



IACUC Guidance:	TAMU-G-040	Title:	Guidelines on Laboratory Rodent Breeding and Weaning	
	Location	Effective Date	Review By	
	College Station/Dallas/Galveston/Kingsville	07/01/2024	6/30/2027	
	Houston	08/01/2024	6/30/2027	

1. PURPOSE

- 1.1. This document provides guidance on breeding, weaning and space allocation for commonly used laboratory rodents.

2. SCOPE

- 2.1. Applies to laboratory rodents used for research, teaching, testing or other purposes under the oversight of the Texas A&M University IACUC.
- 2.2. Does not describe identification (see TAMU-G-014) or genotyping (see TAMU-G-007).

3. RESPONSIBILITY

- 3.1. The **PI** is responsible for:
 - 3.1.1. Describing breeding scheme in the animal use protocol.
 - 3.1.2. Including justification for planned delayed weaning extension in the animal use protocol.
 - 3.1.3. Adherence to minimum allowable cage densities as described in the AWAR and/or the *Guide*.
 - 3.1.4. Training personnel when responsible for colony maintenance.
 - 3.1.5. Providing sufficient monitoring of the animals to prevent overcrowding and associated issues such as cannibalism, fighting, excessively soiled caging, etc.
 - 3.1.6. Ensuring birth dates of all litters are clearly noted on each cage card. (NOTE: Researchers are encouraged to keep additional detailed breeding records separate from the cage cards to prevent accidental loss of important data).
- 3.2. The **IACUC** is responsible for:
 - 3.2.1. Granting exceptions defined in the applicable protocol, as determined by the characteristics of the animal strain or species and/or experimental use.
 - 3.2.2. Semiannual Facility Inspections and IACUC Oversight of Animal Use Locations as described in TAMU-S-010.
 - 3.2.3. Assessing, reviewing, and modifying space allocation as necessary, considering performance indices (e.g., health, reproduction, growth, behavior, activity, and use of space).

4. DEFINITIONS AND/OR ACRONYMS

- 4.1. **Adult:** Animals which are mature, aged beyond weaning date (for the species), of an age able to reproduce, or offspring of egg-laying vertebrates (zebrafish larvae hatch 3 days post-fertilization).
- 4.2. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly, and have delegated authority from, the AV.
- 4.3. **AWAR/AWR:** Animal Welfare Act Regulations. Also known as Animal Welfare Regulations. Regulations administered by the United States Department of Agriculture (USDA). USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations.
- 4.4. Centrally administered support service for animal research and teaching programs at Texas A&M University:
 - 4.4.1. **ARU:** Animal Resource Unit supports the School of Dentistry vivarium
 - 4.4.2. **CMP:** Comparative Medicine Program supports the Texas A&M College Station campus
 - 4.4.3. **PAR:** Program for Animal Resources supports the Institute of Biosciences and Technology vivarium
 - 4.4.4. **PRF:** Pharmaceutical Research Facility supports the Kingsville Pharmaceutical Science Facility vivarium
 - 4.4.5. **Sea Life:** The Sea Life Facility supports the Galveston campus

- 4.5. **Delayed Weaning Extension:** Instances where a litter has passed its weaning date, but is unable to be weaned due to small size or inability to access food or water. All extensions must be administered by the AV or designee, or described and justified in an approved animal use protocol.
- 4.6. **GMA:** Genetically Modified Animals. Animals that have induced mutations that are human-made alterations in their genetic code. This includes both transgenic and targeted mutations that are created to study the expression, overexpression, or under expression of a specific gene.
- 4.7. **Gnotobiotic:** Germ-free animals or formerly germ-free animals in which the composition of any associated microbial flora, if present, is fully defined.
- 4.8. **(The) Guide:** The *Guide for the Care and Use of Laboratory Animals*. Primary reference document for meeting the needs and requirements of animals used in biomedical research. The PHS Policy requires institutions to use the Guide as a basis for developing and implementing an institutional program for activities involving animals.
- 4.9. **IVC (caging):** Individual Ventilated Caging
- 4.10. **Litter:** A group of offspring produced at one birth
- 4.11. **Pup:** Any neonatal or young rodent prior to weaning
- 4.12. **Weaning Date:** Date of separation of weanlings from dam into separate caging. Typically, P21 in rodents.

