McGill

University Animal Care Committee

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1
THE LABORATORY MOUSE

HANDLING AND RESTRAINT

- General Biology and Physiological Data
- Handling and Restraint
- Identification
- Sexing
- Sampling for Genotyping
- Blood Collection: intra-cardiac puncture
- Euthanasia
- Necropsy Explanation with Pictures

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

THE LABORATORY M+OUSE

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.

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

BODY SCORING SYSTEM

Score 1: Muscle wasting is advanced, fat deposits are gone and bones are very prominent. Euthanasia is mandatory.

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

Score 3: The mouse is in optimal condition. Bones are palpable but not prominent.

Score 4: The mouse is well-fleshed, and bones are barely felt.

Score 5: The mouse is obese, and bones cannot be felt at all.

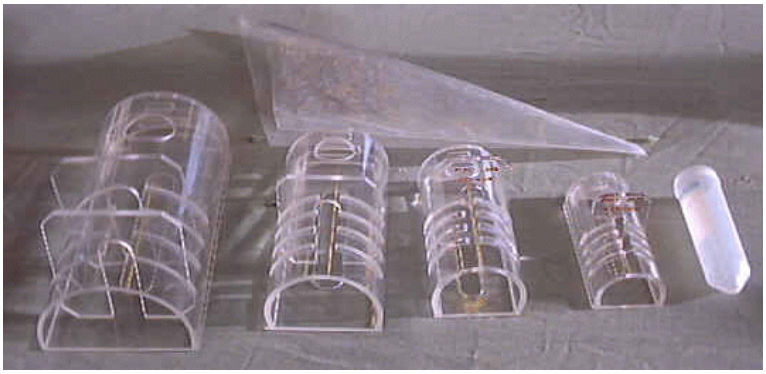
HANDLING AND RESTRAINT

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.

Restraint devices:

Several restraint devices are available in various sizes and materials (e.g., Plexiglas, plastic) and can be used when performing techniques such 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.



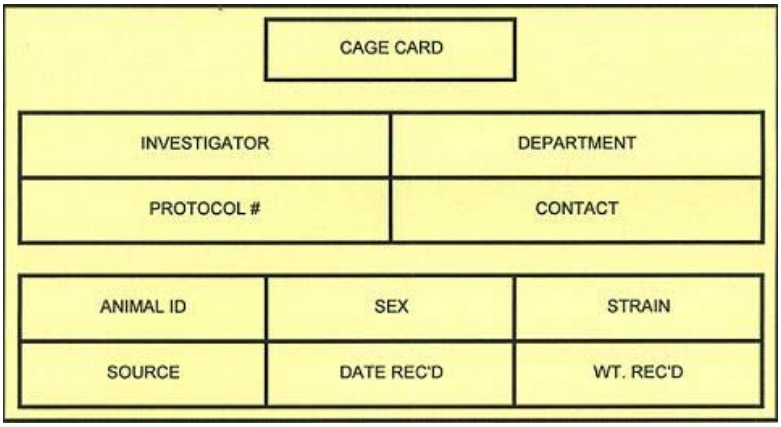
IDENTIFICATION

Mice can be identified by the following methods:

1. Cage cards
2. Temporary markings
3. Ear punching/notching
4. Ear tags
5. Tail tattoo
6. Micro-tattooing
7. Electronic identification with microchips
8. Toe amputation

1. Cage Cards

- Cage cards may be used to identify individually-housed mice or a single breeding pair. They may also be used to identify groups of mice on protocols where individual identification is not necessary.
- All sections of the cage card need to be completed.

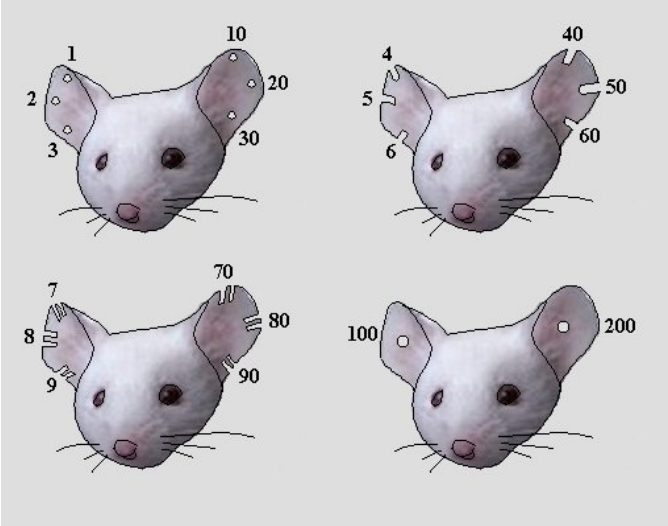


2. Temporary marking

- 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.

