tissue injury can be determined by histopathology. In humans, the hallmark pathologic finding of ALI is diffuse alveolar damage; however, the characteristics of diffuse alveolar damage cannot be reproduced fully in animal models.[14] Instead, various histologic features, such as the presence of hyaline membranes, thickened alveoli, and alveolar spaces with proteinaceous debris and neutrophilic infiltrate are used as markers of the degree of lung injury present in the animal model.

In our study, data for BAL cell counts and protein concentration, ELW, EBA, and MPO showed no significant difference between the 2 groups of mice. Lung histopathology revealed similar, clinically unremarkable findings between the 2 housing conditions. Our findings demonstrate no difference in the respective assays between housing conditions to indicate any alterations in inflammation or permeability, despite differences in ammonia exposure associated with housing systems.

As a result of bacterial metabolism of urea present in mouse urine, mice in microisolation caging are exposed to naturally occurring ammonia levels of increasing magnitude throughout the cage-change period. Our data show that microenvironmental ammonia within static microisolation cages began to increase on day 3 after cage change and peaked at the time of cage change (that is, day 7; Figure 4) to a maximum average of 141.3 ppm. After cage change, ammonia levels returned to below detectable limits. These findings of cyclical fluctuations are consistent with prior studies.[17,24,25]

According to human standards, the 8-h time-weighted averages for ammonia exposure of 25 ppm[3] or 50 ppm[18] are based on reports of sensory, eye, and respiratory tract irritation. Exposure limits to ammonia for mice have not been defined, and applying human exposure standards to mice may be an inaccurate correlate. In one study,[25] mice at high housing densities were exposed to natural, intracage ammonia levels that exceed those in our current study. However, weight gain and growth rates were unaffected, nasal passages and eyes showed no histologic evidence of ammonia damage, and mice did not demonstrate behaviors indicative of discomfort or distress as a result of increased ammonia exposure.[25] In some studies,[5,7,8,27] exposure to natural ammonia caused microscopic changes in upper respiratory epithelium, whereas other studies[1,14,29] offer conflicting evidence by demonstrating rodents with absent to mild histopathologic changes in the nasal epithelium and respiratory tract.

In our study, ammonia levels exceeded the 25-ppm human exposure limit[3] during 5 of the 6 wk and the 50-ppm human exposure limit[18] during 4 of the 6 wk. The 2 consecutive cage–change periods during which ammonia levels did not exceed 50 ppm occurred when the average environmental humidity of animal facility was lower than usual. The connection between macroenvironmental humidity and intracage ammonia has been documented,[22] wherein lower humidity levels result in reduced generation of ammonia. During the study period when

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Figure 3. Representative lung histopathology from a mouse housed in a static microisolation cage (magnification: top left, 4×; top right, 20×) and from a mouse housed in an open-top cage (magnification: bottom left, 4×; bottom right, 20×). No noteworthy abnormal pathology or histologic changes indicative of alveolar damage or inflammation are present.

ammonia levels did not exceed regulatory exposure limits, the average daily macroenvironmental humidity levels ranged from 48.5% to 62.3%. Macroenvironmental humidity averages outside of this time period were slightly higher, ranging between 51.8% and 70.3%. In addition, air temperature can influence the generation of ammonia;[16] however, during the period of decreased humidity in our facility, room temperature remained relatively consistent—from 71.6 to 74.9 °F (22.0 to 23.8 °C)—whereas average temperatures outside of this period ranged from 70.2 to 74.7 °F (21.2 to 23.7 °C).

In our study, no histopathologic differences were present in the lungs of mice housed in static microisolation cages compared with open-top cages, nor was there any histologic evidence of lung injury (Figure 3). Histopathology indicated normal lungs with only minor incidental findings after exposure to cyclical, naturally occurring microenvironmental ammonia during the 6-wk study period. These findings indicate that the ammonia levels recorded in this study are insufficient to cause histologic and pathologic changes in these mice.

To our knowledge, this study is the first to evaluate the effects of intracage ammonia on the integrity of the lower pulmonary endothelium and epithelium by using assays commonly applied in ALI models. The results demonstrate that exposure to the cyclical, naturally occurring intracage ammonia levels

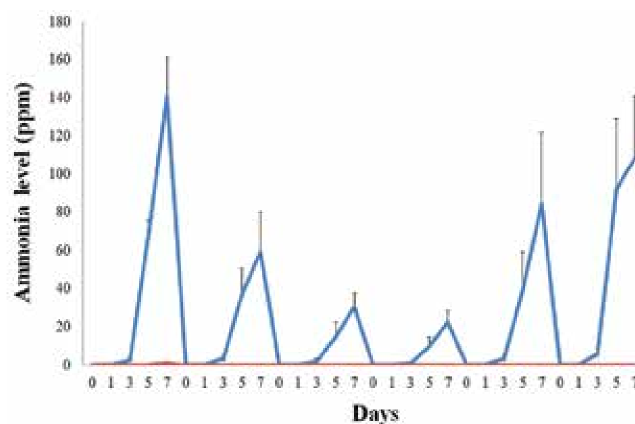

Figure 4. Intracage ammonia concentration (mean ± SEM) in static microisolation and open-top cages. Each time point represents the mean ammonia measurement among 6 cages, with each cage containing 5 mice. The blue line represents the static microisolation cages; the red line indicates open-top cages.

observed in this study within static microisolation caging does not negatively influence murine models of ALI during the

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prestudy period. More specifically, our findings indicate that the ammonia concentrations measured in the static microisolation cages used to maintain mice in this study do not alter the integrity of the lower pulmonary tract nor influence key "very relevant" indicators[14] used to assess ALI, including BAL, ELW, EBA, MPO, and lung histopathology.

