

TITLE: Overcrowding and Breeding

PURPOSE: Standardization of IACUC SOP Procedures

RESPONSIBILITY: Principle Investigators

REVIEW/REVISIONS: IACUC/OAR will review and revise this SOP as needed. Implementation will proceed upon approval of the IACUC Committee.

BACKGROUND:

This SOP addresses overcrowding of rodent cages as required by the *Guide for the Care and Use of Laboratory Animals 8th Edition (Guide)*, AAALAC, and federal regulations. Overcrowding can compromise the health and well-being of the animals. A cage is over-crowded when the number allowed per cage or the weight of the animals in the cage exceeds the maximum criteria as stated in the *Guide*. Principal Investigators (PIs) may request IACUC approval to exceed the permitted number per cage based on scientific justification and must inform the Office of Animal Resources (OAR) of any such exceptions.

RESPONSIBILITY:

PIs 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 and may take action to correct non-compliances as described below. Contact the Veterinary Staff with questions or to receive training on implementing this SOP for your colony.

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 Adults
75	5
150	10

Rat

Cage Size (≈ square inches)	Maximum Number of Adults by Individual Body Weight
139	< 100 g → 8 rats Up to 200 g → 6 rats Up to 300 g → 4 rats Up to 400 g → 3 rats Up to 500 g → 2 rats > 500 g → 1 rat

OAR Protocol for Overcrowded Cages (see the *Guide's* discussion of overcrowding and space allowance):

- Animal technicians will identify an overcrowded cage by placing an “Overcrowded Notice” on the cage, including the date and time of the notice.
- Separation of overcrowded adults must be completed by the morning following the cage flag placement.
- If separation is not completed, animal technicians will separate the animals. OAR will charge for the technician time and a fee of \$25 per cage.
- A cage that is severely overcrowded or if the new litter’s survival is being jeopardized by the older litter (trampled) presents an immediate animal welfare concern and 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 \$25 per cage plus technician time.

Breeding Cages

Breeding schemes 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. The following breeding schemes are allowed with the applicable criteria.

Monogamous Pair Breeding - one male and one female

- This is the preferred method to prevent overcrowding, because it offers extended nursing time for inbred strains where pups are known to be small and slow growing, and 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 to a new cage, the animal care staff will flag the cage and perform an emergency wean the following morning if still present. The PI will be charged the technician time and a \$25 fee per cage needing immediate separation.

Triad Breeding - one male and two females

- This method requires a visible “Triad Breeding Card” on the cage.
- Females must be observed 14 days after initiating the triad breeding scheme and if one is visually identified as pregnant, she must be separated into her own cage. The dates of observation must be written on the triad breeding card. The animals can remain in triad breeding until pregnancy is visually confirmed. Checking for female pregnancy must occur a minimum of every 7 days.
- The OAR staff will flag triad breeding cages that are not properly labeled. The Attending Veterinarian or the IACUC may be contacted to evaluate the protocol status. The OAR staff will either correctly label the cage, or ask the PI to separate the animals and amend their protocol. If there is not an appropriate response (separation of a triad if necessary), OAR will separate the cage and charge accordingly.
- Triad breeding can be performed in large cages (150 square inches) 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

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

Information on Weaning

- 3 Day Rule- To minimize cannibalism, abandonment and trampling of pups, cages should not be changed for 3 days after pup birth. If cages must be changed prior to this time, dirty bedding and the old nestlet should be transferred to the new cage to minimize loss of pheromones.
- 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.
- A few kibbles of feed may be placed on the floor of the cage when pups are around 14 days of age. This will decrease nursing, remove strain on the female, and introduce the pups to mouse chow so when they are weaned they will be used to eating it.
- A veterinary exemption for delayed weaning past day 28 is determined on a case-by-case basis, 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 veterinarian agrees, this would be noted on the Wean Card that is on the cage. The veterinarian and the PI should keep track of the number of animals of each strain that requires this type of exemption. If an exemption becomes frequent, the PI should submit an addendum to the IACUC formally asking to extend the wean range to a specific length and include the collected data as scientific justification. With an approved addendum, the PI will not have to continue to seek a veterinary exemption for delayed weaning periods for specific strains.
- 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 SOP to go past day 28.
- Rodents that are not weaned by day 28, 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.

Approved: 11/12/14

Revised: 4/19/17