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.



4. Ear tag

- Use tags of appropriate size, approximately 5 mm 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. Tattooing

- We recommend performing this procedure under local or general anesthesia.
- Use an electric tattoo machine to write numbers on the tail.
- Ensure that needles are sterile and sharp.

6. Micro-tattooing

- 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.

7. Microchips

- Use appropriate general anaesthesia and analgesia to implant microchip.
- Do not implant microchips in animals less than 3 weeks old.
- Apply disinfectant on the skin (e.g., chlorexidine, betadine).
- Using the implanter, inject the microchip subcutaneously in the neck area between shoulder blades.
- Have available a compatible reader to allow identification of the mice.
- Microchips can be reused after proper cleaning and sterilization (follow manufacturer's recommendation).

8. Toe amputation

- This method can be used only when no other less invasive method is available.
- Toe amputation is only performed on mice.
- The use of this method must be justified and specifically approved by the Facility Animal Care Committee (FACC).

- Toe amputation is acceptable only under the following conditions:
  - The genotype needs to be known before weaning.
  - This method replaces the tail biopsy as a sample for genotyping.
  - Mice must be less tan 10 days old.
  - No more than 2 digits total can be affected, on separate limbs.
  - Only the tip of the digit can be severed (first phalange).
  - Clean, sharp iris scissors or a disposable scalpel blade must be used.
  - A local anesthetic (e.g., lidocaine, bupivacaine, local anesthetic spray, ice) is applied on the site of amputation.

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.
- 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

SAMPLING FOR GENOTYPING

Mice can be genotyped by the following methods:

1. Fecal pellet
2. Buccal epithelial cell
3. Ear punching
4. Tail snipping
5. Toe amputation

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

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

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.

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.
   - 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.
- Check for bleeding. If bleeding occurs, do one of the following:
  - Apply a drop of tissue glue to the cut tip of the tail.
  - Apply a chemical cautery agent such as Kwik Stop® powder or silver nitrate stick.
  - Electric or heat cauterize the cut end of the tail.
- Return the animal to its home cage.

- Procedure for mice over 21 days of age:
  - Requires general anesthesia and analgesia.
  - Administer an analgesic such as carprofen or buprenorphine prior to the procedure and for at least 24 hours thereafter.
  - 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 5% to achieve unconsciousness.
    - Once the animal is unconscious, adjust the flowmeter to 400 to 800mL/min and the isoflurane vaporizer to 2 to 2.5%.
  - 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.

5. Toe amputation

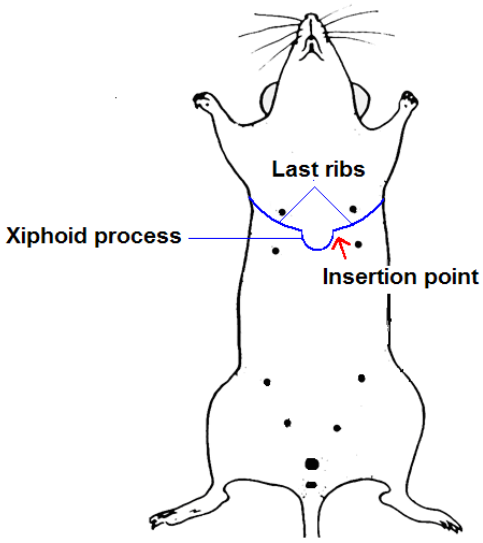
- This method can be used only when no other less invasive method is available.
- Toe amputation is only performed on mice.
- The use of this method must be justified and specifically approved by the Facility Animal Care Committee (FACC).
- Toe amputation is acceptable only under the following conditions:
  - The genotype needs to be known before weaning.
  - This method replaces the tail biopsy as a sample for genotyping.

