



TITLE: **Rodent Weaning Policy**

PURPOSE:

The purpose of this SOP is to ensure that rodent weaning is conducted efficiently and that Public Health Service (PHS) policy and federal regulations governing housing requirements are met. The contents of this SOP apply to all NINDS personnel who are involved in weaning rodents. Personnel from other NIH institutions housing rodents in NINDS-managed shared animal facilities are encouraged to follow this SOP.

Refer to The U.S. Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research and Training, [Guide for the Care and Use of Laboratory Animals, Eighth Addition \(Revised 2011\)](#), U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, March, 1996, Breeding Strategies for Maintaining Colonies of Laboratory Mice (The Jackson Laboratory) and Laboratory Animal Medicine 2nd ed. for further information.

PROCEDURES:

- A. Litters are routinely weaned at 21 days of age. Refer to attachment 3481A Aging Mouse Pups for further information.
 1. Delaying date of wean (DOW).
 - i. If pup(s) appears too small to survive alone, they may be left with the mother for up to another week (or until they have a body weight of at least 10 grams in mice and 30 grams in rats). Litters may be delayed for no more than 7 days (i.e. Day 28). To delay weaning document the word "delay" and the new wean date on a green dot sticker. Place the sticker containing the new wean date on top of the original wean date sticker on the Breeder Flag. If delay of weaning is specifically outlined in the approved Animal Study Proposal (ASP) (**This applies only if the mother has not given birth to another litter**), this will be included in the Supplemental Disposition Form (SDF) by the AHCS staff.
 - ii. In some cases, the pup(s) may benefit from supportive care in addition to or instead of delaying the DOW. Place an orange Watch card on the cage in front of the regular cage card to have the pup(s) assessed by a VT or veterinarian.
 - iii. If a single animal is runt, an orange watch card should be placed on the cage in front of the regular cage card. The vet may suggest that the rest of the litter be weaned into a separate cage and the runt animal may stay with the mother even if there is a new litter present in the cage.
- B. Weaning animals is the investigator's (PIs) responsibility unless a technical request for weaning (https://ahcs.ninds.nih.gov/AHCS_pages/vet.html) has been submitted and accepted.
- C. To maintain compliance with the PHS policy and federal regulations governing animal housing density, the facility staff closely monitor for overcrowding. Overcrowding is an animal welfare concern.
 1. On Mondays, or the first day of the workweek, a VT will email you a pictures of the Weaning Tracking Sheet(s) (WTS) corresponding to your colony. This will be your only notification of the weaning due dates for litters.

2. If litters have not been weaned by age D24, AHCS VT's will resolve the case and complete the Weaning Tracking Sheet. The lab will receive the weaning cage information on the WTS that is emailed weekly.
 - i. If space is not available for new weaning cages, animals may be culled by facility staff if directed by a vet.
 - ii. AHCS staff will not separate animals on weekends/holidays due to time constraints and minimal staffing. Therefore, all overcrowded cages that will reach D24 on a weekend or holiday must be resolved by COB Friday or the last business day prior to the weekend/holiday.
 - iii. If a litter is less than 21 days old on a Friday or holiday, weaning may be delayed until the next business day provided the mother does not give birth to another litter. The weaning of runt pups (less than 10 grams for mice and 30 grams for rats) may be delayed at the discretion of the facility vet.
3. Cages with multiple litters must be separated by close of business (COB) on the day of the second litter's birth or AHCS staff will separate the litters. Exceptions to this multiple litter policy may be approved by the Animal Care and Use Committee (ACUC) or by a vet for health reasons.
 - i. If there is a double litter, separating these litters as soon as possible may prevent competition for food between the oldest and youngest litter thereby pushing the youngest litter away from the food source and the youngest litter being trampled by the oldest litter.
 - ii. The AHCS staff will identify and place an "Attention Required" flag on the cage containing the double litter and record the cage information on the Attention Required log. Refer to [SOP 3201](#) for more information.
 - iii. The VT will notify the investigator (PI) or designated point of contact by email, telephone, or in person, when cages requiring attention are found.
- D. When animals are weaned, a "Food Supplement" flag should be hung on the cage card holder by whoever weans the cage. Refer to [SOP 3202](#) Cage Flag and Card System for Identifying Animal Cages in the NINDS AHCS Managed Animal Facilities for more information.
 1. Whoever weans the animals should fill in the "date to stop" sticker for 4 days from the DOW and check the DietGel box with a permanent marker (not a ball point pen). This ensures that the new weanlings will receive DietGel from the AHCS staff for 4 days after they have been removed from their parents to help support their growth.
 2. If a cage of weanlings is found without a flag you will be contacted by AHCS staff to ask if DietGel can be given for 4 days. If the answer is 'yes' we will hang a cage flag and put a date sticker on with the date of 4 days from when the cage was identified.
- E. How to wean a cage:
 1. Start with two new full cage set-ups (one for males and one for females).
 2. Put Diet Gel and a few pieces of feed on the floor of the cage. Refer to attachment 3300B Supplement Proportions for amount to provide. A diagram is also located in marshalling areas near supplements.
 3. Separate all of the males into one cage and all of the females into the other cage using anogenital distance. Refer to attachment 3481B Sexing of Mice and Rats.
 - i. Anogenital distance is the distance between the animal's anus and genitals. The anogenital distance for males should be sizably larger than that of females.
 - ii. If there is a problem deciphering males from females try a side by side comparison with another animal in the cage.

- iii. If still uncertain of sex, ask for assistance or wean the cage and recheck mice in one week.
- 4. Wean animals per cage space requirements per [SOP 3200](#), Attachment A, unless a greater number is approved in the ASP.
- 5. Fill out cage card labels to place on the weanling cages. Remember to complete the corresponding 'activation label' for each. Refer to [SOP 3631-PI](#) Creating Cage Cards using Cage Card Labels for further information.
- 6. It is important to recheck the sex of the weanlings within a week of weaning in order to identify sexing errors prior to sexual maturity. Refer to attachment 3481B Sexing Mice and Rats for details and pictures.

REFERENCES:

- 1. The U.S. Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research and Training.
- 2. [Guide for the Care and Use of Laboratory Animals, Eighth Addition \(Revised 2011\)](#).
- 3. U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, March, 1996.
- 4. Breeding Strategies for Maintaining Colonies of Laboratory Mice (The Jackson Laboratory).
- 5. Laboratory Animal Medicine 2nd ed.
- 6. [SOP 3202 Cage Flag and Card System for Identifying Animal Cages in the NINDS AHCS Managed Animal Facilities](#)
- 7. [SOP 3200 Cage Space Requirements for Laboratory Rodents](#)
- 8. [SOP 3631-PI Creating Cage Cards using Cage Card Labels](#)
- 9. [SOP 3300 Rodent Environmental Enrichment Program](#) (attachment 3300B Supplement Proportions)

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Attachments:

- 1. 3481A Aging Mouse Pups
- 2. 3481B Sexing of Mice and Rats
- 3. 3200A NINDS AHCS Cage Standards for Laboratory Animals

Updates and/or Changes:

3/5/15:

- Added paragraph two in the Purpose Section.
- Changed the order for Sections A and B. The information was updated to include the cages requiring attention and reorganized to flow with purpose.
- Added Attention Required Flags and updated SOP hyperlinks.

- Changed Section D to replace fruit with Diet Gel for mouse weanlings. Fruit is to be used for rats and as requested for mice.
- Section G2 to exclude the exact amount of Diet Gel provided.

11/17/17:

