Effect of Caging System and Bedding Sterilization on Intra cage Ammonia accumulation with time.

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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.

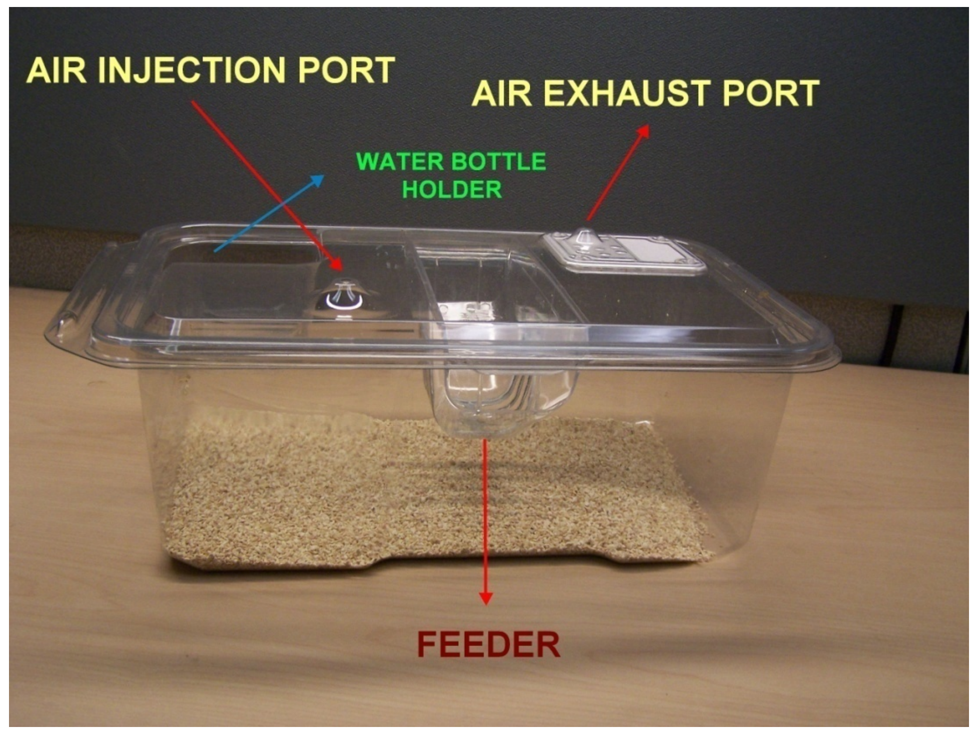
The present study sought to: a) Compare the ammonia removal efficiency of two ventilated cage systems in which the air injection and exhaust ports were either horizontal (CH) or vertical (CV), and b) Determine if using autoclaved bedding (AB) resulted in different intra cage ammonia concentrations than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

Materials

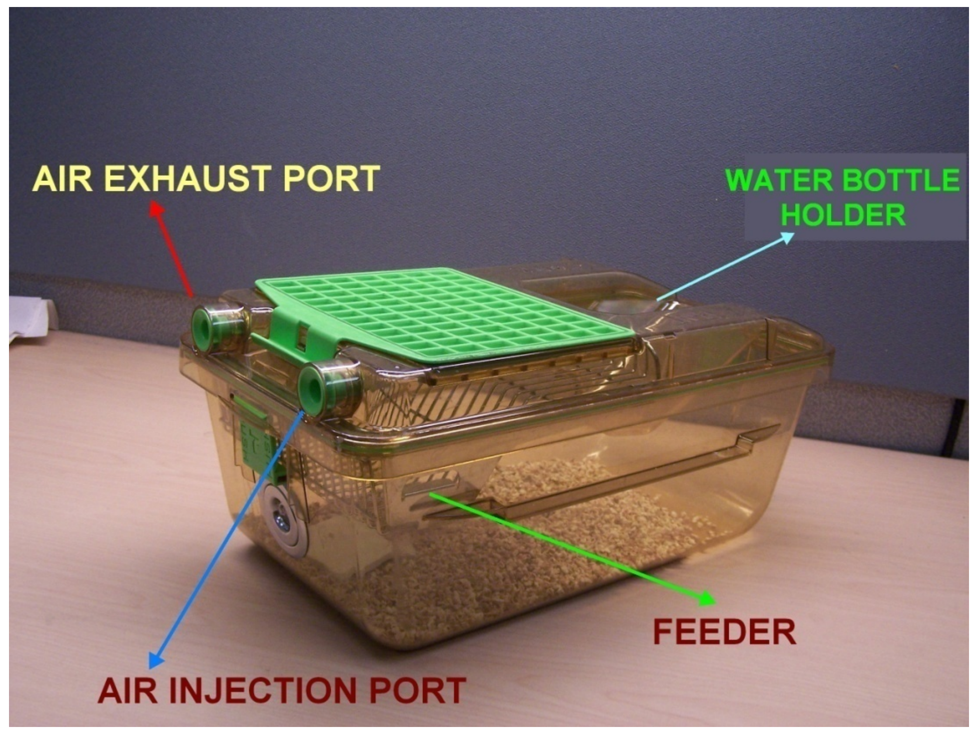
Twenty mouse cages, each containing five CD1 female mice weighing 28-30 g, were allocated to one of four combinations of caging systems and bedding: CV-AB, CV-NAB, CH-AB and CH-NAB, where:

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

CV, pictured below, 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 next column, 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.

