

## MICE

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These frequently asked questions (FAQs) are intended to assist investigators, instructors, and members of animal care committees in the implementation of the [CCAC guidelines: Mice](#) (CCAC, 2019). FAQs provide general responses to comments and questions received by the CCAC during the external reviews of this guidelines document.

If you do not find the answer to your question here, do not hesitate to [contact the CCAC](#) and we will be pleased to provide assistance.

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**1. How was the minimum floor space of 330 cm<sup>2</sup> determined? Is this floor space sufficient to meet the behavioural needs of mice?**

Guideline 3 states: “Cages should provide at least 330 cm<sup>2</sup> of floor space, and occupancy should be based on the minimum floor space required per animal”. The text below states that there should be at least 100 cm<sup>2</sup> per mouse. It also notes that standard group size is 4-5 mice per cage; therefore in most cases, the cage should be at least 400-500 cm<sup>2</sup>. The 330 cm<sup>2</sup> minimum floor size sets a basic lower limit such that if there are only 1 to 3 mice in a cage, the cage size cannot be based solely on the requirement of 100 cm<sup>2</sup> per mouse.

Empirical data concerning the impact of group size and space allocation is not currently available. Bailoo et al. (2018) showed cages of 370 cm<sup>2</sup> were able to accommodate sufficient resources to meet the basic needs of the mice in their study (although their comparisons did not consider cages smaller than 370 cm<sup>2</sup>).

**2. What is the basis for requiring a cage height of 13 cm from the floor to the lip? Aren't mice able to stand in 11 cm cages?**

A cage height of 13 cm is not a change from the CCAC *Guide to the Care and use of Experimental Animals* (CCAC, 1993) (13 cm for mice < 20 g and 15 cm for mice > 20 g). Height requirements are similar in other jurisdictions (e.g., the requirement for mouse cages in the US is 12.7 cm, and in the EU and Australia it is 12 cm). This is the current standard for most mouse cages.

**3. For metabolic cages, the requirement for a resting platform and the means for mice to establish a microclimate when they are held for more than 24 hours would interfere with measurements (e.g., urine collection) and possibly negate the use of metabolic cages—how can this be addressed?**

As described in the guidelines document, there are several aspects of metabolic cages that can have a negative impact on the welfare of mice, including the requirement for single housing and the barren environment. The 24-hour timeframe is motivation for designing studies that do not require mice to be kept in metabolic cages for more than 24 hours, or including innovative collection methods that accommodate the presence of platforms and other resources (e.g., extracting urine from paper towel).

**4. Are both nesting material and bedding material required for mice? What types of material should be used for each?**

Both nesting material and bedding are important to enable mice to create a comfortable microenvironment and allow them to perform some natural behaviours, such as digging, burrowing, and foraging. However, the specific composition of these two types of materials allows them to serve different functions. Nesting material should be easily manipulated by mice for the construction of nests, while bedding material needs to be able to absorb moisture from urine and feces and control ammonia levels. For example, paper is easily manipulated and can be appropriate for nest construction; however, it quickly becomes wet from urine and is not appropriate for bedding. Additionally, it has been found that mice construct more complex nests when provided with more than one type of nesting material.

## 5. Can male mice be successfully group housed?

Fighting among male mice can lead to wounding and possibly death, and therefore when aggression occurs in group-housed males, it must be addressed immediately. However, aggressive behaviour can sometimes be prevented by improving the housing conditions before males are grouped. The guidelines document outline several approaches to reduce or avoid aggressive behaviour among male mice, such as maintaining stable post-puberty groups; providing sufficient cage resources to limit competition; configuring cages to allow multiple escape routes and avoid sightlines of other males; maintaining optimal room temperature and providing nesting material; limiting disturbance from cage cleaning and maintaining scent markings through the transfer of nesting material; not re-housing males back together after mating; and limiting group size in a standard cage to three animals.

## 6. What are cage dividers and in what situations would they be appropriate?

There are two types of cage dividers:

- partial partitions are dividers within cages that increase cage complexity but still allow opportunity for full contact between mice; and
- grid partitions prevent full contact between mice but do allow some degree of social contact.

