ANIMAL EYE CONSULTANTS OF IOWA

Animal Eye Consultants of Iowa	
Instituti	***mittee***
Policy # 39	**"Mouse Colony Maintenance"**
Approval Date: 3/27/2024 (Replacing version 11/1/2021)	

A. Purpose

To outline the AEC IACUC?s expectations for the management of mouse breeding colonies and to ensure space recommendations in the ILAR Guide for the Care and Use of Laboratory Animals are met.

B. Background

The ILAR Guide provides the recommended density of animals permitted in rodent cages. Adult mice and their litters can occupy considerable cage space, produce large amounts of fecal and urinary material, and increase cage temperature, humidity, and ammonia levels, all of which can create sub-optimal housing conditions. Overcrowding caused by multiple litters in a cage and failure to wean on time can have a negative impact on colony health. The ILAR Guide indicates a female mouse with litter should have a minimum of 51 square inches of floor space. Dimensions of standard caging systems used at AEC and guidelines for required space for mice can be found in section D below. The majority of AEC mouse cages are only big enough to support a single dam with a litter and the male (see Table 1).

Exceptions to these standards are recognized as necessary in certain circumstances, such as with strains of low birth weight and slow growth rates, or poor maternal behavior requiring multiple mothers in one cage to assist in nursing (alloparenting). Requests for exceptions for particular strains must be included in the ASAF for IACUC review and approval.

C. Policy

Each PI maintaining rodent breeding colonies must describe their breeding scheme and management plan in their breeding ASAF.

Repeat issues with overcrowded cages or failure to adhere to this policy and/or the management plan approved in the ASAF can result in the loss of privilege to maintain a breeding colony.

Breeding Schemes:

Mice can be set up in trios, harems or pair breeding. Only one male is allowed per breeding cage.

Multiple females may be bred to a single male; however, the total number of mice per cage may not exceed the cage capacity. For trios and harem breeding schemes, a pregnant female must be separated and placed into her own cage (with appropriate nesting/enrichment materials for an individually housed animal) prior to giving birth. This is usually accomplished before E15 (15 days of gestation) when pregnancy is identified, or after mating when a plug is identified. If a female gives birth while in the trio or harem cage, the male and remaining females should be removed to a separate cage leaving the female with her litter undisturbed. When pregnant females are separated, the male may remain with only one female, or he can be moved to another breeding cage or housed separately based on the needs of the colony/protocol.

When breeding in pairs (one male to one female), the dam and sire may remain together throughout gestation and lactation. Breeding pairs often breed immediately following parturition during the postpartum estrus, so pairs with litters near weaning age must be monitored closely for the arrival of a new litter. Ideally, the current litter should be weaned just prior to the birth of the new litter; however, if the new litter arrives early, the older litter must be weaned. Alternatively, the new litter can be euthanized. Comingling slowly developing pups from a pre-existing litter with a new litter can only be managed through a medical directive from the AEC veterinary staff on an individual cage basis. AEC must be contacted to evaluate and approve any comingled cages.

Weaning:

Mice are usually weaned between 19-23 days of age, with 21 days of age being the most common. Litters may also be left with the dam for an extended time when underweight or small of stature, as long as the dam doesn't give birth to another litter. Some transgenic, inbred, or specialty strains do not mature as quickly as normal wild type mice and require an extended nursing period. The weaning age is extended until they are mature enough to be weaned, and a notation is made on the cage card. When strains commonly require an older weaning age (beyond day 23), this exception should be noted in the ASAF and be discussed with the husbandry staff. The weanling mice are separated by sex and housed in a density appropriate for the facility and caging. When genotyping, IACUC SOP #2 on identifying and genotyping rodents must be followed. If the Principal Investigator (PI) or PI's personnel fail to wean a litter or have multiple litters in a cage, the animal care staff will proceed to wean and separate the overcrowded cages as a technical service which can be billed to the investigator. A 24-48 hour notice will be given to the PI if there is no immediate animal welfare concern. Cages with multiple litters and/or more than one pregnant dam should be separated when discovered. Sampling for genotyping is generally completed prior to 21 days of age per the IACUC policy. Lack of genotyping results is not a valid reason to delay weaning a litter.

