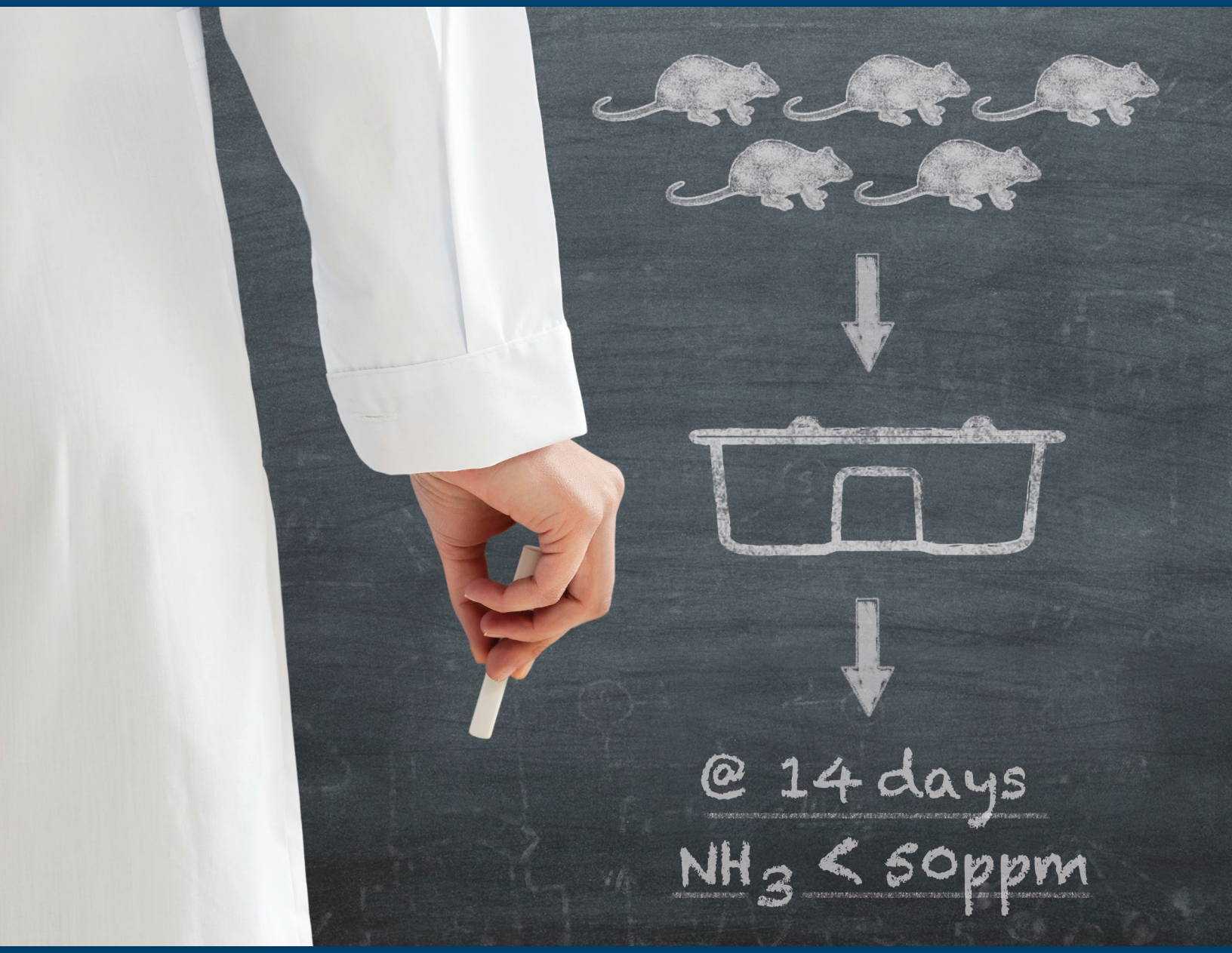
Collection of Ammonia Studies

with the Innovive Disposable IVC System



innovive

Studies have shown that Innovive disposable IVC cages have lower levels of ammonia than traditional systems, which can lead to a 14+ day cage change schedule and better breeding results.

But don’t take our word for it -
Go ahead and draw your own conclusions.

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Start Reading The Studies

Effect of Caging System and Bedding Sterilization on Intra cage Ammonia Accumulation With Time

STUDY ONE

by John A. Maher, Juan P. Rodriguez and Scott A. Mischler
SOURCE: Poster, AALAS National Meeting, Nov 2015.

INTRODUCTION

Cages holding rodents accumulate ammonia and feces over time and need to be periodically changed and washed. Cage changing, however, can cause distress to the rodents and expose the lab personnel to allergens and/or infectious agents. Cage washing, furthermore, is expensive and resource intense. Less frequent cage changes and/or washing are thus desirable.

Using IVCs is helpful: their air supply is continuously renewed and this helps reduce their levels of moisture and noxious gases (i.e. ammonia). Work with IVCs, however, has mainly centered on the frequency of cage air changes. Less attention has been given to how the pattern of air movement within the cage, or other cage design characteristics, affect gas accumulation.

Using the proper bedding is also important: much research has documented differences among bedding materials, as well as the effects of their treatment (i.e. sterilization), on cage changing frequency.

The present study sought to: a) Compare the ammonia removal efficiency of two ventilated cage systems in which the air injection and exhaust ports were either horizontal (CH) or vertical (CV), and b) Determine if using autoclaved bedding (AB) resulted in different intra cage ammonia concentrations than using non autoclaved bedding (NAB).

MATERAILS

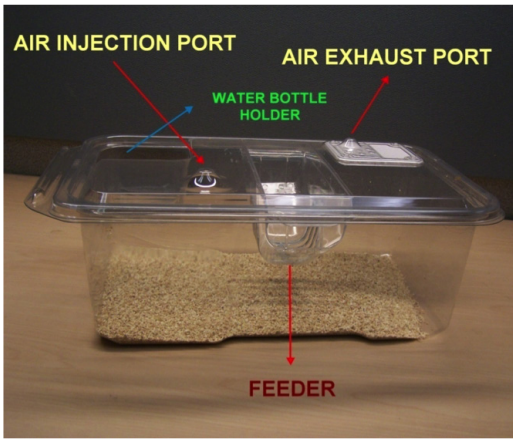
Twenty mouse cages, each containing five CD1 female mice weighing 28-30 g, were allocated to one of four combinations of caging systems and bedding: CV-AB, CV-NAB, CH-AB and CH-NAB, where:

AB: autoclaved ¼ Bed-O-Cob bedding.

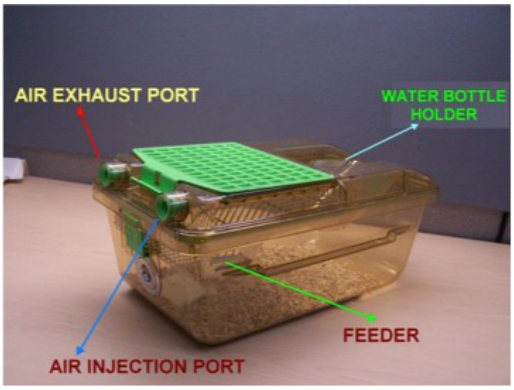
NAB: non-autoclaved ¼ Bed-O-Cob bedding.

CV, pictured right, a caging system where both the air injection and exhaust ports are at the top of the cage and the feeder is in the middle of the cage, between the ports; a built in space for a vertically mounted 300 ml water bottle is located at the cage top near the front.

CH, pictured right, is a caging system where the air injection and exhaust ports are located near the left and right corners of the rear of the cage lid and the feeder is located under the air ports; a built in space for a diagonally mounted 400 ml water bottle is located at the cage front.



PROCEDURES



Double sided racks were used in each caging system. 5 cages were placed on each rack side in an X pattern: Top Left, Top Right, Center, Bottom Left and Bottom Right.

The mice were weighed individually at the start and end of the study; average daily gains were calculated from these weights. The feed provided, and the feed leftovers at each feeding and at the end of the study, were weighed and used to calculate daily feed disappearance. Water was provided in 400 ml (CH) or 300 ml (CV) bottles. The bottles were weighed every morning to measure daily water disappearance. Bedding was weighed at the start (220g) and end of study for each cage. End of study bedding samples were taken and frozen for analysis.

Intra-cage ammonia concentration was used as the criteria of cage environmental quality. Ammonia was measured daily (between 09:30 and 11:30 hours) with a Drager X – am 7000 unit equipped with an sensor calibrated to read from 0 to 200 ppm of ammonia. To measure the ammonia, a 2 minute air sample was drawn from each cage through a brass fitting port in the front of the cage, at about 1" from its bottom. An additional criterium evaluated was the cage's useful life: the number of days that the intra cage ammonia level remained under 25 and 50 ppm.

The study ended for a given cage on the day its ammonia concentration reached (or surpassed) 50 ppm. The end point for cages not yet reaching 50 ppm was when their bedding accumulation, in the view of the veterinary staff, could hinder animal mobility within the cage.

