

## Letter to the Editor

Dear Editors,

The purpose of this letter is to point out several disparities in the article published by Keenan et al. in the September 2022 issue of JAALAS.<sup>1</sup> The results section of the paper is discordant with the data shown in Tables 1 and 2.

The results section states that O<sub>2</sub> levels in cages of group-housed mice under standard temperature conditions with sealed individually ventilated cage (IVC) lids were lower than those with static and unsealed lids. Table 2 shows that O<sub>2</sub> levels in these cages were only lower in sealed cages compared to static cages.

Table 2 also states that O<sub>2</sub> levels were significantly different between unsealed and static cages for group-housed mice in standard temperature conditions, while the results section states that O<sub>2</sub> levels in these cages were not different.

The results section states that in cages with singly housed mice at thermoneutral temperatures had no difference in CO<sub>2</sub> between static and unsealed lids at 1, 4, or 8 h. Table 1 shows that at 4 h, there was a significant difference in CO<sub>2</sub> levels between static and unsealed lids.

Further, the results section states that changes in O<sub>2</sub> and CO<sub>2</sub> were not significantly different over time for single-occupancy cages. However, no statistical analysis was performed to compare changes in CO<sub>2</sub> and O<sub>2</sub> between different time points.

The results section states that O<sub>2</sub> levels for group-housed mice under thermoneutral conditions were significantly lower in IVC with sealed lids compared to IVC with static or unsealed lids and there was no significant difference between sealed and static lids. In contrast, Table 2 shows that there were significant differences between unsealed and static lids and there was no significant difference between sealed and unsealed lids.

Also, the lid type column in Table 2 for thermoneutral conditions is mislabeled. The categories listed are unsealed IVC, ajar IVC, and static; however, the lid categories used throughout the other tables are sealed IVC, unsealed IVC, and static.

Finally, in the results section discussion of microenvironmental conditions of the experimental rat cages, the authors state that 2 pair-housed IVC with exhaust filters removed met study removal criteria at 1h. Later in the paragraph, there is a contradictory statement that IVC with pair housed rats with exhaust filters removed did not meet intervention criteria through the end of the study.

In conclusion, this paper is potentially useful to aid in improving animal welfare and planning for emergencies. However, it is difficult to comprehend the findings of the study as it is currently published due to the discrepancies between the tables and the results section.

Sincerely,

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## References

1. Keenan RA, Rogers RN, Winn CB. 2022. Carbon dioxide, oxygen, and ammonia levels in mouse and rat disposable IVC removed from mechanical ventilation. *J Am Assoc Lab Anim Sci* 61:432–440. <https://doi.org/10.30802/aalas-jaalas-22-000028>.

## Response to Bernardini Letter to the Editor

We appreciate the responder's time to review the results of the paper and, in particular, Tables 1 and 2 regarding the oxygen and carbon dioxide levels in mouse cages thoroughly and providing feedback. We fully support the engagement, review, and constructive feedback from our audience. Indeed, the Tables in this manuscript contain a large amount of data; we wanted to provide the audience with a comprehensive review of the cage conditions over time, if of interest. Of note, there is one discrepancy in the labelling of Table 2 of the "Unsealed IVC" group under the Thermoneutral condition section, and this should instead be labelled as "Sealed IVC," as correctly reflected in the Standard temperature condition section; many of the items pointed out by the writer are resolved by this consideration. However, we assure readers that the information provided in the Tables is accurate and have reviewed point by point the claims in the results compared to the Table as follows:

At 1 h in Table 2 for group-housed mice under standard temperature conditions, it verifies that the mean/SD for group sealed IVC cages was  $17 \pm 0.6\%$ <sup>d,l</sup>. The d and l denote the following:  $P < 0.0001$  significance for lower O<sub>2</sub> levels with sealed IVC lid compared to static lid, and  $P < 0.0001$  for lower O<sub>2</sub> levels with unsealed lid compared to static lid. The same results and similar denotations are included at 4 h and 8 h timepoints in the Table, thus our results are consistent with the Table.

Respectfully, we do not see where O<sub>2</sub> levels are denoted as statistically different between unsealed and static cages for group-housed mice in standard conditions. The statistical analysis for the section mentioned in Table 2 has the following denotations: "b, d, l, l": all illustrate statistical differences between sealed IVC lid and static lid, and not unsealed lids.

Respectfully, the results section does not state that cages of singly housed mice at thermoneutral temperatures had no difference in CO<sub>2</sub> between static and unsealed lids at 1, 4, or 8h. It does however mention under thermoneutral conditions that "CO<sub>2</sub> levels in IVC with sealed lids were significantly higher than those of cages with static or unsealed lids ( $P \leq 0.0001$  and  $P \leq 0.01$  respectively at both 1 and 4 h;  $P < 0.01$  and  $P < 0.05$  at 8 h)..." and that "none of the single-occupancy cages met the criteria for removal from study under thermoneutral conditions."

