I. Background: This policy is based on guidelines provided in the 8th edition of The Guide for the Care and Use of Laboratory Animals (The Guide) (13) and has been designed to ensure that mice are housed in a social manner that promotes their health and wellbeing and avoids overcrowding. Cage overcrowding usually occurs in mouse breeding colonies when: litters are not weaned on time, excessive number of breeder animals are housed in a small cage, or weanling mice are not separated as they get older. Because of the health concern, overcrowded cages are considered to be an animal welfare issue.

This policy balances The Guide recommendations against the style of caging available within the Animal Care Facility (ACF). The two types of mouse caging options currently available provide 51.8 (Thoren #3) and 115.6 (Thoren #2) square inches of floor space. If other caging options are to be used, then the Animal Care (AC) office must be consulted for further guidance.

Where breeding animals are involved, litter numbers rather than body weight of the individual animals are used as the basis for assessing overcrowding to avoid unnecessary handling of neonatal animals and associated distress.

II. Caging Density

The table below outlines the allowable caging density for mouse housed within ACF:

<table>
<thead>
<tr>
<th>Density Guidelines</th>
<th>CAGE</th>
<th>Density Guidelines</th>
<th>Breeding Cages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoren #2*</td>
<td>19</td>
<td>10 – 15 g</td>
<td>Trio breeding w/ up to 2 litters provided:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>• Total litter numbers do not exceed 20 pups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>• There is not more than 7 days age difference between multiple litters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 25 g</td>
<td>• All litters are weaned by day 21</td>
</tr>
<tr>
<td>Thoren #3**</td>
<td>8</td>
<td>10 – 15 g</td>
<td>Monogamous breeding pair w/1 litter provided:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>• Litter size is 10 pups or less by postnatal day 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 25 g</td>
<td>• Litter is weaned by day 21</td>
</tr>
</tbody>
</table>
**Dimensions (in): 12.125 (l) x 12.125 (w) x 5.625 (h) [115.6 sq in TOTAL]**

**Dimensions (in): 12.125 (l) x 4.802 (w) x 5.53 (h) [51.8 sq in TOTAL]**

**BREEDING:** Two different breeding schemes are acceptable. The PI or designee is responsible for carefully monitoring and recording matings, pregnancies and the delivery of litters.

**A. Monogamous Pairs:** Postpartum estrus occurs within 24 hours of parturition; thus if male is left in the cage, the female is likely to become pregnant again while lactating and nursing new the litter, taking advantage of postpartum estrus. One male and one female are housed together for mating and the mice are not separated after the female becomes pregnant and delivers pups. Multiple males in a cage with one female OR multiple females in a cage with one male are not permissible. Litters are born approximately 21 days apart. The older litter **MUST** be weaned prior to the birth of the new litter. This will prevent trampling of newborn pups by the weanling pups, mother rejection of either litter, and prevent the cage from being overcrowded.

**B. Trio Mating:** This method houses two (2) females in a cage with one male and the mice are not separated when the females become pregnant or deliver the pups. Multiple males in a cage are not permissible with Trio Mating. This model takes advantage of postpartum estrus and allows the females to become pregnant and nurse at the same time. This method also allows for the females to share rearing responsibilities and may be useful when there is a history of maternal neglect. Litters are born approximately 21 days apart. The older litter(s) **MUST** be weaned prior to the birth of the new litter(s). This will prevent trampling of newborn pups by the weanling pups, mother rejection of either litter, and prevent the cage from being overcrowded. Only two nursing females and their litters plus one stud male are allowed per cage. When this method is used, females typically give birth within a few days of each other and this situation is ideal for shared rearing responsibilities. If there are multiple litters and they vary in age by more than 7 days, the litters and their respective dams must be separated.

**C. Weaning:** By 21 days unless an exception for poor growth/failure to thrive has been sought from ACF veterinary staff. Under these circumstances, weaning may be delayed until 28 days. The smaller (Thoren #3) cages cannot be used to house more than 1 litter, so if delayed weaning is used, the larger (Thoren #2) cage must be used.

**III. Justification for Departure from the Guide:** The two breeding scenarios illustrated above both represent departures from The Guide. Justification for these departures is provided below:

**A. Thoren #2 Departure:** The Guide requires 117 sq. in. for a breeding trio with 2 litters while the Thoren #2 cage only provides 115.6 sq. in. This difference of less than 2 sq. in. is negligible. With the stipulations stated above, regular monitoring of animal health (through sentinel monitoring and daily observations) and breeding records (provided by PIs) this departure from The Guide is justified and performance based evaluations will be used to support and/or revise this departure going forward.

**B. Thoren #3 Departure:** The Guide requires 66 sq. in. for a monogamous pair with a litter while the Thoren #3 cage only provides 51.8 sq. in. While this is a departure from the current recommendations in The Guide, there is a wealth of recent data that supports that this increased density is not harmful to either the adult animals or the growing pups (15, 16, 17, 19, 21, 23, 24, 25). This recommendation is also supported by the historical observations of the breeding colonies at Brown. With the specific stipulations described above, regular monitoring of animal health (through sentinel monitoring and daily...
observations) and breeding records (provided by PIs), this departure from The Guide is justified and performance based evaluations will be used to support and/or reassess this departure going forward.

IV. Responsibilities: The Principal Investigator or designee has primary responsibility for checking for pregnancy and birth and for recording these events on the “breeding” cage card(s). When the litter is born, the Date of Birth (DOB) and projected weaning date is documented (breeding cage card). However, if ACF staff discovers the birth of litters when checking and changing cages, they will record the DOB on the breeding cage card. After the pups are born, the cage is left undisturbed for at least three (3) days except for replenishing of food and water as needed. In case the bedding gets very dirty or wet and the cage must be changed sooner, the following procedure will be followed: the female will be transferred first, and then the litter plus a small amount of the dirty bedding (so the smell in the clean cage will be familiar) is scooped up altogether with a gloved hand or disposable cup and transferred to the new cage.

In cases where ACF identifies a non-compliant cage, the overcrowding **MUST** be corrected by the research laboratory personnel within two business days of ACF notification. If overcrowding is not corrected within two business days, ACF staff will correct the overcrowding and notify the PI. Charges may be incurred for this service.

V. Requests for Exemption from this Policy

The IACUC shall review requests for exemption to allow higher caging densities based on performance standards. An exemption submitted to the IACUC must be species-specific, appropriate for the animals, and include performance indicators. Performance indicators should include environment (e.g. cage humidity, bedding wetness, etc.), air quality (e.g. ammonia levels at cage change), and animal well-being (e.g., cage mate injury, disease, distress). Data is preferred. Requests (and subsequent approvals) are not global, but are for specific circumstances, activities, conditions, and for a defined period of time. Requests (and subsequent approvals) may be submitted based upon caging system, projects, or procedures. Approved exceptions will be posted on the interior of the animal room door by ACF staff.

References:


Brown University Institutional Animal Care and Use Committee
POLICY on Mouse Housing Density and Overcrowding
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