A) RESPONSIBILITIES

It is the responsibility of all investigators housing rodents at UTSA and the animal care staff to abide by and enforce this policy.

B) APPLICATION

The purpose of this policy is to ensure compliance with the “Guide for Care and Use of Laboratory Animals” and UTSA’s IACUC requirements, as they apply to the breeding and overcrowding of rodents. This policy is based on a standard cage of 75 in² for mice and 140 in² for rats. If larger or smaller cages are needed, please consult LARC for housing requirements.

C) DEFINITIONS:

1) ACUP: Animal Care and Use Protocol (UTSA Animal Use for Research Approval Request Form)
2) IACUC: Institutional Animal Care and Use Committee
3) NRC Guide (or Guide): Most current version of the Guide for the Care and Use of Laboratory Animals
4) ACT: Animal Care Technician
5) OC: Overcrowded Cage
6) OO: Overcrowding occurrence. The discovery by LARC staff of one or more OCs belonging to a particular PI at any one given time
7) Monogamous breeding: 1 male and 1 female breeder per cage
8) Harem breeding: 1 male and no more than 2 female breeders per cage
9) Continuous breeding: Breeders kept together after litter is born to allow breeding during the immediate post-partum estrus
10) Non-continuous breeding: Breeding pair separated when female is visibly pregnant
11) Standard weaning: Weaning by 21 days post-partum
12) Extended weaning: Weaning beyond 21 days of age
13) Symbols: ♂ = male ♀ = female ♀ = pregnant ♀ = female with litter
### REFERENCES
Guide for the Care and Use of Laboratory Animals, 2011, Table 3.2 below:

<table>
<thead>
<tr>
<th>Animals</th>
<th>Weight, g</th>
<th>Floor Area/Animal, in.² (cm²)</th>
<th>Height, in. (cm)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice in groups&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;10</td>
<td>6 (38.7)</td>
<td>5 (12.7)</td>
<td>Larger animals may require more space and meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>Up to 15</td>
<td>8 (51.6)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 25</td>
<td>12 (77.4)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥25</td>
<td>≥15 (≥96.7)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td>Female + litter</td>
<td></td>
<td>51 (330)</td>
<td>5 (12.7)</td>
<td>Other breeding configurations may require more space and depend on considerations such as number of adults and litters, and size and age of litters&lt;sup&gt;d&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Rats in groups&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;100</td>
<td>17 (109.6)</td>
<td>7 (17.8)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>Up to 200</td>
<td>23 (148.35)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 300</td>
<td>29 (187.05)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 400</td>
<td>40 (258.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 500</td>
<td>60 (387.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥500</td>
<td>≥70 (≥451.5)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Female + litter</td>
<td></td>
<td>124 (800)</td>
<td>7 (17.8)</td>
<td>Other breeding configurations may require more space and depend on considerations such as number of adults and litters, and size and age of litters&lt;sup&gt;d&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Hamsters&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;60</td>
<td>10 (64.5)</td>
<td>6 (15.2)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>Up to 80</td>
<td>13 (83.8)</td>
<td>6 (15.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to 100</td>
<td>16 (103.2)</td>
<td>6 (15.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥100</td>
<td>≥19 (≥122.5)</td>
<td>6 (15.2)</td>
<td></td>
</tr>
<tr>
<td>Guinea pigs&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Up to 350</td>
<td>60 (387.0)</td>
<td>7 (17.8)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td></td>
<td>≥350</td>
<td>≥101 (≥651.5)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>The interpretation of this table should take into consideration the performance indices described in the text beginning on page 55.

<sup>b</sup>Singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.

<sup>c</sup>Consideration should be given to the growth characteristics of the stock or strain as well as the sex of the animal. Weight gain may be sufficiently rapid that it may be preferable to provide greater space in anticipation of the animal’s future size. In addition, juvenile rodents are highly active and show increased play behavior.

<sup>d</sup>Other considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.
E) PROCEDURES

1) The housing of rodents must not exceed a density greater than recommended by the “Guide” unless approved by the IACUC in the ACUP.

2) All breeding schemes and practices must be described in the ACUP. Any exceptions to this Policy will need IACUC approval.

3) UTSA’s standard for breeding mice and rats is the *monogamous non-continuous* scheme.

4) Any other breeding schemes must be justified and described in the ACUP and approved by the IACUC.

5) Other possible MOUSE breeding schemes, which must be justified to and approved by the IACUC, are:
   a) **Monogamous continuous**.
   b) **Monogamous continuous with extended weaning**. When weaning beyond 21 days of age is necessary (if pup size is the criteria for weaning), pups must be weighed semi-weekly (two times a week) (or more often) and weight records provided to the LARC Director or designee upon request. Other criteria for extended weaning will be considered by the IACUC.
   c) **Harem breeding**. No more than 1 breeding male and 2 breeding females are permitted per cage.
      (1) A visibly pregnant female must be separated. This leaves the 2<sup>nd</sup> female and male in one cage. This cage can now be treated in one of two ways:
      (a) As a monogamous breeding pair (continuous breeding and extended weaning may be permitted with IACUC approval), or
      (b) Another female may be introduced into the cage (the first female that is found visibly pregnant must be separated).

