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<b>Distribution</b>	<b>RAR/ACUC</b>	

## **HM – 8: Research Animal Resources (RAR<sup>1</sup>) Policy and Procedures for Handling Overcrowded Mouse Cages<sup>2</sup>**

**Purpose:** Procedures for RAR to use to manage overcrowded cages. To provide information to mouse researchers regarding the rules of cage population management. This is not to be used as a notification system for mouse breeders to use to manage their breeding colonies.

**Definitions** - An overcrowded cage is a cage that has:

- more than 5 adult mice, adult being **6 weeks of age or older**
- more than 9 weaned mice that are **less than 6 weeks of age**
- more than one litter of pups where the oldest litter is fully furred and moving about the cage
- an animal welfare concern due to too many animals in the cage

**Options:** When the PI or contact person is called regarding an overcrowded cage the following options are available:

- A person from the lab may separate the animals into additional cages
- A person from the lab may euthanize some or all of the animals in the cage
  - < It is **not** acceptable to mark the cage for euthanasia and leave it overcrowded >
  - < It is **not** acceptable to pool multiple cages of animals into one cage to be euthanized >
  - < It is **not** acceptable to **remove the overcrowded tape** unless the cage is no longer overcrowded >

**Information:**

1. **Maximum adult mice/cage:** The maximum number of adult mice in a cage is 5. This is based on the average weight of an adult mouse and the size of the caging used in the animal facilities. Recommended spaces for commonly used group-housed animals are indicated in the *Guide for the Care and Use of Laboratory Animals*<sup>3</sup>. For the purposes of this document an **adult is defined as 6 weeks of age or older**.
2. **Contact information:** In addition to the PI’s name and protocol number, the name and phone number of the contact person must be on the cage cards. Investigators are responsible for updating this information. RAR uses this information to contact the laboratory if a cage is overcrowded.
3. **Birth/wean dates:** Investigators are responsible for marking the birth and/or weaning dates on their breeding cages. This date is critical for breeding programs with tight timelines for weaning and the birth of subsequent litters, and for accurate determination of overcrowding by RAR staff.

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<sup>1</sup> RAR refers to all mouse housing at Johns Hopkins University

<sup>3</sup> Guide for the Care and Use of Laboratory Animals, NRC. National Academy Press, 2011. P57.

4. **Post-partum estrus:** Post-partum estrus is a 24 hour period after parturition during which the female is fertile and receptive to mating. A dam that is with the male when she delivers litter A will most likely be mated in the 24 hour period. Therefore, she will be due to deliver litter B at 21 days gestation. Litter A should be weaned **before** the expected delivery date of litter B. Once litter B is delivered, the cage is considered severely overcrowded, as the trampling of neonates by the furred litter can result in an animal welfare concern.
5. **Weanling housing:** Mice are not considered adults at weaning. For the purpose of the number of mice per cage, mice are considered adults at 6 weeks of age and therefore are housed with a maximum of 5 mice per cage by that age. Prior to 6 weeks of age, they are still pups. Pups are weaned at 21 days of age, unless there is scientific justification to keep them with the dam longer, such as certain strains of mice that are weaned at 28 days. When weaned prior to genotyping, mouse pups may be housed at greater than 5 per cage with a **maximum of 9**. This is based on the average weight of weanling mice and the size of the cages. The **date of birth must be clear on the cage cards**. If the age of the mice is unclear, they must be housed at a maximum of 5 per cage.
6. **Trio or harem breeders:** If trio or harem breeding schemes are used and more than one female has a litter, each dam with a litter must be separated **before** the oldest litter is moving about the cage, which begins at about 10 days of age. Since this is before weaning age, separation of a trio/harem cage requires that the dam AND her pre-weanling pups are separated to a new cage. The male may be kept with one of the females. If the birth date or wean date are not on the cage card, the cage will be marked as overcrowded when the oldest litter begins to move about the cage.
7. **Timeline for separating:**
  1. Laboratory personnel must separate an overcrowded cage within 2 days of being notified.
  2. A severely overcrowded cage must be separated within 4 hours, or sooner if indicated by the conditions (see below).
  3. RAR personnel will separate the overcrowded cage on the 3<sup>rd</sup> day after notification to the lab, if the lab has not already separated the cage.
8. **Technical service fee:** If RAR must separate the cage due to the lack of availability or action by laboratory personnel, a technical services fee will be charged to the job number on the census sheet.
9. **Chronic overcrowding:** Chronic failure of a laboratory to respond to RAR's request to separate overcrowded cages, or removal of "Overcrowded" tape without separating the cage will be referred to the Institutional Animal Care and Use Committee (IACUC) as a compliance concern.
  1. Chronic cases of labs having frequent overcrowded cages also **may** be referred to the IACUC as a compliance concern.
10. **Clinical call:** If at any time the animals require medical attention, a clinical call should be placed by calling 410-955-3273.
11. **Exceptions** to this policy must be justified in the protocol or in the Mouse Breeding Colony form.

