IACUC Policy on Mouse Breeding and Cage Density

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Background

The purpose of this policy is to address the health and well-being of mice by ensuring safe breeding schemes and population densities. Overcrowding can be a significant animal welfare issue and is in direct violation of SDSU policies as well as *The Guide for the Care and Use of Laboratory Animals*. In keeping with the SDSU PHS Assurance, the SDSU IACUC adopts the breeding and cage density recommendations and procedures outlined in *The Guide* as policy. These recommendations limit mouse breeding density to one lactating female and litter per 51 square inches. Genetically modified animals (GMAs) represent an increasingly large proportion of animals used in research and require special consideration in their population management. Breeding, especially GMAs, takes proper management and record keeping. The principles of reduction (3 Rs) should be utilized in breeding to minimize the generation of animals that are not needed.

Reference: Guide for the Care and Use of Laboratory Animals, 8th edition, pages 56-57, 75-77, 107.

Definitions and Abbreviations

a. **PI -** Principal Investigator
b. **IACUC -** Institutional Animal Care and Use Committee
c. **OLAC -** Office of Laboratory Animal Care
d. **APF -** Animal Protocol Form
e. ***The Guide*** **-** Guide for the Care and Use of Laboratory Animals, 8th Edition
f. **Dam -** female parent of a litter
g. **Sire -** male parent of a litter
h. **Pup -** Neonatal rodents up to 21 days of age; a group of pups from the same mother is called a **litter**.
i. **Weanling -** Young rodent recently separated from its mother; usually 21 - 28 days old.
j. **Weaning -** The moment pups are transferred out of the mothers' cage.
k. **Overcrowding -** More than five (5) mice per cage that are >/= 30g, a litter over 28 days of age, or two litters of any age.
l. **Post-partum estrus -** Female rodents may undergo a fertile estrus 14-24 hours following parturition. Females that are housed with males when they give birth can therefore become pregnant with a second litter that would be delivered around the time of the weaning date of the first litter. This can lead to overcrowding if the weanlings are not separated at 21 days of age.

Responsibilities

PI's are responsible for colony management and ensuring adherence to SDSU policies. PI's must ensure colony management is provided by someone who has received specific training on managing mouse breeding colonies. OLAC will provide initial breeding colony training to lab personnel identified as working with the breeding colony. The PI is responsible for ensuring proper cage card documentation, and for separating and weaning according to the guidelines below. In the event of overcrowding that has not been corrected in a timely manner, OLAC is responsible for separating animals, and the PI may incur OLAC Technician Time fees. PI's may request that OLAC perform breeding colony services in advance, such as weaning, IDing, tailing, etc., on a fee for service basis. Inquiries should be directed to the OLAC Manager. OLAC provides daily breeding colony assistance as part of regular health and husbandry rounds, which may vary depending on the breeding scheme used. Check with the OLAC Manager for current Breeding Colony Assistance SOP(s). Any recurring problems with mouse breeding colony management, such as repeated overcrowding, repeated neonatal mortality due to overcrowding or use of inappropriate dams/sires, etc. will be brought to the attention of the IACUC for corrective action, up to and including the IACUC requiring that the PI use OLAC breeding colony services for any given length of time. Breeding must be adequately described in the PI's IACUC approved APF.

Breeding Schemes that May be Used:

1. Monogamous Interrupted: *Male is removed **BEFORE** the litter is born*
One male and one female are housed together for mating. When the female is noticeably pregnant the male is removed from the cage. This is a preferred method to prevent overcrowding and allows for the litter to remain with the dam for up to 28 days which may benefit some strains that have consistently smaller pups.

2. Monogamous Continuous
One male and one female are housed together for mating. This model takes advantage of postpartum estrus and allows the female to become pregnant and nurse at the same time. Litters are born approximately 21 days apart. Pups must be weaned by 21 days of age (P21), or the same day a second litter is born if the second litter is born early. This method may be preferred if the strain has a shortened breeding life or if there are few females of the genotype needed to produce experimental animals.

3. Polygamous
This method houses two (trio) or three (harem) females in a cage with one male. Visibly pregnant female(s) must be removed and placed in individual cages before they give birth. The male may be kept with one of the females to take advantage of postpartum estrus. Only one nursing female and litter is allowed per cage. This method allows for the production of a larger number of experimental animals that will be approximately the same age.

Weaning

Litters should be weaned between P18 - P28. Some robust strains may be able to be weaned as early as P18, while strains that produce smaller pups may need more time with the dam. All litters must be weaned by P28. Overdue weaning will be corrected by OLAC and the PI will be charged a Technician Time fee (see OLAC's Breeding Colony Assistance SOP(s) for more information).

The act of weaning is separating the litter from the dam and setting up the weanlings in their "adult" cages. Generally pups should be separated by sex at the time of weaning. Pups weaned as early as P18 may benefit from being weaned as one litter into a single cage, however they must be separated by sex into separate cages by P28. It is recommended that the sex of the pups be verified about a week after weaning.

Exceptions

If a litter cannot be weaned by P28 or weaned prior to a second litter being born due to health reasons (runting, etc.), the PI or designee, should notify OLAC and Veterinary staff via email to get approval for delayed weaning. OLAC and/or Veterinary Staff will observe the animals, offer supplemental care if needed, and notify the lab when the pups are ready to be weaned. For strains that consistently require delayed weaning a continuous interrupted or polygamous breeding scheme may be required and consistent delayed weaning past P28 would require an exception to the IACUC policy to be added to the APF. Certain strains may have small litters or do not lactate well so it may be beneficial to house two lactating females together in one cage so they can raise their litters cooperatively which would also require an exception be added to the APF. Veterinary exceptions do not need prior IACUC approval. All other exceptions to this policy must be requested in the IACUC APF and approved prior to use. The request should include justification (scientific, via a performance standard) for extended weaning and should include appropriate documentation (i.e. literature, breeding records, etc.)

Required Documentation and Cage Cards

Animal production records must be maintained. It is the PI's responsibility to accurately report animal numbers to the IACUC annually, including all animals born regardless of if they were weaned or not. Appropriate breeding records and pedigree records should be maintained. Please contact OLAC Manager if you need assistance with animal inventory and breeding log templates or examples.

All breeding cages should have a stock cage card complete with the male's information and a breeder cage card assigned to each female in the cage. The breeder cage card should stay with the female for the life of the animal.

Breeding cage cards **must** include the following information:
- Principal Investigator (last name)
- IACUC protocol #
- Strain or stock and specific GEM or mutant nomenclature
- Date of birth for dam and sire
- Paired or mated date
- Date breeders were separated (if using interrupted breeding)
- # of pups born
- Date weaned