



Duke University
Animal Care & Use Program
Policy



CAGE SPACE REQUIREMENTS FOR MICE

PERFORMANCE STANDARD: Mice will be maintained in a density consistent with this policy.

BACKGROUND:

1. Animals require an appropriate housing environment to thrive and provide a stable biologic model. Barring any scientific evidence to the contrary, the density recommendations of The Guide for the Care and Use of Laboratory Animals (The Guide) should be utilized.
2. This policy uses The Guide recommendations as a basis for defining caging density requirements at Duke. This policy balances The Guide recommendations against the style of caging and resources available. The Duke animal program has rodent caging ranging from 67 - 75 square inches. This policy is based on a standard cage of 75 square inches. If cages are larger or smaller than 75 square inches, then The Guide recommendations shall be used, unless IACUC approval is received for the altered housing density.
3. Numbers rather than body weight of the individual animals will generally be used as the basis for assessing overcrowding. The justification for using 'numbers-based criteria' is:
 - a. To avoid unnecessary handling of neonatal animals and associated distress; and,
 - b. To assure a consistent method of assessing and tracking overcrowding pursuant to this policy.

DEFINITIONS:

1. An **incident** is defined when the following two (2) conditions are met.
 - a. A cage exhibiting excessive density has not been corrected by the PI within two business days of DLAR notification. Excessive density events resolved by the PI within two business days of notification by DLAR are not be counted as an incident; and
 - Any number of cages that occur within a 3 business day period of the first identified incident are considered a single incident. Subsequent unresolved overcrowding events are considered separate incidents if they occur >3 business days from the first identified and unresolved overcrowding incident. For example:
 - Overcrowded cages are identified on a Monday (calendar day 1), and Tuesday (calendar day 2) of the same week. Neither are resolved within 2 business of DLAR notification. These are considered the same incident.
 - Overcrowded cages are identified on Monday (calendar day 1) and Thursday

(calendar day 4) of the same week. Neither are resolved within 2 business of DLAR notification. These are considered two separate incidents.

2. Incidents are tracked on a protocol basis and are not facility specific.
3. Trending analysis may identify investigators with multiple protocols that have frequent overcrowdings (but less than 5 incidences/protocol within a 3-month period) that would also warrant meeting with DLAR, and potentially notification of the IACUC / OAWA.
4. **High frequency** is defined as more than five (5) incidents within a three-month period. High Frequency incidents are reported to the OAWA on the 6th and subsequent incidents within the 3 month period.

ROLES:

1. Research and Division of Laboratory Animal Resources (DLAR) staff will abide by the policy statement (see Appendix A) below unless there is documented PRIOR approval for an exemption by the Institutional Animal Care & Use Committee (IACUC). Exemptions may be granted by the IACUC based on scientific justification provided by the Principal Investigator (PI). The PI is responsible for ensuring that the exemption is marked on the cage card.
2. The DLAR veterinary staff may exempt an animal from these guidelines due to medical concerns or emergencies, but the PI will must subsequently amend the IACUC protocol if and extended weaning date is necessary for maintenance of a mouse line.
3. The IACUC will review high frequency of unresolved overcrowding events and may assess the overcrowding as a non-compliant event (e.g. continuing non-compliance). The potential action that the IACUC could take would include reporting the non-compliance to NIH and/or eliminating a PI's approval to breed mice.

PROTECTIVE POSTURES REQUIRED: Personal Protective Equipment (PPE) will be worn as defined by the operation of the specific facility.

POLICY:

1. The Principal Investigator (PI) and their research staff should monitor animal housing density at a sufficient frequency to allow proper weaning and adherence to the standards of this policy. When deviations from the above standards are noted, the research staff should correct deficiencies immediately. In cases where DLAR has identified overcrowded cages the overcrowding should be corrected by the laboratory staff within two business days of DLAR notification. Failure to correct problems may result in a non-compliance situation.
2. **Maximum Approved Housing Density:**
 - a. **Mice from the Age of Weaning to Adulthood:** Mice of weaning age and above should be housed at a density of 15 square inches per mouse. For the standard Duke housing cage, this equals 5 mice per cage. (Ex: 75 square inch cage /15 square inches per mouse).

b. **Heavy Mice:** If any mouse (breeder or experimental) weighs 45 grams or greater, then that cage is restricted to four (4) mice per cage.

c. **Breeding mice:**

i. No more than three adults in a cage when a litter is born.

ii. No more than two adults and one litter in the cage when any pups in the cage are older than ten days of age. More than one litter and two adults per cage when pups are older than ten days requires IACUC pre-approval. Any exemption must be marked on the designated cage card.

iii. Pups must be weaned by 21 days of age unless an exemption has been approved by the IACUC or the DLAR veterinary staff for health concerns. Cages should be marked with date of weaning.

iv. Breeding cages containing pups 22 days of age or older, and without an IACUC or DLAR exception, are considered overcrowded. Any exemption must be marked on the designated cage card.

v. No more than one litter may be present in the cage. An exception to this policy is appropriate in certain 'failure to thrive' or 'poor breeder' situations:

1. More than one litter can be present in the cage regardless of the mother to which they belong if there is an IACUC or DLAR exemption in place.