- Added in section A1i. rat pup BW of at least 30g

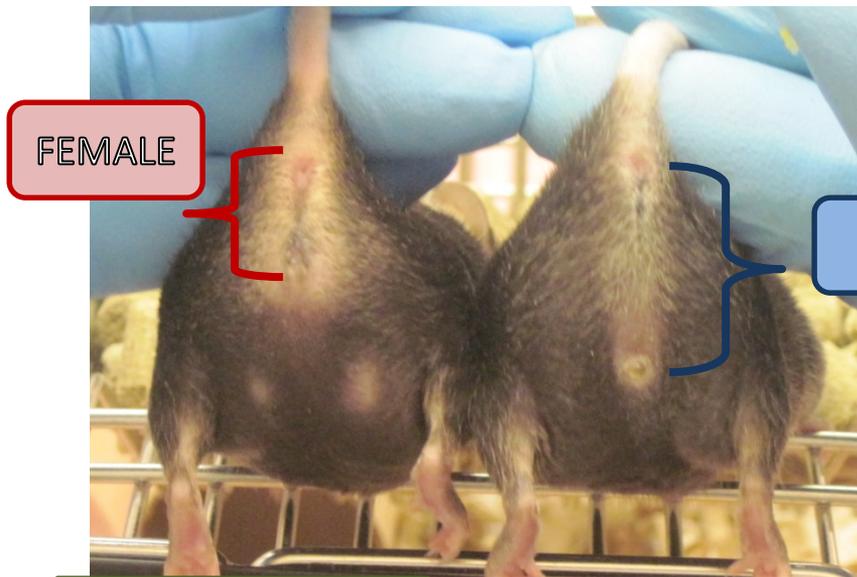
3/2/18

- Removed information indicating that Attention Required flags would be placed on cages containing litters that have reached D21.
- Added information regarding the Weaning Tracking Sheet & communicating litters born, weaning due and litters weaned information to the labs.
- Removed references to the use of cage card request forms.
- Added instructions for how a lab member can indicate that they have delayed the wean date for a litter up to D28.
- Changed number of days that DG is to be provided for weanlings - 4 days instead of 7 days after weaning.
- Added references to attachment 3300B Supplement Proportions
- Added 3200A NINDS AHCS Cage Standards for Laboratory Animals as an attachment to this SOP due to investigators being unable to access SOP 3200.

