Ammonia Levels

in Animal Care Systems Cages in Higher Humidity Conditions

Animal Care Systems' revolutionary approach to individual cage ventilation employs a unique one-pass low-velocity linear air technology which eliminates the introduction of air turbulence and high air velocity, while ensuring maximum cage air quality at a low airflow. Cage ammonia and other environmental parameters were measured in all three Animal Care Systems rack types (Optimice®, Optirat® Plus and Optirat® GenII) at standard recommended airflow rates at higher humidity cage conditions between cage changes.

Introduction

Intracage ammonia levels are influenced by several factors: sex; strain; age of animals housed; number of animals in the cage; temperature; humidity; number of hourly air changes; and amount and type of bedding substrate used. Most rapid ammonia production occurs under conditions of high humidity since high humidity enhances bacterial generation of ammonia.[1] High humidity diminishes fecal and urinary desiccation and provides an optimal climate for bacterial proliferation and subsequent ammonia production.[2] A previous internal study showed low to undetectable levels of ammonia at lower humidity levels.[3] Since ammonia levels are closely influenced by humidity levels, the objective of this study is to measure ammonia levels at higher cage humidity conditions than previously studied, as might be representative in certain areas of the country and worldwide.

Materials and Methods

Ammonia data shown was taken at day 14 for mice and day 7 for rats after initial cage change. Several different categories of bedding types were tested to represent commonly used substrates (Table 1). Equal volumes of bedding were used per cage type except ALPHA Pads® [fixed stamped size] for mice.

Ammonia measurements. Ammonia was measured with either a BW Honeywell GasAlert ammonia sensor (lowest detection threshold > 3 ppm; Honeywell Analytics, Lincolnshire, IL) or a Vivaristat ammonia sensor (lowest detection threshold 0.5 ppm; InPoint Systems, Inc., Los Altos, CA) and an ammonia strip sensor (low 0-1 ppm, medium 1-25 ppm, high 25-50 ppm, danger 50+ ppm; Pacific Sentry LLC Small Animal Ammonia Sensor, Redmond, WA), all placed directly inside the cage for the duration of the tests. Cage temperature and humidity levels were measured with either a Vivaristat temperature/humidity sensor (InPoint Systems, Inc., Los Altos, CA) or an HT1 temperature and humidity smart sensor (SensorPush, Garrison, NY), placed also directly inside the cage.

Rack and cage airflow measurements. Rack airflow was monitored via the Animal Care Systems rack status monitor and cage level airflow with a handheld anemometer (Fieldpiece STA2 In-duct hot wire anemometer, Fieldpiece Instruments, Inc., Orange, CA).

Animals and housing. Animals were sourced from Charles River Laboratories, USA. Rats were Sprague Dawley females, between 6 and 15 months of age. A combination of both CD1(ICR) male and female mice, ages 4 to 23 months of age, were used.

Table 1. Types and sources of bedding used per species.

Bedding Name	Bedding Material	Source	Species
Teklad sani-chips	Aspen chip	Envigo, Somerset, NJ	Mice/Rats
Teklad corncob	Corn cob	Envigo, Somerset, NJ	Mice/Rats
BioFresh™	Cellulose	BioFresh, Ferndale, WA	Mice/Rats
ALPHA Pads®	100% cotton	Shepherd Specialty Papers, Waterton, TN	Mice

Table 2. Summary of ammonia levels in all cage types, number of animals per cage, number of cages, and cage humidity.

Cage type	Average no. animals per cage	Average weight (g) ± SEM	Average NH_3 (ppm) ± SEM	Average cage humidity (%) ± SEM	Total no. cages	Bedding Material			
						Aspen	Corn cob	BioFresh	Cotton
Optimice	4.6	48 ± 1.6	4.9 ± 3.8 (day 14)	56.4 ± 2.6	n=23	n=6	n=6	n=5	n=6
Optirat Plus	3	464 ± 15.3	2.4 ± 1.3 (day 7)	46.6 ± 2.5	n=15	n=5	n=5	n=5	n/a
Optirat GenII	3	340 ± 16.6	1.4 ± 0.8 (day 7)	49.8 ± 2.7	n=14	n=5	n=4	n=5	n/a

Rat cages had a shelter (Rat Retreat™, BioServ, Flemington, NJ) and mouse cages had one Bed-r'Nest® puck (Andersons Lab Bedding, Maumee, OH); in addition, cages with female mice were given one DomiceISLE shelter (Animal Care Systems, Centennial, CO). Cages were stocked at or near capacity for non-breeding animals per allowable floor space according to the "Guide for the Care and Use of Laboratory Animals."[4]

