LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1 – Introduction to the Laboratory Mouse

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1. ANIMALS USED IN RESEARCH

At McGill University, the use of live animals is subject to scientific/pedagogical merit and ethical review to ensure that live animals are used only when necessary and with full commitment to the wellbeing of the animals. McGill University is dedicated to conducting the highest-quality research and to providing animals with the best care. The use of animals in research, teaching and testing is a privilege governed by public concerns, federal and provincial laws and regulations, the Canadian Council on Animal Care (CCAC) guidelines and policies, and McGill University policies, procedures and guidelines.

The CCAC is the national peer-review organization responsible for setting and maintaining standards for the ethical use and care of animals used in science throughout Canada. McGill’s Animal Care and Use Program is certified by the CCAC based on institutional compliance with CCAC standards. This certification is required to receive funding from the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), or the Social Sciences and Humanities Research Council (SSHRC), as well as some provincial funders and charitable organizations.

McGill University’s Policy on the Study and Care of Animals outlines the basic principles for the care of animals involved in research, teaching or testing at McGill University and affiliated institutions.

The privilege of using animals can be withdrawn for individuals who, by their negligence or deliberate actions, establish non-compliance with CCAC guidelines, McGill Policies and SOPs, and the approved animal use protocol. These individuals might face additional disciplinary measures, including reporting the non-compliance to other instances.

1.1. The Animal Use Protocol

All procedures involving the use of animals in research, teaching and testing must be described in an Animal Use Protocol (AUP).

All animal-based protocols must comply with CCAC and McGill University policies and guidelines. AUPs are peer-reviewed for scientific or pedagogical merit and are approved by the local Facility Animal Care Committee (FACC) before animals are purchased and used.

AUPs contain detailed information on:

- All procedures performed on live animals, including euthanasia methods.
- All substances being administered to live animals, including potentially hazardous agents.
- Animal housing and procedure locations.
- The total numbers of animals to be produced and used in a given year, including alternatives for replacement and reduction of animal use.
- Anticipated signs of morbidity or adverse effects on the health and welfare of the animals and monitoring frequency.
- Humane intervention points, which are clear criteria set to prevent or relieve unnecessary pain or distress to a research animal.
- Strategies employed to implement the three Rs – replacement, reduction, and refinement.
- Personnel working with live animals.
1.2. Standard Operating Procedures
McGill University has created over 100 Standard Operating Procedures (SOPs) that provide guidelines for commonly used procedures including analgesia, anesthesia, surgical and experimental procedures, euthanasia, etc. SOPs establish best practices and ensure adherence to all relevant regulations. They are created by the Veterinary Care Subcommittee and routinely reviewed. SOPs are an invaluable resource for any individual working with laboratory animals and should be consulted regularly.

1.3. Quality Assistance Program
The Quality Assistance Program (also known as Post-Approval Monitoring) ensures animal wellbeing, is a resource and assistance to the FACC and to the research community, and ensures adherence to approved procedures. Adherence to Animal Use Protocols is achieved by assessment visits and procedure observations. Assessment visits are carried out by Quality Assistance Advisors.

1.4. Education and Training
All individuals involved in the care and use of animals must receive appropriate training, both theoretical and practical, and adequate preparation before undertaking a procedure, using, or caring for a species. Having a good working knowledge of the AUP is essential.

Individuals using or caring for animals at McGill or its affiliated institutions have a responsibility for the proper stewardship of animals under their care; this includes adhering to protocols, policies, procedures and guidelines. Furthermore, each participant in the Animal Care and Use Program is accountable for reporting animal welfare and compliance concerns. The Guidelines for Animal Welfare and Compliance Concerns describes participants’ responsibilities.

Failure to adhere to McGill policies, procedures and guidelines may result in revocation of access to the animal facility.

In the spirit of promoting humane use of animals in training and applying the principles of the three Rs, animals used in training are subject to a set of specific intervention points detailed in SOP 418 – Humane Intervention Points for Animals Used in Training. The goal being to reduce the total number of animals used in training, reuse animals in a humane and responsible manner, and refine the procedures to minimize discomfort and distress.