We would like to clarify that changes in oxygen were compared at 1-, 4-, and 8-h timepoints and changes in carbon dioxide were compared at 1-, 4-, and 8-h timepoints, thus, the results section is correct. The Material & Methods denotes that for the Statistical analysis, data was analyzed using a longitudinal mixed-effects model with a first-order autoregressive covariance

structure to compare the O<sub>2</sub> and CO<sub>2</sub> levels over time. We did not include these results in the Tables specifically, so understand that this may be potentially confusing.

The Results section as stated is accurate. The Table 2 data is accurate, however, there is an error in the labelling of the Lid Type in the Thermoneutral condition section—as correctly reflected in the Standard temperature condition, it should read as “Sealed IVC, Unsealed IVC, then Static” from top to bottom row. Thus, unsealed IVC in Thermoneutral condition is actually Sealed IVC. Thank you for addressing this point.

As stated in the previous comment, we appreciate the reviewer pointing this out—this was missed in the post approval editing process: In the Thermoneutral condition in Table 2,

“Unsealed IVC” should read as ‘Sealed IVC’ information, and Ajar IVC is equivalent to Unsealed IVC (see below).

We would like to clarify that 2 of the pair-housed IVC with exhaust filters removed met study removal criteria at 1 h, as stated. Indeed, the rest of the cages in the cohort did not meet intervention criteria through the end of the study.

Best regards,

Caroline Bodi Winn, DVM, MS, DACLAM

Rose A Keenan, BS, CVT, RLATg

Renee N Rogers, BS, CVT, RLATg

Pfizer, Inc.

TN	Group	Sealed IVC	20.8 ± 0.1	16.9 ± 0.6 **** ##	16.8 ± 0.8	18.3 (1)	18.1 (1) *** ##	0.1 (1)	19.3 (1)	19.2 (1)	19.5 (1) * #
		Ajar IVC	20.8 ± 0.1	19.2 ± 0.9	19.3 ± 0.3	19.9 ± 0.1	20.0 ± 0.2	19.9 ± 0.1	19.7 ± 0.7	20.5 ± 0.5	20.5 ± 0.4
		Static	20.9 ± 0	20.1 ± 0.1	20.3 ± 0.2	20.4 ± 0.1	20.6 ± 0.1	20.6 ± 0.1	20.7 ± 0.1	20.7 ± 0.1	20.6 ± 0
	Single	Sealed IVC	20.9 ± 0	20.3 ± 0.2 *** ##	20.3 ± 0.2	20.4 ± 0.1	20.6 ± 0.1 **** ##	20.6 ± 0	20.6 ± 0.1	20.6 ± 0.1	20.6 ± 0.1 *
		Ajar IVC	20.9 ± 0	20.7 ± 0.1 †	20.7 ± 0.1	20.7 ± 0	20.8 ± 0.1 †	20.8 ± 0.1	20.9 ± 0.2	20.8 ± 0.1	20.9 ± 0.2
		Static	21.1 ± 0	21.0 ± 0.1	21.0 ± 0.1	20.9 ± 0	21.0 ± 0.1	20.9 ± 0.1	21.0 ± 0.1	21.0 ± 0.1	21.0 ± 0.1

The following symbols denote significantly different values at the same time point: \* = sealed IVC lid compared with static lid; # = sealed IVC lid compared with ajar IVC lid; † = ajar IVC lid compared with static lid; for example, \**P* ≤ 0.05; \*\**P* ≤ 0.01; \*\*\**P* ≤ 0.001; \*\*\*\**P* ≤ 0.0001

## Letter to the Editor

Dear Editor,

I am writing with regard to the article published in the November 2022 issue of JAALAS by Kondo et al., “Effect of Bedding Substrates on Blood Glucose and Body Weight in Mice.”<sup>1</sup>

The purpose of this letter is first to commend the authors on their statistical approach and data presentation, and second, to request clarification on the methods and some of the data presented. Third, a typo seems to be present in the reporting of the statistical data on p. 613, as the Chi-square ( $\chi^2$ ) is printed as a box, squared.

I commend the authors for their statistical analysis and using a *P*-value of 0.01 for significance. This approach increases my confidence in the results and suggests a greater likelihood of reproducibility of the results. I would like a clarification on what software was used for the analysis. Both R (GNU General Public License, R Core Team, Vienna, Austria) and Prism (Prism 7, GraphPad Software, La Holla, CA) are mentioned, but it is not clear which software was used for what purpose. In particular, the data was presented as a box-and-whisker plot. Ideally, however, the legend should provide the reference intervals and the meaning of the circles. For example, does the plot show the minimum and maximum values or the 95% confidence intervals for the whisker measurements?

Sincerely,

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## References

1. Kondo SY, Kropik J, Wong MADLY. 2022. Effect of Bedding Substrates on Blood Glucose and Body Weight in Mice. *J Am Assoc Lab Anim Sci* 61:611–614. <https://doi.org/10.30802/aalas-jaalas-22-000047>.

### Letters to the Editor

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