ISSN: 2583-9365

"What a cool facility!!! " - but how do the inhabitants feel?

A Review on thermal settings in laboratory animal facilities based on published data, national and international guidelines- An archetype approach to evaluate and ensure welfare and to maintain validity of animal studies.

Corresponding author:
Harikrishnan VS,
Scientist- E, Division of Laboratory Animal Science,
Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Biomedical Technology Wing, Poojappura,
Thiruvananthapuram- 695002. E-mail:- harikrishnan@sctimst.ac.in

The temperature paradox

"22±2°C" is a figure imprinted hard and deep into the brain of any facility manager since the very early days of his/her career. The value which got imbibed globally, without much ruckus is however, now a "hot" topic after which scientists and veterinarians in the field of basic laboratory animal science are in hot-trail of.

Mice are housed in laboratories at 20–24°C, which is way below their lower critical temperature which is approximately 30°C where the range of their thermoneutral zone (TNZ) is from 26°C-34°C. Hence, housing these animals at lower temperature ranges will increase the thermal stress and has the potential to alter scientific outcomes (Gaskill BN *et al.,* 2012). This is true for rats as well which are housed at 22-24°C; evidently below their lower critical temperature of 26°C. TNZ of pigs weighing from 1 kg to 5 kg on a maintenance diet, housed in groups of 10 pigs per pen, on concrete floor, is 22-32°C and for 40Kg weighing pigs in similar conditions as stated above but for 15 pigs housed per pen is 13-26°C. In simpler terms, the thermal comfort zone of pigs in their various stages of production are 10-21°C or a mature boar, lactating sow and a gestating sow; 24-30°C for a weaner and 32-38°C for a new-born piglet (for which heating up has to be provided) (Stewart and Cabezón, 2016).

The temperature provided for mouse, rat, hamster, gerbil, guinea pig shall be in the range of 20-26°C and for rabbit it is 16-22°C, whereas 16-27°C is the advisory for farm animals and poultry as issued by "*The Guide"* (Guide for the care and use of laboratory animals, 2011). The CPCSEA guidelines stipulates that the temperature maintained shall be 18-29°C for laboratory rodents and rabbits and for large animals, 18-37°C. The New EU Directives (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes) does not make a reference to the temperature range but broadly states that temperature and relative humidity in the holding rooms shall be adapted to the species and age groups housed and the temperature shall be measured and logged on a daily basis in the case of small animals and that the farm species shall not be restricted to outdoor areas under climatic conditions which may cause distress to them. Thus, the DIRECTIVE 2010/63/EU leaves the issue of technical decision making to the scientists and veterinarians who are supposed to justify their selection of the range based on scientific data and are also supposed to address it and update it from time to time, thereby making the system more dynamic. The advisory is similar in the case of light intensities, photo periods and noise levels where the new EU directives gives a broad basis to their recommendations.

This leaves in question, just the two standards viz *The Guide* and the CPCSEA guidelines to be compared. On a careful observation of the cited statements extracted from respective guidelines, and on a comparison note, it can be comprehended that these guidelines differ in their outlooks on the issue. If a comparative study is done with the thermoneutral zone of each species and the recommended room temperature by guidelines for that particular species, it can be seen that The CPCSEA guidelines 2015 offers more closer and more meaningful recommendation for the small laboratory animal species whereas it is *The Guide* which is closer to the recommended TNZ of that of pigs. In the case of rabbits, it is *The Guide* which is closer to the comfort zone of temperature requirement. However, even though the fact shall not be overlooked that the thermal comfort zone differs based on breed of Sheep, the temperature range recommended by *The Guide* is in closer approximation to the comfort zone of the species rather than the recommendation of the CPCSEA guidelines. It is however, impossible to analyse in this paper a multitude of laboratory animal species used, but as an archetype to the conundrum, mice are being focused, as the species single-handedly contribute towards more than 80% of all the laboratory animals used worldwide.

Findings, data and recommendations by peer groups

In general, it is well documented in humans as well as many animal species that as temperature increases, food consumption comes down where as it is reported in mice that, no significant differences in overall food intake could be observed irrespective of a combination of temperatures from 20, 23, 26, 29, 32, or 35°C (Gaskill *et al.,* 2012). This study used C57BL/6NCrl; BALB/cAnNCrl; Crl:CD1 mice of both sexes equally. Further, this study also found that nesting material can significantly combat thermal distress even at 20°C by providing an option to create a microenvironment within the cage by the animals themselves according to their state of activity during the housing. Previously, it was found out that, from a mouse preference test of home cage temperatures of 20°C, 25°C, or 30°C, the C57BL/6J mice during their inactive and maintenance/resting phase of the day preferred 30°C where as these preferences were not pronounced in active mice (Gaskill *et al.,* 2009). This was even more significant in females rather than in males and the study concluded that, in C57BL/6J mice housed at 20–24°C are not being housed based on their comfort which is evident from the preference tests, and is clearly indicative of the fact that the mice preferences alters based on their gender as well as activity levels. Both these studies point towards the fact when we observe it with the background data of the TNZ of mice that, temperatures below 26°C are not going to do any good for them and it also emphasizes on the necessity of supplying nesting material regularly of an adequate weight (say 6-10g; more evidences required).

