Comparative Medicine
Animal Resources Centre

McGill

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1 – Introduction to the Laboratory Mouse

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1. THE LABORATORY MOUSE

The common laboratory mouse *Mus domesticus domesticus,* the most commonly used animal in biomedical research, is an ideal experimental animal for several reasons: abundance of literature published regarding them, ease of handling, high fertility rate, short gestation period, low maintenance and disease model for various human disorders and diseases.

1.1. General biology and physiological data

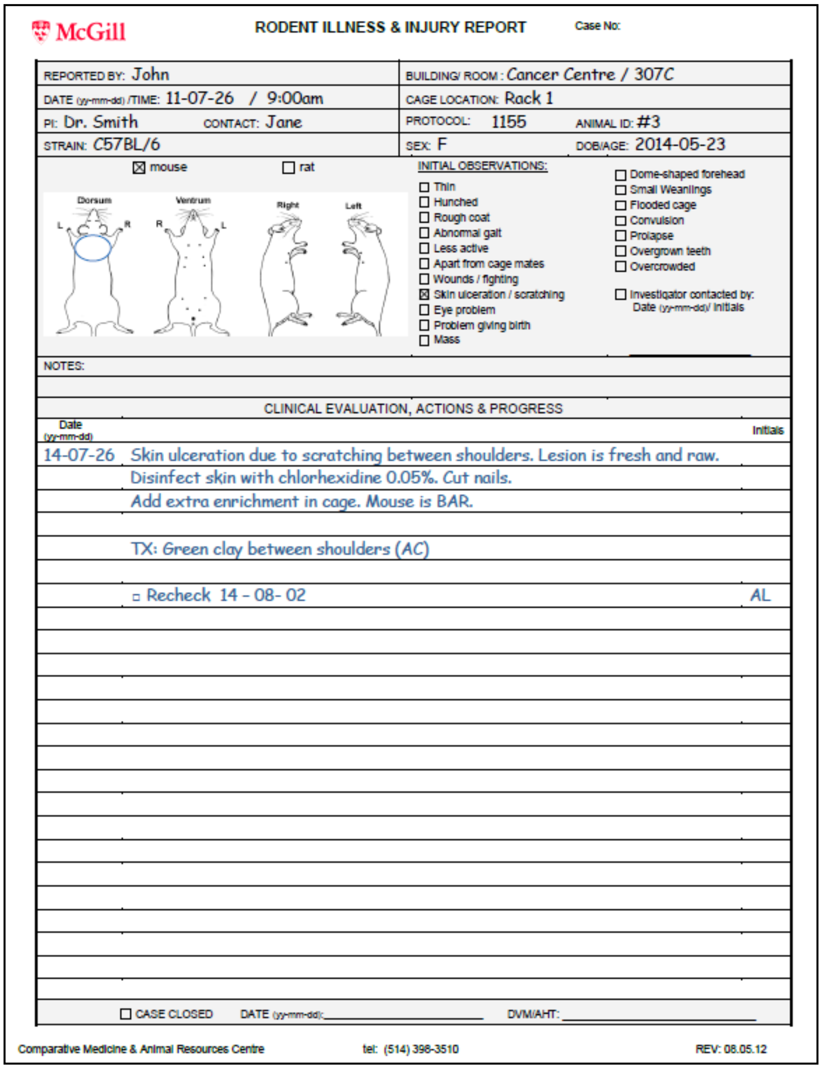
- Most active at night (nocturnal)
- Curious and investigative behaviour
- Poor vision, acute sense of hearing and smell
- Social animals, adult males may require separation if aggressive
- Average body temperature: 37°C
- Respiratory rate: 95-165 breaths/minute
- Heart rate: 325-800 beats/minute
- Daily water consumption: 5 ml
- Daily food consumption: 5 g
- Oestrous cycle length: 4-5 days
- Duration of oestrus: 12 hours
- Average litter size: 6-12
- Gestation period: 19-21days
- Average birth weight: 0.5-1.5 g
- Weaning age: 21-28 days
- Sexual maturity: 6-7 weeks in males; 7-8 weeks in females
- Reproductive span: 7-9 months
- Male adult weight: 25-40 g
- Female adult weight: 20-40 g
- Life span: 1.5-3.0 years

2. VETERINARY CARE PROGRAM

Our Veterinary Care program aims to detect and treat sick or injured animals thus preventing unnecessary pain and distress.

The animal care attendants observe each rodent cage on a daily basis and report any animal that appear ill. A team of animal health technicians and veterinarians then evaluates the animal, provides adequate treatment and follows up to monitor the condition of the sick animal.

2.1. Injury reports and cage cards


McGill RODENT ILLNESS & INJURY REPORT Case No:

REPORTED BY: John	BUILDING/ ROOM: Cancer Centre / 307C
DATE (yy-mm-dd) /TIME: 11-07-26 / 9:00am	CAGE LOCATION: Rack 1
PI: Dr. Smith CONTACT: Jane	PROTOCOL: 1155 ANIMAL ID: #3
STRAIN: C57BL/6	SEX: F DOB/AGE: 2014-05-23

☒ mouse ☐ rat

INITIAL OBSERVATIONS:

- ☐ Thin
- ☐ Hunched
- ☐ Rough coat
- ☐ Abnormal gait
- ☐ Less active
- ☐ Apart from cage mates
- ☐ Wounds / fighting
- ☒ Skin ulceration / scratching
- ☐ Eye problem
- ☐ Problem giving birth
- ☐ Mass
- ☐ Dome-shaped forehead
- ☐ Small Weanlings
- ☐ Flooded cage
- ☐ Convulsion
- ☐ Prolapse
- ☐ Overgrown teeth
- ☐ Overcrowded
- ☐ Investigator contacted by: Date (yy-mm-dd)/ Initials

NOTES:

CLINICAL EVALUATION, ACTIONS & PROGRESS

Date (yy-mm-dd)		Initials
14-07-26	Skin ulceration due to scratching between shoulders. Lesion is fresh and raw.	
	Disinfect skin with chlorhexidine 0.05%. Cut nails.	
	Add extra enrichment in cage. Mouse is BAR.	
	TX: Green clay between shoulders (AC)	
	☐ Recheck 14 - 08- 02	AL