Ammonia in the static microisolation cages reached a maximal mean of 141.3 ppm on day 7; however, prolonged or continual exposure to ammonia levels at higher concentrations may produce effects not detected in this study. Moreover, opportunities for additional studies remain. Genetic polymorphisms in different mouse strains are known to have biologic significance regarding the respiratory effects of air pollution,[21,28] and group-housed male mice may have greater rates of ammonia production.[27] Similarly, the effects of ammonia on biomarkers of vascular permeability have yet to be evaluated in IVC systems. Although our findings can be applied to institutions with static microisolation caging systems, colony pathogen status, the strain of mouse, and the macroenvironment, among other factors, should be evaluated to further determine the effect of the microenvironment–ammonia paradigm on murine models of ALI.

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References

1. **Appelman LM, ten Berge W, Reuzel PG.** 1982. Acute inhalation toxicity study of ammonia in rats with variable exposure periods. Am Ind Hyg Assoc J **43:**662–665.
2. **Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R.** 1994. Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. Am J Respir Crit Care Med **149:**818–824.
3. **Centers for Disease Control and Prevention.** 2007. NIOSH pocket guide to chemical hazards. Washington (DC): Government printing office.
4. **Chen J, Feng G, Song Y, Wardenburg JB, Lin S, Inoshima I, Otto M, Wunderink RG.** 2013. Linezolid exerts greater bacterial clearance but no modification of host lung gene expression profiling: a mouse MRSA pneumonia model. PLoS One **8:**1–12.
5. **Clough G.** 1982. Environmental effects on animals used in biomedical research. Biol Rev Camb Philos Soc **57:**487–523.
6. **DebRoy A, Vogel SM, Soni D, Sundivakkam PC, Malik AB, Tiruppathi C.** 2014. Cooperative signaling via transcription factors NF-κB and AP1/c-Fos mediates endothelial cell STIM1 expression and hyperpermeability in response to endotoxin. J Biol Chem **289:**24188–24201.
7. **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.
8. **Gamble MR, Cough G.** 1976. Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. Lab Anim **10:**93–104.
9. **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.
10. **Hu G, Ye RD, Dinauer MC, Malik AB, Minshall RD.** 2008. Neutrophil caveolin-1 expression contributes to mechanism of lung inflammation and injury. Am J Physiol Lung Cell Mol Physiol **294:**L178–L186.
11. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
12. **Johnson ER, Matthay MA.** 2010. Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv **23:**243–252.
13. **Li WL, Pauluhn J.** 2010. Comparative assessment of the sensory irritation potency in mice and rats nose-only exposed to ammonia in dry and humidified atmospheres. Toxicology **276:**L135–L142.
14. **Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM, Acute Lung Injury in Animals Study Group.** 2011. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol **44:**725–738.
15. **Matute-Bello G, Frevert CW, Martin TR.** 2008. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol **295:** L379–L399.
16. **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.
17. **Mexas AM, Brice AK, Caro AC, Hillanbrand TS, Gaertner DJ.** 2015. Nasal histopathology and intracage ammonia levels in female groups and breeding mice housed in static isolation cages. J Am Assoc Lab Anim Sci **54:**478–486.
18. **Occupational Health and Safety Administration**. [Internet]. 2012. Occupational safety and health standards. Regulations (Standards - 29 CFR), Table Z-1 Limits of Air Contaminants. [Cited 07 August 2017]. Available at: https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=9992
19. **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.
20. **Piegeler T, Dull RO, Hu G, Castellon M, Chignalia AZ, Koshy RGE, Votta-Velis EG, Borgeat A, Schwartz DE, Beck-Schimmer B, Minshall RD.** 2014. Ropivacaine attenuates endotoxin plus hyperinflation-mediated acute lung injury via inhibition of early-onset Src-dependent signaling. BMC Anesthesiol **14:**1–14.
21. **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.
22. **Riskowski GL, Harrison PC, Memarzadeh F.** 2006. Mass generation rates of ammonia, moisture, and heat production in mouse cages with 2 bedding types, 2 mouse strains, and 2 room relative humidities. ASHRAE Trans **112:**134–144.
23. **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.
24. **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.
25. **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.
26. **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.
27. **Vogelweid CM, Zapien KA, Honigford MJ, Li L, Li H, Marshall H.** 2011. Effects of a 28-d cage-change interval on intracage ammonia levels, nasal histology, and perceived welfare of CD1 mice. J Am Assoc Lab Anim Sci **50:**868–878.
28. **Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW.** 2009. Genetic susceptibility to the respiratory effects of air pollution. Postgrad Med J **85:**428–436.
29. **Zissu D.** 1995. Histopathological changes in the respiratory tract of mice exposed to 10 families of airborne chemicals. J Appl Toxicol **15:**207–213.

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