Partial partitions within cages can increase the floor area, facilitate activity, and provide escape routes. There is some evidence that the use of partitions may reduce the aggression typically seen in group-housed male mice (Chamove, 1989; Tallent et al., 2018). Grid partitions do not appear to provide the intended welfare benefits, and have been found to be more stressful for mice post-surgery than individual housing (Van Loo et al., 2007).

The use of cage dividers is recognized to be an area that requires more research. When they are used, there should be careful monitoring to ensure they have a positive impact on the welfare of the mice.

## 7. Is toe clipping permitted as a method of identification?

Toe clipping is a highly invasive procedure and is not permitted solely for the purpose of identification. It is only permitted for neonatal mice up to 7 days of age when no other individual identification method is feasible and the tissue collected is also needed for genotyping or other protocol requirements. Justification for using toe clipping as a means of genotyping, rather than less invasive methods, needs to be provided to an animal care committee. Only one toe is to be clipped.

## 8. What basic needs of mice must be addressed, and what forms of environmental enrichment can provide added welfare benefits?

The guidelines state that the basic requirements for mice are group housing, nesting material, and shelters, and sufficient space to accommodate these elements and behaviours important to their welfare.

As stated in the [CCAC guidelines: Husbandry of animals in science](#) (CCAC, 2017), “Measures should be sought to improve the welfare of the animals by enriching their environment in a manner that further addresses their species-specific and individual physical and behavioural needs.” In determining appropriate

forms of enrichment, consideration must be given to the individual animal and the research requirements, including biosecurity issues, and any enrichment provided should be evaluated to ensure it is providing the intended benefit. Some potential forms of enrichment for mice are listed in the guidelines document, and include features to promote activity, substrates for burrowing, structures to facilitate hiding or avoidance, objects to chew, variety in food, and foraging opportunities.

**9. CCAC guidelines documents now put greater emphasis on welfare assessment. What is expected and what tools are available to assist in welfare assessment?**

Welfare assessment has always been a necessary component of animal-based studies, both for ensuring a good quality of life for the animals within the constraints of the study, and for the quality of scientific data. In recent years, there has been more emphasis on this in the literature, and CCAC guidelines documents have responded by drawing more attention to this important aspect of animal-based science and highlighting the development of tools that can inform welfare assessment.

As noted in the [CCAC guidelines: Mice](#) (CCAC, 2019), the investigator, in consultation with the veterinarian, is responsible for planning and documenting welfare assessment; however, the collection of information should be integrated with the activities of all personnel involved with the animals for more efficient use of resources and less disturbance to the animals. The overall focus of any welfare measurement should be to identify the need for mitigation strategies or endpoints, with the aim of improving the welfare of the animals (i.e., monitoring should be conducted to benefit the animals, not for the sake of monitoring).

Section 8, “Welfare Assessment”, in conjunction with Appendix 6, “Indicators that May be Used to Assess the Welfare Of Mice”, provide a number of references to help investigators and animal care committees identify useful indicators. One reference that may be particularly useful is Spangenberg E.M. and Keeling L.J. (2016) [Assessing the welfare of laboratory mice in their home environment using animal-based measures – a benchmarking tool](#). *Laboratory Animals* 50(1):30-38 (supplementary material, accessed on 2019-07-24).

**10. Since there is evidence that isoflurane is aversive to mice, is it still recommended prior to using carbon dioxide for euthanasia?**

The [CCAC guidelines: Mice](#) (CCAC, 2019) note that exposing mice to carbon dioxide for euthanasia is likely to cause pain and distress (Leach et al., 2004). The guidelines document further states that inhalant anesthetics have also been found to be aversive to rodents, but that isoflurane has been shown to be less aversive to mice than CO<sub>2</sub> (Makowska et al., 2009). However, more recent publications show that aversion to isoflurane can increase with repeated exposure (Wong et al., 2012; Moody and Weary, 2014). Isoflurane is recommended for use if it is the animal’s first exposure to it.