☐ CASE CLOSED DATE (yy-mm-dd):________ DVM/AHT: ________

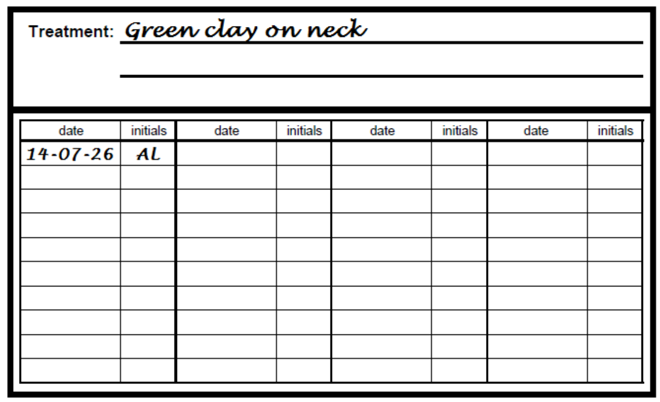
Comparative Medicine & Animal Resources Centre tel: (514) 398-3510 REV: 08.05.12


VETERINARY CARE

CLINICAL CONDITION: Skin ulceration

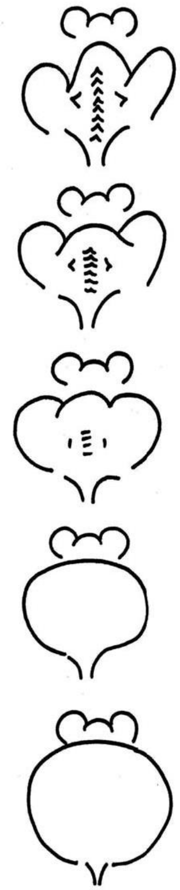
EXAMINED BY (PRINT): Audrée

DATE: 2014-07-26 CASE NUMBER: 14-0562


Treatment: Green clay on neck

date	initials	date	initials	date	initials	date	initials
14-07-26	AL						

2.2. Body condition (BC) scoring system



Score 1: Mouse is emaciated

- Muscle wasting is advanced, fat deposits are gone and bones are very prominent
- Euthanasia is mandatory.

Score 2: Mouse is under conditioned

- The mouse is becoming thin and bones are prominent.
- This category may be further divided subjectively as +2, 2, -2.

Score 3: Mouse is well-conditioned

- The mouse is in optimal condition. Bones are palpable but not prominent.

Score 4: Mouse is over conditioned

- The mouse is well-fleshed, and bones are barely felt.

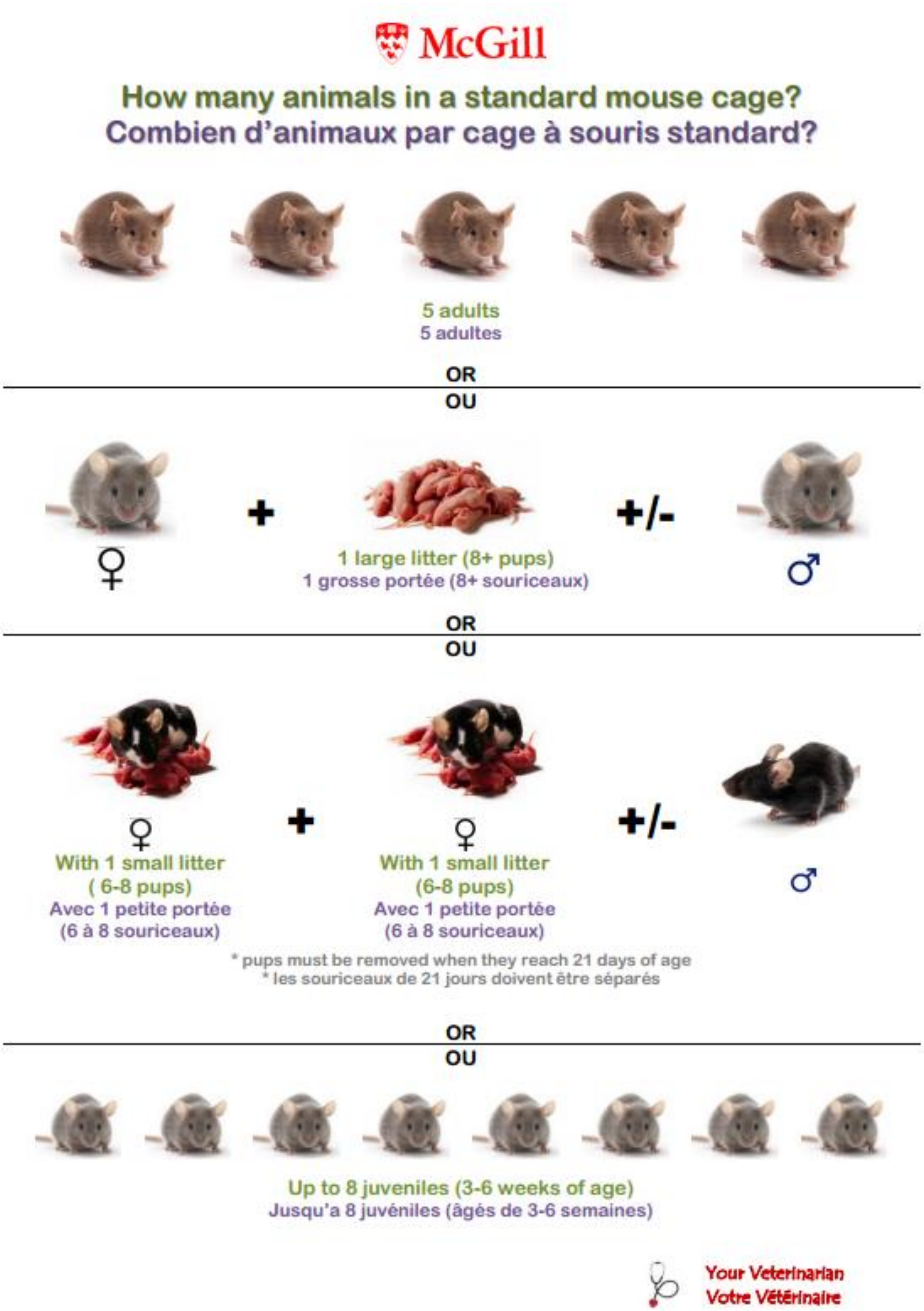
Score 5: Mouse is obese

- The mouse is obese, and bones cannot be felt at all.