- Mice must be less than 10 days old.
- No more than 2 digits total can be affected, on separate limbs.
- Only the tip of the digit can be severed (first phalange).
- Clean, sharp iris scissors or a disposable scalpel blade must be used.
- A local anesthetic (e.g., lidocaine, bupivacaine, local anesthetic spray, ice) is applied on the site of amputation.

BLOOD COLLECTION

Intra-cardiac puncture

- Terminal procedure.
- This procedure must be done on anesthetized animal or one that has been just euthanized.
- Procedure:



- Place the mouse in dorsal recumbency.
- Palpate the xiphoid process between at the end of the sternum. it may help to visualize it prior to starting the procedure.
- Prepare a 1cc syringe with a 25G ⅝" needle.
- 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 keep 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 to the left of the midline.

- When a small quantity of blood will come into the hub of the needle, stabilize your syringe and continue to pull back on the plunger slowly.

  Note: If the blood flow stops, you change the angle of the needle slightly or rotate it.

EUTHANASIA

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

Adult rodents - Chemical methods

1. CO_2 asphyxiation

   - 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)
     - Standard rat cage (12" x 9" x6"): 5.25 LPM
     - Cages of different dimensions:

       Measure the cages width, length and height and multiply them to determine the volume in cubic inches.

       Then divide this by 61 to convert into liters and multiply by 20% to determine flow rate.

       $$\frac{\text{height x width x length}}{61} \times 20\% = \text{flow rate}$$
   - Once 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 heart beat, poor mucous membrane colour, no response to toe pinch, colour change in eyes.

- Following euthanasia by CO2, physical euthanasia such as cervical dislocation is strongly recommended to ensure death.

2. Barbiturate or injectable anesthetic overdose

    - Inject three times the anesthetic dose intra-venously or intra-peritoneally.

    - 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 heart beat, poor mucous membrane colour, no response to toe pinch, colour change in eyes.

    - A physical method of euthanasia, such as cervical dislocation, is recommended on your animals before disposal to ensure that they have been correctly euthanized.

3. 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 or air filter through a nose cone.

    - Sufficient air or O2 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 O2 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 in eyes.

    - Following euthanasia by CO2, physical euthanasia such as cervical dislocation is strongly recommended to ensure death.

<u>Adult Rodents - Physical methods</u>

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 otherwise described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

1. Cervical dislocation

    - For mice, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a narrow, blunt instrument such as the dull edge of a scissor blade, acrylic ruler or cage card holder is pressed at the base of the skull.

    - With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.

2. 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.

3. Exsanguination

   - Animals may be exsanguinated to obtain blood products, but only when they are deeply anesthetized or recently euthanized by CO_2 asphyxiation.

   - Collect blood from the heart. (Procedure described blood collection section of Module 1)

   - 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 in eyes.

   - A physical method of euthanasia, such as cervical dislocation or pneumothorax (the diaphragm is lacerated or the ribcage is opened), is recommended on your animals before disposal to ensure that they have been correctly euthanized.

<u>Neonatal Rodents</u>

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:

1. CO_2 asphyxiation

   - Neonatal animals (up to 10 days of age) are resistant to the effects of CO_2, therefore, alternative methods are recommended.

   - CO_2 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 CO_2 exposure may decrease the time to death

2. Barbiturate overdose

   - Inject 3 times the anesthetic dose IP.

   - May be followed by a physical method of euthanasia (e.g. decapitation using sharp blades).

3. 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).

4. Decapitation

    - Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.