2. OCCUPATIONAL HEALTH PROGRAM
The Occupational Health Program (OHP) for Animal Related Activities addresses the health risks that may result from working with animals. Participation in the OHP is voluntary for personnel in contact with rodent species. However, individuals who are exposed to animals, tissues, body fluids, wastes, bedding, living quarters or equipment involved in the care and use of animals are strongly encouraged to participate in the OHP.

Allergies to animals are a common health issue in research and teaching animal facilities and are recognized as an occupational hazard. Individuals with pre-existing allergic conditions face a greater risk of developing allergies.

Wearing personal protective equipment (PPE) is one way to limit your exposure to rodent allergens.
Always wear adequate attire (long pants, socks, and closed shoes) and PPE to enter areas where laboratory rodents are handled or housed.

Consult the Allergy Prevention factsheet for a list of symptoms and tips on preventing allergies.

3. ANIMAL CARE AND HUSBANDRY

It is essential that all work involving live animals be performed in facilities that ensure the safety of the staff and students while maintaining the health and welfare of animals through high standards of animal care and facility management.

Rodents are housed in facilities where the temperature, humidity, light cycle, and ventilation are continuously monitored and controlled. Husbandry of laboratory animals is the responsibility of the Animal Care team; they make sure animals have clean, comfortable cages, food and water and that the facilities are well maintained. Attendants also observe cages on a daily basis and report any animal that appear ill or injured to the Veterinary Care team.

Laboratory rodents are provided with environmental enrichment substrates or devices which allow for opportunities to express natural behavioural needs and promote physical and mental health. The presence of nesting materials, such as shredded paper and cotton pads, is essential for temperature regulation, social behaviour, reproduction, and the general wellbeing of the animals.

Any deviations from the standard husbandry practices are detailed in the AUP and should be communicated to the facility staff. Cages receiving special diet or water, being deprived of food or water for short periods, or having different husbandry requirements must be clearly labelled. Labelling should include the start and end date/time and your contact information. Standard cage cards are available for common situations, refer to Annex 1.

4. VETERINARY CARE

The Veterinary Care team is composed of animal health technicians and veterinarians; their role is to provide medical and preventive care by evaluating clinical cases, providing treatment and monitoring animals. They also provide training and technical services to the research community, make recommendations and share expertise, monitor the overall health of the colonies, and work to improve the general welfare for animals used in research.

Veterinarians have the authority and responsibility to make determinations concerning animal wellbeing and to assure that this is appropriately monitored and promoted.

You can contact the Veterinary Care team with questions or concerns regarding animal health and wellbeing.

The Veterinary Care team is also responsible for the management of the Veterinary Care Program. The program aims to detect and treat sick or injured animals thus preventing and relieving unnecessary pain and distress.

You may come across a Veterinary Care cage card on one of your cages. This indicates that an illness/injury report has been submitted for one or more animals in that cage. Many of the cases tend to be common conditions such as aggression between mice, excessive scratching or eye infections. A member of the Veterinary Care staff will contact you if they find a more serious case that requires your attention.
5. HUMANE INTERVENTION POINTS

Humane intervention points are clear criteria set to prevent or relieve unnecessary pain or distress to a research animal. Intervention points should be balanced with the experimental endpoints to ensure that animals can be kept on study humanely while they reach the scientific endpoint. In other words, an animal should be euthanized at the earliest possible point that will provide experimental data in order to minimize suffering. Humane interventions are clearly defined in the Animal Use Protocol (AUP) and are defined as actions or instructions including, but not limited to, the following:

- Adequate veterinary treatment, analgesia and/or supportive therapy to the animal(s)
- Termination of painful procedures
- Removal of the animal(s) from the study
- Modification of the experimental procedures to minimize the discomfort to the animal(s)
- Increasing the frequency of animal observations
- Modification to the housing and husbandry practices to improve the comfort of the animal(s)
- Euthanasia

Note that death is never an acceptable endpoint. Every step must be taken to select intervention points that avoid animal death.