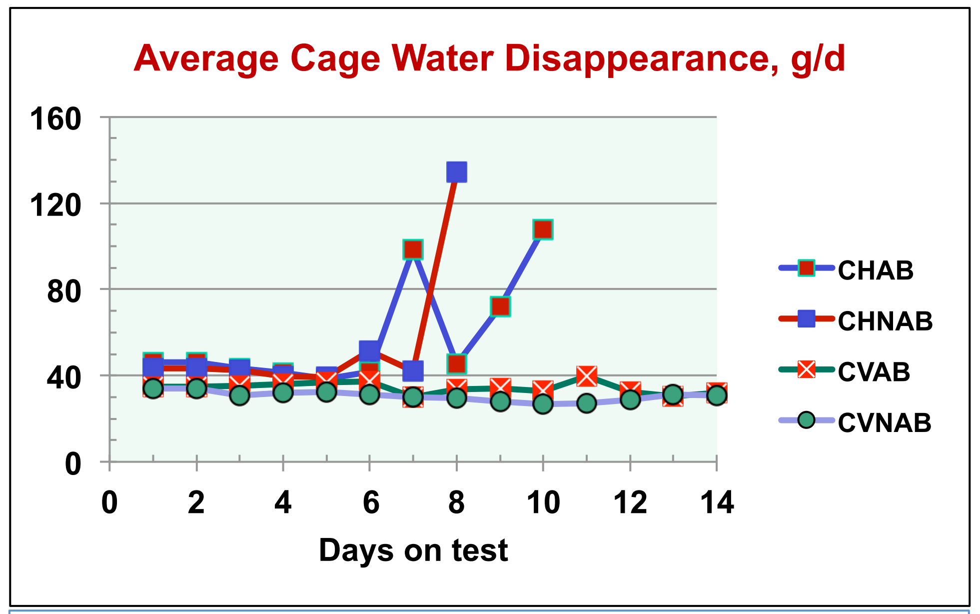
Results

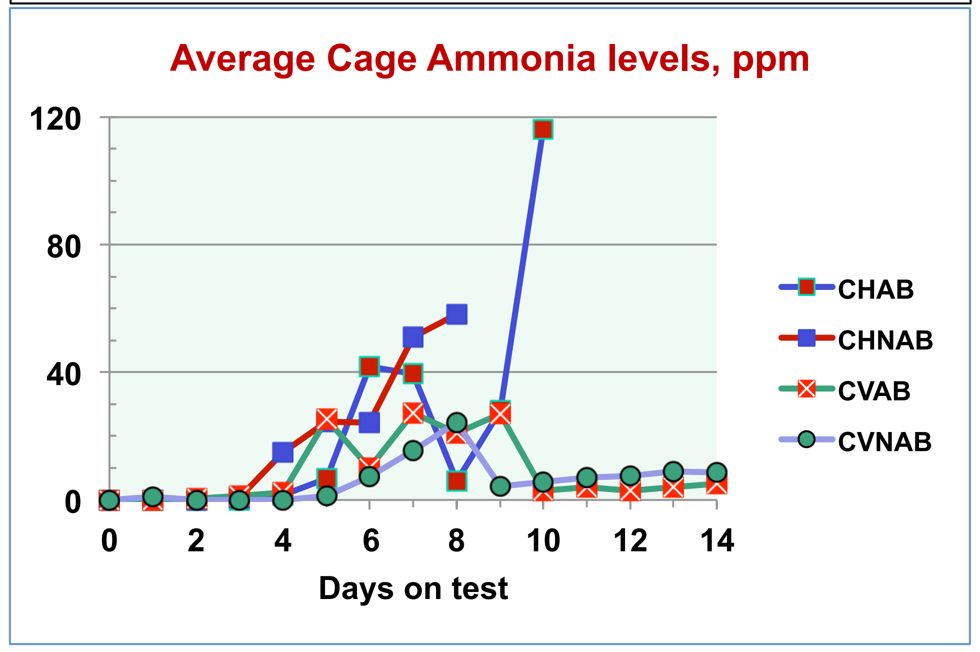
Shown below are averages for main effects in the study:

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 NH3) than CH cages.

