



BROWN

Institutional Animal Care and Use Committee (IACUC)

Policy on Mouse Housing Density and Colony Management

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I. Purpose and Background

The Brown University IACUC has developed this policy to provide guidance to research personnel regarding the housing of mice and management of breeding colonies. It is imperative that mice be housed in a social manner whenever possible that promotes health and wellbeing while avoiding overcrowding. Cage overcrowding usually occurs in mouse breeding colonies when litters are not weaned on time and removed from the cage or there is an excessive number of breeder animals housed in the same cage. This policy is based on guidelines provided in the 8th edition of the *Guide for the Care and Use of Laboratory Animals* (the Guide).

This policy balances the Guide recommendations against the style of caging available within the Center for Animal Resources and Education (CARE) Facilities. The types of mouse caging options currently available provide 51.8 (Thoren #3), 115.6 (Thoren #2) or 75 (Optimice) square inches of floor space.

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 mice housed within the ACF.

Density Guidelines					
Cage	< 10 g	10-15 g	16-25 g	> 25 g	Breeding Cages
Thoren #2* 	19	14	9	7	Trio breeding with up to two litters provided: <ul style="list-style-type: none"> • Total litter numbers do not exceed 20 pups • There is not more than 7 days age difference between multiple litters • All litters are weaned by day 21
Thoren #3** 	8	6	4	3	Monogamous breeding with one litter provided: <ul style="list-style-type: none"> • Litter size is 10 pups or less by postnatal day 14 • Litter is weaned by day 21 Trio breeding is not allowed in these cages.
Optimice*** 	12	9	6	5	Monogamous pair with one litter provided: <ul style="list-style-type: none"> • Total litter numbers do not exceed 15 pups • Litter is weaned by day 21 Trio breeding provided: <ul style="list-style-type: none"> • One of the females is removed as soon as she is identified as being pregnant.

* Dimensions (in): 12.125 (l) x 12.125 (w) x 5.625 (h) [115.6 in² TOTAL]

** Dimensions (in): 12.125 (l) x 4.802 (w) x 5.53 (h) [51.8 in² TOTAL]

*** Dimensions (in): 12.5 (l) x 11 (w) x 5.06 (h) [75 in² TOTAL]

III. **Breeding:**

Males and females should be placed together when they are 6-8 weeks of age. This is when mice reach sexual maturity and mice tend to gain weight and be less productive if mated at later ages. All breeders should be replaced at 7-8 months of age, as this is the window for optimal breeding performance. This window may be shorter for specific inbred strains or transgenic animals. Lab personnel are responsible for setting up, monitoring, and recording matings and pregnancies as well as weaning litters at appropriate ages. Personnel are encouraged to keep additional detailed breeding records separate from the cage cards to prevent accidental loss of important data.

The breeding following breeding schemes are allowed within CARE Facilities, as long as the density guidelines above are followed regarding cage type and size.

- a. **Monogamous Pairs:** This method involves housing a single female with one male. The male can be left with the female in order to take advantage of postpartum estrus, which occurs within 24 hours of parturition. Using this strategy, the female is likely to become pregnant again immediately after delivering her litter. This allows for more efficient breeding. 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 to prevent trampling of newborn pups, maternal rejection of either litter and overcrowding of the cage.
- b. **Trio Breeding:** This method houses two females in a cage with one male. Multiple males in a cage are not permissible with trio breeding. This strategy also takes advantage of postpartum estrus and allows the females to become pregnant again immediately after giving birth. It 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) to prevent trampling of newborn pups, maternal rejection of either litter and overcrowding of the cage.

Thoren# 2 (group cage): 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 which allows 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.

Optimice cage: As this cage only allows for the presence of one litter, if trio breeding is done, one of the females must be removed as soon as she is

identified as pregnant. This will prevent a situation where there are three adults and two litters in the cage, which would constitute overcrowding and represent an animal welfare issue in these cages.

- IV. **Justification for Departure from the Guide for Thoren cages:** The breeding scenarios illustrated above represent departures from the Guide. Justification for these departures is provided below:
- a. Thoren #2 Departure: The Guide requires 117 in² for a breeding trio with 2 litters. The Thoren #2 cage only provides 115.6 in², resulting in a difference of less than 2 in². This difference is negligible, and with regular monitoring of animal health and breeding records this departure from the Guide is justified and performance based evaluations will be used to support and/or reassess this departure going forward.
 - b. Thoren #3 Departure: The Guide requires 66 in² for a monogamous pair with a litter and the Thoren #3 cage only provides 51.8 in². While this is a departure from the current recommendations in the Guide, there are data that supports that this increased density is not harmful to either the adult animals or the growing pups. 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, and breeding records, this departure from the Guide is justified and performance based evaluations will be used to support and/or reassess this departure going forward.
- V. **Genotyping and Identification** should be done in advance of weaning and at the earliest age the method allows. This will allow animals of an undesirable genotype to be euthanized prior to weaning. If animal identification is being performed through the removal of a piece of tissue (e.g., ear punching, toe clipping), that sample of removed tissue should be used for genotyping purposes. Toe clipping can be performed in mice 7-10 days of age and ear punching can be done at 14 days of age. Tail biopsies can also be used for genotyping purposes in mice prior to weaning but another method is required to provide permanent identification. For complete details on all of the available methods, see Brown University's [Rodent Genotyping Policy](#) and [Rodent Identification Policy](#).
- VI. **Weaning:** Weaning must be done at 21 days of age unless an exception for poor growth/failure to thrive has been sought from the Center for Animal Resources and Education (CARE) veterinary staff. Under these circumstances, weaning may be delayed until 28 days. If the female has more than one litter, the larger (Thoren #2) cage must be used as the smaller cages (Thoren #3 and Optimice)

cannot house more than one litter. Males and females must be weaned into separate cages.

- VII. **Responsibilities:** The Principal Investigator or designee has primary responsibility for all aspects of colony management including observing and confirming pregnancy and birth of litter(s); recording these events on the breeding cage card(s) and weaning at appropriate timepoints. When a litter is born, the date of birth (DOB) and projected weaning date must be documented on the breeding cage card. If CARE staff discover a new litter(s) during cage changing procedures, they will record the DOB and projected weaning date. After a litter is born, the cage is left undisturbed for at least three (3) days except for replenishing of food and water as needed. In the rare circumstance where a 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 and nesting material is scooped up altogether with a gloved hand or disposable cup and transferred to the new cage.

In cases where CARE staff identify an overcrowded (e.g., non-compliant) cage, the overcrowding **MUST** be corrected by the research laboratory personnel within two business days of the date of the notification. If overcrowding is not corrected within two business days, CARE staff will correct the overcrowding and notify the PI. Charges will be incurred for this service. In situations where animal welfare is impacted by the overcrowding, CARE staff will correct the overcrowding immediately and charges will be incurred.

CARE offers colony management services to all rodent users on a fee-for-service basis. Services include rodent breeding, strategic planning and maintenance, colony maintenance, animal identification, animal ordering, sample collection for genotyping, euthanasia, tracking animal usage numbers, and record maintenance. The CARE office (care@brown.edu) can be contacted for more information on the program.

VIII. **Requests for Exemptions from this Policy**

The IACUC shall review requests for exemptions 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 driven requests are 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 CARE staff.

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