2.3. The Mouse Grimace Scale (Langford et al. 2010)

The mouse grimace scale is a standardized behavioral coding system that demonstrates facial expressions which can be used to assess pain in the laboratory mouse.

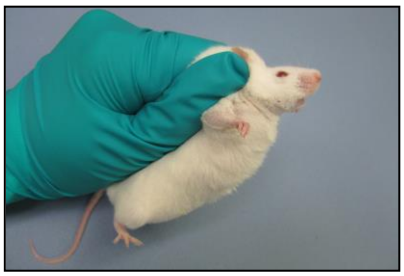
2.4. Cage density



3. HANDLING AND RESTRAINT

3.1. Manual restraint

- Before opening the cage observe the animals within. Nervous or young mice can jump out very quickly and escape.
- For quick transfers from cage to cage, mice can be gently held by the base of the tail with your hand. Alternatively, a pair of long forceps can be used to grasp the base of the tail.
- Place the mouse on the wire-bar lid of the cage while holding the base of the tail with your dominant hand. By applying gentle tension to the tail, the mouse will grasp the wire-bar lid.
- Slide the thumb and index finger of your non-dominant hand over the back of the mouse and quickly grasp the loose skin at the back of the neck as close to the ears as possible.
- The tail can then be tucked under the ring or little finger.

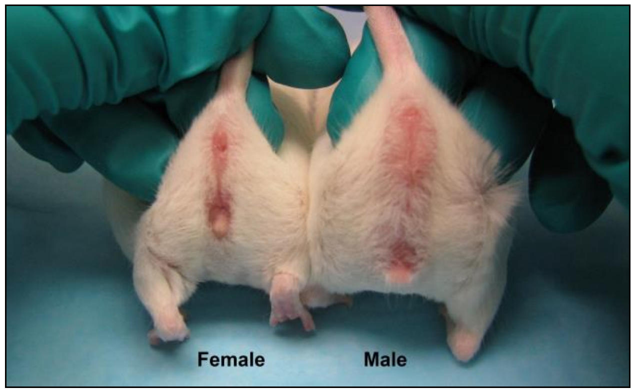


3.2. Restraint devices

- Several restraint devices are available in various sizes and materials (e.g., Plexiglas, plastic) and can be used when performing techniques such as injections or blood collection.
- The restrainer should be small enough so that the animal cannot turn around yet allow the animal to rest comfortably and breathe normally.
- Observe animals to ensure that they do not overheat and never leave an animal in a restrainer unattended.

4. SEX DETERMINATION

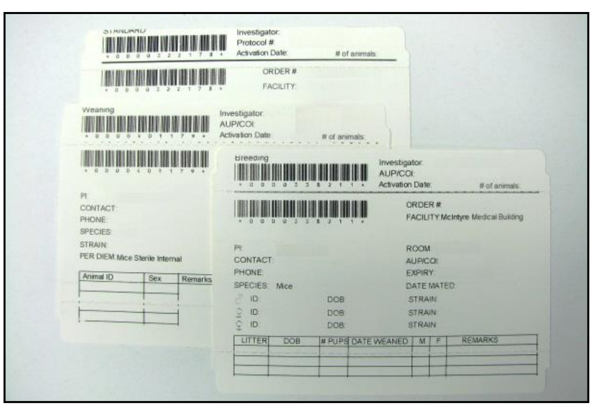
- Sexing of mice is based upon ano-genital distance
- Males have a greater distance between the anus and urogenital opening than females.
- An opposite sex comparison is advisable initially. Compare animals of similar age.
- The testicles can be retracted into the abdomen; therefore, it may be easier to sex a mature male by holding its head up vertically. The genital papilla is more prominent in males than females.

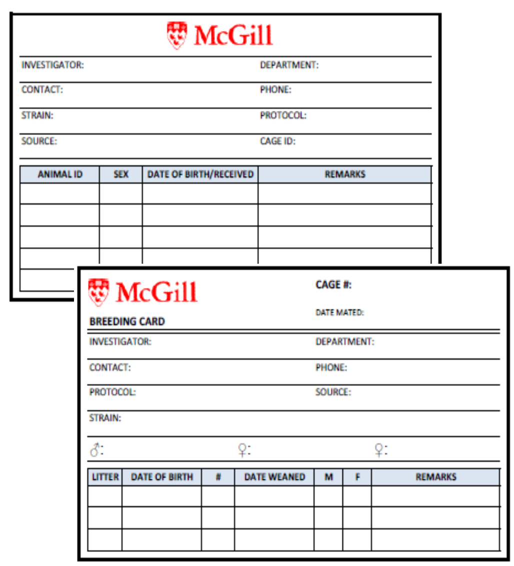


5. IDENTIFICATION

5.1. Cage cards

- All cages must have a Darwin cage card.
- Additional cage cards may be used, however, care must be taken not to cover the Darwin barcode.
- All sections of either card must be completed:

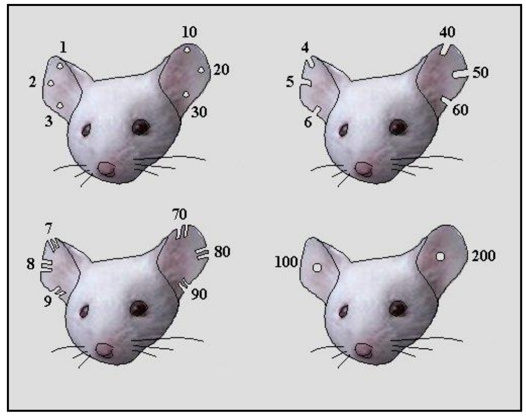




5.2. Temporary markings

- Temporary marking can be used for short term individual identification.
- Use a non-toxic, permanent marker to write numbers, bars or other distinguishable marking on the tail or the ears.
- If temporary marking is to be used for duration exceeding a week, repeat marking at least twice a week.

5.3. Ear punching/notching



- This method cannot be use on rodents under 2 weeks (14 days) of age.
- Restrain the animal securely and using an ear punch, punch a hole and/or notches in the ears following an identification chart.
- Whenever possible, use a simple code to limit the number of notches/punches made to the animal.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals.
- If possible, use the excised tissue as a sample for genotyping.