Results

Data were collected from January to December 2019. Ammonia levels in mice at 14 days after cage change ranged from 0 to 19.5 ppm (total average 4.9 ppm). In Optirat Plus (ORP) cages at 7 days after cage change, ammonia ranged from 0 to 17 (total average 2.4 ppm). In Optirat GenII cages, ammonia levels after 7 days ranged from 0 to 10 ppm (average 1.4 ppm). NH_3 strip color changes ranged from low to medium, consistent with the digital ammonia readings. Cage humidity ranged from 35.4 to 81.8% (average 56.4%) in mice cages and 35.0 to 67.9% (average 46.6%) and 40.1 to 71.9% (average 49.8%) in Optirat Plus and Optirat GenII cages, respectively (Table 2).

The data suggested no main effects of cage and bedding on ammonia ($P = 0.55$ and 0.43, respectively) or relative humidity levels ($P = 0.44$ and 0.41, respectively) for both rat cages (Figure 1) and no main effect of bedding on ammonia ($P = 0.075$) and relative humidity levels ($P = 0.16$) in Optimice cages (Figure 2). However, even though there was no main effect of housing ($P = 0.07$), there appeared to be a significant main effect of relative humidity ($P = 0.008$) on ammonia levels (Figure 3).

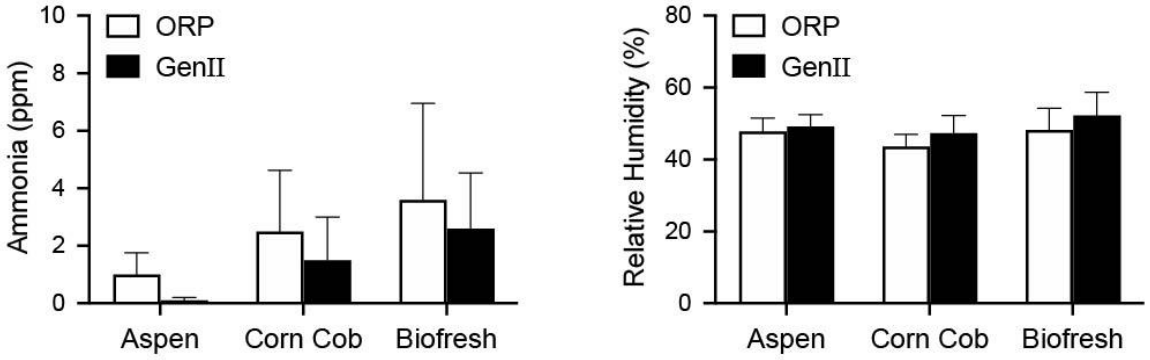

Figure 1 (Left-right). Mean +/- standard error of the mean (SEM) ammonia concentration (ppm) and relative humidity (%) levels in different cage types (Optirat Plus and Optirat GenII) and bedding material (aspen, corn cob and BioFresh). Two-way Analysis of Variance (ANOVA) indicates no main effects of cage and bedding on ammonia (P = 0.55 and 0.43, respectively) or relative humidity levels (P = 0.44 and 0.41, respectively). N = 5 cages/per test condition (3 rats per cage).

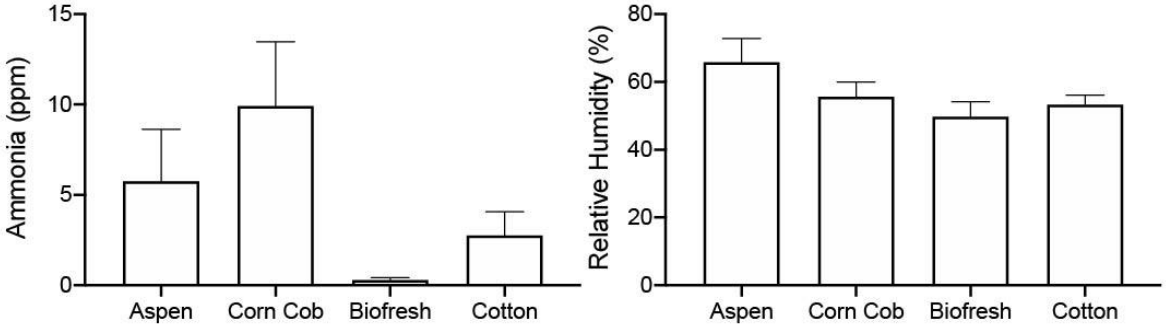

Figure 2 (Left-right). Mean +/- SEM ammonia concentration (ppm) and relative humidity (%) levels in Optimice cage-housed mice under different bedding material conditions (aspen, corn cob, BioFresh and cotton). One-way ANOVA indicates no main effect of bedding on ammonia (P = 0.075) and relative humidity levels (P = 0.16). N = 5-6 cages/per test condition (4-5 mice per cage).