2. Any exemption must be marked on the designated cage card and must include:

- The date of birth of all litters; and
- The IACUC or DLAR Veterinarian approved weaning date, if different than 21 days, on a designated cage card.

3. When litters need to be separated the mothers and litters must be observed sufficiently to determine the appropriate mother for each litter before mice are moved from one cage to another.

4. Maintaining the male in the harem post-breeding increases the risk of having overcrowded cages. Removing the male from harems after breeding has certain advantages, which include:
 - a. Allowing the use of the male in multiple harems (decreasing the total number of animals and sharing the genetics to a larger population of offspring);
 - b. Decrease the risk of cannibalism after birth of the offspring;
 - c. Increase the number of pups that can be maintained in the same cage without exceeding density guidelines;
 - d. Prevent post-partum breeding of the females (which often results in multiple litters from the same female at the same time).
5. Single housing of females from one week prior to gestation until gestation DOES NOT require separate IACUC approval. Refer to the Duke Animal Enrichment Policy for more information: http://vetmed.duhs.duke.edu/documents/iacuc/pdf/policy_on_enrichment_for_species_other_than_NHP.pdf
6. In cases of group housed rodents, where animal(s) exceed 25 grams, the veterinary staff will make a professional judgment based upon The Guide recommendations of evaluating group housed animals to achieve desirable 'individual needs, behavior, compatibility of the animals, numbers of animals, and the goals of the housing situation' (professional veterinary judgment).
7. The IACUC instructs DLAR track the incidences of overcrowding, and report high frequency observations to the OAWA for investigation and IACUC consideration.
8. The IACUC instructs the PI to meet with DLAR representatives when 5 or more instances of overcrowding have occurred to discuss reasons for continuing overcrowding and methodologies which may be employed to lessen the frequency of overcrowded cages.
9. The IACUC instructs DLAR to provide a report of the PI-DLAR meeting as a means of documenting oversight corrective actions.

Appendix A

Division of Laboratory Animal Resources' Activities in Support of This Policy:

1. Cages will be observed daily during normal husbandry procedures to identify overcrowding.
2. Overcrowded cages will be marked and the Principal Investigator will be notified by Email (with return receipt) or fax (with or without accompanying phone call) that corrective action is required.
3. The PI / laboratory personnel will be notified and an "overcrowded card" placed on the cage if:
 - A. There are more than three adults in a cage when a litter is born
 - B. There are more than two adults and one litter in the cage when any pups in the cage are older than ten days of age unless there is an IACUC exemption in place and marked on the designated cage card.
 - C. Pups are 22 days or more of age (and there is no IACUC or DLAR veterinarian- approved exemption in weaning age, which is written on the designated cage card),
 - D. There is more than one litter present in the cage, unless there is an IACUC or DLAR exemption marked on the cage card. More than one litter can be present in the cage regardless of the mother to which they belong if there is an IACUC or DLAR veterinary exemption is in place. Any exemption must be marked on the designated cage card.
 - E. If there are more than 5 total mice of weaning age to adulthood in the cage.
4. If the Principal Investigator does not correct the condition within the two subsequent business days after notification (unresolved overcrowding), DLAR will separate the animals according to the above standards and according to the DLAR SOP on identifying and correcting overcrowded rodent cages.
 - A. DLAR will charge the Principal Investigator a designated fee per new cage generated when animals in an overcrowded cage are separated by DLAR personnel.
5. If there is no space in which to separate the animals, DLAR will make one additional attempt to contact the PI. This notification will consist of an e-mail with an attached read receipt and a telephone call/message to the contact number listed on the cage card. If the PI or lab staff does not respond within an additional 24 hours, DLAR will euthanize animals to correct the cage density and notify the PI by way of e-mail with an attached read receipt.
6. When 5 unresolved or resolved incidents of overcrowded cages occur (a resolved incident is as defined in the policy, but the overcrowded cage(s) was/were resolved), regardless of time period, DLAR management, including the husbandry staff, will meet with the PI and/or their staff to discuss the basis for the overcrowded cages. After this meeting, the PI will be charged a nominal per cage fee for subsequent notices of overcrowded cages.