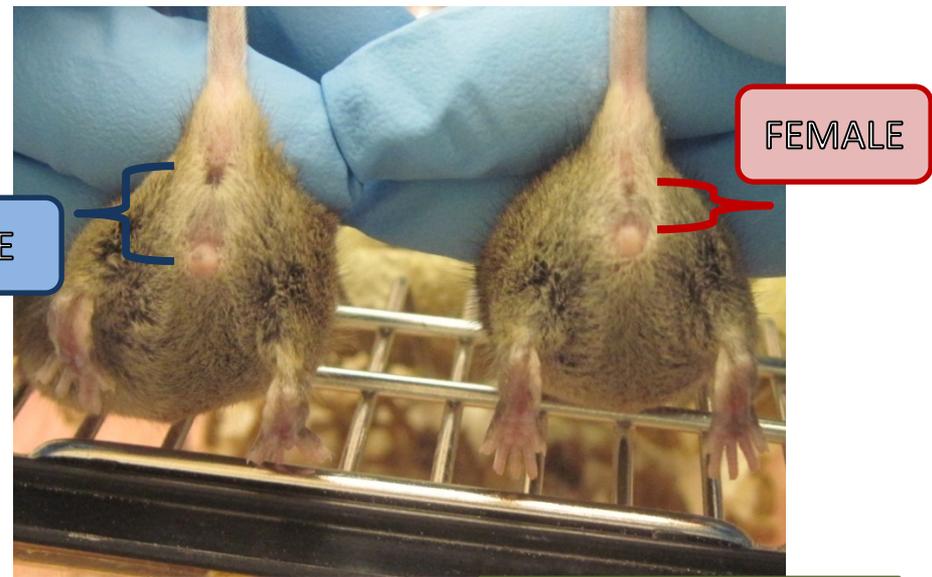
3481A Aging Mouse Pups



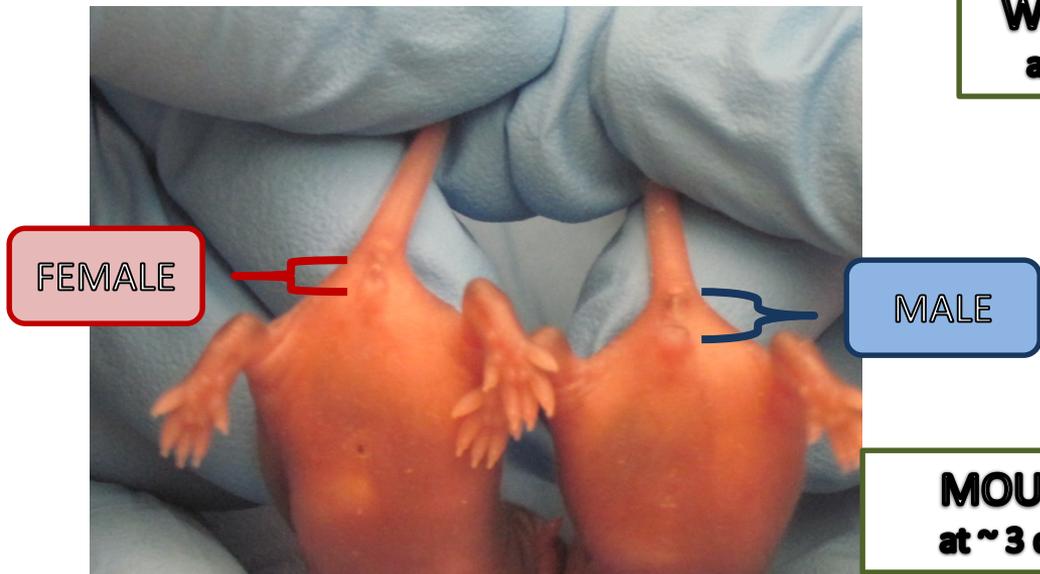
SOP 3481B Sexing Mice



ADULT MICE

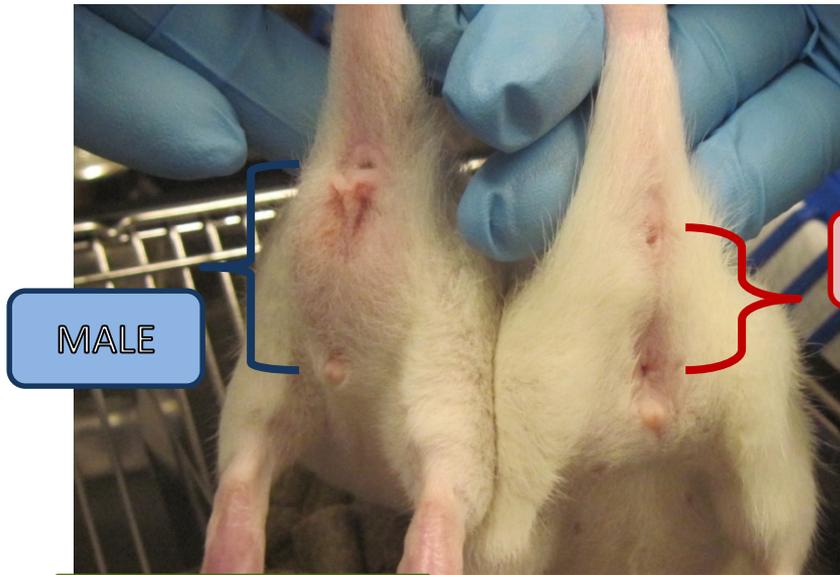


**WEANLING MICE
at ~ 21 days of age**



**MOUSE PUPS
at ~ 3 days of age**

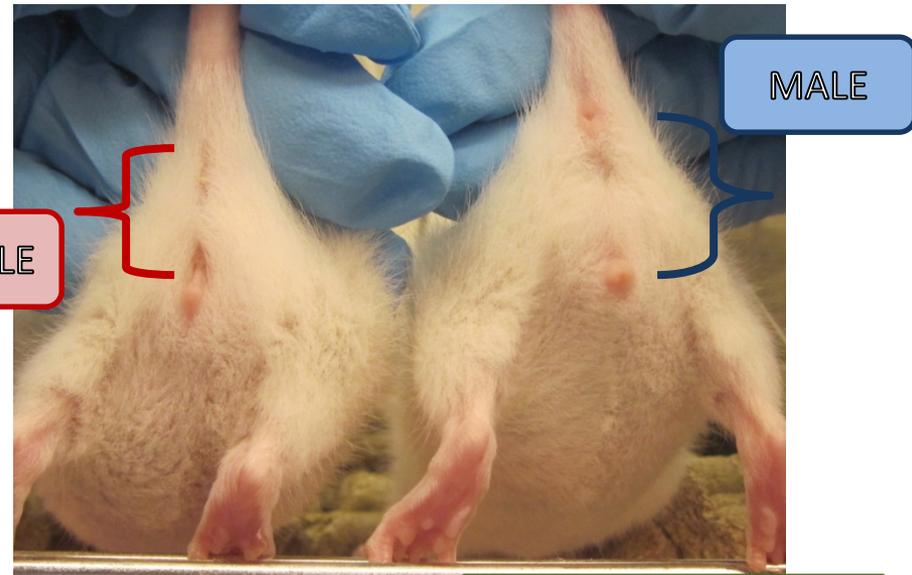
SOP 3481B Sexing Rats



MALE

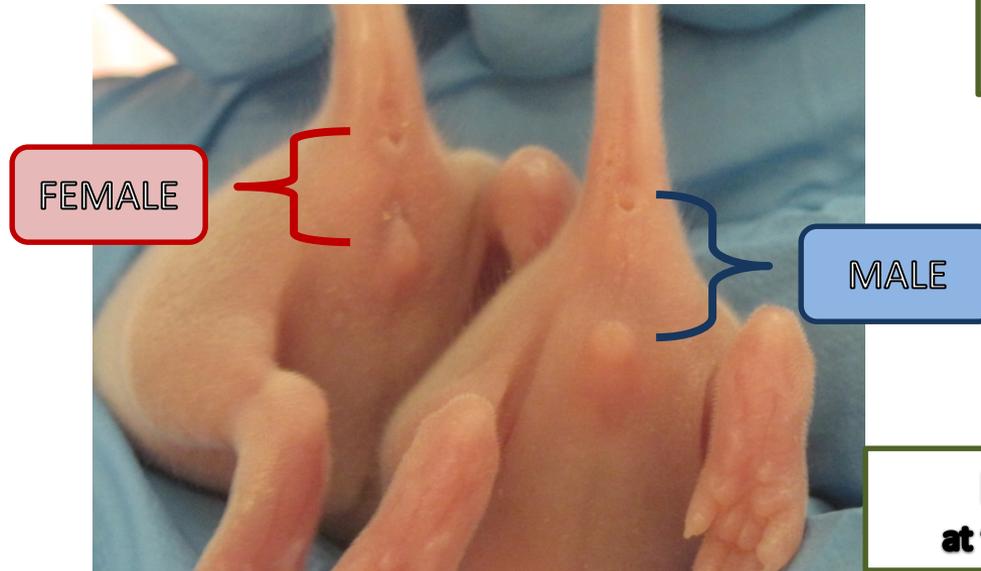
FEMALE

ADULT RATS



MALE

**WEANLING RATS
at ~ 21 days of age**



FEMALE

MALE

**RAT PUPS
at ~ 5 days of age**

SOP 3200A NINDS AHCS Cage Standards for Laboratory Animals

Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups
from *Guide for the Care and Use of Laboratory Animals*, 2011 [Page 57, TABLE 3.2]

MOUSE

Weight	approximate age	Minimum Floor Area/Animal	Maximum Animals/Cage	
			Tecniplast (1145T Sealsafe cage)*	Allentown (NexGen cage)*
< 10 grams	~ < 3wks	6 sq. in.	11	12
11 to 15 grams	~ 3 - 4 wks	8 sq. in.	8	9
16 to 25 grams	~ 5 - 6 wks	12 sq. in.	5	6
> 25 grams	~ 7+ wks	> 15 sq. in.	4	5
