Subject: Rodent Breeding and Weaning

BACKGROUND

The Guide for the Care and Use of Laboratory Animals (the Guide, NRC 2011) lists the minimum space recommendations for laboratory animals. Some of the issues addressed are as follows: Singly-housed animals may require more space per animal than that recommended for group-housed animals, while larger groups may be housed at slightly higher densities. When group housing animals, it is important to take into account population density and ability to disperse, initial familiarity among animals, age, sex, and social rank. Population density can affect reproduction, metabolism, immune responses and behavior. Group composition should be held as stable as possible as mixing groups or introducing new members can alter behavioral and physiological functions. Larger animals may require more space to meet the performance standards.

IACUC Policy

Compliance with these guidelines is required unless written exception has been approved by the Wayne State University Institutional Animal Care and Use Committee (IACUC) on the grounds of accomplishing scientific objectives. Divergences from this policy should be discussed with the veterinary staff.

Mice are routinely housed in two different size microisolator cages (ventilated or static)
- Group I: 60-81 in² floor space
- Group II: 140-164 in² floor space

Rats are routinely housed in cages with 144 in² floor space

Breeding Mice

Breeding pair (1 male:1 female):
The male, female and the resulting litter of pups may be kept together in a Group I cage continuously until the pups are weaned.

Breeding trio (1 male: 2 females):
Mice should be kept in a Group II microisolator cage.

If the trio is in a Group I cage, one of the following options must occur:
- The trio is placed into a Group II cage when one female is observed to be pregnant (at day 18 if conception date is known), which helps to promote compliance with housing standards throughout the period of weaning.
- One of the two females is removed to a separate Group I cage when observed to be pregnant (at day 18 if conception date is known), which helps to promote compliance with housing standards throughout the period of weaning and assists with pedigree documentation.

Other breeding schemes:
- Harem ratios of 1:3 are not recommended; however when implemented the mice must be in a Group II cage. Pregnant females must be moved to another cage to maintain a ratio of 1:1 or 1:2, as described above.
- No breeding ratios other than 1 male to 1/2/3 females are allowed.
- No more than two visibly pregnant females are allowed to be housed together at one time.
- Pregnant females cannot be housed with non-pregnant/non-breeding females (1 or 2 pregnant females: 1+ non-pregnant female).
- Never co-house multiple breeder males in the same cage with multiple females — this will result in competition for the females and fighting.
**Weaning Pups**

Weaning of pups is recommended at 21 days of age.

In cages where the male and female(s) are kept together continuously, all mouse pups must be weaned by 21 days of age or immediately upon observation of the second litter in the cage. This prevents the older pups from injuring the neonates or interfering with the dam's care of the neonates.

When pups are weaned or breeders are separated, a new cage card must be created. **Cage cards cannot be re-used** as the original information on the card will be inaccurate for the new cage/mice. Cage cards should include the genotype information, when applicable, and consistent unambiguous abbreviations may be used when full genotype nomenclature is too lengthy.

**Genetically modified animals (GMAs) - Mice**

Offspring of GMA breeding may have slow development. Therefore, the pups may be not ready for weaning at 21 days of age. Delaying weaning until 28 days of age may be an acceptable alternative. **However, this must be approved in the IACUC protocol.** If a strain requires delayed weaning, the male should be removed prior to parturition, (i.e. when the female is visibly pregnant at ~14 days gestation). This will prevent pregnancy during post-partum estrus and a second litter being born before the first litter has been weaned.

**Breeding Rats**

Breeding pair (1 male:1 female): The male, female and the resulting litter of pups may be kept together in a rat cage continuously until the pups are weaned.

Breeding trio (1 male: 2 females) and other breeding schemes: Breeding trios and other schemes are not permitted with the current size of rat caging at WSU.

**Recordkeeping**

Accurate breeding records should be maintained when managing a rodent breeding colony. These records will help to ensure the maintenance of rodent stocks/strains and provide further information to investigate breeding problems. Records should include the following information:

- Strain
- Generation number
- Animal identification
- Genotype
- Pedigree information - Parents, number of litters/birth date/number of pups born
- Weaning information (number/gender, genotype)

**Preventing genetic drift and sub-strain**

When breeding rodents, it’s important to reintroduce rodents from the original source or background strain after 10 generations to prevent genetic drift and unwanted phenotypes. If a rodent breeding colony goes beyond 20 generations without reintroducing original animals, a sub-strain has been created. It should be noted that the generation gap is cumulative; if the vendor breeds for 10 generations and your laboratory breeds for 10 generations there is now a total of 20 generations between your animals and the original founders.

**Age of breeders**

To maintain breeding efficiency, and help prevent loss of irreplaceable lines and adverse outcomes such as litter loss and cannibalism, it is best practice to replace breeding rodents between 6-12 months. Breeders may need to be retired when they have not produced a litter in 6 weeks or the litter size has dropped below average for that strain. The breeding lifespan varies by strain and should be evaluated using your breeding records to determine the best time to retire breeders.