5.4. Ear tag

- Use tags of appropriate size, approximately 5mm long.
- Rinse tags in 70% alcohol before use.
- Place the tag low on the pinna (distal ⅓) so that it rests against the mouse and does not bend the ear, catch on the cage or cause the mouse to hold its head in a lopsided manner.
- If the tag is placed too tight it can lead to local infection or inflammation. The animal will need to be monitored for these clinical signs and the tag removed if necessary.

5.5. Tattooing

- It is recommended to use local or general anesthesia for the procedure.
- Use an electric tattoo machine to write numbers on the tail.
- Ensure that needles are sterile and sharp.

5.6. Micro-tattooing

- It is recommended to use local or general anesthesia for the procedure
- Use a micro-tattooer to inject tattoo ink in the toe pads and/or the ears.
- Whenever possible, use a simple code to limit the number of toes tattooed.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals.

6. TISSUE SAMPLING FOR GENOTYPING

6.1. Fecal pellet

- Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding.
- Properly identify samples to match animal identifications.

6.2. Buccal epithelial cell

- Firmly restrain the animal by the scruff to maintain its mouth open.
- Using the swab, vigorously scrape both inner cheeks.
- Insert cotton bud into collection tube and snip off excess shaft.
- Properly identify samples to match animal identifications.

6.3. Ear punching

- Do not use this method in rodents under 2 weeks of age.
- Restrain the animal securely.
- Using the ear punch; punch holes and/or notches in the ears following an identification chart.
- Use the excised tissue as a sample for genotyping.
- Properly identify samples to match animal identifications.

6.4. Tail snipping

- Tail biopsy can only be performed twice over the life time of the animal and cannot exceed 5mm total.
- A maximum of 3mm of tail tip can be removed at first.
- Tail snipping is preferably done when pups are 14 to 17 days old.

6.4.1. Procedure for mice 14 to 21 days of age

- General anesthesia is recommended but not required.
- Gently, but securely, restrain the mouse with your hands or with the use of a restrainer.
- Swab the tail with antiseptic (e.g., chlorhexidine, alcohol).
- Snip tail with sanitized scissors or disposable scalpel.
- If you are snipping several mouse tails, clean off any blood or tissues from the scissors and wipe with 70% alcohol or dip in a glass bead sterilizer for at least 30 seconds.
- Place tissue sample into the collection tube.
- Apply pressure on the tip of the tail with a clean gauze and do one of the following:
  - Apply a drop of tissue glue such as Vetbond™ to the cut tip of the tail.
  - Apply a chemical cautery agent such as Kwik Stop® topical styptic powder or silver nitrate stick.
  - Electric or heat cauterize the cut end of the tail.
- Properly identify samples to match animal identifications.

6.4.2. Procedure for mice over 21 days of age

- Requires general anesthesia and analgesia.
  - Administer carprofen 20mg/kg subcutaneously 20 minutes prior to the procedure.
  - Brief general anesthesia is provided with isoflurane:
    - Place the animal in the induction chamber.
    - Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.
    - Adjust the isoflurane vaporizer to 3% to 4% to achieve unconsciousness.
- Remove the animal from the induction chamber and quickly proceed with the tail snipping as described above.
- Return the animal to its home cage once it regains consciousness.
- Properly identify samples to match animal identifications.

7. EUTHANASIA

Mice can be euthanized in a variety of acceptable, effective and humane methods. Euthanasia methods can be either chemical or physical.

7.1. Adult rodents - Chemical methods

7.1.1. CO2 asphyxiation under isoflurane anesthesia

- It is preferable to anesthetize rodents with isoflurane prior to exposure to CO_2 to minimize pain and distress.
- In order to minimize stress animals should be euthanized in their home cage with a maximum of five adult mice or one litter per cage (do not pool mice from different cages).
- Choose an adequately sized induction chamber and connect it to the euthanasia station.
- Place the animal cage, with filter top removed, in the induction chamber.
- Open the oxygen tank and set the flowmeter to maximum flow rate.
- Set the isoflurane vaporizer to 5%.
- Observe the animals closely. Soon after loss of consciousness (when the breath rate is still relatively high) close the vaporizer and the oxygen tank.
- While the animals are still unconscious, promptly open the CO_2 tank and set the flowmeter to maximum flow rate.
- Maintain the CO_2 flow until the animal has stopped breathing. Note that the time required for euthanasia can be several minutes.
- Close the CO_2 flow meter and the valve on the CO_2 tank.
- Leave the animals in contact with CO_2 for an additional 2 minutes, minimum.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.1.2. CO_2 asphyxiation

- CO_2 alone should not be used where other methods are practical for the experiment and the species.
- In order to minimize stress animals should be euthanized in their home cage with a maximum of five adult mice or one litter per cage (do not pool mice from different cages).
- Place the appropriate sized lid on the animal cage with grid removed.
- Connect the regulator hose to lid fitting.
- Do not pre-charge the chamber.
- Plug in the heater unit if necessary (e.g., if euthanizing many cages).
- Open the CO_2 tank valve.
- Set the regulator to the appropriate setting:
  - Standard mouse cage (7.25" x 11.5" x 5"): 2 LPM (Litres per minute)
- Cages of different dimensions: a gradual-fill rate of less than 30% and greater than 20% of the chamber volume per minute should be used.
- After the animals have become unconscious, the flow rate can be increased to minimize the time of death. Please note that the time required for euthanasia can be several minutes.
- Maintain the CO_2 flow until the animal has stopped breathing.
- Close the valve on the tank.
- Leave the animals in contact with CO_2 for an additional 2 minutes, minimum.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.1.3. Barbiturate or injectable anesthetic overdose

- Inject three times the anesthetic dose intravenously or intraperitoneally.
- Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.1.4. Overdose of inhalant anesthetic

- Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanized.
- The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate amount of the anesthetic. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Procedures should be conducted in a chemical fume hood to prevent inhalation of the anesthetic by personnel.
- The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system, air filter or type II B2 BSC.
- Sufficient air or oxygen must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a large container, there will be sufficient oxygen in the chamber to prevent hypoxemia.

- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.2. Adult rodents - Physical methods

Physical methods of euthanasia are also an appropriate means to assure death after euthanasia with CO_2 or anesthetics used as euthanasia agents. Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed.
Anesthesia or sedation is necessary prior to physical methods of euthanasia, unless described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

7.2.1. Cervical dislocation

- Cervical dislocation performed on live animals requires additional training.
- Hold the base of the tail with one hand.
- With the other hand, the thumb and index finger are placed on either side of the neck at the base of the skull. Alternatively, a narrow, blunt instrument such as the dull edge of a scissor blade, acrylic ruler or cage card holder can be used.
- To accomplish the cervical dislocation, quickly push down and forward with the hand or the object pressed at the base of the skull while pulling backward with the hand holding the base of the tail.
- Note: A 2-4 mm space should be palpable at the base of the skull, between the occipital condyles and the first cervical vertebra or within the upper third of the neck.
- To confirm death, monitor animal for the following signs: absence of breathing, pale eyes, no reflexes, animal may urinate.

7.2.2. Pneumothorax

- Cut through the skin and muscle of the abdomen just below (caudal to) the thorax.
- Lacerate the diaphragm with a sharp pair of scissors.

  Note: If the animal is deeply anesthetized, the heart could be removed to accelerate the process and insure death.

7.2.3. Decapitation

- Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.
- The use of plastic cones to restrain animals is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

7.3. Neonatal Rodents

Rodents over 10 days old can be euthanized by the same procedures as adult rodents.

Rodents under 10 days old must be euthanized by one of the following methods:

7.3.1. CO_2 asphyxiation under isoflurane anesthesia followed by decapitation

- Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations and exposure to CO2, therefore, alternative methods are recommended.
- Isoflurane/CO2 may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).
- Keeping neonates warm during isoflurane/CO2 exposure may decrease the time to death.
- Decapitation (using sharp blades).

7.3.2. CO_2 asphyxiation followed by decapitation

- Neonatal animals (up to 10 days of age) are resistant to the effects of CO2, therefore, alternative methods are recommended.
- CO2 may be used for narcosis of neonatal animals but it must be followed by another method of euthanasia (e.g., decapitation using sharp blades).
- Keeping neonates warm during CO2 exposure may decrease the time to death.
- Decapitation (using sharp blades).

7.3.3. Barbiturate overdose

- Inject 3 times the anesthetic dose IP.
- Decapitation (using sharp blades) is recommended on your animals before disposal to ensure that they have been correctly euthanized.

7.3.4. Overdose of inhalant anesthetic followed by decapitation

- Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations, therefore, alternative methods are recommended.
- Inhalant anesthetics may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).
- Decapitation (using sharp blades).

7.3.5. Decapitation

- Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.
- Consider using strong and sharp scissors for decapitation of adult or neonatal mice to reduce the risk of injury to personnel.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

7.4. Gestating rodents

Gestating rodents with foetuses under 17 days old can be euthanized by the same procedures as adult rodents.

Gestating rodents with foetuses over 17 days must be euthanized by one of the following methods:

7.4.1. CO_2 asphyxiation under isoflurane anesthesia

- CO_2 asphyxiation under isoflurane anesthesia of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.

7.4.2. CO_2 asphyxiation of the mother, followed by decapitation or barbiturate overdose (IP) of the fetuses.

7.4.3. Overdose of injectable anesthetics to the mother.

RODENT EUTHANASIA

METHODS OF EUTHANASIA	CHEMICAL				PHYSICAL		
	CO_2 ASPHYXIATION UNDER ISOFLURANE ANESTHESIA	CO_2 ASPHYXIATION	BARBITURATE OR INJECTABLE ANESTHETIC OVERDOSE	INHALANT ANESTHETIC OVERDOSE	CERVICAL DISLOCATION	PNEUMOTHORAX	DECAPITATION
 • Adult rodent • Gestating rodent (under 17 days gestation)	**YES**	**YES**	**YES**	**YES**	**YES** Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC	**YES** Only after a chemical method of euthanasia or under anesthesia	**YES** Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC
 • Gestating rodent (over 17 days gestation)	**YES***	**YES***	**YES**	**YES***	**YES*** Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC	**YES*** Only after a chemical method of euthanasia or under anesthesia	**YES*** Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC
	* Decapitation of pups required after euthanasia of the mother. If barbiturate or injectable anesthetic overdose is used to euthanize the mother, decapitation is not required.						
 • Pups less than 10 days old	Only as Narcosis Followed by another physical method of euthanasia	Only as Narcosis Followed by another physical method of euthanasia	**YES**	Only as Narcosis Followed by another physical method of euthanasia	**NO**	**NO**	**YES**

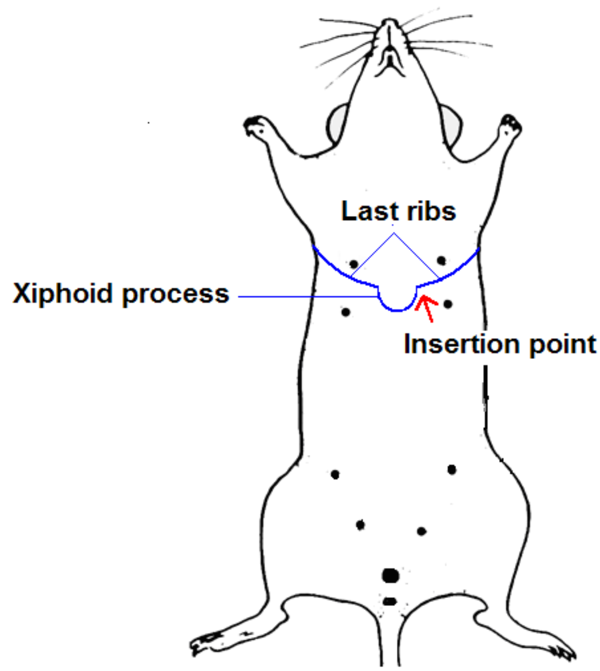
8. BLOOD COLLECTION BY INTRACARDIAC PUNCTURE

- Terminal procedure.
- This procedure can only be done under anesthesia or less than a minute after euthanasia.

