CAGE SPACE REQUIREMENTS FOR MICE

REGULATORY BACKGROUND:

1. The Guide for the Care and Use of Laboratory Animals (The Guide).¹

   NOTE: This policy is based on a standard cage of 75 square inches. If cages are larger or smaller than 75 square inches, then DLAR should be consulted and The Guide recommendations shall be used, unless IACUC approval is received for the altered housing density.

POLICY:

1. The Principal Investigator (PI) is responsible for ensuring adherence to this policy to prevent overcrowding.

2. In cases where DLAR has identified overcrowded cages (i.e., cages not adhering to this policy), the overcrowding must be corrected by the laboratory staff within the timeline identified in the DLAR notification, otherwise the PI will be non-compliant with this policy.

3. Maximum Approved Housing Density per 75 sq inch cage (Figure 1):
   a. Non-breeding mice, post-weaning = 5 mice per cage

   b. Breeding mice:
      i. No more than two adults may be in a cage with a litter(s).
      ii. No more than two litters with their respective dams are allowed in a single cage. For this density ALL the following criteria must be met²:
          1. Mice are housed in an individually ventilated cage.
          2. No more than 14 pups³ in the cage.
          3. Daily monitoring occurs to ensure sufficient space is allocated to mothers with litters to allow the pups to develop to weaning without detrimental effects to the mother or the litter.¹
          4. Weaning is performed as described in this policy.
      iii. Weaning
          1. If only one litter is present in the cage, pups may be weaned up-to 28 days of age.⁴
          2. Two litters present in one cage
             a) One dam – the older litter should be weaned when the new pups are born.
             b) Two dams
i. The oldest litter must be weaned or separated with their respective dam at 21 days of age.

ii. Litters should be separated if one litter can walk and pups of the other litter cannot walk.

iv. Documentation – the litter(s) date of birth must be noted on the cage (on a breeding card in DLAR managed facilities).

4. Exemptions and limitations to this policy:
   a. For scientific or permanent exemptions to this policy IACUC approval is required.
   b. For individual situations in which animal health may be impacted clinical exemptions can be granted. DLAR veterinary staff must approve and document a clinical exemption.
   c. In cases where physiologic parameters (e.g., weight, urine output, litter size, etc.) are excessive PI’s must follow DLAR veterinary recommendations, which may be under the maximum density stated in this policy.

See Appendix I: Important Information Applicable to Research Personnel and DLAR

See Appendix II: Overview of Mouse Breeding Schemes

2 The 8th Edition of the Guide for the Care and Use of Laboratory Animals recommends that a female plus her litter have 51 in² of floor space with a stipulation that other breeding configurations can be used upon consideration of the health and well-being of the mother and the litter. In the standard individually ventilated cages in use at Duke, housing a maximum of two litters with their respective dams may offer the advantage of allowing the mothers to assist each other in nursing the pups and provides the advantage of additional warmth in these cages with increased airflow. Therefore, two litters within the cage can be beneficial to animal welfare. The limitations on the maximum number of total pups per cage and daily monitoring ensures that animal welfare is not compromised.

3 Numbers determined by veterinary judgement based on Duke’s mouse population and breeding schemes.

### Figure 1. Maximum Density of Mice in a Cage***

| Maximum number of adults in a cage: The maximum number of adults (including weaned mice) is 5 of either sex. |
| Maximum number of adults with a litter: There can be no more than two adults and a litter(s) in the cage. |
| 21 Day weaning or separation: Two dams and their litters, are allowed in one cage (without IACUC approval) when at least one litter can be weaned at 21 days or moved to a separate cage with their dam and weaned up to 28 days. One litter with their dam must be separated into a different cage if there are pups in one litter that can walk and pups in the other litter that cannot walk or there are more than 14 total pups. |
| 28 Day weaning: When only one dam and her litter is present, with or without one male, the pups can be weaned up to 28 days without an IACUC approval |
| Two litters belonging to the same dam: When two litters belong to the same dam, the older litter must be weaned when the second litter is born. NOTE: extended weaning cannot be used for first litter. |

| O | O | O | O | O | O = Adult mouse (including weaned mice) of either sex, ♀ = Adult female mouse, |
| O | O | O | O | O | O | O | O = Litter of pups, ♀ = Adult Male Mouse, ♀ +/- = With or without a male |

*** Situations may apply where housing density requirements under maximum stated in this figure apply and that the PI MUST follow. See section 4.c.
APPENDIX I: Important Information Applicable to Research Personnel and DLAR

1. Notation of the accurate date of birth is required by the PI/laboratory staff on the breeding card (not cage card) or a birth date will be designated by DLAR must be accepted.

