Journal of the American Association for Laboratory Animal Science
Copyright 2008
by the American Association for Laboratory Animal Science

Vol 47, No 5
September 2008
Pages 8–9

Letters to the Editor

Dear Dr. Toth,

In the paper entitled "Ammonia and Carbon Dioxide Concentrations In Disposable and Reusable Ventilated Mouse Cages,"[1] the authors compare daily intracage concentrations of ammonia (NH_3) and carbon dioxide (CO_2) in 2 disposable independently ventilated cages (IVCs) with levels found in 3 reusable IVCs. Both types of caging were equipped with sampling ports in the front and back. Samples were collected once daily (at the same time each day), first for determination of NH_3 and then for determination of CO_2. Each of the 5 cages had 5 mice in residence. There were an equal number of unoccupied cages, and sampling was conducted for 9 consecutive days. The authors found differences in the concentrations of NH_3 and CO_2 in the front of cages compared to the back. What I found unusual were the reported levels of NH_3 obtained from reusable IVCs. On day 9 of occupancy, the authors report NH_3 concentrations between 59 to 77 ppm at the front sampling port and greater than 120 ppm at the rear ports of reusable IVCs. Figure 2, illustrating average daily NH_3 levels, depicts concentrations at the front of the reusable cages of around 50 ppm and at the rear port greater than 200 ppm. The authors indicate that the rapid rise in NH_3 occurring in reusable cages after day 5 differs from previous findings. They appropriately identify some of the many variables which can contribute to NH_3 production inside the cage and believe they have controlled all except the cage and rack design and function. Although they did not recognize any clinical manifestations of illness in mice housed under these conditions, they state, "Currently there are no upper level NH_3 exposure guidelines for mice; for humans, the 8-hr time weighted average exposure limit is 50 ppm." The reader is left to wonder if mice held in reusable IVCs for greater than 5 d are exposed to any significant threat due to the NH_3 levels in the cage.

I have several problems with this study that I would like to share. First I believe there was an insufficient number of cages sampled per cage type (given that ammonia was only generated in 2 disposable and 3 reusable cages) to reach statistical significance in any comparison of mean/median NH_3 concentration per cage type. This is important because, the statement on page 60 reads: "when occupied, disposable IVCs had lower NH_3 concentrations than reusable IVCs (P = .0176)", and the Discussion states that "significantly higher NH3 concentrations (were found) in the reusable IVCs". Although numerous observations of the same variable, conducted in the same cage, may demonstrate statistically significant differences over time, the issue of interest is not whether 1 or 2 individual cages showed higher NH_3 concentrations at later sampling times (differences within individual cages) but whether the mean/median NH_3 concentration of 1 cage type (reusable or disposable) differed significantly from the other cage type. The fact that the sample size of the disposable cage type only consisted of 2 cages prevents the calculation of any measure of variability and, therefore, comparisons between cage types is unfeasible. Furthermore, this low sample size precludes determination of whether the data collected at any time is normally distributed, an assumption used in the calculation 274 ppm NH_3 for any level exceeding 150 ppm (instrument sensitivity limit).

Second, although the authors acknowledge that the distribution of NH_3 and CO_2 is spatially non-homogeneous (concentrated in the front of one cage type and the rear of the other), they do not use spatial statistics in their analyses. Third, while they cite a body of work showing different results for intracage NH_3 concentrations in IVCs, they fail to cite one important study that compared IVCs with dimensions similar to those described in their paper.[2] The authors of the study not cited conclude: "there was no significant average increase in ammonia concentration in units 1 and 3, as determined by regression analysis."[2] These IVCs (similar in size to the reusable cages in the study subject to this editorial) had less than 20 ppm NH_3 after 12 d of occupancy. Although it is true that testing conditions differed between the 2 studies (4 mice per cage versus 5 mice per cage in the present study, different bedding, and so forth), the remarkable differences in NH_3 concentration between the 2 studies should give the authors pause.

In the present study the authors detected NH_3 in some unoccupied cages and attributed this to "carry over from the previously sampled cage". They also indicate that on days 5 through 7, one of the reusable IVCs had much less detectable NH_3 than the other 2 cages. Furthermore, they describe differences in the way the mice use the cage; in reusable cages, the mice congregate in the front rather than the back of the cage. I think it reasonable to assume from this that site used for a latrine within the cage may also differ and contribute to the non-homogeneous NH_3 concentrations. Therefore, perhaps not all of the variables contributing to NH_3 production in reusable cages were controlled. Increasing the number of cages studied in both groups (reusable and disposable) should minimize effects associated with sampling technique and animal behavior and may very well lead to a different outcome in terms of NH_3 concentration in reusable IVCs.

