UNIVERSITY STANDARD

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
STANDARD ON MOUSE CAGE DENSITY

Introduction

PURPOSE
To ensure sufficient space for mice with litters that will allow pups to develop to weaning without detrimental effects, and to house adult mice in space that is sufficient for their needs. This Standard is a blend of the requirements in the 8th edition of the Guide for the Care and Use of Laboratory Animals and the long standing, performance based UNC policy.

Multiple factors contribute to successful breeding and welfare of mice. Additionally, different mouse strains can exhibit variations in breeding behaviors and physiological parameters, making it difficult to make a “one size fits all” rule for regulation of mouse breeding. The purpose of this standard is to allocate sufficient space for mice with litters that will allow for optimal breeding and welfare, and to indicate appropriate space for adult mice.

SCOPE OF APPLICABILITY
All Principal Investigators, laboratory members, and DCM personnel involved in the breeding and maintenance of mouse colonies.

Standard

Mouse Breeding Cages:

1. Nesting material must be added to all breeder cages at the time of set-up. Approved types of nesting material for mice can be referenced on the IACUC standard: Environmental Enrichment Program for Animals
2. Research staff are required to record date of birth on the DCM Weaning Notice (pink) card within three (3) days of birth.(Note- see the DCM instructions for use of the pink card)
‘Pink Card’ Standards (see image of the card under ‘Definitions’):

- When a new litter is noted in the cage, document the pups date of birth (DOB) on the pink card using your best conservative estimate of age.
- Record the wean by date for day 23 using a china marker.
- This pink card should be used by DCM and the labs to record the litter’s DOB.
- As each litter is born, record DOB and wean by date on a separate line, if applicable.
- If there are no litters, remove the pink card and wipe clean, saving for further use.

3. UNC mouse breeding cages are permitted to contain the following maximum numbers of animals:

<table>
<thead>
<tr>
<th></th>
<th>1 Adult</th>
<th>2 Adults</th>
<th>3 Adults**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single litter</td>
<td>no limit on pups</td>
<td>no limit on pups</td>
<td>no limit on pups</td>
</tr>
<tr>
<td>Multiple litters, after one litter reaches 14 days of age*</td>
<td>14 pups</td>
<td>11 pups</td>
<td>9 pups</td>
</tr>
</tbody>
</table>

* Pup numbers are not limited until the oldest litter reaches 14 days of age.

** Cages with more than 3 adult mice, plus pups, will be flagged for correction with an ‘Action Required’ (yellow) card.

4. Wean pups by 23 days old. (Typically, pups will have reached 10 grams by 23 days old for most litters.

Do not wean pups that are < 10 grams. If pups are smaller than 10 grams at 23 days, consult with DCM Vet Services for how to handle these special cases.

5. After pups are weaned, cage density guidelines are based on body weight as shown in the table below.
Recommended Minimum Space for Mice Housed in Groups – Stock cages:

<table>
<thead>
<tr>
<th>Animals</th>
<th>Weight of mice (grams)</th>
<th>Floor Area of cage needed per animal, (inches²)</th>
<th># Adult mice per static microisolator 70 inches²</th>
<th># Adult mice per green/blue line 78-82 inches²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice in Groups*</td>
<td>&lt;10</td>
<td>6</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Up to 15</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Up to 25</td>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;25</td>
<td>≥ 15</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Female + litter</td>
<td></td>
<td>51 recommended space for the housing group</td>
<td>See chart above</td>
<td>See chart above</td>
</tr>
</tbody>
</table>

*Non-breeding cages allow a maximum of 5 adults of the same sex including no more than 5 weaned (23 day old) animals per cage.

‘Action Required’ Standards:

- On Day 24, (24th day after DOB) DCM will mark cages exceeding the recommended minimum space requirements with a yellow “Action Required” card. The date that the corrections must be made by will be listed on the card. Corrections must be made by the PI/lab staff by the end of the day listed on the card (two calendar days), including weekends and holidays.
  - Example- DCM flags a cage with a yellow “Action Required” card on Wednesday, corrections must be completed by end of the day Friday (two calendar days). On Saturday morning DCM staff will separate according to the standard. For a cage marked on a Friday, DCM will separate the following Monday.
- A list will be made by DCM of cages flagged with yellow “Action Required” cards. If the lab corrects the density by the end of business on the day the card is placed, there will be no charge assessed for flagging the cage. If the density is not corrected on the day the card is placed, the following day, DCM will assess a charge for flagging the cage.
- Cages that exceed the densities listed in this standard would be considered out of compliance the day after the date on the “Action Required” card has expired. (Therefore, DCM will correct the issue at this time so that the density is in compliance.)
  - Options for reducing numbers: wean older litter, remove male, move mother/litter.
- On the morning after the expiration date, DCM is authorized to correct the overcrowded cage, separate animals, and assess a separation charge without notifying the investigator. DCM will correct by removing a male or separating litters. If
there is no space in which to separate, DCM may euthanize animals to correct the cage density.