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 on of the following methods:

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

2. Overdose of injectable anesthetics to the mother.

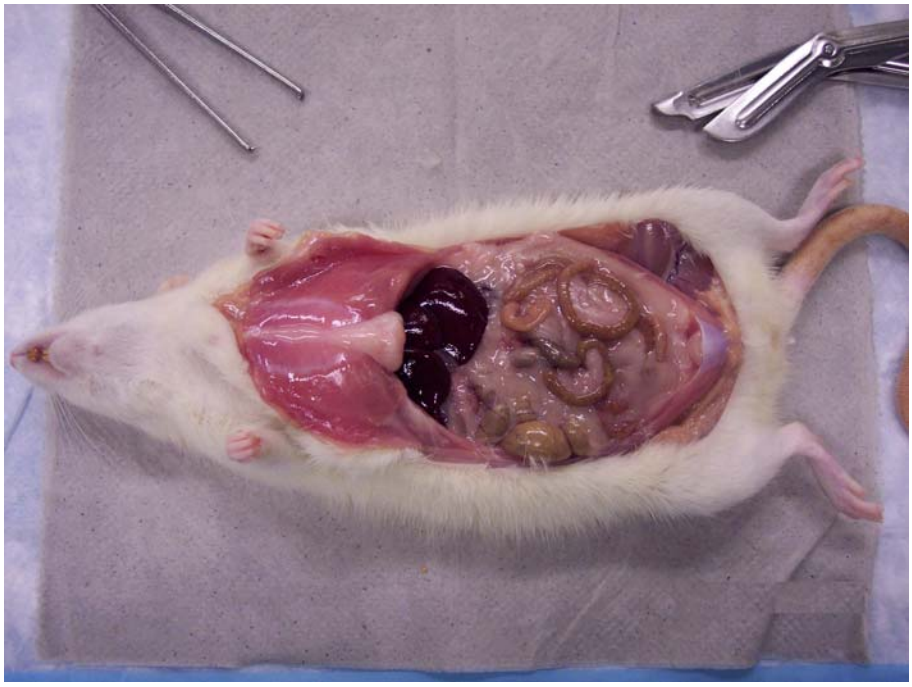
Unacceptable Euthanasia Techniques for Rodents

- Decompression
- Asphyxiation
- Air embolism
- Rapid freezing
- Carbon monoxide
- Methoxyflurane
- Ether
- Nitrogen
- Nitrous oxide
- Chloroform
- Chloral hydrate
- Poisons (strychnine, cyanide)
- Household products and solvents (acetone, alcohol)

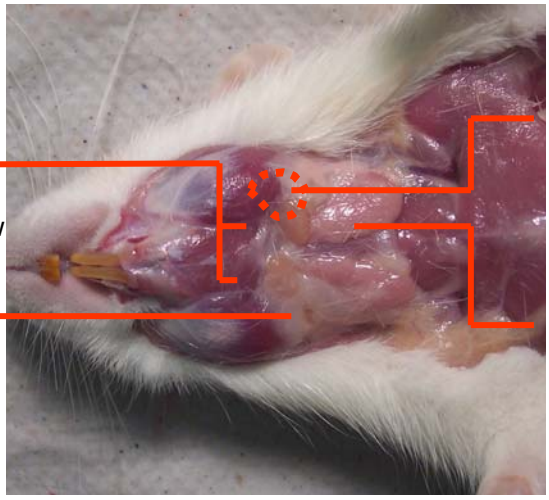
MOUSE EUTHANASIA

Method of Euthanasia	CO_2 Asphyxiation	Barbiturate or Injectable Anesthetic Overdose	Inhalant Anesthetic Overdose	Exsanguination	Cervical Dislocation	Decapitation
 Adult mouse and gestating mouse (under 17 days gestation)	**YES**	**YES**	**YES**	**YES** After CO_2 or Under General Anesthesia	**YES** After CO_2 or Under General Anesthesia	**YES** After CO_2 or Under General Anesthesia
 Gestating mouse (more than 17 days gestation)	**YES**	**YES** decapitation of pups not required	**YES**	**YES** After CO_2 or Under General Anesthesia	**YES** After CO_2 or Under General Anesthesia	**YES** After CO_2 or Under General Anesthesia
	Decapitation of pups required after euthanasia of mom					
 Pups under 10 days old	**Only as Narcosis**	**YES**	**Only as Narcosis**	**NO**	**NO**	**YES**

NECROPSY



SALIVARY GLANDS



Lymph Nodes

Brown solid nodes found near the jaw line.

Parotid

This is the white, half-moon shaped tissue lying on top.

