

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON RODENT BREEDING AND CAGE DENSITY

GENERAL POLICY

The goal of this policy is to define appropriate cage density for laboratory rats and mice, including animals that are used for breeding. The primary objectives of cage management are to ensure a healthy environment for the animals housed therein and to assure compliance with state and federal regulations as well as current accreditation standards. Rodent welfare can be impacted by higher than acceptable cage densities because they can affect the cleanliness of the cage and the frequency of required cage changes.

Additionally, excessive cage densities may adversely affect breeding colonies. All of these situations can cause unnecessary stress for the animals and may also compromise intended research objectives. All personnel involved with animal research are responsible for ensuring that the cage environment is appropriately maintained.

While the Center for Comparative Medicine (CCM) staff provides basic husbandry to most rodents housed at MGH, it remains the responsibility of the Principal Investigator (PI) to maintain his/her animals in compliance with Institutional Animal Care and Use Committee (IACUC) policies and approved protocols. Rodents are housed in accordance with guidelines provided in the [Guide for the Care and Use of Laboratory Animals](#). Those guidelines are described below for housing mice and rats, respectively. Genetically modified animals, that have a new phenotype which may express an adverse health condition, may require increased monitoring or husbandry practices and notification to the IACUC. If an investigator is found to have overcrowded cages resulting from any of the causes described below, the IACUC may withhold approval to breed or use animals until appropriate oversight procedures have been established.

CAGE CAPACITY GUIDELINES

Adult Mice and Rats

Adult mice are defined as any sized mouse that is weaned and at least 28 days of age. Adult rats are defined as any sized rat that is weaned and at least 21 days of age. The IACUC has adopted the minimum space dimensions listed in the *Guide* (p. 57) for the standard shoebox mouse cage (6.5" x 10.5"), as listed in Table 1, and the standard shoebox rat cage (17" x 8.5"), as listed in Table 2.

Table 1. Mouse Housing Guidelines*

Weight of Largest Mouse	Maximum Number of Adult Mice
Less than 25 grams	5
25 grams or more	4

Table 2. Rat Housing Guidelines*

Weight of Largest Rat	Maximum Number of Adult Rats
Less than 300 grams	4
300 to 400 grams	3
400 to 500 grams	2
More than 500 grams	1

** To address the impact of cage density on adequate veterinary care, CCM Veterinary Staff may require lower number of animals to ensure the welfare of the mice involved. Examples include diabetic animals, obesity models, aging models, chronic fighting, and animals needing to be isolated for veterinary purposes.*

For any cage of adult mice or rats found outside of the parameters described in Tables 1 and 2, CCM staff will notify the PI or his/her designee on the need to reduce animal density. Any cages remaining unchanged after three consecutive calendar days following notification will be separated immediately by CCM staff to bring the cage within compliance, and fees may be incurred by the investigator. Potential CCM interventions to possible overcrowding scenarios are described in [Table 5](#).

Breeding Colony Management

1. Breeding Pairs:

For any approved protocol that involves breeding, it is the responsibility of the PI to assure the breeding colony is properly maintained. This includes the selection of breeding pairs, pairing for mating, separation of breeders after mating, and weaning of offspring.

The PI is also responsible for assuring animals are weaned at appropriate times. The latest acceptable weaning dates are outlined in Tables 3 and 4. It is not necessary to wait until the last day for weaning. Mice and rats can be successfully weaned as early as Day 18 if the pups satisfy the criteria described below.

Table 3. Latest Acceptable Mouse Weaning Day

Mice in the Cage	Latest Weaning Day
Dam with litter, +/- sire	Day 28

Table 4. Latest Acceptable Rat Weaning Day

Rats in the Cage	Latest Weaning Day
Dam with litter	Day 21
Dam and sire with litter*	Day 21

**For rats, dam and sire with a litter is only allowed with special larger caging that is only available in some CCM facilities. Please contact your facility manger regarding availability.*

2. Criteria for Weaning

For weaning at any age, pups must satisfy the following requirements:

- Erupted front teeth: the teeth must be present to allow the animal to eat solid food
- Sufficient size: the animal must be of a sufficient size to be able to reach the food and water supply. General minimum size guidelines are as follows, but may vary depending upon the strain:
 - Mice: 2.5” in length and 8-12 g
 - Rats: 4” in length and 25-30 g

In the case of strains with litters that are too immature to wean by Day 21 (rats) or Day 28 (mice), delayed weaning must be approved by the IACUC for the protocol covering the strain in question.