#### **Procedures for Severely Overcrowded Cages:**

1. A severely overcrowded cage is one in which delay could result in harm, illness, or extreme discomfort to the animals in the cage, such as: trampling or cannibalizing of pups, fighting, or moisture build up inside the cage.
  1. RAR will use the contact information on the cage card to notify the laboratory **immediately** of the presence of a severely overcrowded cage.
  2. Animals in a severely overcrowded cage must be separated **within 4 hours**. In cases where a 4 hour period could result in animal death, the cage will be separated immediately, and a technical services fee will be charged to the job number on the census sheet.

3. Examples of severely overcrowded cages include:
  1. Two litters in one cage, where one litter is furred and moving about the cage (See Information, Section 4 and Section 6)
  2. Excessive populations of juvenile or adult mice, especially when visible fighting or wounding is seen
2. When responding to a notification of an overcrowded cage, the lab **must personally separate or euthanize** the animals. It is **not acceptable to mark the cage for euthanasia and leave it overcrowded** for RAR to euthanize.
3. After notifying a lab of a severely overcrowded cage, RAR will record whether they reached a person or left a voicemail, the name and phone number of the individual in the laboratory that was contacted, and the date & time of the call on the overcrowded cage log for the room/suite.
  1. A follow-up email will be sent to document the severely overcrowded cage notice.
4. RAR will mark the cage with an “OVERCROWDED” label and record the date, time and their initials on the label.
5. If someone from the lab cannot be located or does not respond within 4 hours, or if the severely overcrowded cage is deemed to pose an animal welfare concern before 4 hours, then the supervisor or designee will proceed with separating the animals and notify the supervisor.
  1. If the cage contains multiple litters, refer to Information Section, Item 6. If the older litter is not yet at weaning age, then one litter must be separated **with the dam** to a new cage.
  2. Males will be placed in separate cages from females.
  3. A technical services fee will be charged to the job number on the census sheet.
6. Cage cards with information identifying the home cage will be placed on the new cages.

**Procedures for Mild to Moderately Overcrowded Cages:**

1. A mild to moderately overcrowded cage is one in which the number of animals exceeds the number identified in this policy.
2. On Day 1, using the contact information listed on the cage card, RAR will notify the laboratory that there are overcrowded cages that must be separated within 2 days.
3. RAR care staff will report overcrowded cages to the area supervisor, and the supervisor will contact the lab via phone or email.
  - a. RAR will record whether they reached a person or left voice mail, the name and phone number of the individual in the laboratory that was contacted, and the date & time of the call on the overcrowded cage log for the room/suite.
4. RAR will mark the cage with an “OVERCROWDED” label and record the date, time and their initials on the tape.
5. The lab will have 2 days to separate or euthanize the animals.
6. If the lab responds to the overcrowded cage notification to request that the animals not yet be separated, the following guidelines will be followed:

Example	Action
Overcrowded cage with a breeding male and female, and a single litter of pups with <b>no date of birth listed</b> .	The case is considered resolved and noted on the overcrowded cage log, and the “OVERCROWDED” label is removed.
Lab subsequently provides date of birth indicating animals are not yet 21 days old.	The lab must write the DOB and wean dates on the cage cards.  If the cage is not separated on the wean date, the cage will be marked

	Overcrowded again, and the process will restart at step 2.
Overcrowded cage with a breeding male and female, and a single litter of pups with a <b>date of birth that indicates pups are over 21 days old.</b>	RAR staff will refer the case immediately to the veterinary team to determine next steps.  Overcrowded tape will not be removed unless directed by the veterinarian.
Lab subsequently requests that pups be allowed to stay with the dam longer.	
<b>Any other situation</b> in which the lab requests that a cage, which RAR has determined meets the overcrowded cage criteria, not be separated	RAR staff will refer the case immediately to the veterinary team to determine next steps.  Overcrowded tape will not be removed unless directed by the veterinarian.

7. On Day 3, if the cage has not been separated, with supervisor or designee approval RAR will proceed with separating the animals and notify the supervisor.
  - a. If the cage contains multiple litters, refer to Information Section, Item 6. If the older litter is not yet at weaning age, then one litter must be separated **with the dam** to a new cage.
  - b. Males will be placed in separate cages from females.
  - c. A technical services fee will be charged to the job number on the census sheet.
8. Cage cards with information identifying the home cage will be placed on the new cages.

I acknowledge that I have read and understand the JHU Animal Care and Use Program document “**Research Animal Resources (RAR) Policies and Procedures for Handling Overcrowded Mouse Cages**” and I will follow this procedure. I agree to bring any deviations in this procedure to the attention of my supervisor/GPS Working Group.

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Name (Print)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature