

Rodent Breeding and Weaning in LASP Animal Facilities

Rodent Breeding

In an effort to maximize production, minimize stress on the animals, and adhere to animal welfare policies and regulations, it is important to establish an appropriate breeding program. The objective(s) of the study, the peculiarities of the strain(s) involved, and the experience of the individual(s) responsible for maintaining the breeding program must be taken into consideration when developing a breeding program.

Several mating systems can be used but the most common are a single male:female, trio, and harem breeding. Trio breeding (one male and two females) can increase breeding efficiency within a colony and may work well to facilitate production of offspring for lines that are difficult to breed and maintain. Harem breeding (one male with 3-4 females) is generally avoided unless it is desirable to maximize the progeny of an individual male.

It is important to note that both trio and harem breeding can lead to overcrowding and subsequent changes in metabolic and physiologic processes, alterations in disease susceptibility (Broderson and others 1976, Schoeb and others 1982; Vesell and others 1976), and may adversely affect scientific research if care is not taken to avoid overcrowding. Harem breeding should be reserved for situations where intense production is required.

In all instances, investigators, laboratory staff, and LASP personnel must abide by the cage size requirements delineated in the *Guide for the Care and Use of Laboratory Animals*¹, other applicable regulations, or ACUC approved performance standards as described in this document. Adhering to the table below may help avoid overcrowding.

For trio mating cages may not house more than one adult male and two adult female mice that are left together continuously. In an ideal situation, each pregnant female should be moved to a separate cage to have her litter. If this is not possible, the next best method to help reduce overcrowding is to separate at least one of the moms and her pups immediately after birth. At any time, no more than one male and two females with litters can be housed in a cage. Pups from these litters should not be more than 10 days apart. Occasionally, breeding trios will produce large litters that will strain the caging system. If at 14 days there are over 14 animals in a cage the two litters must be separated.² If pups are not weaned in a timely manner, severe overcrowding may occur. This is due to postpartum breeding, which will produce another litter when it is time to wean the first litter. This is an ACUC approved performance standard.

For harem mating one male and up to 4 females may be housed in a breeding cage. According to ARAC guidelines, in a harem mating situation females must be separated prior to parturition.

The ACUC recommends removing plugged or pregnant females to separate cages upon identification of the pregnancy.

This policy applies to all breeding programs, including transgenic mouse production colonies. Any modifications to this policy must be made to the Animal Care and Use Committee and receive approval. An appropriate scientific justification will be required to obtain an exemption.

Rodent Weaning

Accepted weaning age for most strains of mice and rats, regardless of mating system, is 21 days. However, some genetically altered animals benefit from weaning at a later age. If a later age of weaning is required or anticipated for the strain, it must be described in an approved ASP or modification.³ For individual litters, the veterinarian may be consulted to approve a later weaning time. Regardless of the system used or the approved weaning age, it is the PI's responsibility to ensure prompt disposition of overcrowded cages. Details are provided below:

1. Investigators and their staff have the primary responsibility for weaning litters on time as described in this guideline.
2. The animal care staff is responsible for identifying overcrowded cages, notifying investigators of overcrowded cages, and separating animals in overcrowded cages if not resolved by the investigative staff. A maximum of 5 animals should be weaned into a single cage. Exemptions from this guideline must be described in an approved ASP or modification.⁴
3. Animal care personnel/Investigator will record the date of birth on the cage card at the time each litter is born in their respective animal rooms. The litter should not be minimally disturbed immediately after birth; therefore, the number of pups is recorded at the next cage changing.
4. All litters, regardless of mating system used, must be weaned by 21 days of age unless otherwise specified in the Animal Study Proposal³. If a longer weaning time is specified in the Animal Study Proposal, the mother and litter must be separated into a clean cage, or a mating system of one female to one male must be used. In such cases, litters must be weaned no later than 28 days of age.
5. If animals are not weaned by 23 days of age (or 29 days if approved in the Animal Study Proposal), the investigator/technician is notified by facility management by e-mail, fax, and/or phone call.
 - a. If there is no response within 24 hours, a second attempt at notification is made.
 - b. If the cage is not weaned by the next business day, the investigator will be notified that they have until 2pm that day to wean their mice, or the mice will be weaned by the facility and they will be charged for technical services.
 - c. This same notification system will be used if the number of animals exceeds 14 at 14 days of age.²
6. Weaning procedures performed by the animal care staff are **charged to the investigator's account** at the current rate for technical service.
7. Failure to adhere to this guideline adversely affects animal welfare, may influence study results, and creates a state of noncompliance with recognized animal care standards.
8. Continued noncompliance will be brought before the NCI ACUC and may result in loss of facility privileges.

Footnotes

¹From the Guide for the Care and Use of Laboratory Animals 8th edition.

TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups*

Animals	Weight, g	Floor Area/Animal, ² in. ² (cm ²)	Height, ^b in. (cm)	Comments
Mice in groups ^c	<10	6 (38.7)	5 (12.7)	Larger animals may require more space to meet the performance standards.
	Up to 15	8 (51.6)	5 (12.7)	
	Up to 25	12 (77.4)	5 (12.7)	
	>25	≥15 (≥96.7)	5 (12.7)	
Female + litter		51 (330) (recommended space for the housing group)	5 (12.7)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters. ^d
Rats in groups ^c	<100	17 (109.6)	7 (17.8)	Larger animals may require more space to meet the performance standards.
	Up to 200	23 (148.35)	7 (17.8)	
	Up to 300	29 (187.05)	7 (17.8)	
	Up to 400	40 (258.0)	7 (17.8)	
	Up to 500	60 (387.0)	7 (17.8)	
	>500	≥70 (≥451.5)	7 (17.8)	
Female + litter		124 (800) (recommended space for the housing group)	7 (17.8)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters. ^d
Hamsters ^c	<60	10 (64.5)	6 (15.2)	Larger animals may require more space to meet the performance standards.
	Up to 80	13 (83.8)	6 (15.2)	
	Up to 100	16 (103.2)	6 (15.2)	
	>100	≥19 (≥122.5)	6 (15.2)	
Guinea pigs ^c	Up to 350	60 (387.0)	7 (17.8)	Larger animals may require more space to meet the performance standards.
	>350	≥101 (≥651.5)	7 (17.8)	

*The interpretation of this table should take into consideration the performance indices described in the text beginning on page 55.

²Singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.

^bFrom cage floor to cage top.

^cConsideration should be given to the growth characteristics of the stock or strain as well as the sex of the animal. Weight gain may be sufficiently rapid that it may be preferable to provide greater space in anticipation of the animal's future size. In addition, juvenile rodents are highly active and show increased play behavior.

^dOther considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.

² The 14 heads at 14 days is an ACUC approved performance standard.

³ When requesting delayed breeding (i.e. wean at 28 days) a justification should be provide. Acceptable justifications typically include a statement regarding the small size of the animals at birth and how delaying the weaning will improve pup survival.

⁴ Exemptions from the 5 mouse per cage requirement should be described in an approved ASP or modification. Exemption requests should include the following information:

1. Justification for the increased number of animals per cage. i.e. to permit additional time for genotyping or experiments will occur prior to mice reaching a specific weight as indicated in *the Guide*.
2. Schedule for how often mice will be weighed and indication of who will weigh the mice (i.e. a specific investigator or LASP staff). Mice should not exceed the weight guidelines as detailed in the Guide (see footnote 1 for table)
3. Indication that the weight record will be kept in the animal room or other location easily accessible to Facility Staff.