

University of Colorado Boulder
Office of Research Integrity
Institutional Animal Care and Use Committee
SOP # 28
Overcrowding and Breeding Policy

Background

This policy addresses overcrowding of rodent cages as required by the *Guide for the Care and Use of Laboratory Animals (8th Edition)*, AAALAC, and federal regulations. Overcrowding can compromise the health and well-being of the animals. A cage is overcrowded when the number allowed per cage or the weight of the animals in the cage exceeds the described maximum criteria. The only exception is when an investigator has received IACUC approval, based on scientific justification, to exceed the permitted numbers per cage. For any exception to the IACUC policy, it is the responsibility of the researchers to inform the Office of Animal Resources (OAR).

Responsibility

Principal Investigators (PI) and their staff are responsible for ensuring that the number of animals per cage and breeding schemes comply with this policy. Principal Investigators are responsible for ensuring that their personnel are appropriately trained to manage their breeding colonies. Animal care technicians are responsible for monitoring the number of animals per cage during routine cage checks.

Training

If you have any questions about this policy or would like to be trained on how to appropriately implement this policy in your colony, contact the Veterinary Staff to receive training.

Requirements and Procedures

Non-breeding Cages (see page 57 of the *Guide for the Care and Use of Laboratory Animals 8th Edition*)

Mouse

Cage Size (≈ square inches)	Maximum Number of Adult Mice
75	5
150	10

Rat

Cage Size (≈ square inches)	Maximum Number of Adult Rats by Individual Body Weight
175-200	<300 g → 5 rats 300 - 400 g → 4 rats >400 g → 3 rats
250	<400 g → 5 rats 400 - 500 g → 4 rats >500 g → 3 rats

OAR Protocol for Overcrowded Cages (see the Guide’s discussion of overcrowding and space allowance on pages 55, 56, and 57):

- Animal technicians will identify an overcrowded cage by placing an “Overcrowded Notice” on the cage, including the date and time of “Overcrowded Notice” card placement. The technician will notify the PI and the contact person (if listed) via e-mail.
- Separation of overcrowded adults must occur within 24 business hours of initial notification.
- If separation is not completed within 24 hours of notification that a cage is overcrowded, animal technicians will separate the animals. A \$25 fee per cage needing separation and technician time will be charged atop that.
- Any cage that is severely overcrowded (>150% maximum) and an immediate animal welfare concern will be reported to the veterinarian and the PI/contact via phone. If the researcher is unable to be contacted, the animals may be separated into different cages immediately at the discretion of the veterinarian and the PI will be charged, per above (\$25 fee per cage plus technician time).

Information on Breeding Cages

The following breeding schemes are allowed with the applicable criteria. **A breeding scheme must be described in a protocol and approved by the IACUC before breeding takes place. If the breeding scheme is not described in as much detail in your protocol as outlined in this SOP, please submit an addendum to your protocol to clarify your breeding scheme.**

Monogamous Pair Breeding - one male and one female

- Advantages: **a)** Preferred method to prevent overcrowding, **b)** Offers an extended nursing time for inbred strains where pups are known to be small and slow growing, **c)** Maximizes reproductive productivity of females by utilizing post-partum estrus
- Males may be continuously housed with females provided that there are never multiple litters in a cage. The researcher must actively manage the colony to monitor for pregnancy and wean a litter prior to the birth of a new litter. If a new litter is born and the older litter is not yet weaned, the animal care staff will perform an emergency wean (weaned upon discovery of the cage). The PI will be charged a \$25 fee per cage needing immediate separation and technician time.

Triad Breeding - one male and two females

- A visible "Triad Breeding Card" must be placed on the cage if this breeding scheme is taking place.
- The first observation date must be documented 14 days after initiating the triad breeding scheme. If a female is visually identified as pregnant, she must be separated into her own cage. The animals can remain in triad breeding until pregnancy is visually confirmed. Checking for female pregnancy must occur every 7 days (at a minimum), and those dates of observation must be written on the triad breeding card.
- If triad breeding is set up without a properly labeled card, OAR will flag the cage, the PI will be contacted, and the protocol status will be evaluated at that time by the Attending Veterinarian or the IACUC. OAR can either assist with correctly labeling the cage, or we can ask the PI to separate the animals and amend their protocol. If there is not an appropriate response (separation of a triad if necessary), the OAR will separate the cage and charge accordingly.
- *Large cages* (150 square inches) – Triad breeding can be performed in large cages without separating animals such that the maximum number in a cage is 1 male, 2 females, and 2 litters. Each female may still only have 1 litter (multiple litters from the same female are not allowed).

Other Breeding Scheme

- Other breeding schemes other than those listed above must be described in an animal protocol or addendum, and a system must be put in place for flagging those cages so they are not mistakenly separated (like for triad breeding).

Information on Weaning (for rats and mice, indicated here as "rodents")

- Accurate weaning requires the accurate recording of date of birth. The first person to discover a litter is responsible for recording the date of birth and range of weaning.
- Rodents are generally not able to be weaned prior to day 18, and are generally weaned between 21 and 28 days of age depending on strain.
- If rodents require delayed weaning past day 28, a veterinary exemption is required.
 - A veterinary exemption for delayed weaning is a case-by-case situation, as this is considered an animal health issue. The veterinarian should be called around day 26-28 (prior to day 29) and asked to evaluate the animals for being too small to wean. If the vet agrees, this would be noted on the Wean Card that is on the cage. The vet and the PI should keep track of the number of animals of each strain where this type of exemption needs to occur. If an exemption is happening frequently, then it would be recommended that the PI submit an addendum to the IACUC formally asking to extend the wean range to a specific length and include the data that have been collected as the scientific justification. After the addendum is approved, the protocol has a formal exemption associated with it and they do not have to continue to seek vet approval to wait longer to wean the specific animal strain.
 - **IMPORTANT NOTE:** Contact the veterinarian if you see any health issues with offspring such as repeatedly small litter sizes or if you would like to request a veterinary exemption for any other reason than already described in this policy to go past day 28.
- Rodents that are not weaned by day 29, and do not have a veterinary exemption, will be separated on day 29 by animal care technicians and the PI will be charged \$25 per cage and technician time.

Exceptions: Exceptions to the policy can be made on an individual protocol basis with adequate scientific justification. For example, a strain with poor maternal care may do better with two females in a cage to communally raise pups.

Implementation: Charging for overcrowded cages will not begin immediately. OAR will notify the research community when charging for any service in this SOP will begin.