

# OVERCROWDED MOUSE CAGE GUIDELINES

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## Background

According to the *Guide for the Care and Use of Laboratory Animals (Guide)*, up to five adult mice may be housed in either static or individually ventilated mouse cages ( $\geq 15$  square inches of floor space per animal). For breeding purposes, the size of the standard mouse cage is designed to accommodate one adult female mouse, one adult male mouse, and their litter (51 square inches of floor space). Mice 21 days and older are considered to be adults and thus each individual counts as 1 mouse unless justified in the Animal Use Protocol (AUP) and approved by the Animal Care and Use Committee (ACUC). Animal overcrowding compromises humane animal care and goes against the principles of the *Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals*.

## Purpose

These guidelines were established to assist Principal Investigators (PIs), their staff, and personnel from the Office of Laboratory Animal Care (OLAC) in identifying an overcrowded mouse cage. Additionally, these guidelines will define how overcrowded cages will be identified and how incidents of overcrowding will be rectified.

## Definitions

1. **Overcrowded Cage**
  - a. Greater than 5 adult (post-weaning) mice of the same sex in a standard mouse cage
  - b. Any cage with two litters more than 7 days apart (i.e., a litter of newborn pups with a second older litter that is 7 days disparate in age)
  - c. Trio breeding cages containing  $> 12$  pups
2. **Immediate Separation:** Disparate litters in which all or some of the newborn pups are dead will be separated as soon as they are identified.

## Procedures

1. OLAC staff monitors animal health daily (e.g., food, water, health concerns) but is not responsible for the management of rodent breeding colonies. When overcrowded cages are identified by OLAC staff during morning health checks, a tag will be placed on those cages at the time that they are identified. These tags will have the date and the

- initials of the OLAC staff member who tagged the cage.
- a. Any tagged cages that have **not** been addressed by the associated PI lab by 8 am on the following workday will be separated by OLAC staff.
  - b. A per-cage charge will be levied for each cage required to house animals at appropriate densities.
  - c. Exception: Immediate separations. See definition above.
2. Lab members hold the primary responsibility for checking for pregnancies and births and for recording these events on the appropriate cage card(s). When a litter is born, the date of birth (DOB) and projected weaning date **must be documented on the card for that cage.**
- a. OLAC personnel are not responsible for contacting lab members to inform them of new litters or the need to wean a litter.
  - b. The date of birth for a new litter may be recorded on the cage card by OLAC staff, but this would be done as an aid to the investigator, **not** as a required OLAC staff duty.
3. Weaning of litters must be performed by 21 days of age. The day of birth is counted as day zero. OLAC staff will tag cages with 21-day-old pups during morning health rounds and the animals will be separated the following day as outlined above in section 1a.
- a. Cages that do not have birth dates noted on the cage card may be tagged as overcrowded and animals may be weaned prematurely based on visual assessment made by OLAC staff.
  - b. OLAC facility supervisors and veterinary staff can approve an immediate separation if the litters are disparate. See definition above.
4. When a new litter is present, cage changing will be delayed no more than 3 days.
- a. Cages for delayed change must be clearly identified with a “new litter” tag with the date and responsible person’s initials.
  - b. Cages will be changed by the lab within the 3 days.
  - c. Cages will be changed regardless of litter age if a cage is flooded or overly soiled. The determination to clean that cage will be made by the OLAC area supervisor based on animal welfare concerns.
5. Trio breeding (two females and one male per cage) **must be** described and justified in the PI’s approved AUP.
- a. When specifically outlined and approved in the AUP, trio-breeding groups and their non-disparate litters of no more than 12 pups may remain in the same cage until weaning by 21 days of age. Pups will not be counted individually until 7 days of age.
  - b. To avoid overcrowding, lab members are strongly encouraged to genotype pups as early as possible and cull those that do not meet the desired genetic profile.
  - c. To avoid disparate litters, a visibly pregnant dam should be separated to her own

- cage prior to giving birth. If they are not separated to their own cage, once the litter of pups reaches 7 days of age, this litter and dam must be separated to their own cage to avoid the possibility of harm to a younger litter.
- d. All of above guidelines and procedures regarding overcrowded cages must be followed.
6. Harem breeding (up to four females and one male per cage) **must be** described and justified in the PI's approved AUP.
    - a. To avoid multiple litters per harem cage, any visibly pregnant dam **must** be moved to her own cage prior to giving birth.
    - b. All of above guidelines and procedures regarding overcrowded cages must be followed.
  7. The need to wean any mice later than the 21-day standard **must be** described and justified in the PI's approved AUP.
    - a. Approval will be based on data documenting smaller than standard pup size despite food supplementation at 14-21 days.
    - a. Breeding males **must** be removed from the cage so that the post-partum estrus does not result in additional pregnancies.
    - b. Allowing a 3-week-old litter to stay in the cage with a lactating female who also has a newborn litter is **not** permitted.
    - c. Investigators should submit a "Request for Special Services" on the OLAC website to ensure that OLAC staff is aware of this deviation from standard mouse husbandry.
  8. Investigators may request additional training and breeding colony consultations via the OLAC Training Coordinator and Veterinary Staff.

## References

- Bayne K and Turner PV eds. Laboratory Animal Welfare. 2013. Chapter 12, Rodent and Rabbit Welfare in the Research Environment. Pp 184-185
- Office of Laboratory Animal Welfare. (2002). [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#).
- UC Berkeley ACUC Guideline on [Cage Card Notification System Guidelines](#)
- UC Berkeley OLAC Guideline on [Quick Reference: Improving Mouse Breeding Success](#)