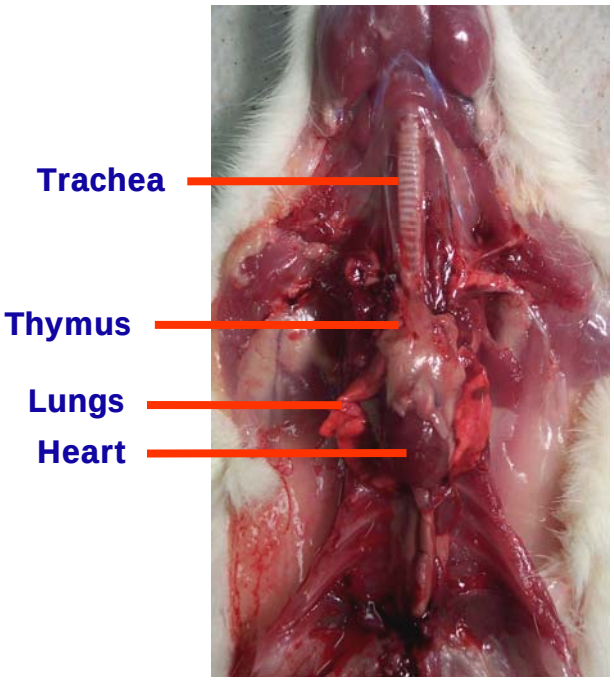
Sublingual Gland

Also known as Submaxillary gland is found attached to the corner of the submandibular gland.

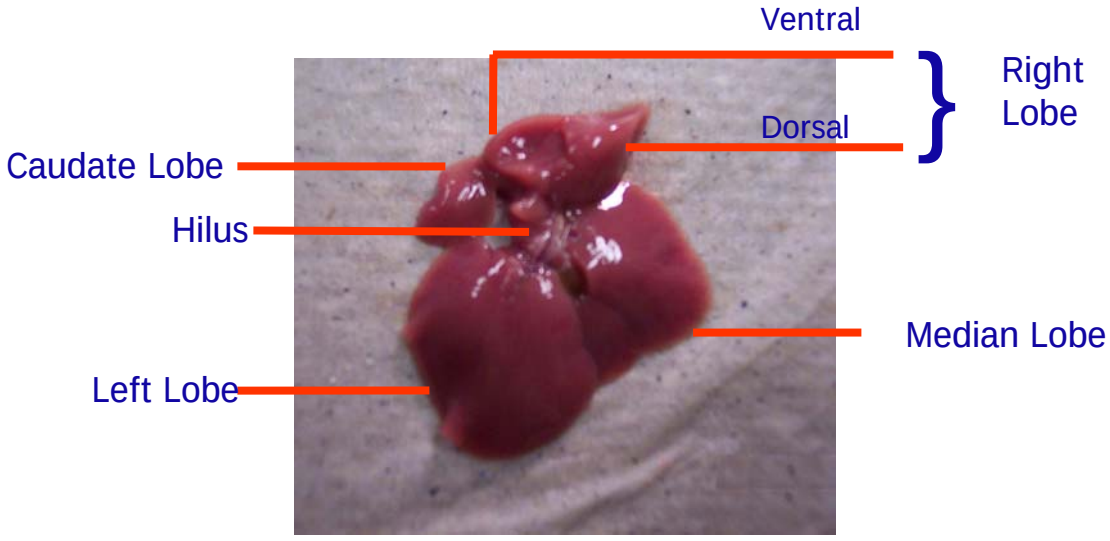
Submandibular Gland

The largest salivary gland, responsible for most of the secretions.

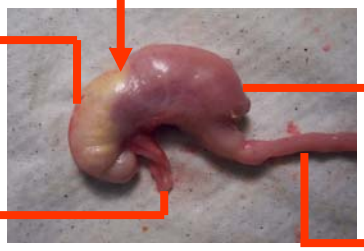
TRACHEA, THYMUS, LUNGS AND HEART



LIVER



STOMACH



Limiting Line

The division between the (beige) non-glandular and the (red) glandular

Non-Glandular

(a.k.a. Cardiac Stomach)
Same striations as esophagus.

Glandular

Where most of the digestion occurs.