5.1. General intervention points:

- Weight loss exceeding 20% of baseline bodyweight. For young animals, failure to maintain normal weight gain within 15% of age-matched control animals.
- Body condition score (BCS) less than 2.
- Uncontrolled seizures.
- Impaired mobility which interferes with normal eating, drinking, ambulating or grooming.
- No or weak response to external stimuli.
- Hypothermia.
- Mass that is ulcerated, necrotic or impairing normal function (e.g., eating, drinking) or exceeding acceptable size endpoints: 2cm³ or 10% of the baseline bodyweight
- Respiratory distress: labored breathing, increased or decreased respiratory rate, cyanosis
- Hunched posture, lethargy and lack of grooming.
- Incoordination, paralysis
- Abnormal vocalizations
- Pale eyes and/or extremities (rodents) or mucous membranes
- Uncontrolled hemorrhaging
- Self-mutilation
- Specific organ failure assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc.)
5.2. Study-specific intervention points:

- Study-specific humane intervention points have been determined for aging animals, animals used as cancer models, animals used in the production of antibodies, etc. These intervention points are detailed in McGill University’s Standard Operating Procedures.

5.3. Monitoring

Regular monitoring of rodent colonies and experimental animals will allow for timely assessment of the health of the animals.

It is the responsibility of the research staff to monitor animals according to the frequency indicated in the Animal Use Protocol. It is recommended to monitor animals at least once per week. However, the frequency of monitoring should be increased as health status declines or as the endpoints are approaching. Post-operative animals and animals undergoing invasive or frequent procedures should be monitored daily.

Careful observation of animals in their home cage can provide a wealth of information about the health and welfare of the animals. Activity, such as grooming, eating, drinking, nest building, interaction with cage mates, and general appearance are indicators of general health and well-being. Simple cage side observations can detect wounds, changes in movement or posture, decreased activity levels, presence of tumors, etc.

Hands-on physical examination provides an assessment of the animal’s hydration, body condition score, observable abnormalities, and the presence of palpable anomalies in the abdomen.

Measuring the body weight can be a helpful tool to monitor general health. It is recommended to weigh animals before the start of experimental procedures and once per week thereafter.

Observations and measurements, frequency of monitoring, and interventions should be carefully recorded.
6. THE LABORATORY MOUSE

The common laboratory mouse, *Mus musculus*, 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
- Poor vision, acute sense of hearing and smell
- Social animals, although 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
- Estrous cycle length: 4-5 days
- Duration of estrus: 12 hours
- Average litter size: 6-12
- Gestation period: 19-21 days
- 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: 12 months
- Male adult weight: 25-40 g, Female adult weight: 20-40 g
- Life span: 1.5-3.0 years

6.2. Body condition (BC) scoring system

Scoring the body condition of rodents is a non-invasive method for assessing health and establishing endpoints where body weight is not a viable monitoring tool, such as with tumor models, ascites production, pregnancy, or in young growing animals.

Body condition scores (BCS) range from 1 (emaciation) to 5 (obesity).

Scores are determined by frequent visual and hands-on examination of each animal. The hands-on evaluation is done by palpating over the vertebral column and sacroiliac bones. The findings are matched to the descriptions and diagrams below to determine a score.
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.
- A body condition score of less than 2 is considered a humane intervention point requiring euthanasia.

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.
6.3. 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. Animals should be evaluated by quietly observing them without
moving the home cage.
6.4. Cage density

McGill University adheres to national and international guidelines that determine how many mice can live in a standard cage. Overcrowding cages is not only detrimental to the wellbeing of the mice, these cages also require more frequent changing.

Per single, standard mouse cage, you can house:

- **Up 5 adult mice** (aged over 6 weeks old or weighing >16g). Mice should be of the same sex.
- **1 breeding female (+/- the male) with one litter**
- **2 breeding females (+/- the male) with two litters as long as each litter has less than 8 pups**
- **Up to 8 juvenile mice (weanlings) between 3 and 6 weeks of age.**
7. HANDLING AND RESTRAINT

7.1. How to pick up a mouse

- Gentle handling will minimize stress. The stress of handling mice may affect physiological parameters and behavior, and therefore have a significant impact on scientific outcomes.

- Whenever possible, consider using alternative methods to avoid handling mice by their tail to reduce handling stress, e.g., using cupped hands or tubes to scoop mice.

