

Institutional Animal Care and Use Committee

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IACUC GUIDELINE: RODENT BREEDING

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Purpose:

This guideline establishes the expectations of the IACUC on: 1) managing rodent breeding colonies, 2) breeding and weaning rodents, 3) collecting tissues for genotyping rodents, and 4) identifying and alleviating painful or distressful phenotypes that may arise from breeding rodents with spontaneous or induced genetic mutations. If the procedures described below are not appropriate for your specific rodent species or strain, or your unique experimental requirements, then you need to describe your specific breeding plan in your animal use protocol. Genetically engineered mice or rats with a known or expected distressful phenotype must be carefully monitored by appropriately trained and qualified personnel according to the humane endpoint monitoring plan in the approved protocol.

1. Breeding Management and Census Tracking

- The PI should designate one or more members of the research team to manage the rodent breeding colony, and monitor the animals on a regular basis. The ARC staff will also monitor rodent breeding colonies in the ARC, and the lab should respond in a timely manner (within 48 hours) to a request from ARC staff to separate litters in overcrowded cages. However, ARC staff may, immediately and without investigator approval, separate or wean any animals when a serious welfare concern exists.
- The person(s) managing the breeding colony should track the number of animals that are produced by the breeding colony, including animals that are euthanized because they do not meet the experimental requirements (e.g. because they lack the necessary genotype). More importantly, the person(s) responsible for the breeding management must ensure that the number of animals produced by the breeding colony does not exceed the number approved on the protocol.

2. Breeding and Weaning

Mice of the genus Mus

- A female mouse and litter should have 51 in² of cage floor space¹, and each additional adult mouse in the cage should have 15 in² of floor space. The standard “shoebox” mouse cage (75 in² of floor space) can therefore accommodate one adult female mouse, one

adult male mouse, and their litter. There should only be one litter at a time in a cage of this size.

- A larger, or “double-wide” (180 in² of floor space) mouse cage, is available and should be used for triad breeding configurations (two females and one male). Two litters, from different dams, are permitted in the larger cage at one time.
- Pregnant mice should be disturbed as little as possible a couple days leading up to birth and for three days after giving birth in order to minimize stress to the animal and the possibility that she will reject or cannibalize her litter.¹³ If it is necessary to remove an animal from a cage with a pregnant animal, for example because the cage is over-crowded, then remove the animal(s) that is (are) not pregnant.
- If the male mouse is left in the cage with the female continuously, then the female may become pregnant during the postpartum estrus, which occurs within 24 hours of parturition. This continuous mating scheme can result in the production of large numbers of animals in a short period of time (i.e. the second litter is born before the first litter is weaned), and therefore needs to be vigilantly monitored to avoid over-crowding or over-production. Litters that are greater than 48 hours apart in age are less likely to survive until weaning (i.e., the younger pups will die).¹⁴
- Mouse pups should be weaned when they are ready and when they are large enough to reach the food and water in the hopper. Typically, mice are able to be weaned between 21 and 28 days of age (date of birth = day 0). In some cases, however, such as with genetically modified or mutant strains, weaning at an older age (≥ 28 days of age) may be more appropriate for the health and well-being of the animals. If necessary, pups may be provided with additional nutritional support (e.g., by offering DietGel in addition to the regular rodent chow if the pups were part of a large litter and are slightly smaller than normal/expected) or a warmer environment (e.g., by placing the cage on a warming pad if there was only one pup of either sex in the litter). Pups receiving this additional support should be monitored daily. If there is no continued improvement in their condition (i.e., if they are not growing steadily), then the AV must be promptly notified. Similarly, notify the AV if the weanlings (day 21-28) are very small in size (< 7 g BW). The AV must also be notified, if the animals, at any age, have a new or **unexpected** phenotype negatively affecting their health or well-being.
- At weaning, mice should be separated by sex, male and female mice in separate cages.
- Replace breeders at appropriate intervals or conditions: As a general rule, female breeders should be replaced (retired or culled) by 34 weeks of age, and both sexes should be replaced if they fail to produce a litter 60 days after the last litter or fail to wean any pups for two successive litters.