2. Overcrowding cards placed on cages by DLAR can only be removed by the PI/laboratory staff when the overcrowded situation is corrected. Otherwise consultation with animal husbandry personnel, the Operations Manager for the facility or the veterinarian is required. If the consultation reveals that the card was placed on the cage in error, the cage will not be considered overcrowded.

3. There will be a significant fee assessed and the IACUC will be notified if an overcrowded cage is manipulated in such a way as to conceal the overcrowded nature without correcting the condition (e.g., removing overcrowded cards without consultation with DLAR).

4. Any exemptions must be designated on a special husbandry conditions card listing the specific exemption(s) and placed on the cage unless all of the cages in the room or rack are under the exemption. If the entire room is under exemption a sign can be posted to reference the exemption(s). Regardless of the method used, the specific cages with the exemption must be clearly defined or the cages will be considered overcrowded.

5. PI/Laboratory staff must preemptively manage cages to prevent overcrowding as defined by this policy. Waiting for DLAR to place an overcrowded card on the cage before taking action is not acceptable.

6. DLAR is not responsible for loss of research data due to the separation of mice in an overcrowded cage. The PI/laboratory staff must not write study information on the DLAR cage card as these cards are replaced when the IACUC protocol expires.

7. If the overcrowded situation is not corrected within the timeline identified in the DLAR notification (generally within 2 business days), DLAR will separate the cages and charge a fee.

8. Fees –for- Service: DLAR will charge a fee for separating overcrowded cages. DLAR will also charge a fee for notification of overcrowded cages beginning October 1, 2016.

DLAR Responsibilities: Unless an IACUC exemption applies, DLAR will generally place an overcrowded card on the cage and notify the PI when (see Figure 1):

- There are more than 5 adults or weaned mice in a cage.
- There are more than two adults and a litter
- There are more than two litters and their respective dams in a cage
- There are more than 14 pups in a cage with two dams
- Pups in a cage with a single litter have not been weaned by 29 days of age
- Pups in a cage with two litters have not been weaned or separated by 22 days of age
- There are pups in one litter that can walk and pups in the other litter than cannot walk

For any other overcrowding concerns the cage will be marked with and processed as an animal health abnormality. The veterinarian will be contacted and alternative breeding schemes will be discussed. DLAR staff should contact their Operations Manager, or designee, if they have any questions concerning overcrowding.
Appendix II: Overview of Mouse Breeding Schemes

- **Monogamous breeding (one male and one female in a single cage):** Monogamous breeding schemes can be continuous or interrupted. Continuous monogamous breeding is a breeding scheme where the male and the female remain in the cage and takes advantage of the post-partum estrous, but can lead to a dam delivering a second litter requiring the first litter to be weaned. The continuous scheme is useful when mice are viable for weaning by the time that any second litter is born.\(^4\) Interrupted monogamous breeding consists of removing the male before the delivery of pups so that breeding does not occur on the post-partum estrous, which occurs from 14 to 28 hours after delivery. This breeding scheme is useful when pups need an extended weaning up to 28 days.

- **Trio Breeding (one male and two females):** Trio breeding can consist of removing one of the females to a separate cage before giving birth or leaving two females together and removing the male. Allowing two females to remain in the cage with two litters may be beneficial for some strains due to the fact that the two dams aid each other in caring for the pups. This scheme may be well-suited for mice that can be weaned at 21 days. Alternatively one litter, with their dam, may be separated into another cage on Day 21 and weaned up to Day 28. Caution should be used in strains of mice that produce over 7 pups per litter as density is restricted to no more than 14 pups in a cage. The older litter must be separated when there are pups of one litter that can walk and pups of the other litter that cannot. Removing one female to a separate cage prior to delivery is better suited for pups that need extended weaning up to 28 days. NOTE: the policy has specific requirements for housing two dams with their respective litters in one cage. See section 3.b.ii.