THE MOUSE BREEDING POLICY
The IACUC approved mouse breeding policy is available for reference on the IACUC website. Click HERE
When a litter is born, the policy allows no more than 3 adults in a cage.

≥ 14DAYS after birth, the policy allows:

- 9 pups with 3 adults, or
- 11 pups with 2 adults, or
- 14 pups with 1 adults

When pups have reached ≥ 10 GRAMS, the policy requires the pups to be weaned.
Mandatory Exception Situations

- Multiple males in the space with a single female.

- A particular strain consistently delivers a litter of 14 or more neonates.
Investigator Responsibilities

- Place nesting material in all breeding cages at time of set-up.

- **Date of Birth** - Indicate the date of birth for each litter on all cages. This information should be recorded within 3 days after birth of the litter. The information must be **legible**.

- If a DLAM scan card is NOT available, write the PI name, Protocol Number and Date Cage Cards Requested on a regular white card. The information must be **legible**.
Investigator Responsibilities (cont)

- Remove dead pups from cages as soon as identified
  - Place in a carcass bag, labeled with the protocol number

- Complete genotyping early to prevent overcrowded cages

- Do not rely on DLAM to manage your mouse colony
Differentiating Sexes

• Males have greater anogenital distance
• Males reach sexual maturity at 8 weeks
• Males breed up to one year

• Females reach maturity at 7-8 weeks
• Females breed for approximately 6-8 months
Weaning Tutorial

Dr. Nirah Shomer DVM, PhD, DACLAM created a PowerPoint that is an excellent aid in sexing young pups. Please view the presentation if you are having trouble determining the sex of young rodents.

http://www.lawte.org/materials/nirah/weaning_mice.ppt
WHAT DOES THE YELLOW “ACTION REQUIRED” CARD MEAN?
Example #1

The cage has too many adults
More adults than UNC allows

OR

4 or more adults with pups

<table>
<thead>
<tr>
<th>Weight</th>
<th>Floor area per animal</th>
<th>Tecniplast # per cage 78-82 sq in</th>
<th>Conventional # per cage 70 sq in</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 g</td>
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<td>5</td>
</tr>
<tr>
<td>&gt; 25 g</td>
<td>15 sq. in</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
Example #2

At day 14 or greater there are more pups than the policy allows

Overcrowding if there are more than
- 9 pups with 3 adults, or
- 11 pups with 2 adults, or
- 14 pups with 1 adult
Example #3

Pups weigh greater than 10 grams

*Cards will be put on when pups REACH 10g and not before*
DLAM is NOT required to contact investigator prior to placement.

Repeated or ongoing problems w/ policy will be reported to the IACUC.

Contact IACUC if you cannot comply w/ policy.

Investigator’s responsibility to ensure mouse cage densities are maintained!

Extreme cases of overcrowding!

- DLAM is authorized to correct the overcrowded cage w/o prior notification to PI.
THERE ARE 3 INSTANCES WHERE THE PINK CARD IS USED
4 or more adults in a cage and any of the females are pregnant
New litters are born and the # of pups is more than the policy allows after day 14.
Instance #3

Pups weigh greater than 10 grams
HOW CAN THE AGE OF YOUNG MICE BE ESTIMATED?
Days 1 - 4 pups go from bright red to pink
Days 5 - 8: Pups ears are up and they begin to grow fur.
Days 9-12 pups become fully furred and will have top and bottom teeth
Days 13-14 eyes and ears will open
<table>
<thead>
<tr>
<th>DAY</th>
<th>EVENT</th>
<th>DAY</th>
<th>EVENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin real red; short curly whisk; umbilical cord</td>
<td>8</td>
<td>Fuzz on belly</td>
</tr>
<tr>
<td>2</td>
<td>Straight, fine whisk; skin lighter red</td>
<td>9</td>
<td>Fur back; last day belly translucent, milk bands</td>
</tr>
<tr>
<td>3</td>
<td>Ear “nub” prominent</td>
<td>10</td>
<td>Top teeth erupted; belly slight fuzz</td>
</tr>
<tr>
<td>4</td>
<td>Pink smoother skin; ear flaps ½ up, down</td>
<td>11</td>
<td>Fully furred; fur short &amp; smooth</td>
</tr>
<tr>
<td>5</td>
<td>Ears up; white fuzz across shoulders</td>
<td>12</td>
<td>Nipples visible</td>
</tr>
<tr>
<td>6</td>
<td>Bottom teeth erupted</td>
<td>13</td>
<td>Eyes open</td>
</tr>
<tr>
<td>7</td>
<td>Entire back slightly furred</td>
<td>14</td>
<td>Ears open</td>
</tr>
</tbody>
</table>
ENFORCEMENT

WHEN A YELLOW
“Action Required” CARD
IS PLACED ON THE
CAGE........

Cage density should be addressed by research staff within 48 hours.
DLAM DATES CARDS AS FOLLOWS:

The correction must occur before the end of the stipulated day.