Rats of the genus Rattus

- Three different cage sizes are available for housing rats in the ARC, an 80 in² (floor space) cage, a 140 in² cage, and a 210 in² cage. Since the recommended¹ minimum floor space for a female rat and litter is 124 in², only the 140 in² and the 210 in² cages should be used for breeding rats. When rats are bred in the 140 in² cage, the male should be removed before the litter is born. If rats are bred in the larger 210 in² cage, then the male

can be left in the cage with the female and litter. There should only be one litter at a time in either cage (140 or 210 in²).

- If the male rat is left in the cage with the female continuously, then the female may become pregnant during the postpartum estrus, which occurs within 24 hours of parturition. This continuous mating scheme can result in the production of large numbers of animals in a short period of time (i.e. the second litter is born before the first litter is weaned), and therefore needs to be vigilantly monitored to avoid over-crowding or over-production.
- Rat pups should be weaned at or near 21 days of age (date of birth = day 0).
- At weaning, rats should be separated by sex – male and female rats in separate cages.

3. Tissue Collection from Transgenic Rodents for Genetic Analysis

Toe or tail tip amputations may be performed on genetically-modified rodents to collect tissue for molecular analysis to determine the presence or absence of a particular gene (i.e. genotyping). However, less invasive alternative sampling methods (ear punch biopsy, blood, or saliva) are available, and should be considered.² An ear punch biopsy offers the advantage of simultaneously permanently marking (identifying) a mouse and providing a tissue sample for genotyping. If done correctly, it should cause only slight or momentary pain to the animal and can be performed without anesthesia or analgesia.¹⁵ A 2mm punch biopsy size is recommended, and the procedure should be performed when the mice are 2-3 weeks of age.

The general opinion on amputation, whether of the toe (toe-clipping) or tail (tail-clipping), is that it is a potentially painful procedure. Further, as a method of identification of small rodents (mice), toe-clipping should be used only when no other individual identification method is feasible.³ Toe-clipping is permitted when it is used to simultaneously identify and genotype neonatal mice, and when the following practices to minimize the potential for pain and detrimental long-term effects are followed:

Toe-clipping

- A single partial amputation per foot, at the level of the second phalange is recommended.
- It should be performed when the mice are 7 days of age, and anesthesia is not required.
- It should not be performed in neonates younger than 7 days of age, because it is difficult to precisely cut the toe at the desired location, and if too much tissue is amputated it can result in physical impairment (i.e. diminished grip strength).⁴
- Sharp sterile microsurgery scissors (Vannas Spring Scissors, 4 mm blades) should be used, and they should be disinfected with alcohol between animals.

Tail-clipping, or tailing, is often the preferred method for obtaining tissue for genotyping mice, but as with any amputation the resulting tissue injury may cause pain. The coccygeal vertebrae of laboratory mice (C57BL/6J) are innervated between 3 and 7 days of age, and ossified by 17 days of age in the same strain.^{5,6} In both cases (innervation and ossification), development proceeds in a proximal to distal fashion, so amputation of the distal tail tip should produce less pain and trauma. Similarly, neuronal development of the brain is not complete at birth in the

mouse, and the ability to feel pain may not arise until 12-14 days of age (at least in the rat).^{7,8} In both pre-weanling, weanling, and adult mice behavioral changes indicative of pain or distress, specifically excessive tail grooming, have been consistently observed following tailing, and last up to 60 min.⁹⁻¹² Unfortunately, the anesthetic most commonly used for tail biopsy (i.e. isoflurane) provides no or little benefit in alleviating pain or distress following tailing.⁹⁻¹¹ However, at least for pre-weanling mice (7-15 days of age), topical anesthesia in the form of ice-cold ethanol may provide analgesic relief.¹² Given this information, the following practices should be followed in order to minimize the potential for pain and detrimental long-term effects.

Tail-clipping

- The tail amputation should be performed as soon after birth as practical, but if it's performed after the rodent is 7 days of age then the following anesthetic and analgesic recommendations should be followed:
 - For pre-weanling mice, tail tip immersion for 10 seconds in ice-cold ethanol **prior to** tail biopsy may provide pain relief.
 - For weanling or adult mice, either immersion in 0.75% bupivacaine for 30 seconds **post-biopsy**, or administration of buprenorphine (0.05 mg/kg SC) should provide pain relief from tailing.
 - General anesthesia (isoflurane) is recommended only for weanling or adult mice, and should be supplemented with an analgesic agent (bupivacaine or buprenorphine). When anesthesia is used, the mouse should be closely monitored until it recovers fully from anesthesia.
- As little tissue as possible, but <5 mm, should be amputated from the distal tail tip using a pair of sharp sterile scissors or a sterile scalpel blade (preferred), which should be disinfected with alcohol between animals and a new sterile blade or scissors should be used for each litter.
- In contrast to toe-clipping, tail-clipping will result in bleeding that should be controlled by applying gentle and direct pressure with a sterile gauze.
- Repeat tail-clippings on a single mouse are discouraged.