In another study (Speakman and Keijer, 2012) performed in single housed and in group housed mice, the idea of housing mice at more or less 30°C to best mimic human thermal physiology is challenged. This work is a review and meta-analysis based one where data on lower critical temperatures from more than 17 mice strains and even more studies than the number of strains done by different groups are analysed. Even though this is not a primary study, which compiles data from other studies, the authors claim that the optimal temperature to achieve a comparable human thermal range is in the range from 23 to 25°C for single housed mice, and around 20–22°C for group housed mice. Keijer *et a.l*, 2019 in 10-12 weeks old and 27-30g weighing C57BL/6 "all male mice" study concluded that "We concur with Fisher *et al.* and others that 21°C is too cool, but we continue to suggest that 30°C is too warm. We support this with other data. Finally, to mimic living environments of all humans, and not just those in controlled Western environments, mouse experimentation at various temperatures is likely required. Here, they have findings in contrast with the previous meta-analysis review and in their latest study, it is being found out that 21°C is too cold for these animals and a room temperature of 25-26°C is now being suggested. The basic hypothesis is based on the observation that humans under normal life conditions display energy expenditure values of around 1.6–1.8 times that of the basal metabolic rate and so, the authors points out that the mice should be housed below thermoneutrality at 1.6 times their basal metabolic rate so that they can effectively dissipate their extra heat. Secondly, the authors argue that normal activity generates heat above resting metabolic rates and that, as a consequence, humans (and implicitly all mammals) would prefer exposure to temperatures below thermoneutrality in order to dissipate the extra heat.

Fischer *et al.*, (2018) in his study substantiates with data that, housing of mice within their thermoneutrality zone and concludes that, thermoneutral temperatures remain the preferred method of modeling human conditions for metabolic research – and probably for many other types of medical research. The statement from studies of Speakman and Keijer, 2012 concludes that to mimic, simulate and extrapolate data to suit a global population, experimentation at variable temperature ranges is required is in a big way.

A sample comprehensive literature search

This very limited review of just a single factor viz "room temperature" from a multi-factorial array of factors of housing requirement like relative humidity, air changes, pressure differentials, filtration and particle size specification etc. that too from a multitude of guideline documents and standards the pharma and medical device industries globally base their testing upon, on a single species, the mice. It will be very interesting to populate the entire list of research on laboratory animal housing (here I customised the search on rats, mice, guinea pigs and rabbits for a sample comprehensive search) so far attempted and documented. An advanced search in PubMed using MeSH (Medical Subject Heading) terms and MeSH database were used to combine studies of relevance and to populate work so far published in peer reviewed journals and as books or book chapters. ("Housing, Animal"[Mesh] AND "Rats"[Mesh]) OR ("Mice"[Mesh] AND "Housing, Animal"[Mesh]) was the search string used to construct a "comprehensive search" on 13th December 2019 fetched 2542 papers in PubMed. It has to be kept in mind that, the literature searching is a combination of an art and a science which requires practice, intuition, and trial and error. Extensive searches are required and will consume enormous time for a systematic review to be performed and it involves many databases and a combination of advanced search strategies in order to be methodologically sound. Even if this cannot be considered as an entire historical population of work done in the field, this can be well considered to be a sample cross section of what can be expected from similar searches using multiple databases. This shows the multitude of data hunting attempts in this "hot-topic" and a meta-analysis (not attempted within the scope of this paper) will reveal the results obtained from attempted scientific works. As of now, the preference of mice to remain in hotter temperatures deserves due consideration.

Conclusion

Even if comments are carefully made based on data evolving from studies encompassing the aspects like "of which strain/ stock of mice are we speaking about" or "of which age group" or "of which gender" or "during dark phase or light phase" or "during rest or during activity" or "of which weight range"; welfarists and scientists shall consider the fact that most of the facilities at some point of time will have to house animals in both the sexes, many strains and of all weight ranges and age groups to suit the needs of the end-users. This points towards the requirement to maintain a temperature setting which can hold most of these animals comfortably. This also welcomes scientists to undertake more studies which will have an impact on animal welfare and add strength to the scientific basis behind setting the condition to closely simulate human thermal metabolism so that the pharma research world can extract data which can be extrapolated with maximum fidelity.

References

1. National Research Council. Guide for the care and use of laboratory animals. National Academies Press; 2010 Dec. 27.
2. CPCSEA guidelines, 2015; As extracted from the Compendium of CPCSEA 2018.
3. Directive EU. 63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union. 2010 Sep;276(33).
4. Ecker, E. D., & Skelly, A. C. (2010). Conducting a winning literature search. *Evidence-based Spine-care J.*, *1*(1), 9–14. doi:10.1055/s-0028-1100887
5. Speakman JR, Keijer J. Not so hot: Optimal housing temperatures for mice to mimic the thermal environment of humans. *Mol Metab*. 2012;2(1):5–9. Published 2012 Nov 8. doi:10.1016/j.molmet.2012.10.002
6. Jaap Keijer, Min Li, John R. Speakman. What is the best housing temperature to translate mouse experiments to humans? Molecular Metabolism. Volume 25, July 2019, Pages 168-176.
7. Fischer AW, Cannon B, Nedergaard J. Optimal housing temperatures for mice to mimic the thermal environment of humans: An experimental study. *Mol Metab*. 2018;7:161–170. doi:10.1016/j.molmet.2017.10.009