On Monday: date is for Wednesday
On Tuesday: date is for Thursday
On Wednesday: date is for Friday
*On Thursday: date is for Monday
*On Friday: date is for Monday
If no action is taken by the morning of the 3rd day, then DLAM is required to act.
SPACE AVAILABLE:

• DLAM will separate the animals

• DLAM fills out back of yellow card and gives to facility supervisor

PI Name: Bellinger
Protocol#: 04.004
Building: McGavran
Room#: 2116
Date: 2/3/05
Card#: 123456
Your Initials: TRH

Keep for 3 months
IF **NOT ENOUGH SPACE IS AVAILABLE** DLAM WILL

- Separate the cages they can

- **euthanize animals in the remaining overcrowded cages**
  (starting with the oldest pups)
• DLAM will fill out a euthanasia form

• DLAM will euthanize only the number of animals that will bring the cage into compliance (starting with the oldest pups)

• DLAM fills out back of yellow card and give it and the euthanasia form to supervisor

PI Name: Bellinger
Protocol#: 04.004
Building: McGavran
Room#: 2116
Date: 2/3/05
Card#: 123456
Your Initials: TRH

Keep for 3 months
Division of Laboratory Animal Medicine
University of North Carolina

BREEDING POLICY NON-COMPLIANCE CHARGES

For Business Office Use Only:

<table>
<thead>
<tr>
<th>Email Subject Line:</th>
<th>NON-COMPLIANCE CHARGES</th>
</tr>
</thead>
</table>

THIS IS BEING SENT TO YOU FOR INFORMATION ONLY
THESE CHARGES WILL BE BILLED ON THE NEXT DLAM MONTHLY BILLING

<table>
<thead>
<tr>
<th>Email Notice To PI and Admin Staff:</th>
<th># of Cages Out of Compliance:</th>
<th>Total Charges @ $50 per Cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date Sent:</td>
<td>Billing Ref:</td>
</tr>
</tbody>
</table>

To Be Completed by Husbandry Supervisor:

Send to: dlamadmin@unc.edu  Subject Line: NON-COMPLIANCE CHARGES

<table>
<thead>
<tr>
<th>Investigator Name:</th>
<th>Protocol #:</th>
<th>Building &amp; Room #:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date:</th>
<th>Separated By Whom:</th>
<th>Form Completed By:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Item #</th>
<th>Over Crowded Cage Card Number(s):</th>
<th># of New Cages:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
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<tr>
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CASE EXAMPLES IN WHICH ANIMALS WILL BE EUTHANIZED BY DLAM TECHNICIANS IN ORDER TO BRING A CAGE INTO COMPLIANCE:

This is after the 2 day period and there’s no space to separate animals.
If you find 7 adult mice in a Tecniplast cage that are over 25 grams, how many should be euthanized?

2 ADULT MICE WILL HAVE TO BE EUTHANIZED
If you find 2 adult mice and 10 pups more than 10 grams but less than 15 grams in a Tecniplast cage, how many should be euthanized?

4 PUPS WILL HAVE TO BE EUTHANIZED

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You find 3 adults and 3 litters in a Tecniplast cage, what needs to be euthanized?

The 4 (18 day olds) will have to euthanized, and 4 of the 5 (14 day olds) will have to be euthanized.

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Additional Scenarios for Cage Overcrowding:

- The following 10 examples are to provide an understanding of how DLAM technicians will bring an unattended, overcrowded cage into compliance.
- Possible options for the researcher are also indicated.
Conventional Cage: 3 adult mice, 7 pups ≥ 10 grams and 1 pup < 10 grams

Solution?

DLAM: Ideal: All 8 pups weaned into one cage. If no extra cage space was available, “wean” 3 pups into the existing cage, euthanizing 5 pups.

PI: The PI can do the same thing.

Comment: The floor space (square inches) of each animal must be known: 3 x 15 = 45 sq. in. for the adults. Total floor space is 70 sq. in. 25 sq. in. remains for the pups. Each pup requires 8 sq. in.; therefore only 3 pups may remain in the cage. If the specific strain puts on weight quicker than it matures, an exception must be approved by the IACUC to allow heavier pups to remain with the adults.
Conventional Cage: 1 adult + 17 pups

Solution?

**DLAM:** At day 14, DLAM places the yellow “Action Required” card. Receive veterinary approval to separate 3 pups at day 16, or euthanize them.

**PI:** Receive veterinary approval to separate 3 pups at day 16, or euthanize them.

**Comment:** An exception can be submitted to the IACUC for approval allowing > 14 pups with 1 adult after 14 days of age. Pups to be weaned prior to 10 grams need veterinary approval in order to ensure viability.
Tecniplast Cage: 6 adults (>25 grams) + 1 female pup (15 grams and 21 days old)

Solution?