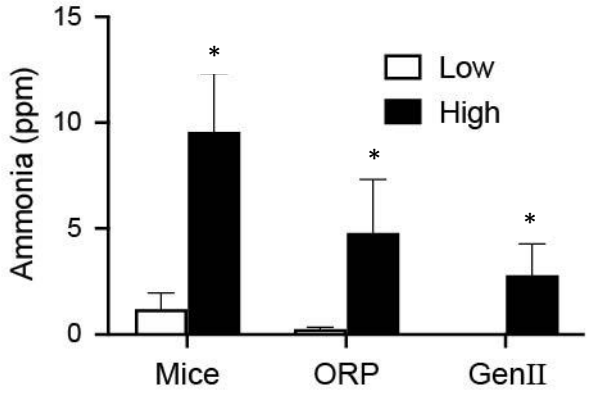

Figure 3. Mean +/- SEM ammonia levels (ppm) in Optimice-housed mice and in Optirat Plus/GenII-housed rats as a function of relative humidity (%) levels, rank-ordered as high and low, collapsed across bedding conditions. Two-way ANOVA indicates no main effect of housing (P = 0.07), but a significant main effect of relative humidity (P = 0.008). N = 7/8 cages per humidity ranking.

Discussion

Currently there are no upper level ammonia exposure guidelines for mice; for humans, the 8-h time-weighted average exposure limit is 25 ppm or 50 ppm maximal exposure.[5] Numerous studies have described detrimental health effects of increased ammonia levels on laboratory rodents.[6,7] Most of these effects are subclinical but often include histologic and immunologic changes[8] that may impact animal welfare and variably alter research results. Based on results of our previous study at lower environmental humidity levels where ammonia remained below 5 ppm throughout the course of a cage change interval, this study showed higher ammonia levels in higher humidity conditions. The average ammonia in higher humidity cage conditions was greater than at lower humidity in this study and that previously tested[3] but still remained much lower than other published studies using the same strain of mice that have shown much higher (in some instances greater than 150 ppm) levels of ammonia in other individually ventilated cages.[9,10]

Several different types of bedding were tested to represent some of the most used substrates in the industry. We found that all bedding types that we evaluated were acceptable choices for use in Animal Care Systems cages and that the practice of changing bedding every 2 weeks in mice cages and every 7 days in rat cages is acceptable.

References

1. **Gamble MR, Clough G.** 1976. Ammonia Build-up in Animal Boxes and its Effect on Rat Tracheal Epithelium. Laboratory Animals **10:**93-104.
2. **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**(3):93-8.
3. https://animalcaresystems.com/images/uploads/pdfs/Ammonia_Values_in_ACS_Cages_040119a_Q2_summarized.pdf (Cited 2020)
4. **Institute for Laboratory Animal Research.** Guide for the Care and Use of Laboratory Animals 8th ed., 2011.
5. **American Conference of Governmental Industrial Hygienists (ACGIH).** 2007. Threshold limit values (TLV) and biological exposure indices (BEI). Cincinnati (OH): ACGIH.
6. **Broderson JR, Lindsey JR, Crawford JE.** 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am J Pathol **85**:115–130.
7. **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.
8. **Sanford AN, Clark SE, Talham G, Sidelsky MG, Coffin SE. 2002.** Influence of bedding type on mucosal immune responses. Comp Med **52**:429–432.
9. **Levy, DRM, Flores R, Garcia GE, Craig SL, Jensen VB.** 2018. Effects of Extruded Compared with Pelleted Diets on Laboratory Mice Housed in Individually Ventilated Cages and the Cage Environment. JAALAS **57**(6):686-694.
10. **Silverman J, Bays DW, Cooper SF, Baker SP.** 2008. Ammonia and Carbon Dioxide Concentrations in Disposable and Reusable Ventilated Mouse Cages. JAALAS **47**(2):57-62.