- 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, use cupped hands or a tunnel. Picking up mice by the tail may induce aversion and anxiety and should be avoided whenever possible. When mice are to be picked up by the tail, the base of the tail should be used; never hold a mouse by the tip of the tail.

7.2. Manual restraint

- To allow for procedures or health assessments, mice may need to be manually restrained.

- Restrain should be gently yet secure. When adequately restrained, the mouse remains calm and does not struggle.

- To securely restrain a mouse:
  - 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 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.

View the Handling & Restraint Instructional Video
7.3. 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.

7.4. Animal-related injuries

- Minor bites or scratches occur occasionally when handling laboratory mice. Standard first aid should be applied. All injuries should be reported to McGill Environmental Health and Safety. Refer to SOP 702 – Animal-Related Injuries for additional information.

8. SEX DETERMINATION

- Sexing of mice is based upon a comparison of the ano-genital distance
- Males have a greater distance between the anus and urogenital opening than females (approximately double). The genital papilla is more prominent in males than females. Note that the testicles can be retracted into the abdomen.
- An opposite sex comparison is advisable. Always compare animals of similar age.
9. IDENTIFICATION METHODS

9.1. Selecting an identification method

The selection of identification methods should take into consideration:

- Whether the animals will be single or group housed
- The age of the animals at the time of identification
- Whether the method needs to be temporary or permanent
- Whether the collection of tissue for genotyping is required
- The least invasive identification method available should be selected

9.2. Cage cards

- All cages must have a barcoded Darwin cage card. Additional cage cards may be used, however, care must be taken not to cover the Darwin barcode.
- Use cage cards to identify individually-housed mice or a single breeding pair.
- Use cage cards to identify groups of rodents on protocols where individual identification is not necessary.
- Cages cards must include, at a minimum, the following information:
  - Principal Investigator
  - Protocol number
  - Species
  - Strain
  - Sex
  - Number of animals in the cage
9.3. 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.

9.4. Ear punching/notching

- Rapid and minimally invasive method that also yields a tissue sample for genotyping.
- Does not require anesthesia and analgesia.
- This method cannot be used on rodents under 2 weeks (14 days) of age.
- 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.
- Has the advantage of using the excised tissue as a sample for genotyping.
- Procedure:
  - Ensure the ear punch apparatus or scissors are sharp.
  - Disinfect the ear punch or scissors with 70% alcohol and wipe dry before use.
  - Restrain the animal securely by the scruff.
  - Using the ear punch or scissors, punch holes and/or notches in the ears, following an identification chart.
  - Whenever possible, use a simple code to limit the number of notches/punches.
  - Use the excised tissue as a sample for genotyping. Place in well-identified collection tube.
  - Disinfect ear punch or scissors between animals with 70% alcohol.

9.5. Ear tag

- Provides a unique identification number for each animal.
- Does not require anesthesia and analgesia
- This method should not be used in rodents under 2 weeks of age.
- Proper care in placing ear tag is important and improperly placed tags can result in a loss of a tag, inflammation, injury, or excessive scratching.
- This method can be combined with ear punching on the opposite ear for tissue collection for genotyping or to avoid issues that arise if two or more animals lose an ear tag in the same cage.
- Procedure:
  - Use tags that are approximately 5 mm long. Tags should be appropriately sized according to the species.
  - Rinse the tags in 70% alcohol before use.
Place the tag low on the pinna (distal 1/3) so that it rests against the animal and does not bend the ear, cause the animal to hold its head in a lopsided manner, or catch on the cage.

- Monitor site of implantation for local infection or inflammation. The animal should be monitored for clinical signs and the tag removed if necessary.

### 9.6. Micro-tattooing

- Use a micro-tattooer to inject tattoo ink in the toe pads or the ears or the tail.
- This method is suitable for both neonates and adults.
- Local or light general anesthesia can be used for the procedure.
- 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.