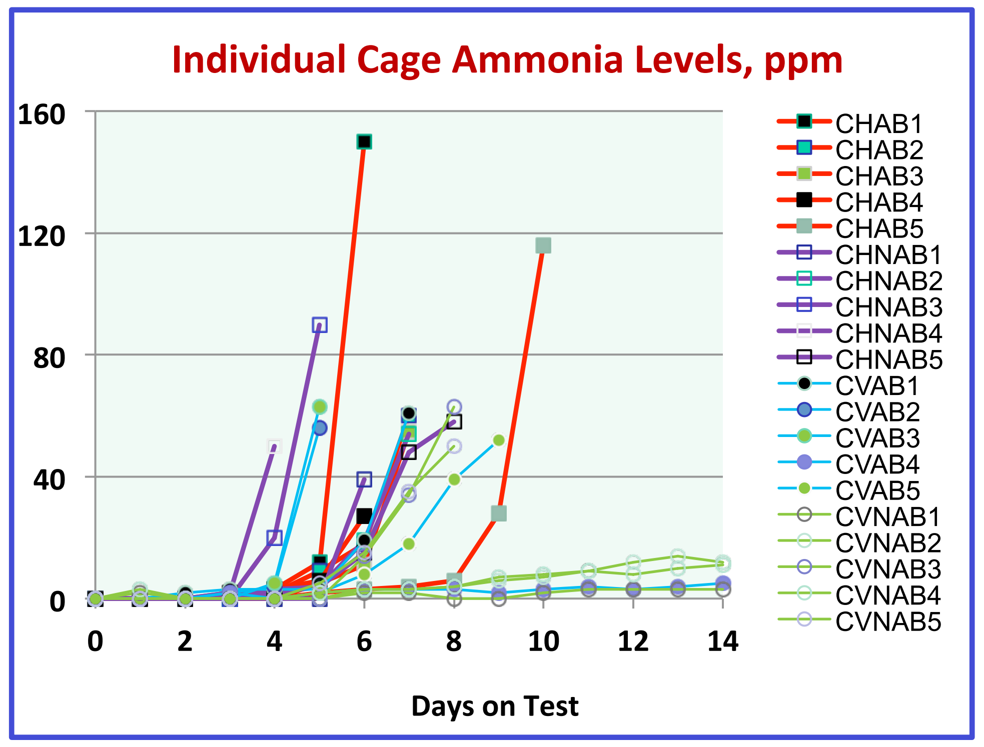
Statistical analysis indicated that the cage ammonia levels were closely related to the rate of water disappearance from the cages. Water disappeared to a much lesser extent from CV cage bottles than from CH cage bottles.





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.

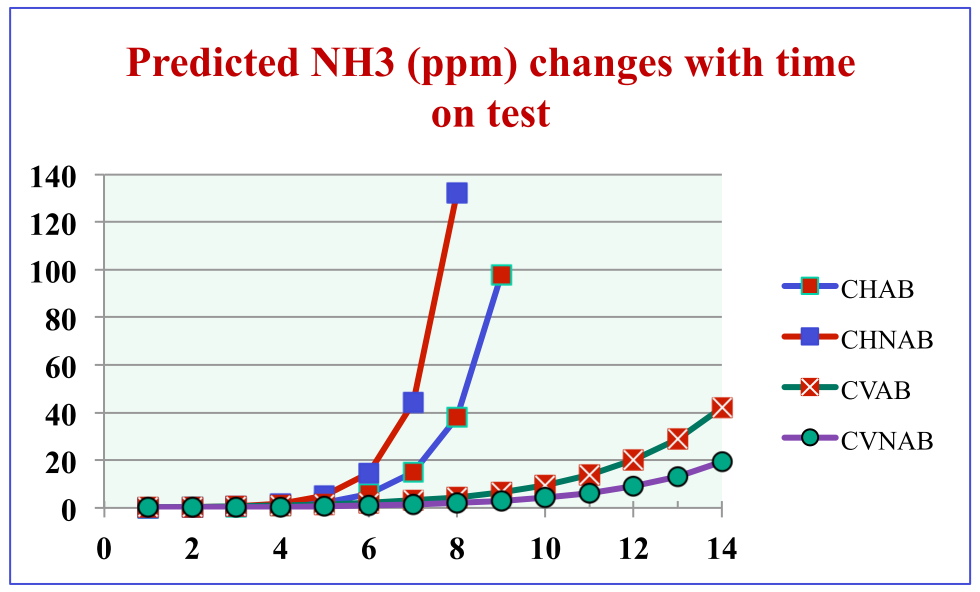
Ammonia Levels:
Average values for the intra-cage ammonia concentration are shown above whereas the individual NH3 readings for each cage are shown below.



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 NH3 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: NH3 production was assumed to change at a rate as that off the bacterial population that produces it; that rate is described by the equation $N^t = N^0 e^{kt}$ where N^0 and N^t are the bacterial population (or NH3 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 NH3 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 NH3 values, and showed different rates of change in NH3 level with time (k) for CH and CV cages. The equations (graphed below) predict that CH cages reach NH3 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 below 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.