Record Keeping for all rodent colonies:

Breeding records should be kept by the Principal Investigator. Cage cards for breeding cages should include the regular information (PI, ASAF #, DOB, strain/genotype, etc.) as well as relevant information about the breeding such as breeding/pairing dates, plug dates (if known), birth dates and wean dates.

Animals born to a breeding protocol should be recorded in breeding logs kept by the Principal Investigator or the colony manager. The animal numbers should be provided to the Facility Manager so they can be entered into the IACUC database in accordance with IACUC Policy #36.

Acceptable Group Housing Practices for Male Mice and Management of Fighting Mice

Fighting amongst male mice is a well-documented behavior, which can result in severe injury and death. Unfamiliar male mice will almost always fight, especially those that

have been used as breeders. Per AEC IACUC Policy #30 , adult male mice are not considered a social species and can be single housed.

When group-housing mice, the following practices must be followed to minimize fighting:

- Do not combine male mice that are >28 days of age.
- Do not recombine male mice (>28 day of age) that have been separated.
- Weaned male mice should only be group-housed with littermates.
    - Males from different litters may be combined if within seven days of weaning and if the age difference is seven days or less apart. A clean cage should be used when combining mice to minimize territorial behavior.
- Mice that are observed to be aggressive should be housed individually and never be recombined with other males.

Examples of When Combining Male Mice is Acceptable or Not	
A male mouse that is weaned at 21 days of age with a male mouse weaned at 23 days of age.	Acceptable to combine
Male mice that are 36 days old with a cage of male mice that are 40 days old	Not acceptable to combine
40-day old male mice that are littermates that have been separated for seven days.	Not acceptable to combine
50-day old male mice that are littermates and have been housed together since birth	Acceptable to keep combined
A male mouse that is 45 days old with a male mouse that is 21 days old	Not acceptable to combine

It should be noted that some strains of mice are more aggressive than others and may fight regardless of age. These mice should be monitored closely for signs of fighting and males should be housed individually.

Even when best practices are followed, fighting may still occur. While uncommon, it is also possible for female and male mice or groups of females to fight. If mice are observed to be fighting, the aggressive mouse (or mice) should be moved to a different cage and any fighting wounds should be reported to AEC so that a treatment plan can be determined (See SOP 9).

D. Resources

Table 1: Mouse Cage Sizes at AEC

Cage Type	Species	Area sq. inches	Environmental Enrichment Options
Allentown IVC Cage	Mouse	75	Shelter
Ancare Static cage	Mouse	67	Shelter
Techniplast IVC	Mouse	77.6	Loft/Shelter
One Cage™ 2100 single cage	Rat/Mouse	90	Shelter/Loft
One Cage™ 2100 double cage	Rat/Mouse	204	Shelter/Loft

Table 2: Mouse Cages Size Requirements from The Guide

			Required area (sq inches)	
Mouse female + litter			51	
		Weight (g)	Floor area/animal	
	Mouse	<10	6	
		up to 15	8	
		up to 25	12	
		>25	>15	
**see ILAR Guide Table 3.2, p. 57 for additional information*				

E. References

1. PHS Policy on Humane Care and Use of Laboratory Animals https://olaw.nih.gov/policies-laws/phs-policy.htm
2. ILAR Guide
3. OLAW FAQ F10 Can performance standards be used to determine rodent housing practices including management of rodent breeding colonies? https://olaw.nih.gov/faqs#/guidance/faqs?anchor=questionuseandmgmt_10
4. The Guide for the Care and Use of Laboratory Animals (2011 Edition) https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf
5. The Jackson Laboratory: Breeding and Husbandry Support