- The IACUC expects that research staff will manage colonies such that yellow ‘Action Required’ cards are not required on a frequent or excessive basis. Colonies with greater than 10% of total breeding cages flagged with the cards at any given time will be reported to the IACUC for further action.

**Additional Standards:**

- Research staff should avoid placing mice prone to fighting in the same cage. Housing conditions such as certain mouse strains known for persistent fighting, mice of different ages, males not weaned together, or males that are not littermates are to be avoided. The IACUC considers incompatible housing resulting from fighting/injury an animal welfare concern that must be remedied as soon as it is identified.

Note: Breeding Software Program - Conditional Allele Mouse Planner (CAMP) – created at NIH National Heart, Lung, and Blood Institute and published in Transgenic Res. 2011 Aug 26 to help scientists set up a breeding strategy and calculate the costs of generating mice with conditional alleles. To access the free application, go to [http://helixweb.nih.gov/CAMP/](http://helixweb.nih.gov/CAMP/)

**EXCEPTIONS**

The IACUC will consider requests for an “Exception” to this standard on a case-by-case basis if the investigator provides scientific justification with documentation. The exception will not be in effect until the PI has received written notification from the IACUC.

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**Definitions**

**IACUC:** Institutional Animal Care and Use Committee  
**DCM:** Division of Comparative Medicine  
**Yellow “ACTION REQUIRED” Card:** Yellow card placed by DCM technicians on rodent cages that are out of compliance for cage density, indicating a specific date that the issue must be remedied by. Pictured below.  
**Pink Wean Card:** This pink card is placed by either DCM or research staff, whomever identifies a new litter first. The date of birth (DOB) and the wean date (DOB + 23 days) is to be written on the card as soon as the new litter is noticed. Instructions for this card’s use, and an image of the card are included in this Standard. Pictured below.  
**Cage Density:** The maximum number of rodents allowed in a given cage space based on the animal’s weight and cage type.
Weaning: The transfer of pups out of the mother’s cage

University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

DO NOT REMOVE FOR DCM USE ONLY

A few common resolutions for separating cages to maintain compliance:

1. Separate weanlings at 23 days of age
2. 1 adult: remove oldest litter if second litter drops
3. 2 adults: remove pups >14 days if there are 2 litters in cage or if there are more than 11 pups. Keep the pups with an adult female if <23 days old.
4. 3 adults: remove pups >14 days old if there are more than 9 pups in the cage. Keep the pups with an adult female if <23 days old.
5. Separate pregnant dam

Related Requirements

EXTERNAL REGULATIONS AND CONSEQUENCES (if applicable)
The Guide for the Care and Use of Laboratory Animals, 8th ed. 2011, pp. 56-58
UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more detailed guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.
Contact Information

<table>
<thead>
<tr>
<th>Subject</th>
<th>Contact</th>
<th>Telephone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol or Standard Questions</td>
<td>Office of Animal Care and Use</td>
<td>919-966-5569</td>
<td><a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a></td>
</tr>
<tr>
<td>Rodent Housing Questions</td>
<td>Division of Comparative Medicine (DCM)</td>
<td>919-962-5335</td>
<td></td>
</tr>
</tbody>
</table>

Important Dates

- Effective Date and title of Approver: 10/09/03, IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: Revised 07/16/04, Revised 01/12/05, Revised 01/26/05, Revised 01/27/06, Revised 04/17/09, Revised 10/08/10, Revised 06/06/12, Revised 07/10/15, Revised 04/08/16; Revised 03/01/2019. Veterinarians reviewed, new pink card and yellow action card added with descriptions on utilization.

Approved by:

Dr. Roland Tisch
UNC IACUC Chair

3/2019