RESULTS

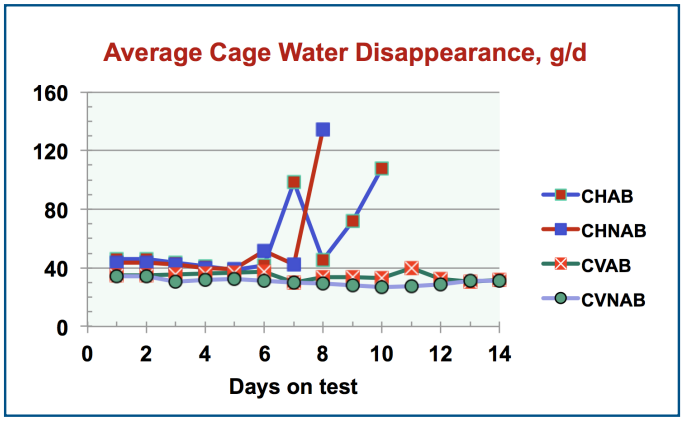
Shown below are averages for main effects in the study:

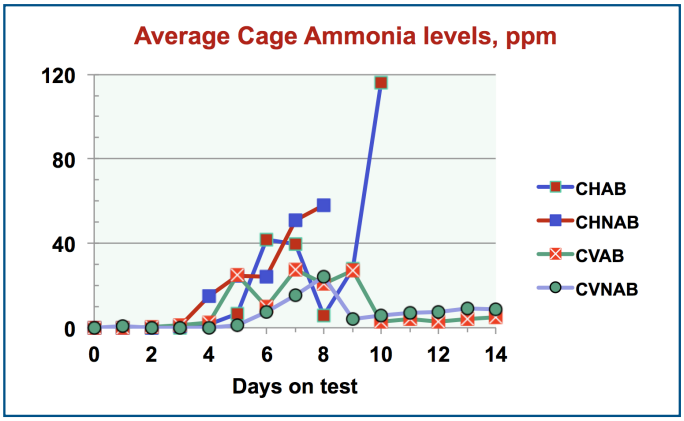
PARAMETER	CH	CV	P	AB	NAB	P
Initial Weight, g	26.5	26.12	0.11	26.3	26.3	0.97
Weight gain, g/d	0.17	0.31	**0.0001**	0.22	0.25	0.27
Feed disappearance, g/d	61.5	31.0	**0.0003**	50.3	42.2	0.24
Water disappearance, g/d	47.8	31.3	**0.0001**	42.0	37.1	0.14
Bedding accumulation, g/d	66.2	30.4	**0.0003**	51.1	45.5	0.48
Cage days < 25 ppm ammonia	5.2	7.8	**0.03**	6.05	6.75	0.51
Cage days < 50 ppm ammonia	5.5	7.5	**0.02**	6.21	7.45	0.27

Bedding sterilization did not affect any of the parameters considered. Mice in CV cages gained more weight but used less feed and water, and produced less waste (bedding) than CH mice. CV cages also had a longer useful life (days under 25 and under 50 ppm NH_3) than CH cages.

Statistical analysis indicated that the cage ammonia levels were closely related to the rate of water disappearance from the cages. Water disappeared to a much lesser extent from CV cage bottles than from CH cage bottles.

Feed “grinding” occurred at a higher rate in CH cages likely because their feeder design and position allowed the mice greater playing access. This larger feed wastage contributed to the greater bedding accumulation observed in the CH cages.

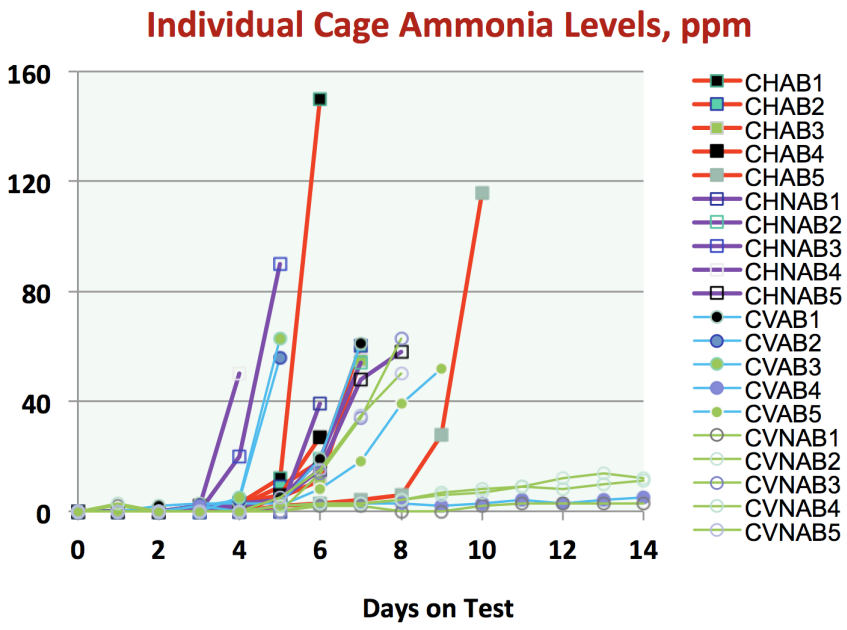




AMMONIA LEVELS

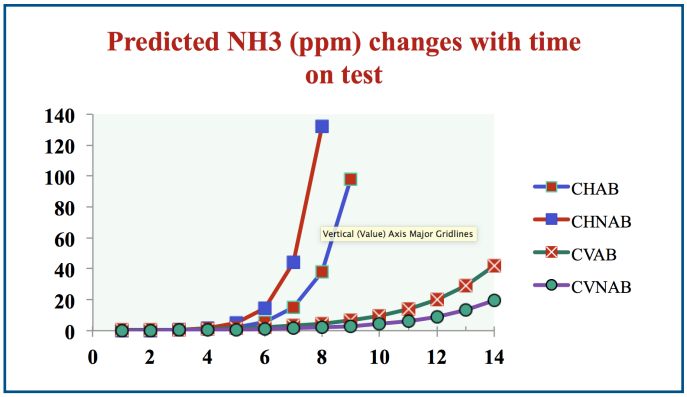
Average values for the intra-cage ammonia concentration are shown above whereas the individual NH_3 readings for each cage are shown below.

Intra-cage ammonia values were larger ($P<0.01$) for the CH than for the CV cages. All other things being equal, the moister, more nutrient rich environment in the CH cages may have favored a larger bacterial population, enhanced bacterial activity and thus greater NH_3 levels.



Because of the use of artificial end points, studies like this one are not amenable to analysis using standard statistical methods. Two different descriptive approaches were therefore used to compare the treatments:

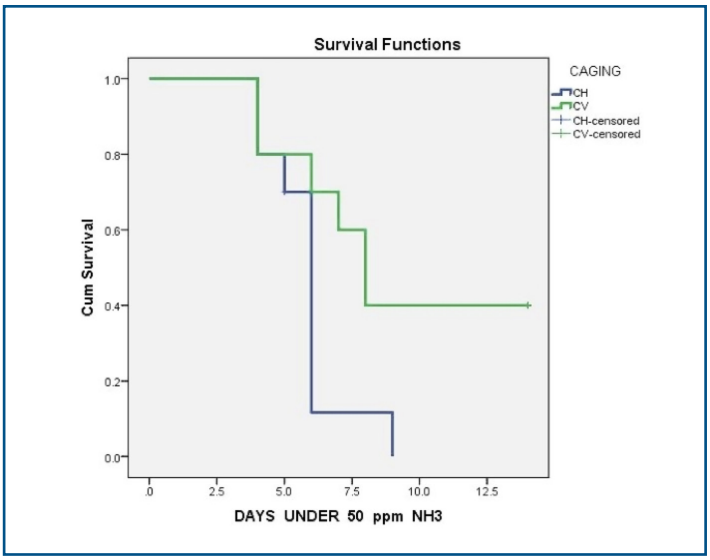
Exponential growth and decay model: NH_3 production was assumed to change at a rate as that off the bacterial population that produces it; that rate is described by the equation $N^t = N^0e^{kt}$ where N^0 and N^t are the bacterial population (or NH_3 levels) at time 0 and t, t is a time measure, and k is the rate of change per unit of time t.



The natural log values of the NH_3 levels were regressed against the days on test for the cages in each treatment and the intercept and slopes of the resulting regression lines were compared to ascertain if they were different or the same. The results indicated that the model fitted the data closely ($P<0.01$), accounted for about 55% of the variation in NH_3 values, and showed different rates of change in NH_3 level with time (k) for CH and CV cages. The equations (graphed below) predict that CH cages reach NH_3 levels over 50 ppm within 8 days while CV cages still remain under that value after 14 days.

Survival Analysis (Kaplan and Meyer): Survival analysis models the time to the occurrence of an event (a failure, i.e. a death, the end of a cage's useful life, etc.). It allows for censoring: the handling of objects in which the failure did not occur before the end of the test. It also allows to compare the time to the event among several groups (i.e. treatments, caging systems). The Kaplan-Meyer model was chosen because is non parametric and thus less sensitive to non-normal and non homogenous variance data like the one in this study.

The analysis indicated differences in useful life to both 25 and 50 ppm between the caging systems. The results shown (right) are for survival days under 50 ppm: the probability of CH cages surviving to day 10 is 0; the probability of the CV cages surviving to day 14 is 0.4.



Go To Next Study

Ammonia Levels and Cage Change Frequency For Mice Housed on 3 Different Types of Ventilated Racks

STUDY TWO

by Mary Rainey, Ollie Dalisay, Eleanor Gonsalves,
Evelyn Jamie, Roberto Leal, Rafael Soares, Jerik Toloza

BACKGROUND

The BMS BDC vivarium is currently utilizing three different types of caging to maintain our mouse colonies. These are:

1) Innovive Innorack™ IVC Mouse 3.0 ventilated rack with Innocage™ Mouse Pre-Bedded cage bottoms with Papertwist-enriched corncob bedding, Innocage IVC Mouse Dual Filter lid, and mouse metal feeder, in which we put Lab Diet Isopro 5P75 pellets. On these cages we use the Hydropac® Watering System pouches by Lab Products. (Hydropac AWS 2500, kept at pH range 2.3-2.8, tested at each start-up and after every -500 pouches made.) Per manufacturer recommendations, the bottoms are changed every 14 days, and the lids are changed every 28 days.