Sincerely,
Fred Quimby

Dr. Quimby disclosed that he has purchased equipment from Thoren Inc., Allentown Caging Inc. and Lab Products and he personally knows the owners of all 3 companies. He has given talks in the past that referred to Thoren and Allentown cages. However, he has no financial interest in any of these companies.

References

1. **Silverman J, Bays DW, Cooper SF, Baker SP.** 2008. Ammonia and carbon dioxide concentrations in disposable and reusable ventilated mouse cages. J Am Assoc Lab Anim Sci **47:**57–62.
2. **Perkins SE, Lipman NS.** 1996. Evaluation of micro environmental conditions and noise generation in three individually ventilated rodent caging systems and static isolator cages. Contemp Top Lab Anim Sci **35:**61–65.

Response:

Dear Dr. Toth,

We appreciate the detailed analysis of our paper[1] in the letter to the editor from Dr. Fred Quimby. However, we are not in agreement with the interpretations and conclusions offered.

The writer notes that Figure 2 shows mean NH_3 levels of >200 ppm at the rear sampling ports whereas the text simply states that all NH_3 levels were >120 ppm. As we noted in the text, the upper limit of the detection chip used was 150 ppm. With values outside of the range of detection, it is easy to introduce biases. Our goal was to impute values in those ranges that were reasonable estimates of those values so as to minimize biases. Because the upper end of the range was open ended (>150 ppm) and because the log concentrations approximated a normal distribution, we were able to estimate a point in the open ended interval where the median value or midpoint would be in that interval. Using the midpoint in an interval as an estimate for points in the interval is standard statistical practice with solid theoretical justification. Our assignment of estimated median values based on the normal distribution of log-transformed values was described in the Statistical Methods section.

The writer also states that the sample sizes used prevented us from making a valid determination of the normal distribution of the data and that spatial statistics were not used. Whereas the former issue is a concern with any research, for our study the writer is mistaken because maximum likelihood estimates of all the variance and covariance parameters were readily made by fitting a general linear mixed model to the data from this complex designed experiment. The methodology we used was described in the text, and the normal distribution of the data was noted earlier. Spatial effects were addressed explicitly through main and interaction effects during modeling.

We must also respectfully disagree with the inference that an uncited publication[2] contradicts our findings. That study found that 2 of 3 mouse IVC cage types maintained relatively low levels of NH_3 after 12 days. However, one cage type had a NH_3 level of approximately 125 ppm. In fact, those findings support our statement that significant methodological differences preclude valid inter-study comparisons. The referenced article used different cage racks, different rack air flows, a different NH_3 measurement system, different bedding, automatic watering, a different number of mice with a greatly different biomass, and so forth. Further, for inclusiveness, in our paper we cited studies that reported NH_3 levels lower than ours and other studies where high NH_3 levels were found.

There is also a suggestion that the accumulation of feces and urine in one part of a cage might have resulted in higher levels of ammonia in that area. Such an accumulation is possible, particularly if there are a small number of mice in a cage. Had we found "latrine areas" we would have reported the same. In our study, there were 5 large mice per cage and their frequent digging and scattering of the bedding appears to have nullified this possible effect.

Lastly, the writer states that our study leaves readers wondering about the effects on mice from long term exposure to NH_3 at levels >50 ppm in reusable IVCs. Since we did not study the health effects of either short or long term NH_3 exposure, it would be inappropriate for us to comment on that opinion. However, a previously published study[3] can provide information to interested readers.

Sincerely,
Jerald Silverman,
Stephen P Baker

References

1. **Silverman J, Bays DW, Cooper SF, Baker SP.** 2008. Ammonia and carbon dioxide concentrations in disposable and reusable ventilated mouse cages. J Am Assoc Lab Anim Sci **47:**57–62.
2. **Perkins SE, Lipman NS.** 1996. Evaluation of micro environmental conditions and noise generation in three individually ventilated rodent caging systems and static isolator cages. Contemp Top Lab Anim Sci **35:**61–65.
3. **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.