

WVU IACUC POLICY: Breeding and Weaning of Rats and Mice

Purpose and Background

Federal regulations establish the density of animals permitted in rodent breeding cages. Adult rats and mice and their litters can occupy considerable cage space, produce large amounts of fecal and urinary material and increase cage temperature, humidity, and ammonia levels, all of which can create sub-optimal health conditions. This overcrowding is particularly noticeable near the time of weaning and in harem breeding schemes although the use of ventilated caging systems partially compensates for these increases in waste gases, temperature and humidity. When litters of two different ages are present in the same cage, the younger litter often receives insufficient milk due to the older, more competitive, suckling pups nursing from both post-partum females. Finally, the presence of more than one male in a breeding cage often results in aggression between competing males and commonly leads to reabsorption of pregnancies (an occurrence in rodents known as the Bruce effect). The following standards describe breeding schemes, weaning of litters, and numbers of litters allowed per rodent cage, in concurrence with the 2011 *Guide*.

Exceptions to these standards are recognized as necessary in certain circumstances, such as with weanlings of low birth weight and slow growth rates, or poor maternal behavior requiring multiple mothers in one cage to assist in nursing (cross-fostering). Requests for perpetual exceptions for particular strains should be submitted to the IACUC through revision of your animal use protocol, in the non-surgical procedures section. Exceptions should be justified by provision to the IACUC of published citations or data demonstrating poor post-natal strain survival for which the exemption is requested. Potential reasons for exemption include: 1) Experimental design (e.g. studying psychological imprinting of pups); 2) Need to extend suckling in strains that fail to thrive (requests benefit from submitting supporting data); 3) Need to prevent loss of litters due to cannibalism, which is diminished by cross-fostering (benefits from submitting supporting data).

General Requirements

1. No more than one breeding age male is allowed in a breeding cage. Female rodents must be at least six weeks old before they are placed in a breeding cage with a sexually mature male. The only exception to this is superovulation of 3-4 week old female mice for collection of zygotes or embryos.
2. A cage will be considered overcrowded if there is more than one litter in a cage.

Breeding Schemes

1. One male x one female

Mouse and rat litters should be weaned by post-natal day 21, or before birth of another litter, whichever comes first. **A cage is considered overcrowded if a new litter is born before the older litter from the same female is weaned.** The date of birth must be recorded on the cage card. Animal care technicians will wean the litter if the research staff does not perform this by postpartum day 21, or if a new litter is born. Investigators will be charged for this service. If there is no prior agreement with the OLAR staff to routinely perform weaning by day 22 (or with the birth of a new litter by that female, whichever comes first), failure of the research staff to wean in a timely manner will be considered a non-compliance issue, and is reportable to the IACUC.

2. Harem Breeding (one male x two or more females)

If breeding is conducted in a 1 to 2 or more ratio, each female must be removed to a separate cage when she is observed to be pregnant (usually by 15 days gestational age). Date of birth for the litters must be recorded on the cage card by the animal care staff (or possibly by the investigator if they discover it first). **A cage is considered overcrowded if a new litter is born before an older litter from the same or a different female is weaned.** Animal care technicians will wean the litter if the research staff does not perform this by postpartum day 21, or if a new litter is born. Investigators will be charged for this service. If there is no prior agreement with the OLAR staff to routinely perform weaning by day 22 (or with the birth of a new litter by that female, whichever comes first), failure of the research staff to wean in a timely manner will be considered a non-compliance issue, and is reportable to the IACUC.

Exception for non-thriving litters

Even with wild-type mice, occasionally litters or individuals within a litter do not thrive. If a litter of normal weaning age (as defined in this policy) is not judged to be weaning ready, the supervisor of the vivarium where the animals are housed should be consulted. Each vivarium supervisor has the authority to provide a one-time exemption delaying weaning up to seven days in length for any specifically identified litter scheduled to be weaned on day 21, if in the supervisor's opinion, that litter will fail to thrive. In such an instance, the supervisor can also decide to separate females as needed to avoid overcrowding or losses from excessive competition. In such a scenario, the male must be removed until weaning is complete to avoid impregnation of the female during her post-partum estrus. If a new litter is born during this period of exemption by accident, this newborn litter must be fostered and removed immediately to avoid inter-litter interference when ages are disparate among litters; alternatively, the exempted litter can be weaned at that time. Vivarium supervisors will maintain a log of weaning exemptions in their office for inspection by the IACUC. (A sample log is appended.) Permission for continuing exceptions for a particular line or strain must be obtained from the IACUC by submitting a protocol amendment rather than continually asking the vivarium supervisor for exemptions for a particular line/strain.

Similarly, in instances where "aunting" by a second female is known (by presenting data to that effect) to increase survival, harem breeding may be allowed as justified and approved by the IACUC on a protocol by protocol basis.

Counting animals

PIs must include all animals produced from breeding, even if they will cull those animals. We ask that PIs make a thoughtful effort at estimating the number of additional animals that they need considering "wastage" animals in their totals. We recommend that you make a generous estimate of litter sizes in this calculation. To minimize human contact with new litters, we have traditionally counted animals starting at 5 days postpartum, which no longer comports with the requirement to count from birth. In addition, the traditional delay of 5 days when compounded with harem breeding with multiple litters on the ground at one time (each with their own 5 day waiting periods for cage changes), means we are out of federally mandated compliance with the requirement to change cages every 14 days (or sooner based on performance standards). Hence, unless specifically justified in a protocol, counting will routinely be done before 5 days of age.

Types of animals to be counted:

1. Animals purchased from a vendor or imported from a research institution

For example: Twenty animals arrive from a vendor; only 15 were used in the experiment. All 20 animals are counted at their arrival to OLAR.

2. Animals on breeding protocols

- a. All breeders involved and all of the offspring produced, even if only a sub-set of the offspring were used for actual experimentation will be counted.

For example: Ten mice are produced from a selected mating of one male and one female. However, genotyping reveals that only 2 of the offspring are of the right genotype and those 2 animals are transferred to a research protocol and used for experimentation. All 12 animals (one male, one female, ten offspring) are counted.

- b. Animal produced on a breeding protocol that are transferred to a research protocol are reported and counted on BOTH the breeding protocol AND the research protocol. In the example above, 12 animals should be counted on the breeding protocol and 2 of these animals will also be counted on the research protocol. Therefore, animals produced on a breeding protocol and are not transferred to a research protocol are reported on only the breeding protocol.

3. Breeding on an experimental protocol

- a. All breeders involved and all of offspring produced, even if only a sub-set of offspring were used for the actual experimentation will be counted.

References:

Guide for the Care and Use of Animals. 8th ed. NRC, NAS 2011.

RODENT WEANING EXEMPTION LOG

Vivarium _____ Room _____

Date of Exemption:

Principal Investigator:

Protocol ID:

Number:

Species:

Date of Birth:

Date to be weaned:

Reason for Exemption:

Vivarium:

Room:

Supervisor's Initials:

Note: Exemption from weaning can be no more than seven days beyond the original date to be weaned (i.e. normally 21 days). Beyond this, IACUC or individual veterinary approval is required.