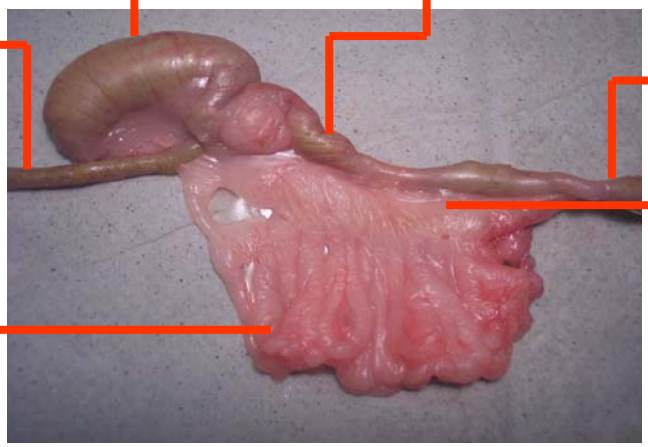
Esophagus

The lining is striated to prevent regurgitation.

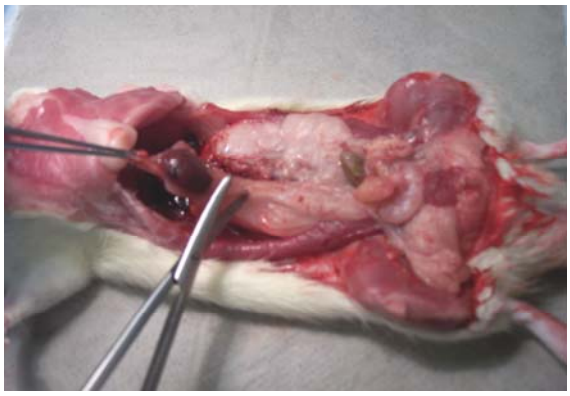
Duodenum

First section of the small intestines.

CECUM, MESENTERIC LYMPH NODES, COLON AND RECTUM.

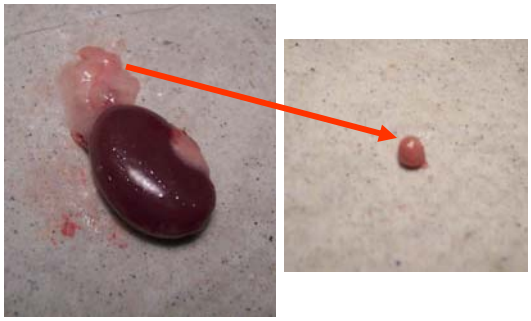


KIDNEY



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.



THE FEMALE REPRODUCTIVE SYSTEM

Uterine Horn

You may find black dots along the horns. These are past implantation sites, where 8-12 offspring will develop for approx. 21 days.

Ovary

Found embedded in fat attached to the uterine horn. The oviduct, a small coiled tubule, may be found attached to the ovary.

Urinary Bladder

May be found full or empty. Will hold up to 2.5ml of urine each day in the mouse and 15ml in the rats.

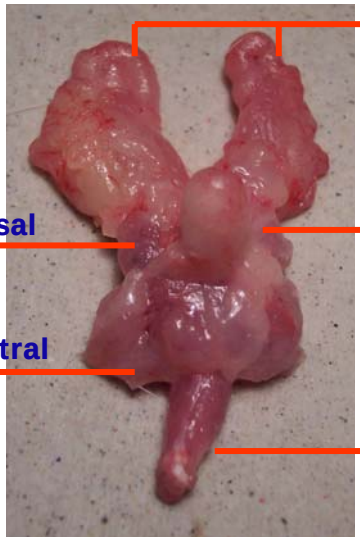
Cervix

You may find black dots along the horns. These are past implantation sites, where 8-12 offspring will have developed for approx. 21 days.

Vagina and Vulva

Used for copulation.

THE MALE REPRODUCTIVE SYSTEM



Prostate

The most common organ in the reproductive system to be collected.

Dorsal

Ventral

Seminal Vesicles

Care should be taken when examining these organs; they are very fragile and full of seminal fluid.

Urinary Bladder

May be found full or empty. If full, be very careful when removing as to not soil your workstation.

Penis

Used for copulation and urination.

Necropsy photos courtesy of Douglas Hospital facility Animal Health Technicians.