4. Breeding Rodents with a Spontaneous or Induced Mutation

If the phenotype of a novel mutant strain is not well characterized, then the first offspring of a newly generated line/strain should be carefully observed from birth into early adulthood for signs of disease, pain, or distress. Timely identification of animal health and well-being concerns requires daily observation of the animals by appropriately trained personnel. If this monitoring reveals a painful or distressful condition, then the lab should report this condition to the veterinarian, and in consultation with the veterinarian, develop a plan to alleviate the painful or distressful condition, which should include establishing well-defined humane endpoints for the affected breeding colony.

References:

¹ *Guide for the Care and Use of Laboratory Animals, 8th Edition.* Table 3.2, page 57

- ² Pinkert CA. Transgenic animal technology: Alternatives in genotyping and phenotyping. *Comp. Med.* 53(2):126-139. 2003
- ³ *Guide for the Care and Use of Laboratory Animals, 8th Edition.* page 75
- ⁴ Schaefer DA, Asner IN, Seifert B, Bürki K, and Cinelli P. Analysis of physiological and behavioral parameters in mice after toe clipping as newborns. *Lab Anim.* 44:7-13. 2010
- ⁵ Silverman J, and Hendricks G. Sensory neuron development in mouse coccygeal vertebrae and its relationship to tail biopsies for genotyping. *PLOS ONE*
<http://journals.plos.org/plosone/article/?id=10.1371/journal.pone.0088158>
- ⁶ Hankenson FC, Garzel LM, Fischer DD, Nolan B, and Hankenson KD. Evaluation of tail biopsy collection in laboratory mice (*Mus musculus*) vertebral ossification, DNA quality, and acute behavioral responses. *J Am Assoc Lab Anim Sci* 47:10-18. 2008
- ⁷ Dierch TJ, Mellor DJ, Johnson CB, and Lentle RG. Electroencephalographic responses to tail clipping in anaesthetized rat pups. *Lab Anim* 43:224-231. 2009
- ⁸ Barr GA. Formalin-induced c-fos expression in the brain of infant rats. *J Pain* 12:263-271. 2011
- ⁹ Hankenson FC, Branden-Weiss GC, and Blendy JA. Behavioral and activity assessment of laboratory mice (*Mus musculus*) after tail biopsy under isoflurane anesthesia. *J Am Assoc Lab Anim Sci* (50(5):686-694. 2011
- ¹⁰ Jones CP, Carver S, and Kendall LV. Evaluation of common anesthetic and analgesic techniques for tail biopsy in mice. *J Am Assoc Lab Anim Sci* 51(6):808-814. 2012
- ¹¹ Branden GC, Brice AK, and Hankenson FC. Adverse effects of vapocoolant and topical anesthesia for tail biopsy of preweanling mice. *J Am Assoc Lab Anim Sci* 54(3):291-298. 2015
- ¹² Dudley ES, Johnson RA, French DEC, and Boivin G. Effects of topical anesthetics on behavior, plasma corticosterone, and blood glucose levels after tail biopsy of C57BL/6NHSD mice (*Mus musculus*). *J Am Assoc Lab Anim Sci* 55(4):443-450. 2016
- ¹³ Wharton KN, Walsh C, Bauer-Pisani TJ, Smith PC, and Wilson SR. Effect of cage change frequency on perinatal mortality in C57BL/6J mice. *J Am Assoc Lab Anim Sci* 63(2):148-153. 2024
- ¹⁴ Hickman DL, and Swan MP. Effects of age of pups and removal of existing litter on pup survival during cross-fostering between multiparous outbred mice. *J Am Assoc Lab Anim Sci* 50(5):641-646. 2011
- ¹⁵ Taitt KL, and Kendall LV. Physiologic stress of ear punch identification compared with restraint only in mice. *J Am Assoc Lab Anim Sci* 58(4):438-442. 2019