2) Allentown® individually-ventilated racks with a 160 cage capacity, with JAG75 mouse cage bottoms containing a mixture of Sanichip 7090A and Paperchip Soft Texture bedding with filter-top lids and metal grids that hold Lab Diet Isopro 5P75 pellets, and utilize the in-house Edstrom Chlorine Injector Station Repressurization Automatic Watering System, kept at 2.3mg/L chlorine in the system water, and checked weekly. These cage bottoms are changed weekly, except for breeding cages with pups over 10 days old, which are changed on a more frequent, as needed basis. The metal grids are changed every 3-4 weeks, and the filter tops are replaced as needed.

3) Ventilated Rack #3 (undisclosed vendor) with individually-ventilated mouse cages with filter-top lids. On these, we use the mouse metal grids to hold Lab Diet IsoPro 5P75 food and fitted with a plastic sleeve to hold the Hydropac® Watering System pouches. These cage bottoms are changed every 7 days, except for singly housed mice, which are changed every 2 weeks. The metal grids are changed every 3-4 weeks, and the filter tops are replaced as needed.

The air supply and exhaust for all cage types is HEPA filtered in and out. All ventilated racks are exhausted through a thimble connection to the building HVAC system. All racks are set for 30-35 air changes per hour, and the rooms are set for 10-12 air changes per hour.

The Guide recommends cage change frequency for solid-bottom mouse cages of at least once per week, which may be extended in certain circumstances[1]. Our goal is to see if we can standardize our cage change intervals and to determine an appropriate cage change frequency for all caging systems based on ammonia monitoring within the cage environment. This will enable us to have more efficient use of equipment, technician husbandry time, and available space.

PURPOSE AND HYPOTHESIS

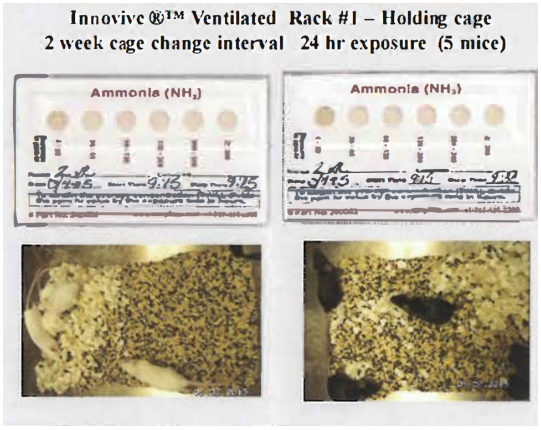
Currently, our cage change SOP slates that all non-disposable caging is to be changed on a weekly basis, except for singly-housed mice and breeding cages. Since it is also required for a minimum of 2

technicians to check each cage daily, we spend a significant amount of time observing the mice and the cage environment. Based on our observations, we have found that the amount of fecal material and moisture that accumulates in the cage after 1 week is minimal, therefore, we feel it may be possible to extend the cage change frequency for non-breeding cages to once every 2 weeks.

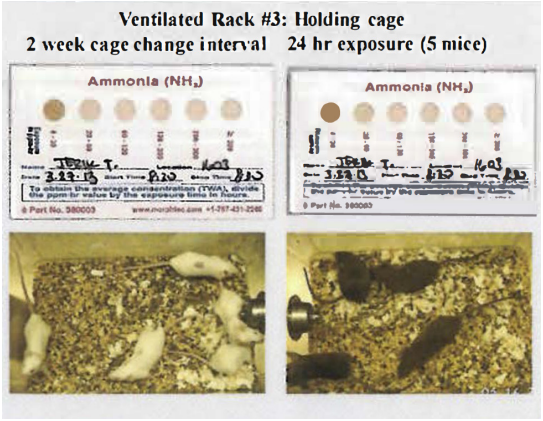
MATERIALS AND METHODS

We utilized the Chroman System Ammonia Monitor by Morphix™ Technologies (Part Number 380003), which is a patented, passive, direct-read autogenic exposimeter. “The device is constructed from six cells attached on one side to a flat indicator layer and on the other side to a series of different diffusive resistances. Ammonia gas diffuses to the cells through the different diffusive resistances and reacts with the indicator layer, producing color change from beige to black. The color produced on the indicator layer is a direct measure of the exposure dose.” The monitor measures an exposure range of 4 -300 parts per million per hour via color change from beige to black. Using 3M Scotch-Blue™ Painter’s Tape for Multi-Surfaces #2090, these monitors were taped inside the lids of the IVCs. On the Innovive cages, the monitor was taped directly to the inner surface of the cage lid, near the feed bin, so that the mice could not chew on the monitor.

For the other 2 cage types, the monitor was taped to the filter of the cage lid, with the metal grid denying mice access to the monitor. We also took photographs of the cages at the various time points, once the cages were opened to remove the ammonia monitors from the lids. For all 3-cage types, we initially placed monitors in cages 1 week after a cage change and left them in for 1 hour. We tested cages containing 1, 2, 3, 4 and 5 adult mice, and cages containing breeding pairs with a varying number of pups at different ages. Except for the breeding cages, we saw no color change on the monitors. We repeated these same density parameters again, leaving the monitors in for 4 hours. Again, this resulted in no color change except in the breeding cages. We then repeated the tests in all densities, leaving the monitors in for 24 hours. We chose this 24-hour time frame to capture the nocturnal “activity” period of the mice, not just the daytime “quiet” period.

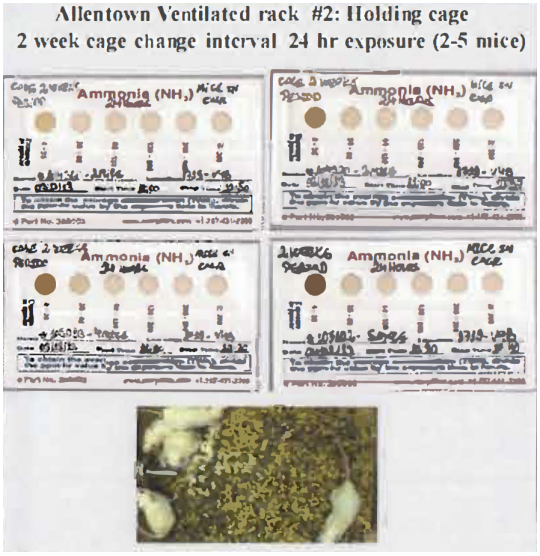


For all cage types, on the 1-week tests, the monitors did not register a color change, indicating an ammonia level of less than 4 ppm. The only color change observed was in breeding cages with pups. These cages are changed on an individual schedule, depending on the age of the pups, which may turn out to be more than once a week, after the pups reach 10 days of age. For all other cage types and densities except breeding cages, after waiting 14 days from the previous cage change, a monitor was placed in the lid of the closed cage for 24 hours, and then removed for reading. For the 2-week tests, there was only a color change at the 4-20 ppm/hour range indicator.



RESULTS

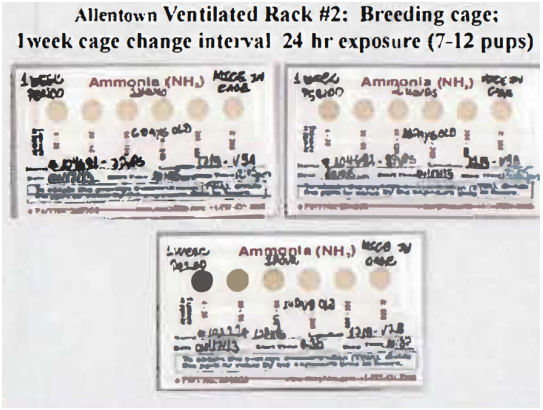
As seen in the photos of the ammonia monitors and their corresponding cage pictures, the color change is only in the area of the 4-20 ppm/hour circle. Dividing this ppm/hr exposure by the 24 hours the monitor was in the cage, the mice exposure to ammonia is less than 1 ppm/hour. We used the human OSHA Permissible Exposure Limit (PEL) - General Industry of 50 ppm/hr. (8 hr. TWA)[2] as a reference. The RD50 of ammonia exposure in mice is 303ppm[3]. We are well below these reference points at the 14-day cage change timeframe.



CONCLUSIONS

Based on the readings we obtained, we feel it will be safe to allow a 14-day cage change interval for all caging systems, except for breeding cages with litters, and those visually deemed in need of change during their daily check.

We presented this to our IACUC, received approval of the extended interval, and have incorporated these new standards into our Cage Changing SOP.



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1 Guide for the Care and Use of Laboratory Animals, 8th edition. Washington (DC): National Academics Press [US]; 2011 (pp.70-72)

2 United States Department of Labor. Occupational Safety & Health Administration Exposure Limits and Health Effects. OSHA Permissible Exposure Limit (PEL) - General Industry (Ammonia) 29 CFR 1910 1000 Table 2-1

3 Centers for Disease Control and Prevention. Doc. for Imm. Dangerous To Life or Health Cons (IDLHs). Ammonia CAS number: 7664-41-7

*Product names used with permission from vendors

Go To Next Study

Effect of 2 Bedding Materials on Ammonia Levels in Individually Ventilated Cages

STUDY THREE

By: Jason M Koontz,[1*] David M Kumsher,[2] Richard Kelly III,[3] and Jonathan D Stallings[1] SOURCE: Journal of the American Association for Laboratory Animal Science Vol 55, No 1, January 2016, Pages 25-28

This study sought to identify an optimal rodent bedding and cage-change interval to establish standard procedures for the IVC in our rodent vivarium. Disposable cages were prefilled with either corncob or ex-cellulose bedding and were used to house 2 adult Sprague-Dawley rats (experimental condition) or contained no animals (control). Rats were observed and intracage ammonia levels measured daily for 21 d. Intracage ammonia accumulation became significant by day 8 in experimental cages containing ex-cellulose bedding, whereas experimental cages containing corncob bedding did not reach detectable levels of ammonia until day 14. In all 3 experimental cages containing ex-cellulose, ammonia exceeded 100 ppm (our maximum acceptable limit) by day 11. Two experimental corncob cages required changing at days 16 and 17, whereas the remaining cage containing corncob bedding lasted the entire 21 d without reaching the 100-ppm ammonia threshold. These data suggests that corncob bedding provides nearly twice the service life of a-cellulose bedding in the IVC system.