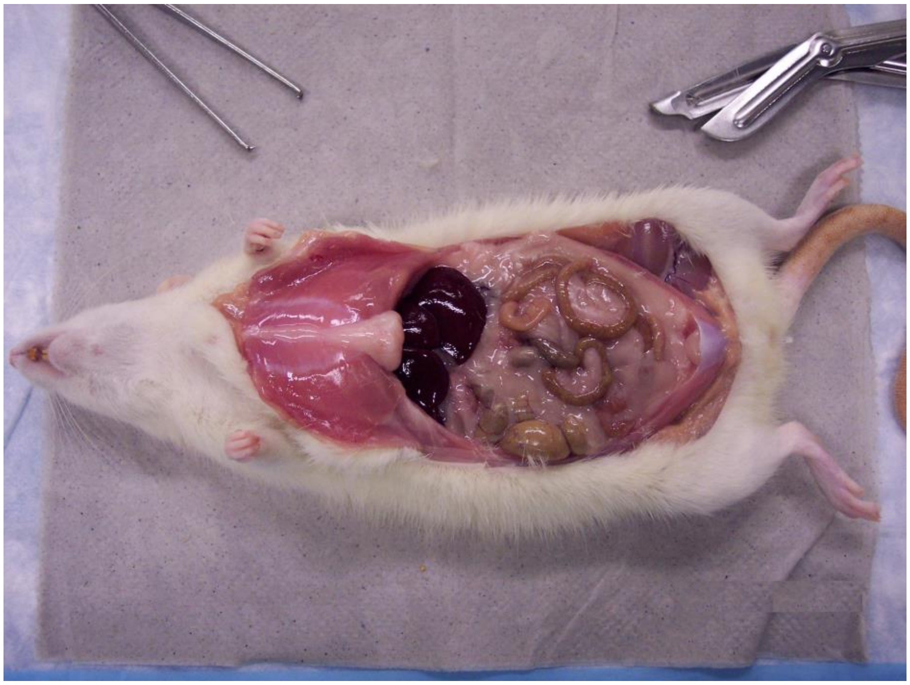
8.1. Procedure

- Prepare a 1cc syringe with a 25G ⅝" needle.
- Place the mouse in dorsal recumbency.
- Palpate the xiphoid process between the last two ribs at the tip of the sternum.
- Insert the tip of the needle between the left side of the xiphoid process and the last rib.
- Once you puncture the skin, gently pull back on the plunger to create a minimal amount of negative pressure within the syringe and maintain it.
- Penetrate the thoracic cavity slowly while directing your needle toward the heart at an angle of approximately 40-45 degrees.
- Note: The heart is slightly left of the midline.
- When a small quantity of blood flows into the hub of the needle, stabilize your needle and continue to pull back on the plunger slowly. The blood should flow into the syringe at a steady rate.

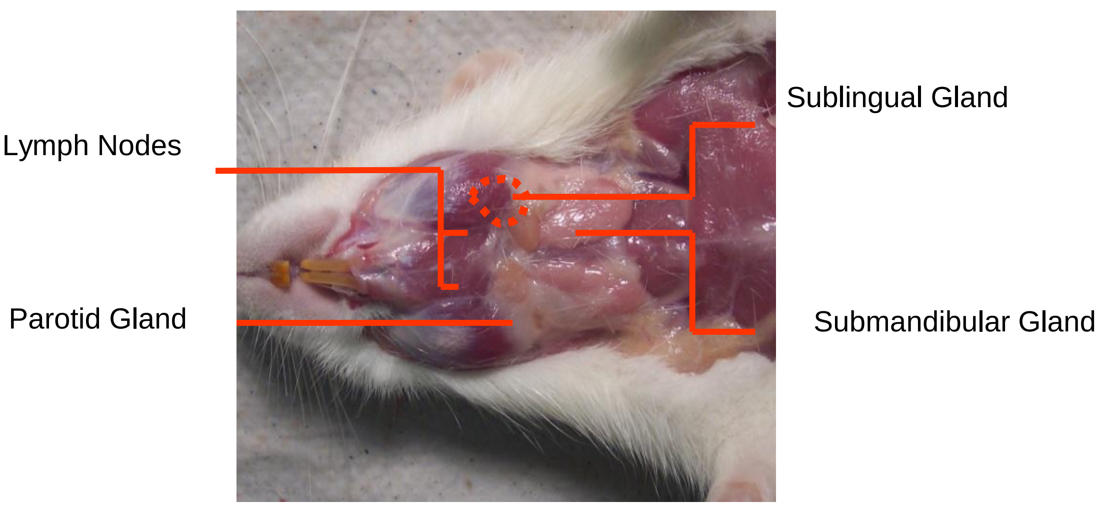
  Note: If the blood flow stops, you change the angle of the needle slightly, rotate it or make very small movements to alter the needle placement.