### 9.7. Microchips:

- Do not implant microchips in animals less than 3 weeks old.
- Implant microchips subcutaneously according to the manufacturer’s instructions.
- A compatible reader is required to allow identification of the mice.
- This method requires the use of general anesthesia and analgesia. Refer to corresponding analgesia and anesthesia SOPs.
- Procedure:
  - Clean the site of microchip implantation with skin disinfectant, e.g., 2% chlorhexidine solution.
  - Using the implanter, inject the microchip subcutaneously in the neck area.
  - Reuse microchips only after proper cleaning and sterilization (follow manufacturer’s recommendation).

### 9.8. 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.

### 10. TISSUE SAMPLING FOR GENOTYPING

To maximize the efficiency of the breeding colony, genotyping samples should be processed in a timely manner and animals of the undesired genotype removed quickly.

#### 10.1. Selecting a tissue collection method

The selection of the tissue collection method should take into consideration:

- The age of the animals at the time of tissue collection
- The least invasive method available should be selected
10.2. Fecal pellet
- This method is minimally invasive and may be useful with rodent strains prone to bleeding disorders.
- Procedure:
  - Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding for a few minutes.
  - Identify animal as per Rodent Identification SOP.
  - Place fecal pellet in an identified collection tube.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

10.3. Skin swabbing
- This method is minimally invasive and may be useful with rodent strains prone to bleeding disorders.
- Procedure:
  - Restrain the animal.
  - Using a cotton-tipped swab, stroke the ventral and dorsal skin against the direction of hair growth.
  - Perform a minimum of 3 strokes of 3cm in length each.
  - Insert cotton bud into collection tube and snip off excess shaft.
  - Identify animal as per Rodent Identification SOP.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

10.4. Buccal epithelial cell
- This method is minimally invasive and may be useful with rodent strains prone to bleeding disorders.
- Procedure:
  - Firmly restrain the animal by the scruff to maintain its mouth open.
  - Using a cotton-tipped swab with a <2mm bud, vigorously rub the inner cheeks while rotating the swab, avoiding the tongue.
  - Insert cotton bud into collection tube and snip off excess shaft.
  - Identify animal as per Rodent Identification SOP.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

10.5. Ear punching
- Refer to section 9.4

10.6. Whole blood
- Collect as per SOP 403-Guidelines Blood Collection Volumes and Frequency.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.
10.7. Tail biopsy

- Tail snipping should be performed on mice between 14 and 21 days of age (ideally between 14 and 17 days).
- Tail biopsy can only be performed twice over the life time of the animal and cannot exceed 5mm total.
- Identify animal as per Rodent Identification SOP.
- Tail snipping procedure for mice less than 21 days of age:
  - General anesthesia is recommended but not required.
  - Gently, but securely, restrain mouse (manual or mechanical).
  - Swab the tail with antiseptic (e.g. alcohol).
  - Snip 2-3mm off the tip of the tail with sharp, sanitized scissors or disposable scalpel.
  - Remove biologic material and sanitize the scissors after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if you are snipping several mice tails.
  - Place tissue sample into an identified collection tube.
  - Check for bleeding before returning mouse to its cage. If bleeding occurs, do one of the following:
    - Apply a drop of tissue glue to tip of tail.
    - Apply a chemical cautery agent (e.g. Kwik Stop® powder or silver nitrate stick).
    - Electric or heat cauterize the cut end of the tail.
- Tail snipping procedure for mice over 21 days of age:
  - Requires general anesthesia and analgesia.
  - Brief general anesthesia is provided with isoflurane, by placing animals in an induction chamber to achieve unconsciousness. Refer to Rodent Anesthesia SOPs.
  - Analgesia must be provided for 72 hours as per Rodent Analgesia SOPs.
  - Perform the tail snipping as described above.

10.8. Distal phalanx biopsy (toe amputation)

- This method is acceptable in circumstances where the genotype needs to be known before weaning. This method replaces the tail biopsy as a sample for genotyping and ear notching as an identification method.
- Specialized training is required for this procedure.
11. EUTHANASIA

Mice can be euthanized in a variety of acceptable, effective and humane methods; these methods can be either chemical or physical. Chemical methods of euthanasia should always be followed by a physical method to ensure death.

Only the approved euthanasia method described in the Animal Use Protocol can be used.