For many animal facilities, IVC are an increasingly popular rodent housing option. These cages offer several benefits over traditional cage systems, including better containment, simplified handling, and increased protection from allergens.[9] Disposable IVC systems might also provide labor and cost savings by eliminating the need to clean and sanitize reusable cages. In addition, IVC systems have been shown to reduce cage ammonia levels and extend cage change intervals compared with static cage systems.[7]

As facilities make changes to IVC systems, the type of bedding to use and cage change frequency are important considerations. In accordance with the *Guide for the Care and Use of Laboratory Animals*,[10] bedding must be replaced and the microenvironment cleaned often enough to keep animals clean and dry and to keep pollutants (for example, ammonia) below irritating levels.[10] Due to a lack of directly comparable published data on this topic, conflicting advertising by bedding and IVC manufacturers, and marked differences in design and performance among IVC systems, choosing the right bedding and cage-change interval can be difficult.[3] Although high bedding absorbency is often associated with its ability to better neutralize ammonia, this situation is not always the case, and few published data are available to support these claims.[13] The absorbencies of some bedding types have been measured, but results vary greatly depending on whether absorbency is measured relative to the mass or the volume of bedding.[2]

In the current study, we sought to compare the accumulation of intracage ammonia between IVCs using 1/4-in. of corncob or an a-cellulose paper bedding in a commercially available IVC system for 21 d. Corncob and a-cellulose beddings were selected for this study because they are available in prefilled disposable cages directly from the IVC rack manufacturer. This study sought to identify the optimal bedding choice and cage-change interval for use in the IVC system in our vivarium. Similar studies have been performed by using various types of bedding and cage systems but report inconsistent results. Two studies report significant accumulation of intracage ammonia in IVC after only 1 wk when using recycled

paper bedding,[12,15] whereas another reports no measureable intracage ammonia after 2 wk when a similar bedding was used.[7] In addition, many bedding and cage combinations have not been tested in IVC systems. It is important to note that the manufacturer of the a-cellulose paper bedding claims significant performance differences between recycled paper beddings and those of engineered a-cellulose paper.[14]

MATERIALS AND METHODS

Rats and husbandry. This study was approved by the IACUC of the US Army Center for Environmental Health Research, an AAALAC-accredited facility. The 2 contact beddings used in this study were corncob and a-cellulose beddings (ALPHAdri, Shepherd Specialty Papers, Watertown, TN). Innocage Rat Pre-Bedded cages (141-in.^{2} of floor space; Innovive, San Diego, CA) with external water bottles were used, which were filled to a depth of 1/4 in. with the selected bedding. All cages were housed in the Innorack IVC Rat 3.5 system (Innovive) at 60 air changes hourly in negative pressure mode, in accordance with manufacturer's recommendations. Male Sprague-Dawley rats (Hsd:Sprague-Dawley SD; n = 14; weight, 450 g; age, 18 wk; Harlan, Indianapolis, IN) were used for this study, to maximize cage biomass. All rats were screened by using the institution's health monitoring program and were free from the following pathogens: Kilham rat virus, rat parvovirus, Toolan HI virus, Sendai virus, pneumonia virus of mice, reovirus type II, murine encephalomyelitis virus, sialodacryoadenitis virus, rat minute virus, Hantaan virus, lymphocyctic choriomeningitis virus, cilia-associated respiratory bacillus, mouse adenovirus types 1 and 2, rat rota virus, rat corona virus, *Mycoplasma pulmonis, Clostridium piliforme, Pasteurella* spp., fur mites, and pinworms. Each cage housed 2 randomly distributed rats. Rats of this size were chosen to maximize the amount of animal biomass per cage, following the animal mass and space guidelines described in the *Guide*.[10]

Rats were conscious and freely moving for the duration of the experiment and were given an irradiated, certified chow designed for toxicological studies (Harlan 2016, Teklad Global, Harlan, Indianapolis, IN) and water (prefilled 500-mL water bottles, Innovive, San Diego, CA) ad libitum. Animal holding rooms were maintained at 69.8 ± 0.1 °F (21.0 ± 0.1 °C), 49.5% ± 4.4% humidity, and a 12:12-h light:dark cycle, as recommended by the *Guide*.[10] Two rolls of certified and irradiated thick rolled tissue paper (Diamond Twists, Harlan, Indianapolis, IN) were provided as enrichment and destructible bedding material. For the duration of the study, enrichment was added only during cage changes, to minimize cage opening. All cages and cage materials were new at the beginning of the study.

Cage setup and ammonia measurement. Six experimental cages, each containing 2 rats, were used: 3 with corncob bedding and 3 with a-cellulose bedding. To establish baseline ammonia levels, 6 control cages, not containing any animals, were used: 3 with corncob bedding and 3 with a-cellulose bedding. We placed 2 control cages, 2 corncob cages, and 2 a-cellulose cages on each level of the rack. The cage types were put into the rack in alternating order so that no 2 adjacent levels were the same, to avoid any bias resulting from position in the rack due to differences in light, air flow, or noise. A small (1/2-in.) hole was drilled into the upper right hand corner of each cage, to use as a sample collection port. The sample-collection ports were sealed with white laboratory tape between samplings. Adding collection ports allowed ammonia measurements to be performed daily without opening the cage or removing it from the rack. Intracage ammonia levels were measured (nos. 6400000 and CH20501 5/a), Accuro pump and ammonia tubes (Item numbers respectively, Draeger Safety Diagnostics, Irving TX) once daily, for each experimental and control cage, between 0800 and 1000. The ammonia tubes have a range of Oto 70 ppm or 5 to 600 ppm, depending on the scale used. The lower range was used until intracage ammonia exceeded 70 ppm, at which time another measurement was taken by using the 5 to 600 ppm scale. The pump and ammonia tubes were used according to the manufacturer's instructions.

The tip of the ammonia tube was inserted approximately 3 inches into the cage, at an upward angle so that the tip of the tube was above the wire bar at the top of the cage, to prevent the rats from chewing on the ammonia tube during sampling (FIGURE 1).

Figure 1. Ammonia measurement through sample port. Pump and ammonia tube are shown, with ammonia tube inserted through the sample port and into the cage, above the wire bar, for ammonia measurement.

Once a cage reached an ammonia level of 100 ppm, it was changed immediately. There are no ammonia exposure limits or guidelines for rodents, and we chose 100 ppm because of other studies reporting adverse health effects in rats exposed to higher levels of ammonia.[1,6] In addition, 130 ppm ammonia is highly irritating to humans and can cause adverse respiratory and pulmonary health effects.[4,5]

Water consumption and cage biomass. All rats were weighed on a digital scale (Olympia Plus, Solenhe, Hamburg, Germany) prior to beginning the experiment. Rats were randomly placed into experimental and control cages, and the total cage biomass did not differ between cages. Water consumption was measured over a 1-wk period; fresh water bottles were placed at the beginning of the study on a Monday, and water consumption was measured by carefully weighing the water bottle from each experimental cage on a digital scale (Solenhe) daily, for Tuesday through Friday (4 data points total). Water weights were recorded to the nearest whole gram for all experimental cages, but no water consumption data were taken for control cages. Water bottles were handled carefully to avoid spillage, and cages were observed daily and monitored for leaks. Water consumption was then averaged for each bedding group. Removing the water bottles did not require the cages to be opened and did not interfere with ammonia measurements.

Data analysis. Data, ammonia levels, rat weights, and water consumption was averaged for each experimental bedding group. The Student t test was used to determine whether 2 groups of data differed significantly from each other. AP value of 0.05 was chosen as the threshold for significance.

RESULTS

Intracage ammonia levels. Ammonia levels were measured daily for each experimental and control cage in both the corncob and a-cellulose groups. By day 8, all 3 experimental a-cellulose cages had significantly ($P < 0.05$, Figure 2) elevated ammonia levels, relative to the corncob cages, whereas all 3 experimental corncob cages maintained undetectable ammonia levels until day 11. All 3 experimental a-cellulose cages had significantly higher levels of intracage ammonia than did the experimental corncob cages from days 8 through 11. Although all 3 a-cellulose cages exceeded 100 ppm ammonia by day 11, all 3 experimental corncob cages had undetectable ammonia levels until day 14. Please note that no measurements were taken on days 5, 6, 12, 13, 19, and 20, which fell on weekends, because measurements were taken on weekdays only (Monday through Friday). After 14 d, 2 of the corncob cages registered very low levels of ammonia (3 and 17 ppm), which slowly increased until day 17, when they both exceeded 100 ppm. The final corncob cage had its first measurable ammonia level (5 ppm) on day 17; the ammonia level within this cage rose to 90 ppm by day 21. There was no detectable ammonia in any control cage over the course of the 21 d experiment (FIGURE 2). Although intracage ammonia levels

varied significantly between the 2 beddings types, no adverse signs were observed in any of the rats.