Breeder pair with litter (assumes male is >25g)		>65 sq. in.	1 (pair)	1 (pair)

* Maximum number of adult mice that can be housed per cage - Tecniplast: 4; Allentown: 5

RAT

Weight	approximate age	Minimum Floor Area/Animal	Maximum Animals/Cage		
			Tecniplast (1291H Sealsafe cage)	Tecniplast (GR900 cage)	Tecniplast (1500U cage)
< 100 grams	~ < 4 wks	17 sq. in.	7*	8	13
101 to 200 grams	~ 5-6 wks	23 sq. in.	5*	6	10
201 to 300 grams	~ 7-8 wks	29 sq. in.	4*	4	8
301 to 400 grams	~ 9-10 wks	40 sq. in.	3	3	5
401 to 500 grams	~ 11+ wks	60 sq. in.	2	2	3
> 500 grams		> 70 sq. in.	1	2	3
Breeder pair with litter (assumes male is > 500 g)		> 124 sq. in.	by approved exception only	1 (pair)	1 (pair)

* Due to rapid growth, if space is available, housing 2 per cage will eliminate frequent separation of rats.

Tecniplast cage size

Mouse (1145T) = 67.43 sq. in.

Rat (1291H) = 124 sq. in.

Rat (GR900) = 140 sq. in.

Rat (1500U) = 233 sq. in.

Allentown cage size

Mouse = (NexGen) 75 sq. in