**DLAM**: Separate male and females into two cages. If no space available, euthanize female pup and 1 male.

**PI**: Separate male and females into two cages. If no space available, euthanize 2 mice.

*Comment: CAGE OUT OF COMPLIANCE WHEN LITTER BORN, SINCE ONLY 3 ADULTS ALLOWED WHEN PUPS ARE BORN.*

Maximum of 5 adults (>25 grams) allowed per Tecniplast cage. **Exception for more than 1 male together with a single female per cage is required.**
Tecniplast Cage: 4 adults (>25 grams) + 2 pups (10 grams; 14 days old \{atypical scenario\}) and 2 new litters of pinkies (11 total)

Solution? Several acceptable options are available

**DLAM:** Remove 1 adult (preferably male). Separate 2 10 gram pups. Since mice at 14 days are not generally 10 grams, veterinary approval for pup viability is required. If no space is available, euthanize all the pups (2 10 gram and 11 pinkies).

**PI:** Remove 1 adult (preferably male). Separate 2 10 gram pups. Since mice at 14 days are not generally 10 grams, veterinary approval for pup viability is required. If no space is available, euthanize all the pups (2 10 gram and 11 pinkies).

**Comment:** Cage already out of compliance when pups born 14 days ago. No more than 3 adults and a total of 9 pups ≥ 14 days old allowed. Exception is required if animals > 10 grams are to remain in the cage with adults.
Tecniplast Cage: 6 adults @ 20 grams

Solution?

**DLAM**: No action required.

**PI**: No action required.

**Comment**: The floor space (square inches) of each animal must be known: 6 x 12 = 72 sq. in..
Conventional Cage: 2 adults @ 30 grams, 3 pups @ 14 grams and 2 pups @ 9 grams

**Solution?**

**DLAM**: No action required. Ideally, if space is available, the pups > 10 grams would be weaned into a new cage.

**PI**: No action required. Ideally, if space is available, the pups > 10 grams would be weaned into a new cage.

**Comment**: The floor space (square inches) of each animal must be known: $2 \times 15 + 3 \times 8 + 2 \times 6 = 66$ sq. in. Technically, the 14 gram juveniles are ‘weaned’ into their existing cage.
Transferring from Tecniplast Cage to Conventional Cage: 5 adults >25 grams

Solution?

**DLAM:** Separate into 2 cages. Either 2 females (pregnant and not pregnant); male housed alone or with a female, or 1 female removed.

**PI:** The PI can do the same thing.

**Comment:** Only allowed 4 adults in a conventional cage.
Tecniplast Cage: 3 adults (trio breeding) and 8 pups (<10 grams, 15 days old)

Solution?

**DLAM:** Initially, no action required. When pups weigh ≥ 10 grams, all pups must be weaned. If no space is available, 6 pups are euthanized.

**PI:** The PI can do the same thing.

**Comment:** 3 adults and 9 pups after 14 days of age allowed.
Conventional Cage: 3 adults (trio breeding) and 12 pups (<10 grams, 14 days old)

Solution?

**DLAM:** Ideally would remove 2 adults. If no space available, 3 pups must be euthanized.

**PI:** The PI can do the same thing.

**Comment:** 3 adults and 9 pups after 14 days of age allowed until pups are $\geq$ 10 grams.
Tecniplast Cage: 3 adults + 14 pups (16 days old)

Solution?

**DLAM:** If space is available, separate 2 adults (leave mother with pups). If no space is available, euthanize 5 pups.

**PI:** 1) move two adults into cages with other mice not at capacity, 2) leave the adults and cull at least 5 pups with an unusable phenotype, 3) leave the adults and choose 5 pups >10 grams to euthanize or wean into other caging not at capacity, 4) continue to relocate or cull pups from cage as they reach 10 grams using floor space calculations for mice 10-25 grams, or 5) request exception for future breeding.

**Comment:** No more than 3 adults and 9 pups after 14 days of age allowed until pups are ≥ 10 grams.
Euthanasia: Rodent Pups and Adults

- Unweaned pups that are euthanized prior to weaning do not count towards the total animal number in section 4-0-3.

- Remember! Unweaned pups are vertebrate animals and must be treated humanely and in compliance with federal and institutional policies.

- The method of euthanasia of pups and adults must be described in the application.

- Ensure only methods approved in the application are used.
WHEN THE RESEARCH GROUP CORRECTS THE CAGE DENSITY THEY NEED TO INDICATE THIS BY INVERTING THE YELLOW CARD AND LEAVING IT ON THE CAGE.
The Office of Animal Care and Use conducts voluntary Mouse Colony Management lectures on an as needed basis.

To Sign up for the next Colony Management Lecture, go to http://research.unc.edu/Offices/NLAC/index.htm. Click “Mouse Related Topics” on the left-hand side of the screen under “Inside the Office”. To Sign Up for the next lecture, please contact Sandra O'Buckley, Rene Cutting or Emily Hearne.
ANY QUESTIONS, THOUGHTS OR CONCERNS?