Water consumption. Water consumption in each experimental cage was measured over 1 wk (5 business days) by weighing the water bottles daily. Daily water consumption was calculated and averaged for each bedding group. Although the rats in the a-cellulose cages consumed slightly more water daily (64 g) than did the rats in the corncob cages (58 g), the difference was not statistically significant (TABLE 1). Food consumption was not measured by weighing or counting pellets; due to the brittle nature of the pellets, it is common for a partially eaten pellet to break or crumble and fall from the wire feeder onto the cage floor. However, food levels were checked daily by an animal technician, and no noticeable differences in food consumption were reported for any of the experimental cages.

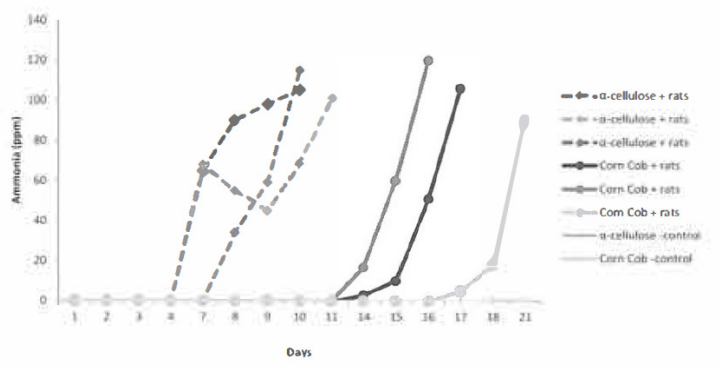

Figure 2. Effect of corncob and a-cellulose bedding on intracage ammonia levels (ppm) in IVC. Intracage ammonia levels were measured once daily for 21 d. Each bedding group contained 3 control cages (without animals) and 3 experimental cages, each of which housed 2 adult rats each. All three experimental cages with a-cellulose bedding had significantly ($P < 0.05$, 2-tailed t test) higher ammonia levels than the experimental corn cob cages from day 8 to the last day that the a-cellulose cages were used. Once the ammonia level reached 100 ppm, the cage was changed immediately, and subsequent ammonia levels are not shown. All 3 experimental a-cellulose cages exceeded 100 ppm ammonia by day 11. One corncob cage lasted the entire 21 d without reaching the 100-ppm ammonia threshold; however the other 2 corncob cages required changing at days 16 and 17. Although each· individual experimental cage is plotted separately, the corncob and a-cellulose control groups are represented by one line each, because all control cages remained at 0 ppm ammonia throughout the experiment.

Cage biomass. All rats were weighed at the beginning of the study (day 1); all had approximately the same mass. Rats were randomly distributed throughout experimental cages, and total cage biomass was calculated for each group. Total cage biomass at the beginning of the study did not differ between groups. All rats were weighed again at the conclusion of the study (day 21), with no significant difference between groups. Consistent cage biomass between the corncob and a-cellulose groups rules out biomass as a factor contributing to the intergroup differences in intracage ammonia levels.

DISCUSSION

In this study, we compared the ability of corncob and a-cellulose beddings to control ammonia levels in IVC over a 21-d period. Our data suggest that, when biomass is maximized, a-cellulose bedding was effective in the IVC systems for a maximum of 7 d. After 1 wk, the levels of accumulated intracage ammonia will be high (100 ppm or greater). Whether such a level of ammonia causes adverse effects in the rats or confounds experiments has yet to be determined.[1,4,5,6], In contrast, all 3 experimental corncob cages had relatively low levels of intracage ammonia after 2 wk (14 d), therefore doubling the interval between cage changes compared with that for a-cellulose.

We cannot definitively account for the drop in intracage ammonia seen in an experimental a-cellulose cage on days 8 and 9, but this cage still exhibited higher ammonia levels than any of the corncob cages during this time window. The bedding type, amount, and cage airflow were the same as those for all other cages in the a-cellulose group. This cage was housed in the same rack in the same room as the other cages, so an external factor such as temperature, humidity, or disturbances can be eliminated. The cage

Table 1. Water consumption.

		Water consumption (g)				
Bedding	Cage no.	Day 1	Day 2	Day 3	Day 4	Average
α-Cellulose	1	64	64	78	37	61
α-Cellulose	4	59	72	85	65	70
α-Cellulose	6	61	56	63	63	61
Corncob	2	59	61	67	66	63
Corncob	3	52	48	63	65	57
Corncob	5	54	50	58	53	54

Water consumption was an indirect way to assess amounts of urination in each experimental group, to determine whether differences in production between groups affected the ammonia levels reported. Water consumption did not differ between the bedding groups (α-cellulose, 64 g; corncob, 58 g).

was not removed from the rack or opened during this time. We hypothesize that the drop in intracage ammonia can be attributed in some way to animal behavior; the rats' activity level or waste production likely affected the ammonia levels.

The 2-wk cage-change interval will not only markedly reduce labor time and material costs, especially when using disposable cages in an IVC system, but it will also allow studies that require prolonged exposures, treatments, or observations without disturbing the animals. In our study, we maximized the biomass in each cage to create a 'worst-case scenario' for ammonia accumulation and soiled bedding. We will base our bedding choice and cage change interval for our entire rodent vivarium on these data, rather than having different change intervals for every different situation, to simplify and streamline planning and ordering. In light of these data, we will be using corncob bedding and a cage change interval of 2 wk for our entire rodent vivarium.

If fewer or smaller rats were used, we would expect to see an increase in service life for each bedding type. Increased cage biomass (that is, more or larger rats) results in increased ammonia levels,[16] therefore we hypothesize that using fewer or smaller animals might potentially extend the service life of corncob bedding to 3 wk (21 d). In our study, 1 of the 3 experimental corncob cages lasted 21 d without reaching 100 ppm ammonia; however the other 2 cages in this group needed to be changed on days 16 and 17, due to high ammonia levels.

Water consumption was measured in each experimental cage as an indirect way to assess amounts of urination in each group, to determine whether differences in urine production affected the ammonia levels reported. Intracage ammonia results primarily from urease-positive bacteria, which metabolize urea from the urine and feces of the animals.[8] Therefore, ammonia levels are proportional to the amounts of wet urine and urease-positive bacteria present in the cage. IVC systems help to reduce the levels of both urine and urease-positive bacteria by providing sufficiently frequent air changes to dry the cage bedding.[11,7] We found that there was no statistically significant difference in water consumption, and presumably urine production, between the a-cellulose and corncob groups in our study. Therefore, we conclude that the significant difference in intracage ammonia levels between the 2 groups was not due to differences in urination.

Because increased cage biomass results in increased intracage ammonia levels,[16] we ensured that each cage had the same total biomass before the experiment began. We weighed all of the rats at the conclusion of the experiment to see whether the animals in each group had similar growth rates over the course of the 3-wk experiment. A difference in growth rates between the groups might account for some of the difference observed in intracage ammonia levels. We found that total cage biomass did not

differ between the 2 groups. Again, this finding suggests that the differences in ammonia levels between the a-cellulose and corncob groups were due to the bedding material and not another external factor. Choosing the right bedding and cage change interval are important for the wellbeing of the animals and for minimizing the time and expense spent on unnecessary cage changes. Determining the optimal bedding and cage-change interval for a particular study, animal species, and cage setup can be challenging, given the lack of published information, conflicting reports from bedding manufacturers, and differences in the performance of various IVC systems. Although other published studies have compared different bedding materials in both static and IVC cages, we are unaware of any study that has compared the effect of a-cellulose and corncob beddings on intracage ammonia levels in an IVC system for an extended time period. Other factors to consider when choosing bedding type and change interval for a particular facility or study might include intracage carbon dioxide levels and fecal cortisol but were not included in this study.

The cages used in this study were purchased prefilled with bedding and are designed for a specific commercial rack system. The design of the rack system we used is fairly new, and its popularity is growing quickly, but only a few relevant data are available in the literature. One study compared IVC cages with static cages over 9 d[15] and described various advantages of the IVC system. However, to our knowledge, long-term studies that compare the 2 bedding options (corncob and ex-cellulose) available directly from the rack manufacturer are unavailable. Our current study fills this gap and will help other animal facilities to make educated decisions that are based on empirical data rather than common practice, when they need to choose a type of bedding and a cage-change interval.

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Go To Next Study

Ammonia and Carbon Dioxide Concentrations in Disposable and Reusable Ventilated Mouse Cages

STUDY FOUR

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This study compares resuable and disposable individually ventilated mouse cages in terms of the formation of intracage CO_2 and NH_3. Crl:CD-1(ICR) female mice were placed in either disposable or reusable ventilated cages in a positive pressure animal rack. Intracage CO_2 and NH_3 were measured once daily for 9 d; temperature and relative humidity were monitored for the first 7 d. Results indicated higher CO_2 levels in the rear of the disposable cages and in the front of the reusable cages. This pattern corresponded to where the mice tended to congregate. However, CO_2 concentrations did not differ significantly between the 2 cage types. Average CO_2 levels in both cage types never exceeded approximately 3000 ppm. Intracage NH_3 began to rise in the reusable cages on day 4, reached approximately 50 ppm by day 5 and by day 9 was greater than 150 ppm at the cages' rear sampling port while remaining at approximately 70 ppm at the front sampling port. Intracage NH_3 levels in the disposable cages remained less than or equal to 3.2 ppm. Intracage temperature and relative humidity were approximately the same in both cage types. We concluded that the disposable ventilated cage performed satisfactorily under the conditions of the study.