11.1. Adult rodents - Chemical methods

11.1.1. CO₂ asphyxiation under isoflurane anesthesia

- It is preferable to anesthetize rodents with isoflurane prior to exposure to CO₂ 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 CO₂ tank and set the flowmeter to maximum flow rate.
- Maintain the CO₂ flow until the animal has stopped breathing. Note that the time required for euthanasia can be several minutes.
- Close the CO₂ flow meter and the valve on the CO₂ tank.
- Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
- Confirm euthanasia before disposing of the carcass by observing that there is no respiratory movement for at least 20 minutes, or follow up with a physical method of euthanasia, such as cervical dislocation or pneumothorax.
- 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.

**NOTE**: You may be exposed to low levels of waste anesthetic gases (isoflurane) during this procedure. If you are currently pregnant, please consult your physician or McGill University’s Environmental Health and Safety department before using isoflurane.
11.1.2. **CO₂ asphyxiation**

- CO₂ 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 CO₂ 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 CO₂ flow until the animal has stopped breathing.
- Close the valve on the tank.
- Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
- Confirm euthanasia before disposing of the carcass by observing that there is no respiratory movement for at least 20 minutes, or follow up with a physical method of euthanasia, such as cervical dislocation or pneumothorax.
- 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.

11.1.3. **Barbiturate or injectable anesthetic overdose**

- Inject pentobarbital at a dose of 120mg/kg intravenously or intraperitoneally.
- 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.
11.1.4. 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.

**NOTE:** You may be exposed to low levels of waste anesthetic gases (isoflurane) during this procedure. If you are currently pregnant, please consult your physician or McGill University's Environmental Health and Safety department before using isoflurane.

11.2. Adult rodents - Physical methods

Physical methods of euthanasia are also an appropriate means to assure death after euthanasia with CO₂ or anesthetics used as euthanasia agents. Anesthesia or sedation is necessary prior to physical methods of euthanasia.

11.2.1. Cervical dislocation

- Cervical dislocation may be performed without anesthesia or sedation only when described and scientifically justified in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC). Cervical dislocation performed on live animals requires specialized 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.
11.2.2. Pneumothorax

- After the animal has been euthanized using a chemical method, or once the animal is deeply anesthetized:
  - 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.

[View the pneumothorax instructional video]

11.2.3. Decapitation

- Decapitation may be performed without anesthesia or sedation only when described and scientifically justified in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC). Decapitation performed on live animals requires specialized training.
  - 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.
  - Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.
  - Consider using strong and sharp scissors for decapitation of adult 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.

[View the adult decapitation instructional video]

11.3. 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:

11.3.1. CO₂ 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.

**NOTE:** You may be exposed to low levels of waste anesthetic gases (isoflurane) during this procedure. If you are currently pregnant, please consult your physician or McGill University’s Environmental Health and Safety department before using isoflurane.
11.3.2. CO$_2$ asphyxiation followed by decapitation

- 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.

11.3.3. 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.

11.3.4. 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).

**NOTE:** You may be exposed to low levels of waste anesthetic gases (isoflurane) during this procedure. If you are currently pregnant, please consult your physician or McGill University’s [Environmental Health and Safety](#) department before using isoflurane.

11.3.5. Decapitation

Consider using strong and sharp scissors for decapitation of 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.

**View the neonate decapitation instructional video**

11.4. Gestating rodents

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

Gestating rodents with fetuses at or above 17 days must be euthanized by one of the following methods:

11.4.1. 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.

**NOTE:** You may be exposed to low levels of waste anesthetic gases (isoflurane) during this procedure. If you are currently pregnant, please consult your physician or McGill University’s [Environmental Health and Safety](#) department before using isoflurane.
11.4.2. **CO₂ asphyxiation**
- CO₂ asphyxiation of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.

11.4.3. **Overdose of injectable anesthetics to the mother.**

11.5. **Confirmation of death**

All rodents must be subject to a confirmation of death before disposal of the carcass.

To confirm death, monitor animal for the following signs:
- No rising and falling of chest
- No palpable heart beat
- Poor mucous membrane color
- No response to toe pinch
- Color change in eyes.