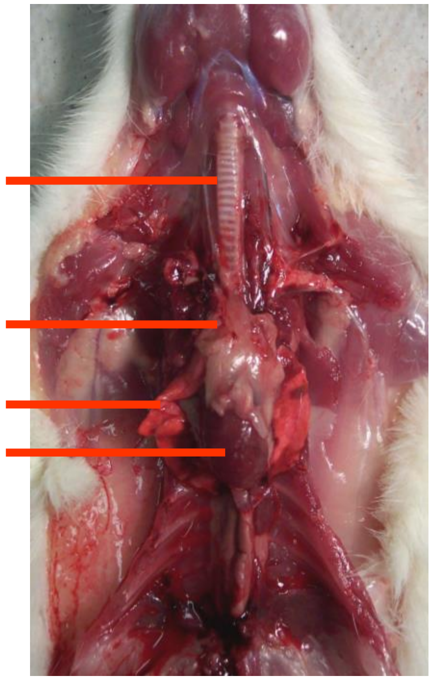
9. NECROPSY



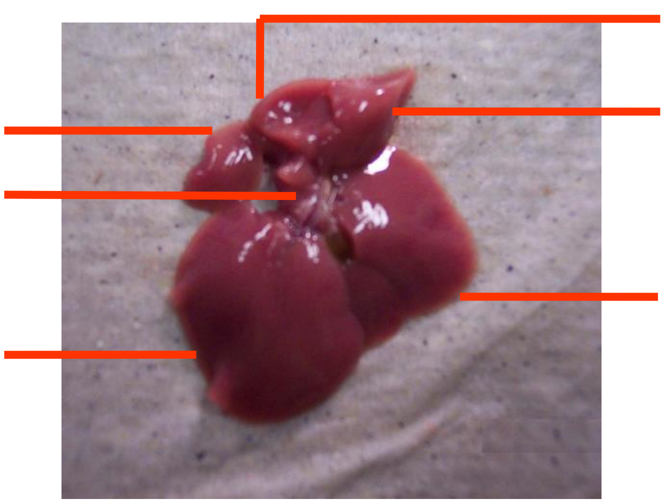
9.1. Salivary glands

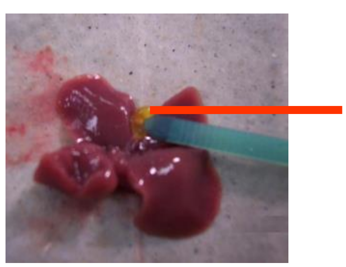


9.2. Trachea, thymus, lungs and heart

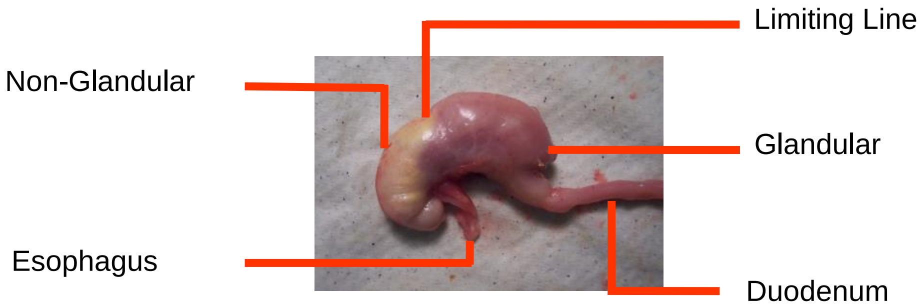


9.3. Liver

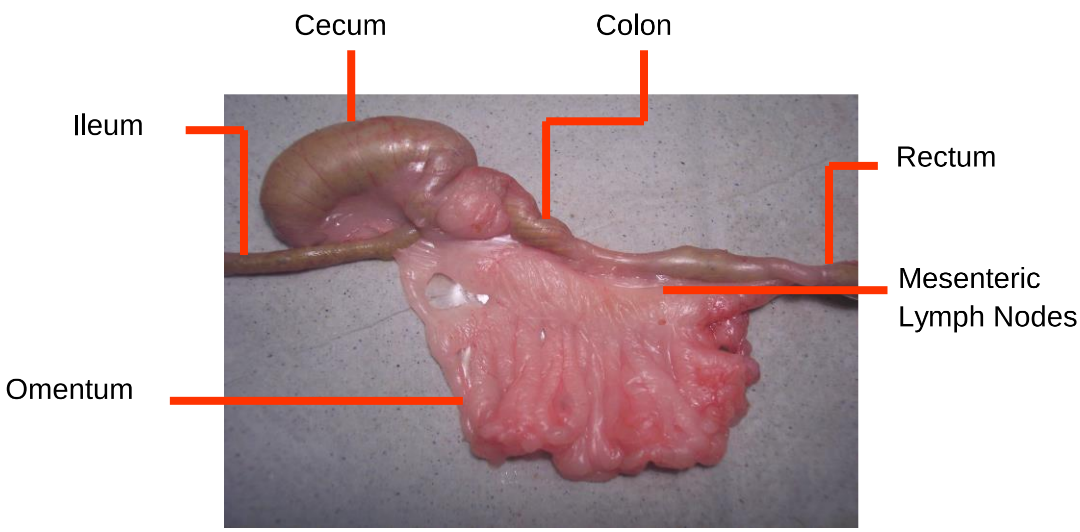




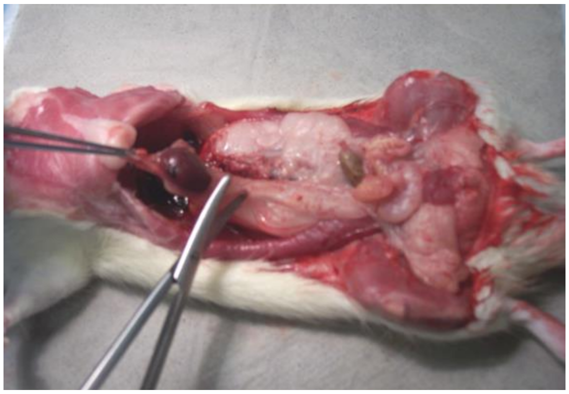
9.4. Stomach



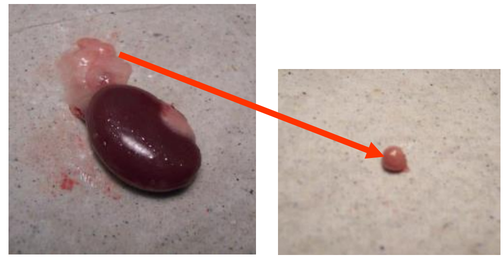
9.5. Cecum, mesenteric lymph nodes, colon and rectum