Abbreviations: ACH, air changes per hour; IVC, individually ventilated cage

A growing body of literature documents the health effects on laboratory animals of intracage gases such as NH_3 and CO_2.[2,4,13,21,22,29] In general, static containment cages (that is, cages with filtered tops that are not connected to, or in the path of, a directional air flow) are more likely to create conditions conducive to the production of high concentrations of intracage NH_3 and CO_2 than are individually ventilated cages (IVCs).[15] Nevertheless, few published studies address the comparative efficacy of different styles of IVCs in evacuating these gases. Recently, disposable rodent cages have been developed for both routine and nonroutine animal housing (for example, biocontainment) and offer animal facilities potential cost savings in labor and equipment. We therefore studied the levels of CO_2 and NH_3 produced by mice housed in disposable IVCs placed in a positive-pressure animal rack and compared them with the NH_3 and CO_2 concentrations developed in a reusable IVC. We hypothesized that both cage types would be similarly efficacious in controlling CO_2 and NH_3 levels.

MATERIALS AND METHODS

Disposable caging and rack. Polyethylene terephthalate cages and cage tops were used. Cage bottoms measured 27.3 × 18.0 cm at the level of the top of the bedding. The cage top, which had a small filter area, snap-fitted tightly to the rim of the cage bottom. The top also had 2 preformed plastic ports; one for air to enter and another to exhaust cage air. These ports were located approximately 14 cm above the cage bottom and directly articulated with air supply and exhaust ports on the rack. The disposable water bottle was largely outside of the cage, fitting into an indentation in the cage top. The total interior volume of the bottom and top (including preformed indentations in the cage top) was 5876 ml.

The disposable IVCs were placed in a positive-air–pressure double-sided rack that was capable of holding 112 mouse cages when full, although not all cage slots had to be occupied for proper operation. The rack was equipped with exhaust and supply blowers. The rack provided HEPA-filtered air, was set at 60 air changes/h (ACH), and exhausted into the room through a HEPA filter built into the exhaust blower.

Reusable caging and rack. Polysulfone cages were used. Cage bottoms measured 28.0 × 17.3 cm at the level of the top of the bedding. The cage top overhung the cage bottom and had a relatively large filter area. The total interior volume (excluding the portion of the cage top that overhung the bottom of the cage) was 6320 ml. The space occupied by the water bottle and the wire-bar top was excluded from the volume determination. There was 1 air entry port at the rear of the bottom section, approximately 3 cm above the cage floor. Most exhaust air escaped around the interface between the cage top and bottom, was collected by an air-exhaust plenum on the cage rack (with exhaust vents located immediately adjacent and horizontally parallel to the rear of the cage top), and subsequently was evacuated from the rack through a direct connection to the building's exhaust system. The reusable IVC's water bottle was entirely enclosed within the cage.

Reusable IVCs were placed in a positive-air–pressure mouse rack that was capable of holding 90 cages when full, although not all cage slots had to be occupied for proper operation. The rack was equipped with a supply blower providing HEPA-filtered air. The rack was set for 60 ACH and connected to the building exhaust, which was set at 50 ft^3/min.

Animals. Retired breeder female Crl:CD-1(ICR) mice (Charles River Laboratories, Wilmington, MA) were used. Serologic monitoring while they were in our animal facility (Charles River Laboratories Diagnostic Services, Wilmington, MA) confirmed freedom from murine norovirus, mouse parvoviruses, mouse hepatitis virus, reovirus (types 1 and 3), lymphocytic choriomeningitis virus, lactate dehydrogenase-elevating virus, mouse rotavirus, Theiler murine encephalomyelitis virus, *Ectromelia*, hantavirus, mouse adenovirus, Sendai virus, and *Mycoplasma* spp. Animals also were free of common pathogenic mouse ectoparasites and endoparasites.

Husbandry. Mice were housed at 5 animals/cage in a room free of other animals. They were fed a commercial irradiated laboratory mouse diet (Purina LabDiet 5P76, Ralston Purina, St Louis, MO). A reversed 12:12-h light:dark cycle was used so that the dark cycle occurred during working hours. This adjustment was done to help ensure maximal animal activity during the sampling periods.[12,29] Animals were acclimated to this cycle for 2 wk prior to initiating testing. Lights were turned on during sampling. Each cage had 270 g of food placed on its feed tray. Animals were given 275 ml of acidified reverse-osmosis water in a water bottle for reusable IVCs and disposable water bottles for the disposable IVCs. Bottles were placed carefully to minimize spillage. Paper chip bedding (160 g; Paperchip Soft Texture, Shepherd Specialty Papers, Kalamazoo, MI), approximately 1 cm deep, was placed in each cage. Paper chip bedding was used because studies suggested that it would be somewhat less effective in maintaining cage homeostasis than corncob bedding.[3,19]

Control cages (without mice) had the same amount of food, water, and bedding as did cages with mice. The room was ventilated at approximately 22 ACH. Room supply airflow was approximately 1163 ft^3/min, and exhaust was approximately 1064 ft^3/min.

Gas, temperature, and humidity measurements. The stainless steel gas sampling ports were commercial bulkhead fittings with barbed tubing connections (MBHA-1332-316, Beswick Engineering, Greenland, NH). They were placed in the front and left-rear of each cage through holes drilled in the plastic. The bottom of the port was 1.9 cm above the top of the bedding, which was the approximate height above the bedding of a mouse's nose. A small piece of plastic intravenous tubing (about 4 cm

in length) was attached to the exterior of the sampling port, and a standard Luer adapter was placed on the other end of the tubing (FIGURE 1). Air flow in or out of the port was effectively stopped by a standard pinch clamp on the tubing and a Luer lock plug placed over the end of the Luer adapter. The plug provided an additional barrier against air escape or entry and was removed during sampling. A stainless steel mesh sink strainer (manufacturer unknown) was secured over the inside of each sampling port to prevent the mice from breathing directly on the port during sampling. The strainer was 4.0 cm in diameter and extended 2.5 cm into the cage. It kept mice at least 2 cm from the ports. All screws used had rubber washers on the outside of the cage and were secured by standard steel nuts.

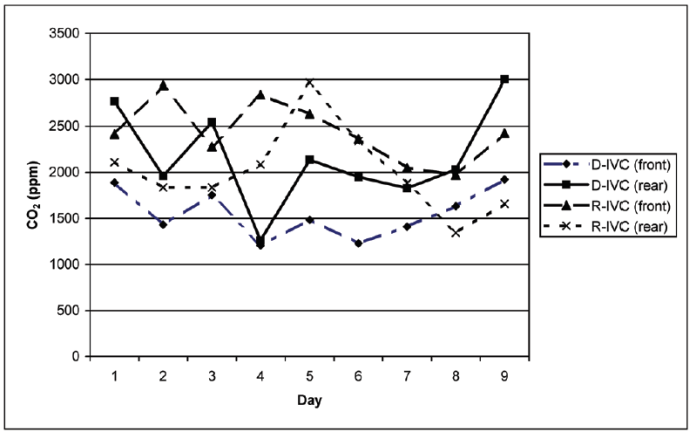

Figure 1. Average daily CO2 concentrations from front or rear sampling ports of 2 disposable (D-IVC) and 3 reusable (R-IVC) ventilated cages.

NH_3 and CO_2 measurements were made with a chip measurement analyzer (model 6405300, Dräger Safety, Pittsburgh, PA). A small piece of rubber tubing (approximately 5 cm long) was attached to the sampling port of the analyzer. During testing, the other end of this tubing was attached to the Luer adapter on the sampling port of the cage. The gas analysis system was self-calibrating in that all calibration information was prestored on the bar code of the NH_3 or CO_2 analysis chip that was inserted into the analyzer. An electronic system self-test was performed every time the analyzer was switched on. When the chip was inserted into the analyzer, the calibration information from the chip was automatically downloaded to the analyzer. A sampling system test was automatically performed before each gas concentration analysis, and the analyzer was flushed with room air after testing each cage.

For NH_3 measurements, chips with a detection range of 2.0 to 50 ppm or 10 to 150 ppm were used (the higher range chip was used only if a value greater than 50 ppm was detected initially by using the lower range chip). Chips were accurate to 7% to 8% of the measured value and reproducible at 10% to 12% (SD).[7,8]

For CO_2 measurements, chips with a detection range of 200 to 3000 ppm or 1000 to 25,000 ppm were used (the higher range chip was used only if initial findings with the lower range chip indicated a value greater than 3000 ppm). Chips were accurate to 5% to 7% of the measured value and reproducible at 7% to 10% (SD).[7,8] For either NH_3 or CO_2 gas analysis, the analyzer withdrew 15 ml air/min. Maximum sampling time was 2.75 min (which occurred when very low levels of NH_3 or CO_2 were detected). More typically, sampling times were 45 to 60 s.

Intracage temperature and humidity were measured and recorded hourly for the first 7 d by using a remote data logger (HO8-004-02, Anset Computer, Bourne, MA) preprogrammed for 7 d of recording and attached to the inside of the cage above the food hopper. The study was initially designed to terminate in 7 d, but because we allowed it to progress for a total of 9 d and because the cage could not be opened during the study, temperature and humidity recordings are for the first 7 d only. Ambient room temperature and humidity were recorded once daily at the beginning of the testing session by using a sling psychrometer (Bacharach, Pittsburgh, PA).

Animal randomization. Each mouse was marked for identification and weighed; those with weights outside of 1.5 SDs were excluded. All included animals were then placed in rank order, by weight, from lowest to highest. This weight ranking was divided into 5 groups with 6 animals in each group. By use of a random number generator, 1 animal from each of the 5 weight groups was placed in either a disposable or reusable IVC. This process was repeated until each of 6 cages had 5 animals. When the weights of all the mice in any 1 cage were averaged, the 6-cage average ranged from 38.6 to 38.9 g.

