Objective

The purpose of this policy is to outline recommendations set forth for successful colony maintenance to comply with new rodent housing requirements in the *Guide for the Care and Use of Laboratory Animals, Eighth edition* (Guide, NRC, 2011).

Overview

USC’s Assurance of Compliance with Public Health Service Policy (on file with the NIH Office of Laboratory Animal Welfare) and AAALAC, International accreditation standards require strict compliance with federal animal welfare regulations and the *Guide*. Previous editions of the *Guide* have always specified the minimal amount of cage floor space required for each species based on size and/or weight. However, the eighth edition of the Guide now has specific floor space recommendations for breeding mice and rats with preweanling litters. According to the *Guide*, mice less than 10 grams (e.g., pre-weanlings) require 6 square inches each and mother mice with litters require 51 square inches. Given that the standard mouse cage at USC has 75 square inches of floor space, strict adherence to this guidance would require separation of all females with litters into single cages and would preclude trio-breeding schemes housing two adult female mice with their litters. The IACUC acknowledges that this level of adherence may not be in the best interests of researchers developing specialized genetically-modified animals. The aim of this policy, therefore, is to meet the intent of the *Guide* using performance based standards of animal care and use while keeping in mind the special needs of investigators maintaining transgenic mouse breeding colonies.

Breeding and Weaning Issues

- A standard-sized filter-top mouse cage (75 square inch floor space) can house up to 5 adult mice.
- Accepted breeding schemes include paired (one male, one female), trio (one male, two females), and harem (one male, three or four females) mating.
- Average litter size for mice is between 1 and 10 pups and is highly variable due to genetic and environmental factors.
- Female mice experience a post-partum estrus and can become pregnant within 24 hours of delivering a litter. Consequently, leaving a male mouse in the cage until the time of delivery can result in the production of a subsequent litter when the first litter is 21 days old. This can result in the second younger litter becoming trampled and/or not properly cared for if the first litter is not weaned promptly.
- Significant overcrowding and unintended breeding activity may result if the weanling mice are not separated at 21 days of age.
- Research staff members are responsible for weaning and separating mouse litters.
Policy Statements

- Pair mating (1m & 1f) is allowed and is the standard practice. When a litter of mice is born, there can be no more than 2 adult mice in the cage. If the PI proposes a different breeding strategy, the PI must provide justification based on average litter size, difference in age of the litters, growth rates of the strains.

- For a “trio” mating scheme with one male and two females per cage, adult animals may remain in the home cage as long as the average litter size of the mouse line is 6 pups or fewer. Maximum allowed number for pups is 12 per cage. Exceptions for lines with average litter size of more than six pups must be specifically justified with a reasoning based on the specific mouse line and health or wellbeing of the animals along with data showing that trio mating with large litter sizes is necessary.

- For a “harem” mating scheme with up to four females and one male per cage, pregnant female mice must be separated prior to birth of the litter to one per cage (for mouse lines with average litters larger than six pups) or two females (for lines with average litter sizes equal to or smaller than six pups).

- Mice from strains such as CD-1, FVB, Swiss Webster, or ICR are known to produce litters in excess of 10 pups per litter. These mice should be mated as permanent pairs or as trios or harems with separation of pregnant females as stated above. If there is a large litter in a breeding cage with a second pregnant female, the females must be removed to separate cages prior to the birth of the second litter.

- When multiple litters of mice are present in one cage, the age difference between litters must be less than 6 days. If a subsequent litter is born at an interval greater than 6 days, the mother and litter (of the oldest pups) must be moved to a separate cage.

- All litters must be weaned and separated at 21 days of age unless a specific exemption is listed on the approved IACUC protocol.

- Birth dates of all litters must be clearly noted on each cage card; this information must be provided by research staff.

The IACUC acknowledges that based on the strain’s unique growth and development characteristics, some mouse strains require that procedures in exception to this policy may be required. For example, weaning of mouse pups earlier or later than 21 days of age may be appropriate depending upon health status and body weight of the pups. Requests for exception to this policy must be described in detail in the IACUC protocol associated with the breeding colony and will be considered on a case-by-case basis.

Adherence to this Policy

The Principal Investigators responsible for adhering to this policy and must ensure that all research personnel responsible for colony maintenance are appropriately trained and experienced. Research personnel must provide sufficient monitoring of the animals to prevent overcrowding and deal with associated issues such as cannibalism, fighting, excessively soiled caging, etc.

If significant overcrowding is noted by DAR staff or if a litter is more than 21 days old, an “overcrowding” card will be placed on the cage. Animals must be separated within two days of the posting; failure to comply may result in additional charges to the researcher. In extreme cases, such as when insufficient space in the housing room precludes separation of litters into separate cages, DAR veterinary staff will be instructed to notify the PI immediately and recommend euthanizing excess animals.

Additional References

1. The Guide for the Care and Use of Laboratory Animals, 8th Ed. 2011 NRC, page 57
2. AAALAC interpretation of new Guide policies (http://aaalac.org/about/guidelines.cfm)
3. UC Irvine policy document (http://www.research.uci.edu/ora/acup/mousebreedingcolony.htm)