5. GUIDELINES OR PROCEDURE

5.1. Breeding

- 5.1.1. Outbred animals are widely used in biomedical research. Founding populations should be large enough to ensure the long-term genetic heterogeneity of breeding colonies. Inbred strains of various species, especially rodents, have been developed to address specific research needs. When inbred animals or their F1 progeny are used, it is important to periodically monitor genetic authenticity.
- 5.1.2. Generation of animals with multiple genetic alterations often involves crossing different GMA lines and can lead to the production of offspring with genotypes that are not of interest to the researcher (either as experimental or control animals) as well as unexpected phenotypes. Carefully designed breeding strategies and accurate genotype assessment can help to minimize the generation of animals with unwanted genotypes. Newly generated genotypes should be carefully monitored and new phenotypes that negatively affect well-being should be reported to the IACUC per TAMU-G-015 and managed in a manner to ensure the animals' health and well-being.
- 5.1.3. The first offspring of a newly generated GMA line should be carefully observed from birth into early adulthood for signs of disease, pain, or distress. Investigators may find that the phenotype precludes breeding of particular genotypes or that unexpected infertility occurs, situations that could lead to increases in the numbers of animals used and revision of the animal use protocol.
- 5.1.4. Breeding colonies should be established based on need and managed according to principles of animal reduction such as cryopreservation for rodent stocks or strains.

5.2. Breeding Success

- 5.2.1. Improved in enriched environments. Breeding animals should have adequate nesting materials and/or substitute structures.
- 5.2.2. Breeding animals will require more space, particularly if neonatal animals will be raised together with their mother or as a breeding group until weaning age. Space quality also affects its usability. Enclosures that are complex and environmentally enriched may increase activity and facilitate the expression of species-specific behaviors, thereby increasing space need.
- 5.2.3. Average litter size for mice is between 1 and 10 pups and is highly variable due to genetic and environmental factors.
- 5.2.4. Female mice and rats experience a post-partum estrus and can become pregnant within 24 hours of delivering a litter. Consequently, leaving a breeding male in the cage until the time of delivery can result in the production of a subsequent litter when the first litter is between 19-21 days old. This can result in the second younger litter becoming trampled and/or not properly tended.



5.2.4.1. Prior progeny must be separated from the mother before she gives birth to a new litter to avoid overcrowding and to maximize success of the new litter.

5.3. Breeding Schemes

5.3.1. Breeding scheme(s) used must be described in the approved animal use protocol.

5.3.1.1. Monogamous or Pair Breeding: One (1) adult male and one (1) adult female

5.3.1.2. Trio Breeding: One (1) adult male and two (2) adult females

5.3.1.3. Harem Breeding: One (1) adult male and up to four (4) adult females

5.3.1.3.1. **Note:** Harem breeding is discouraged due to challenge of monitoring for overcrowding cages and injury to pups

5.4. Space Allocation

5.4.1. Space recommendations and housing density vary extensively with the species, age/size of the animals, and type of research.

5.4.2. There is no ideal formula for calculating an animal's space needs based only on body size or weight. Performance indices should also be considered, and the AV (or designee) is available for consultation.

5.4.3. Variables that may affect the animals' response to different cage sizes and housing densities include, but are not limited to, species, strain (and social behavior of the strain), phenotype, age, gender, quality of the space (e.g., vertical access), and structures placed in the cage.

5.4.4. Consideration should be given to the growth characteristics of the stock or strain as well as the sex of the animal.

5.4.5. Intensive management such as culling or separation of litters may be utilized to reduce housing density and allow desired offspring to develop to weaning without detrimental effects for the mother or the litter.

5.4.6. Mice

5.4.6.1. Minimum space requirements based on the *Guide*:

Weight or Stage of Maturity	Minimum Floor Space Per Animal in in ² /cm ²
Note that singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.	
<10 gm	6 / 38.7
Up to 15 gm	8 / 51.6
Up to 25 gm	12 / 77.4
>25 gm	≥ 15 / 96.7
Female + litter*	51 / 330

*Recommended space for the group. 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.

5.4.7. Rats

5.4.7.1. Minimum space requirements based on the *Guide*:

Weight or Stage of Maturity	Minimum Floor Space Per Animal in in ² /cm ²
Note that singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.	
<100 gm	17 / 109.6
Up to 200 gm	23 / 148.35
Up to 300 gm	29 / 187.05
Up to 400 gm	40 / 258
Up to 500 gm	60 / 387
>500 gm	≥70 / 451.5
Female + litter*	124 / 830



*Recommended space for the group. 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.

5.4.8. Guinea Pig

5.4.8.1. Minimum space requirements for primary enclosures based on AWR:

Weight or Stage of Maturity	Minimum Floor Space Per Animal in in²/cm²
Note that singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.	
Weaning to 350 grams	60 / 387.12
> 350 grams	101 / 651.65
Nursing females with their litters	101 / 651.65

5.4.8.2. Except where harem breeding is practiced, preweanling guinea pigs shall not be housed in the same primary enclosure with adults other than their parents.