General study design. The study was designed to be performed in triplicate (3 animal-containing cages and 3 non-animal control cages of each cage type), but due to a technical problem, we only used duplicate data for the disposable IVCs. One cage (n = 5 mice) was placed in each of the 3 middle rows of their respective ventilated rack. Cages with mice were on the outer edge of the rack, and each cage had an unoccupied control cage next to it. The 2 racks faced each other, being separate by approximately 1.3 m. At the same time each day, air samples were taken from each sampling port of each cage and analyzed for either CO_2 or NH_3 concentration. All cages were sampled first for NH_3. After all NH_3 samples were taken from all cages, the same cages then were sampled for CO_2. The pattern was to first take samples from a disposable IVC with animals (front then rear port), then from the associated control cage (front then rear ports). After all disposable IVCs were sampled, the reusable IVCs were sampled. Intracage temperature and humidity were recorded continually remotely once every hour. Cages were otherwise left undisturbed during the course of the study. The study was ended after 9 d. All work was approved by the University of Massachusetts Medical School's institutional animal care and use committee.

Preliminary testing. Preliminary testing suggested that NH_3 and CO_2 concentrations might differ between the front and back of the cages, therefore sampling ports were placed at those 2 locations. We did not find significant gas concentration differences between higher and lower levels of the cages. To test for the air tightness of the sampling ports, smoke sticks were placed within cages (Tel-Tru Smoke Stick, Liberty Industries, East Berlin, CT). We found no overt escape of smoke through closed ports or screw holes.

To confirm the consistency of the remote temperature and humidity monitors (described earlier), we tested the monitors against the sling psychrometer and then against each other. All readings were nearly identical.

Although the original intent of this study was to use the mouse racks as supplied by the manufacturer (that is, without further validation of air flows), we nevertheless performed an initial verification of rack air flows by using a digital manometer for the disposable IVC rack and a specialty manometer for the reusable IVC rack. Both racks performed at or near 60 ACH.

Statistical methods. For the evaluation of CO_2 data, we used actual measured values. For NH_3 data evaluation, we used actual measured values, but when measured concentrations were less than 2.0 ppm, we assigned a value of 1.0 ppm (the midpoint in the range of 0 to 2 ppm). For values greater than 150.0 ppm, we assigned a value of 274.5 ppm (an estimate of the median value in this range based on the normal distribution of log-transformed values). As indicated earlier, all disposable IVC data came from 2 animal-containing or control cages, whereas data from reusable IVCs came from 3 animal-containing or control cages. The effects of cage type, sampling port location, and time were evaluated by using general linear mixed models[16] to fit repeated measures growth curve models for NH_3 and CO_2. In the presence of significant differences among means, pairwise comparisons were made by using the Tukey Honestly Significant Difference test (using the estimated covariance matrix to account for correlated observations).[11] The distributional characteristics of outcome measures were evaluated both graphically and by the Kolmogorov–Smirnov Goodness of Fit Test for Normality.[6] Natural logarithms of outcomes were applied

to better approximate normally distributed residuals. All computations were performed by using the SAS Proc Mixed procedure[24] and SAS version 9.1.3[25] statistical software package. Statistical significance was defined as present when associated P values were less than 0.050. For this study design, power analyses showed that the sample sizes provided 85% power for detecting a true difference of 20 ppm NH_3 between cage types and greater than 90% power for detecting a true difference of 75 ppm CO_2.

RESULTS

CO2. All unoccupied cages maintained CO_2 concentrations near ambient room levels (440 to 530 ppm) throughout the study. Unoccupied cages showed no significant differences in CO_2 concentrations between the front and rear sampling ports or between the 2 types of cages.

At the first sampling time point, which occurred approximately 60 min after placing animals in the cages, CO_2 concentrations were higher in both disposable and reusable IVCs as compared with the ambient level (FIGURE 1). Over the 9 d of the study, CO_2 concentration differed significantly between samples taken from the front sampling ports compared with the rear sampling ports of reusable IVCs ($P = 0.0064$, with 1.126 times more CO_2 at the front port). For the disposable IVCs, differences approached but did not reach statistical significance ($P = 0.0511$, with 1.103 times more CO_2 at the rear port). For the disposable IVCs, mice tended to cluster in the back half of their cages, where CO_2 was detected at higher levels. The opposite was found for reusable IVCs, where higher CO_2 levels usually were detected from the front ports, and the animals tended to congregate in the front of those cages. When the CO_2 concentrations from either of the sampling ports were compared between disposable IVCs and reusable IVCs, there were no significant differences between the 2 cage types. The lowest CO_2 concentration recorded in an individual animal-containing cage was 1040 ppm, and the highest was 4000 ppm.

The disposable IVC cages showed no clear pattern of temporally changing CO_2 concentrations over the 9 d of the study, however, the reusable IVC cages showed a significant reduction in CO_2 over the same 9 d ($P = 0.0305$). The reason for this is not known.

NH3. Ambient (room) NH_3 levels remained below 2.0 ppm. There were no statistically significant differences between front and rear sampling ports of either cage type. Mean intracage NH_3 levels in reusable IVCs were occasionally greater than 25 ppm and, in some instances, were greater than 150 ppm (FIGURE 2). Animals in the cages with high NH_3 concentrations were closely monitored, but no overt problems (that is, sneezing, rubbing of eyes or nose, erythema, changes in behavior patterns) were noticed, and with approval from the institutional animal care and use committee, studies were continued for an additional 2 d. Unoccupied cages maintained negligible NH_3 concentrations throughout the study. Infrequent low concentrations of NH_3 were found in 4 of 90 unoccupied cage measurements (maximum, 3.8 ppm, data not shown) and most likely occurred from NH_3 carryover from the previously sampled cage (which contained animals) due to incomplete flushing of the analyzer between cages. In unoccupied cages, NH3 concentrations did not differ significantly between front and rear ports or between the 2 types of cages.

In cages with mice, NH_3 levels did not rise above the minimal detectable concentration (2.0 ppm) until day 4. At that time, concentrations of approximately 2 to 3 ppm were detected from both sampling ports of 1 reusable IVC and the rear port of another reusable IVC. By day 5, the average NH_3 concentration in the reusable IVCs was near 70 ppm (FIGURE 2) with the highest concentrations found in 2 of the 3 cages. In the 1 remaining reusable IVC, NH_3 concentrations remained at 5.1 ppm or less until study day 8, at which time concentrations rose to 33 and 48 ppm at the front and rear ports, respectively. By day 9, all reusable IVCs had NH_3 concentrations between 59 and 77 ppm at the front sampling ports and greater than 120 ppm at the rear ports.

Throughout the course of the study, the disposable IVCs with animals maintained NH_3 concentrations that were no greater than 3.2 ppm in any cage. Reusable IVCs with animals had higher NH_3 than did unoccupied cages (P = 0.0046). In disposable IVCs, NH3 did not differ significantly between occupied and unoccupied cages. When occupied, disposable IVCs had lower NH_3 concentrations than reusable IVCs (P=.0176).

Temperature and humidity. During the study, the mean daily ambient (room) temperature at the time of sampling was 21.4 °C, and the mean relative humidity was 43.9%. These readings closely correlated with the recordings from the control cages. The ranges of room ambient temperature and relative humidity at the time of sampling were 20.6 to 21.6 °C and 41% to 50%, respectively.

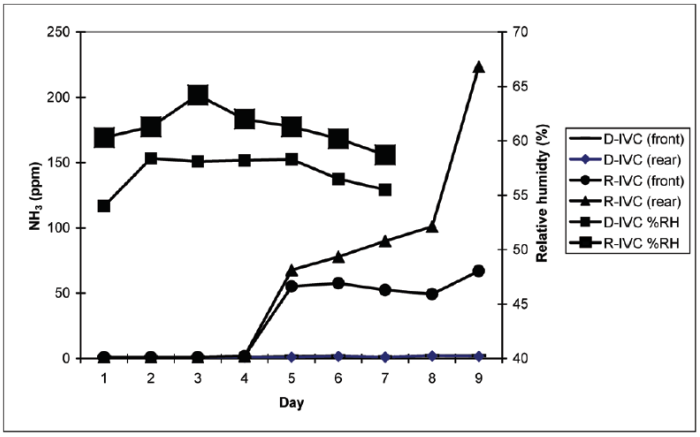

Figure 2. Average daily NH3 concentrations from front and rear sampling ports of 2 disposable (D-IVC) and 3 reusable (R-IVC) ventilated cages and associated average relative humidity (%RH) at the time of sampling.

The mean high and mean low intracage temperature and humidity readings for the entire study (TABLE 1) indicate minimal differences between disposable and reusable IVCs. At the actual time of NH3 sampling, humidity ranged from 54.0% to 58.4% in the disposable IVCs and 58.7% to 64.2% in the reusable cages. For both cage types, at the time of sampling, mean relative humidity peaked near day 3 and then gradually decreased.

DISCUSSION

The primary intent of this study was to evaluate intracage NH_3 and CO_2 levels that developed in a disposable IVC as compared with a reusable IVC that we had used for many years. Specific effects on animal health or behavior were not evaluated. Based on our observations from routine husbandry and the findings of others who used various types of mouse cages to study NH_3 and CO_2,[17,27] our working hypothesis was that concentrations of these gases would not significantly differ between disposable and reusable IVCs. Nevertheless, over the course of the study, we found significantly higher NH_3 concentrations in the reusable IVCs. We also found significant differences in CO_2 concentrations between front and rear sampling ports of the cages, although overall there were no CO_2 concentration differences between the disposable and reusable IVCs. Given that animals in the disposable cages congregated near the rear of their cages and mice in reusable cages congregated near the front of their cages at the time of sampling, it was not surprising that CO_2 concentrations were higher where the animals were located.