3. Breeding Trios and Harem Breeding Cages

Breeding cages containing a male and multiple females are referred to as “trios” (1 male and 2 females) or “harems” (1 male and up to 3 females). If utilizing a breeding strategy involving multiple females in the same cage, it is the responsibility of the PI to assure that females are separated as they become pregnant (detectible visually by embryonic day 18) to ensure there can only be one litter born to a cage.

For rats, trio or harem breeding is only allowed with special larger caging that is only available in some CCM facilities. Please contact your Facility Manager regarding availability.

4. CCM Interventions for Overcrowded Cages

Table 5. Possible CCM interventions for overcrowded cages

Possible Separation Scenario	CCM Action
Dam with 1 litter (with or without sire)	Notify PI or designee 3 days in advance of weaning date; wean and separate by sex if animals reach weaning date and are not yet weaned
Dam with 1 litter + multiple other animals	Separate other animals if not corrected within 24 hours after discovery (unless approved in protocol)
Dam with older litter and new litter	Separate older litter by sex if not corrected by 1:00 PM on the calendar day after discovery
2 dams plus 2 litters	Separate 1 dam and 1 litter if not corrected by 1:00 PM on the calendar day after discovery (unless approved in protocol)
More than 5 adult (weaned) mice in cage	Separate immediately
Exceeding maximum number of adult animals	Notify PI or designee; separate if not corrected within 3 consecutive calendar days
Special health-related conditions	Notify PI or designee; separate if not corrected by the time required

DOCUMENTING AND TRACKING RODENT BREEDING

- The use of a breeding colony management system is integral to the reduction of animals unnecessarily produced in the research environment and preservation of genetically modified strains. The *Guide* (p. [76](#)) states “Care should be taken to preserve such resources through standard genetic management procedures, including maintenance of detailed pedigree records and genetic monitoring to verify the presence and zygosity of transgenes and other genetic modifications.”
- The use of a breeding colony management system is required for all rodent breeding.
- Rodent breeding colony management systems should:
 - Document and track current and past breeding pairs

- Document and track individual offspring produced
 - Include sufficient information to calculate breeding performance indicators
- See [Appendix 1](#) for the minimum data required to be tracked in the selected colony management system
- The number of animals produced by breeding, including breeders, animals used for experimental purposes and animals euthanized without being used (e.g., incorrect genotype), together with the number of animals purchased should not exceed the number of animals approved by the IACUC. Tracking approximate numbers of animals produced by breeding is required to avoid overproduction relative to the numbers approved on the protocol.

REFERENCES

National Research Council of the National Academies. [Guide for the Care and Use of Laboratory Animals, 8th edition](#). National Academies Press: Washington, D.C., 2011.

U.S. Department of Health and Human Services. [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#). Office of Laboratory Animal Welfare, National Institutes of Health: Bethesda, MD, 2015.

U.S. Department of Health and Human Services. *Public Health Service Policy on Humane Care and Use of Laboratory Animals – Frequently Asked Questions*. [Animal Use and Management, Question No. F.2. Is the IACUC responsible for tracking animal usage?](#)

v1.1, 15 June 2005

v1.2, 20 September 2006

v1.3, 23 October 2013

v1.4, 18 June 2014

v1.5, 15 September 2021

v1.6, 17 August 2022

Appendix 1 – Required data for colony management system

Current and Past Breeding Cages:

1. Room/rack/cage location/identifiers
2. Strain being generated if different from breeding pair
3. Animal identifiers
4. Dates of birth (DOB) of breeders
5. Strain/genotype
6. Date of pairing/mating
7. Estimated # of pups born and DOB for all litters produced
8. Date of tissue collection for genotyping if applicable
9. Weaning date & number of pups weaned for all litters produced
10. Date of disposition (removal of breeding program by transfer to studies, retirement, euthanasia, death)

Current and Past Progeny (Replacement Breeders):

1. Room/rack/cage location identifiers
2. Animal identifiers
3. Date of Birth (DOB) of breeders
4. Strain/genotype(s)
5. Date of tissue collection for genotyping (post weaning, if applicable)
6. Weaning date
7. Date of disposition (removal of breeding program by transfer to studies, retirement, euthanasia, death)

Optional helpful information for breeding management

Primer sequence and protocol or qPCR

Date strain last “managed” or evaluated for performance

Mating goals

Source of strain (commercially available, collaborator)

Breeding performance indicators

- Average litter size
- Average number weaned/litter
- Pups weaned per female per week
- Age in mating or weeks in breeding