9.6. Kidney and adrenal gland

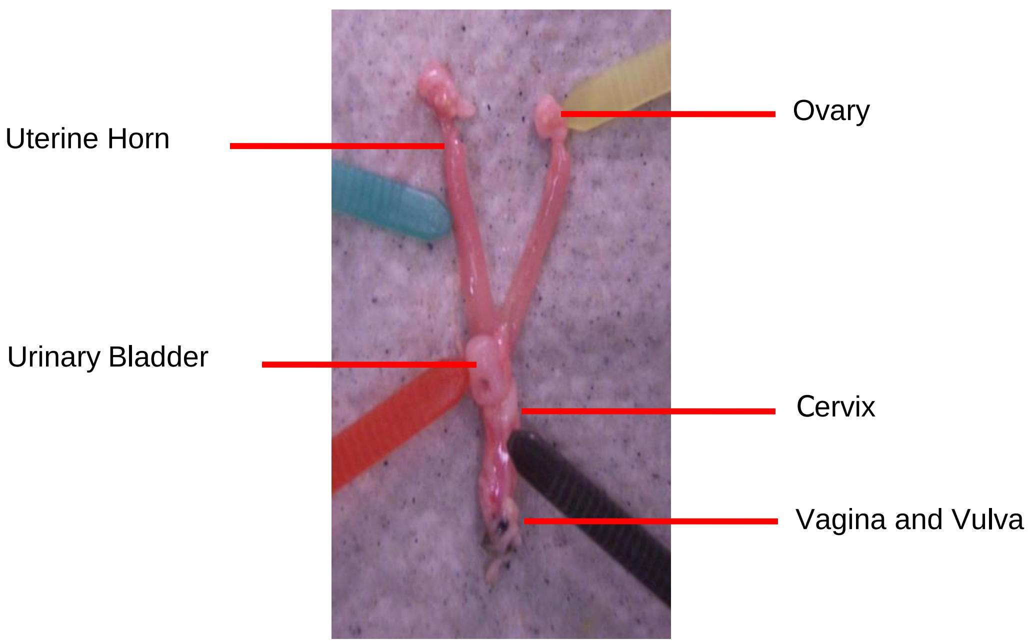


It is normal to find the right kidney displaced a little more cranially than the left in both rats and mice.

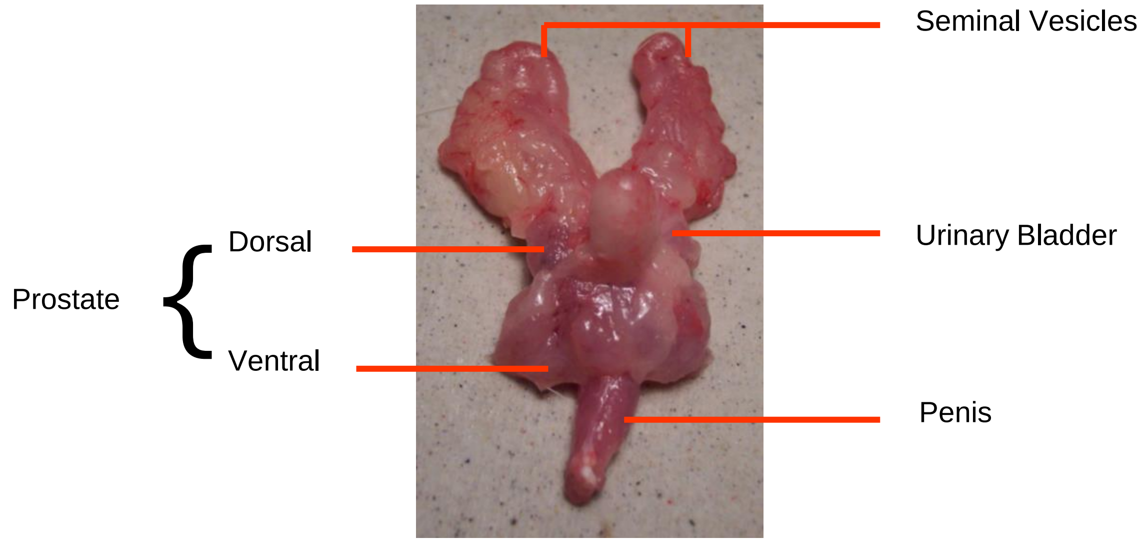


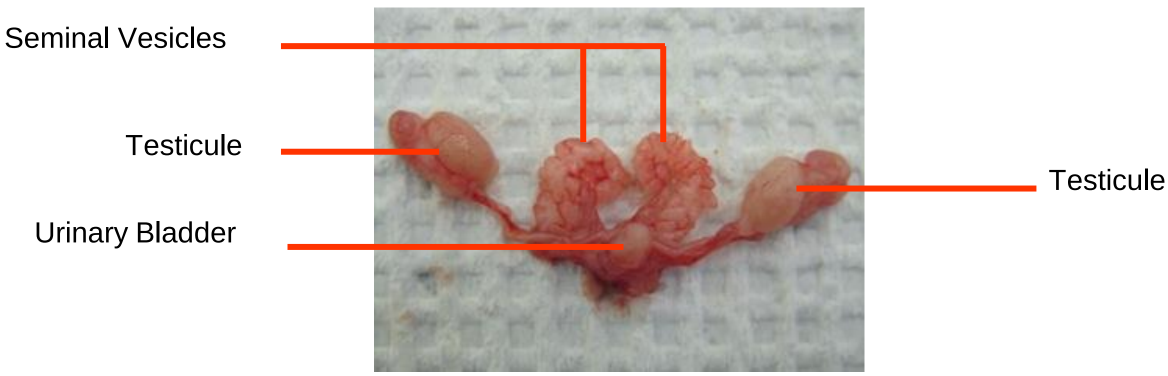
The adrenal gland may be found within the adipose tissue above the kidney, so be careful when dissecting the fat away.

9.7. The female reproductive system



9.8. The male reproductive system





Necropsy photos courtesy of Animal Health Technicians from the Douglas Hospital animal facility and from the Comparative Medicine Animal Resources Centre of McGill University.

10. REFERENCES

10.1. CMARC website

www.mcgill.ca/cmarc

10.2. CMARC emails

Veterinary Care	aht.arc@mcgill.ca
Technical Services, Equipment rental (Anesthetic machines)	rts.arc@mcgill.ca
Imports, Transfers and Quarantine	import.cmarc@mcgill.ca
Imaging Services	imaging.cmarc@mcgill.ca
Irradiator Services	irradiator.cmarc@mcgill.ca
Workshop and Training	workshop.cmarc@mcgill.ca
Polyclonal Antibody Production	antibodyproduction.cmarc@mcgill.ca
Materials and drug sales	drss@mcgill.ca
Comparative Pathology	comparative.pathology@mcgill.ca

10.3. McGill Standard Operating Procedures (SOP)

http://www.mcgill.ca/research/researchers/compliance/animal/sop

10.4. Animal compliance online theory course

- In order to be approved on the animal use protocol, participant must complete the online theory course.
- Basic level: For participants performing techniques shown in Module 1 only.
- Advanced level: For participants performing techniques shown in Modules 2 and above.
- Link to theory course: http://animalcare.mcgill.ca/
- Email: animalcare@mcgill.ca

The UACC would like to acknowledge the invaluable help of the Comparative Medicine and Animal Resources Centre technicians in preparing this handout.