CO2. CO_2 concentrations were greater at the end of the cage where animals congregated. At this time, we can only hypothesize why the mice tended to congregate at different ends of the 2 cage types. The reusable IVC used a low-velocity air flow with a perforated metal air inlet located at the back of the cage at the level of the mice. The air is pushed downward and forward toward the front of the cage. For the disposable IVC, the supply air port is on the top of the cage toward the front, and air is exhausted through a port near the top rear of the cage. There is a high-volume, low-pressure air flow (0.2 to 2.5 in. H2O), which is further interrupted by the presence of the food tray. We speculate that these differences may influence preferred areas of animal congregation within the cage. In a study with BALB/c female mice housed in IVCs with 60 ACH, the animals preferred having the air flow enter from the top of the cage.[1]

Table 1. Mean intracage temperature and percentage relative humidity over 7 d in 2 disposable and 3 reusable IVCs.

	Mean low temperature (°C)	Mean high temperature (°C)	Temperature range (°C)	Mean low relative humidity (%)	Mean high relative humidity (%)	Relative humidity range (%)
Disposable, with mice	22.4	25.0	22.0–25.0	50.9	63.0	50.7–64.0
Reusable, with mice	22.1	23.6	22.0–24.4	52.8	70.4	51.0–72.5
Disposable, no mice	20.9	21.7	20.9–21.7	37.5	48.0	36.0–48.0
Reusable, no mice	20.6	21.3	20.6–21.7	38.3	46.4	31.6–50.0

Although considerable differences in methodology (for example, different cages, cage racks, gas analysis methodology, animal strains and stocks) do not allow for detailed comparisons with other studies, the CO_2 levels detected in this study were in general agreement with those found by others using IVCs.[17,21,22,27] Room ventilation rates will affect CO_2 levels in cages, but even with static caging in rooms with 20 ACH, CO_2 concentrations similar to those in the present study have been reported.[20] Further, after 6 d, IVCs at 60 cage ACH demonstrated CO_2 levels similar to those in the present study, although NH_3 levels were lower (approximately 1.2 ppm).[22] Intracage temperature was approximately the same as in the current study, whereas intracage relative humidity was slightly lower. In our study CO_2 levels in the reusable IVCs fell over time, but a reason for this was not apparent in a review of hourly intracage temperature and humidity recordings and animal activity observations made during the sampling times.

The present human occupational exposure limit for CO_2 is 5000 ppm for an 8-h time-weighted average exposure duration,[30] although continuous around-the-clock exposures of 2500 to 5000 ppm may cause headaches.[10] Currently, there are no recommended CO_2 limits for mice. Even though mice are continuously exposed to CO_2 in a cage environment, human limits may not be appropriate for laboratory animals due to evolutionary changes resulting from the adaptation of many rodent species to spending a portion of their lives underground.[13] The authors of the previous study[13] suggested that until further evidence is provided, a CO_2 concentration of 1.5% (15,000 ppm) should be considered the experimental limit, requiring a few days of recovery after exposure. That recommendation may have been based on the work of others who found CO_2 levels as high as 1.4% in artificial rat burrows.[28] More recently, levels greater than 50,000 ppm have been deemed inappropriate for animal welfare.[14] In the present study, the mean daily CO_2 levels ranged from approximately 1250 to 3000 ppm.

NH3. Intracage NH_3 levels are potentially subject to multiple variables, such as the bedding used, cage design, cage ventilation (including air changes, filter cleanliness, and methods used to evacuate cage air), cage and room temperature and humidity, number and size of animals in the cage, cage cleaning frequency, animal health, and so forth. In the present study, we controlled all of these variables except the cage and rack design and function (which were specific to each manufacturer) and found that NH_3 levels in 2 of the 3 reusable IVCs began to increase on day 4 and were greater than 50 ppm by day 5. This rapid rise differs from findings in other studies using different methodology [21,22] and highlights the difficulty in making direct comparisons. Similarly, a study involving rats indicated that bedding type did not have an effect on NH3 concentrations,[4] whereas other experiments[21,23] suggested the opposite conclusion for mice. Intracage NH_3 concentrations for the 2 disposable IVCs with mice never rose above 3.2 ppm.

In both the CO_2 and NH_3 experiments in the current study, intracage temperature remained within the recommended secondary enclosure (that is, room) range of the *Guide for the Care and Use of*

Laboratory Animals.[18] Intracage humidity was almost always within the *Guide's* recommended range (30% to 70%), although a single reading from a reusable cage was just outside of that range (72.5%, Table 1) and returned to an acceptable level in approximately 2 h (data not shown). Each day, at the time of NH3 sampling, the intracage humidity was 3 to 4 percentage points higher in the reusable cages than the disposable ones. Relative humidity is well known to affect NH_3 production,[15,17,23] but whether the somewhat small humidity differences we recorded significantly affected NH_3 production is unclear. Intracage humidity in 4 different types of ventilated cages showed statistically non-significant differences, and the associated NH_3 levels were essentially the same.[17]

In the current study, the mice did not demonstrate clinical abnormalities, although other authors have reported health problems due to high or prolonged NH_3 exposure.[2,4,9,26,29,30] In contrast, no atypical clinical findings were associated with NH_3 levels as high as 140 ppm,[27] and another study reported that NH_3 concentrations as high as 241 ppm had no effect on nasal passage histology.[22] These previous findings are in line with results from another study,[5] which demonstrated no significant clinical problems in rats exposed to levels of NH_3 greater than the maximum in our study. Currently, there are no upper level NH_3 exposure guidelines for mice; for humans, the 8-h time-weighted average exposure limit is 50 ppm.[30]

In summary, our findings indicate that the disposable IVC studied performed satisfactorily under the conditions used. NH_3 levels were equal to or less than 3.2 ppm over the course of 9 d, and CO_2 levels never rose above 0.3% (3000 ppm). Temperature and humidity remained within the secondary enclosure boundaries of the *Guide*.[18] Extrapolation of our results should be made with caution, because published reports indicate extensive variations in findings depending on the methodology used.

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Editor's note: Vendor donation of the ventilated rack and disposable caging represents a potential conflict of interest regarding the data presented in this article. *Corresponding author. Email: jerald.silverman@umassmed.edu

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Innorack® 3.0 Intracage Ammonia (NH_3) Concentration Report

STUDY FIVE

INNOVIVE PERFORMANCE REPORT | 2012

INTRODUCTION

A primary goal of individually ventilated cage (IVC) housing systems is to provide animals with higher quality living conditions than that of the traditional static containment cages. The Innorack® 3.0 ventilation system employs transversal airflow that provides efficient evacuation of moisture and gases from the Innocage®, resulting in a cleaner environment for the mice and more time in between cage change-outs1. This study was performed to evaluate daily intracage ammonia (NH_3) levels produced by mice housed in the Innorack® 3.0 IVC system.

MATERIALS AND METHODS

Test Location. This test was performed at Explora Biolabs in San Diego, CA in a temperature and humidity controlled vivarium. Average temperature was 74˚F and average humidity was 45%.

Equipment. A single–sided, 77-cage position Innorack® 3.0 served as the test IVC rack. The rack was fully populated with cages. Rack airflow was set to 60 ACH @ POS differential pressure. Test cages were modified to include a sampling port located 3.4 inches from the front surface of the cage and 1 inch from the bottom (FIGURE 1). NH_3 measurements were taken with a Sperian Biosystems PHD6™ Gas detector with a PID sensor.

figure 1. Ammonia Test Cage

Study Design. Adult CD-1 male and female mice were housed separately at 1 animal per cage, 3 animals per cage, and 5 animals per cage. Average weight of mice ranged from 36g to 40g. Mice were housed in 40 cages: 10 cages contained 1 animal; 10 cages contained 3 animals; and 20 cages contained 5 animals - placed randomly in the middle of the rack. Each test cage contained 450mL of corn cob bedding, 270g of Harlan Teklad® 18% Protein Rodent Diet, and 275mL of acidified water in polyethylene terephthalate water bottles.

The test was administered for 14 days. Measurements were taken each day between 8:00 AM and 9:00 AM. Prior to collecting data, the PHD6 device underwent a self-test and fresh air calibration. Measurements were then taken by inserting tube of the PHD6 over the test probe of the cage and waiting 30-90 seconds for the NH_3 reading to stabilize. NH_3 recordings were immediately logged after the reading stabilized. During the study, food trays were topped off and water bottles were filled as needed while cage bottoms remained undisturbed. Anytime a cage lid was removed, it was noted on the testing sheet.

DISCUSSION

Mean ammonia levels for 14-day cage change cycle are shown in (FIGURE 2). Under all test conditions, low or undetectable levels of NH_3 were present. Even with the highest density measured, average NH_3 levels were less than 30 ppm. These results indicate a 14-day change out, and in most cases even a longer period, is suitable for all the tested configurations.

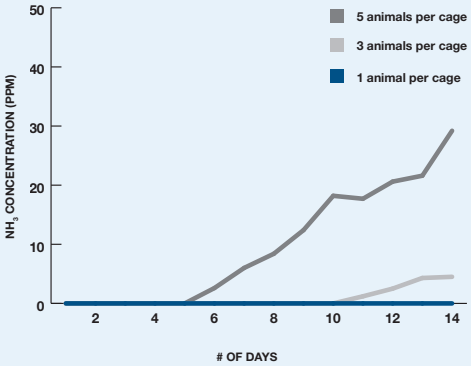

FIGURE 2. MEAN NH_3 LEVELS
The figure shows mean levels of intracage ammonia concentration during a 14-day cage change cycle.

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