5.4.9. Hamster

5.4.9.1. Minimum space requirements for primary enclosures based on AWR:

5.4.9.1.1. A nursing female hamster, together with her litter, shall be housed in a primary enclosure that contains no other hamsters and that provides at least 121 square inches of floor space.

5.4.9.1.2. In the case of nursing female dwarf hamsters such floor space shall be at least 25 square inches.

5.4.10. **Other species** – please consult with the AV, or designee.

5.5. Offspring Viability

5.5.1. Unless required by study aims and described in the approved protocol, physical manipulation of the mother or pups until at least day 3 postpartum is not recommended.

5.5.2. Weaning is a critical period in an animal’s life. Without maternal care, newly weaned rodents must be able to survive. Transgenic and knockout animals often require special attention during weaning as they may be smaller and less vigorous than the normal animal. Some suggestions for successful survival of weaned rodents:

5.5.2.1. Culling of litters

5.5.2.2. Separation of litters from the breeding group

5.5.2.3. Allocation of sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning

5.5.2.4. Cage enrichment/nutritional enrichment

5.5.2.5. Readily available water source for newly weaned rodents (e.g. water-gel supplement in small container on cage bottom, primed/reachable lixits)

5.5.2.6. Readily available food source (e.g. food pellets on cage bottom) until rodents are capable of eating from the food hoppers

5.5.2.7. Avoid weaning small weanlings on Fridays or before holidays unless a lab member will be present on the weekend or holiday to observe the animals.

5.5.3. If an animal care technician observes overcrowded cages, vivarium staff will notify the investigator or designee and/or separate cages to comply with density allowances.

5.6. Recordkeeping

5.6.1. Birth and weaning dates of all litters must be clearly noted on each cage card or breeding card/record.

5.6.2. Cage card holders should be kept to a minimum of no more than two per cage to not impede visibility of the animals.



- 5.6.3. The number of animals used is limited to the number necessary to obtain valid results. This requires the documentation of animals acquired and used, including animals that are generated and then euthanized (i.e. offspring of the wrong genotype/offspring produced in excess are still counted against the total number of animals approved by the IACUC)
- 5.6.4. Research records, including breeding records, must be maintained consistent with TAMU SAP 15.99.03.M1.03.

6. EXCEPTIONS

- 6.1. The PI may request an exception to the above standards by describing the departure in the AUP
- 6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

7.1. References

- 7.1.1. OLAW:
 - 7.1.1.1. [Guide for the Care and Use of Laboratory Animals](#)
 - 7.1.1.2. [FAQ #F2](#) When and how should animals be tracked on approved protocols?
- 7.1.2. USDA: [Animal Welfare Act Regulations](#)
- 7.1.3. TAMU SAP [15.99.03.M1.03](#) The Responsible Stewardship of Research Data

7.2. [IACUC/AWO Referenced Documents](#): (requires TAMU NetID authentication)

- 7.2.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards
- 7.2.2. TAMU-G-007 Guidelines on Genetically Modified Animals and Genotyping
- 7.2.3. TAMU-G-014 Guidelines for the Identification of Research Animals
- 7.2.4. TAMU-G-015 Guidelines for Reporting Animal Concerns
- 7.2.5. TAMU-S-010 Semiannual Facility Inspections & IACUC Oversight of Animal Use Locations

7.3. Contacts

- 7.3.1. [CMP](#): at (979) 845-7433
- 7.3.2. [ARU](#): at (214) 828-8149
- 7.3.3. [PAR](#): at (713) 677-7471
- 7.3.4. [PRF](#): at (361) 221-0770

7.4. Acknowledgements

- 7.4.1. This document was partially prepared using materials obtained from Emory University.

8. HISTORY

Effective Date	Version #	Description
10/01/2021	000	College Station/Galveston: New document. Reviewed and approved via email.
11/01/2021	001	Houston/Kingsville: Replaces IBT-207.03. Reviewed and approved via email.
01/18/2022	002	Dallas: Replaces CD-207.02
03/24/2022	003	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	004	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.
07/01/2024	005	College Station/Dallas/Galveston/Kingsville: Renewal; updated Scope, Responsibility, Definitions and References sections; added expectation related to weaning prior to weekend/holiday, and additional record keeping requirements.



08/01/2024	006	Houston: Renewal; updated Scope, Responsibility, Definitions and References sections; added expectation related to weaning prior to weekend/holiday, and additional record keeping requirements.
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