6) Overcrowding exists in a MOUSE cage when:
   a) More than five mice over 21 days of age are present, unless extended weaning has been approved by IACUC, and the cages have been identified as a breeder cage with weanlings.
   b) Any of the following breeding conditions occur in any given cage:
      (1) ◊ ◊ + ◊ - Two pregnant females
(a) **Action required**: Separate each into individual cages.

(2) ♂️ + ♀️♀️ - A pregnant female + a female with litter

(a) **Action required**: Separate pregnant female into individual cage.

(3) ♂️♀️♀️ + ♂️♀️♀️ - Non-pregnant female + female with litter + male, or

♀️♂️♀️♀️ - Non-pregnant female + pregnant female + male

(a) **Action required**: 

(i) Separate male ♂️ and non-pregnant female ♂️ (they may continue breeding in a separate cage), or

(ii) Separate male ♂️ (only if 2nd female ♂️ is not visibly pregnant), or

(iii) Separate non-pregnant female ♂️ (if approved for continuous breeding).

c) Multiple (i.e. two or more) litters are found.

(1) If approved for continuous breeding, pups must be weaned promptly at 21 days of age to avoid having multiple litters at the same time. Extended weaning beyond 21 days must be approved by the IACUC.

(2) Multiple litters for fostering purposes require IACUC approval (for recurring or planned cases) or veterinary approval (for individual cases).

7) **Overcrowding exists in a RAT cage when**:

a) There are more than three rats over 21 days of age per cage, provided the total weight of the animals in the cage does not exceed the maximum allowed by the *Guide*. It is the PI’s responsibility to document the weights (in grams) twice a week on the pertinent cage card.

b) There is more than one rat in a cage when one of the rats is > 500 grams. Rats weighing > 500 grams must be single housed.

c) Multiple litters are present.

d) A male is housed with a visibly pregnant female. Pregnant females must be single housed.

8) **Investigators are responsible for checking their colonies and cage densities daily as well as and recording the date of birth (DOB) for new pups. If the LARC ACT discovers a litter with no recorded DOB they will estimate the date to the best of their knowledge and record it on the back of the cage card or the PI’s indicated area. LARC is not responsible for miscalculated DOB’s.**
9) When an overcrowded cage is found, the LARC will take appropriate action to correct the overcrowding problem.
   a) Cages that contain situations that, under the judgment of the University Veterinarian or designee, may be detrimental to the health and welfare of the rodents may be separated immediately.
   b) Three OOs within a 30-day period, if not corrected within three calendar days of notification will be reported to the IACUC and the LARC Director.

10) The number of animals separated into newly generated cages to alleviate overcrowding will be added to the applicable protocol’s census at the time new cages are generated.
    a) If the new cages (actual number of animals) cause the protocol to exceed its IACUC approved animal use number, the excess animals will be transferred to the LARC Holding Protocol until this situation is remedied by the Investigator.
    b) Animals transferred to the LARC Holding Protocol may not be used, handled or manipulated without the consent of the LARC Director. Per diems are still charged to the PI during this holding period.

11) PI notification
    a) LARC personnel notifies PI/lab personnel if overcrowded cages are identified and sets a 3-calendar-day grace period for lab staff to correct the overcrowding. LARC notifies PI/lab personnel by placing a tag on overcrowded cages with CDLARC008 – Overcrowded Cage card (white card with red letters).
    b) The grace period for separating the animals begins at the time notification is made.

12) LARC actions
    a) If PI/lab personnel fail to correct the overcrowded cage(s) by the end of the grace period, LARC personnel will separate the animals from overcrowded cage(s) and the PI will be charged for this service based on established LARC services rates for technician time.
b) LARC tags overcrowded cages with *CDLARC008 – Overcrowded Cage* card (white card with red letters) to notify investigators. PI/lab personnel are required to visit their animals on a daily basis, so no other form of PI/lab personnel notification is needed.

c) After overcrowding is corrected, LARC staff remove the *CDLARC008 – Overcrowded Cage* card from the cage. The removal of this card by LARC staff serves as notification to PI/lab personnel of the separation. A fee for this service is then charged to the PI.

d) While every effort will be made by the LARC staff to identify separated animals correctly, their only mission in this instance is to ensure that full compliance with federal mandates is maintained. If LARC staff has to separate animals from overcrowded cages after deadline time & date, LARC assumes no liability in the maintenance of research data.

13) IACUC intervention

a) After receiving notice of 3 or more PI-uncorrected overcrowding incidents (within a 30-day period) from the LARC, the IACUC will take appropriate action, which may include but is not limited to:

1. A formal warning letter
2. Remedial training
3. Restricted access to the animal room/vivarium
4. Reassignment of the investigator’s animals in part or in whole to the LARC for management or to help investigator with colony breeding. Fees for special services will apply.
5. Suspension of research activities in part or in whole.

14) Deviations from this Policy must be justified in writing (generally in an Amendment or New/Renewal Protocol application) and may be administratively approved as long as there is sufficient assurance that the weight/number of animals in any one cage will not exceed the provisions of the ‘Guide.’ Other deviations from this Policy must also be justified in writing and will need committee approval.