The guidelines document acknowledges that there is currently a substantial amount of research being conducted on inhalant techniques for euthanasia, and encourage careful evaluation of any new evidence that becomes available. A number of recent papers in this area are cited in the [CCAC guidelines: Mice](#) (CCAC, 2019).

### 11. If I add a shelf-type structure to my mouse cage, does it count towards the total floor space available to the mice?

The [\*CCAC guidelines: Mice\*](#) (CCAC, 2019) states that cage floors should be at least 330 cm<sup>2</sup> and cage occupancy should be based on the minimum amount of cage floor space required per animal.

Sufficient cage floor space for mice is needed to accommodate the following (Bailoo et al., 2018):

- room for shelter and rest;
- choice for determining thermal comfort;
- opportunities for physically avoiding or being removed from the visual sightline of other mice in the same cage;
- ability to nest on dry bedding; and
- opportunity to perform important behaviours without necessarily touching other animals (e.g., rearing, burrowing, grooming).

The [\*CCAC guidelines: Mice\*](#) (CCAC, 2019) states that each mouse should have 100 cm<sup>2</sup> of space (i.e., a maximum of four mice should be housed in a cage with 400cm<sup>2</sup> floor space and a maximum of five mice should be housed in a cage with 500cm<sup>2</sup>). This is thought to be the minimum amount of space for mice to be able to carry out important behaviours listed above without touching other animals, and is in line with international norms. However, these numbers are not precise as there is limited research on the impact of cage floor space on mouse welfare, though there is evidence that no commercially-available cages are large enough to permit separation of activities deemed important for mouse welfare (Makowska et al., 2019).

**Addition of shelf-type structures may be used to provide additional space to accommodate an additional mouse, providing that there is only a small discrepancy in actual floor space of the cage itself and the space required by the CCAC guidelines.** CCAC guidelines are performance-based, and therefore, some leniency in the actual space allowance for each mouse may be acceptable when justified by the research requirements, provided there is evidence that the animals have sufficient space to carry out the important behaviours noted above. The addition of a shelf-type structure may increase the opportunity for mice to perform these behaviours. This variance is permissible until such time as cages are replaced with cages of the appropriate floor space. It is also important to note that mice can vary in size, and activity levels, and the height and surface area of the shelf must be designed for access and use by the type of mice in the cage. Additionally, larger mice and more active mice may require more space, and in such cases, fewer animals should be housed in the cage.

**Where shelf-type structures are inserted, they must be evaluated to ensure that they contribute to the cage environment, and the behaviour of the animals monitored to ensure that the structures:**

- 1) provide additional opportunity for some of the mouse's needs (mentioned above) to be met;
- 2) encourage positive behaviours such as nest building;
- 3) discourage, or at least not contribute to, negative behaviours such as aggression;
- 4) ensure access to food and water;

- 5) permit escape from dominant individuals; and
- 6) provide sufficient space for at least two mice to occupy the space together.

Where shelf structures are used, the individuals involved in caring for the animals are encouraged to share their practical experience with the CCAC and other users, to assist in providing appropriate guidance.

## References

Bailoo J.D., Murphy E., Varholick J.A., Novak J., Palmer R. and Würbel H. (2018) Evaluation of the effects of space allowance on measures of animal welfare in laboratory mice. *Nature Scientific Reports* 8:713.

Makowska I.J., Franks B., El-Hinn C., Jorgensen T. and Weary D.M. (2019) Standard laboratory housing for mice restricts their ability to segregate space into clean and dirty areas. *Scientific Reports* 9(1):6179.

## 12. What are the implementation timelines for the new housing requirements?

The CCAC has determined that all existing, non-compliant mouse cages within certified institutions must be replaced by the following schedule: 33% by April 21, 2025, 66% by April 21, 2028, and 100% by April 21, 2031. This decision is based on fairness, consistency, animal welfare considerations, and current supply of larger cages. Please note that all newly acquired mouse cages must meet the housing guidelines described in the [CCAC guidelines: Mice](#) (2019). Moving forward, the CCAC will actively seek updates from program participants on their progress toward these goals during assessment visits and communications.