Death can be confirmed using one of the following methods:

11.5.1. **Neonates and fetuses over 17 days, >E17:**
- Decapitation

11.5.2. **Adult rodents:**
- Physical method as described in section 11.2.
- After prolonged exposure to CO₂ (minimum of 15 minutes), allow animals to remain inside the cage used for euthanasia for an additional 15 minutes minimum. Observe animals for signs described in 8.2 and muscle stiffening signifying the onset of rigor mortis. If death cannot be confirmed, repeat exposure to CO₂.
## RODENT EUTHANASIA

<table>
<thead>
<tr>
<th>METHODS OF EUTHANASIA</th>
<th>CHEMICAL</th>
<th>PHYSICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂ ASPHYXIATION UNDER ISOFLURANE ANESTHESIA</td>
<td>CO₂ ASPHYXIATION</td>
</tr>
<tr>
<td>Adult rodent</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Gestating rodent</td>
<td>YES*</td>
<td>YES*</td>
</tr>
<tr>
<td>(under 17 days gestation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestating rodent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(over 17 days gestation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pups less than 10 days old</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Only as Narcosis Followed by another physical method of euthanasia</td>
<td>Only as Narcosis Followed by another physical method of euthanasia</td>
</tr>
</tbody>
</table>

* 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.
12. REFERENCES

12.1. Comparative Medicine & Animal Resources Centre

CMARC website  www.mcgill.ca/cmarc
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


https://www.mcgill.ca/research/research/compliance/animals/animal-research-practices/sop

12.3. University Animal Care Committee (UACC) 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.
- McGill University Animal Care Committee Theory Course
- Email: animalcare@mcgill.ca

12.4. Photographing/filming guidelines

- McGill Social Media Guidelines
- McGill University Animal Care Committee’s Guidelines for photography/filming of animals for research purposes

12.5. Useful links

- Canadian Council on Animal Care
- Quality Assistance Program
- Policy on the Study and Care of Animals
- Animals in Research and Teaching
- McGill Occupational Health Program

The UACC would like to acknowledge the invaluable help of the Comparative Medicine and Animal Resources Centre Veterinary Care group in preparing this handout.
Contact **aht.arc@mcgill.ca** to obtain special cards

<table>
<thead>
<tr>
<th>FRONT</th>
<th>BACK</th>
<th>WHEN TO USE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPECIAL FOOD</strong></td>
<td><strong>SPECIAL FOOD/WATER</strong></td>
<td>When feeding a diet different than the standard chow.</td>
</tr>
</tbody>
</table>

- Administration of special food or water must be described in the approved protocol.
- Research staff are responsible for administering special food/water.
- All the information on the front of this card must be completed.
- Food and water should be stored in labeled containers.
- Water bottles must be replaced at least once a week.
- Special food should be provided by adding one piece of food coloring to each bottle (when not using colored water bottles).
- If the food/water level is critically low and you cannot be reached, Veterinary Care staff will be notified. Regular food or water may be administered at the discretion of the veterinarian.

<table>
<thead>
<tr>
<th><strong>SPECIAL WATER</strong></th>
<th><strong>SPECIAL FOOD/WATER</strong></th>
<th>When administering treated or medicated water.</th>
</tr>
</thead>
</table>

- Administration of special food or water must be described in the approved protocol.
- Research staff are responsible for administering special food/water.
- All the information on the front of this card must be completed.
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- Water bottles must be replaced at least once a week.
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<table>
<thead>
<tr>
<th><strong>FOOD DEPRIVATION</strong></th>
<th><strong>WATER DEPRIVATION</strong></th>
<th>When food is removed for any period of time.</th>
</tr>
</thead>
</table>

- Food deprivation for its duration must be described in the approved protocol.
- The duration of deprivation that will achieve scientific objectives should be indicated.
- Food and water should be provided by adding one piece of food coloring to each bottle (when not using colored water bottles).
- If the food/water level is critically low and you cannot be reached, Veterinary Care staff will be notified. Regular food or water may be administered at the discretion of the veterinarian.

<table>
<thead>
<tr>
<th><strong>FOOD DEPRIVATION</strong></th>
<th><strong>WATER DEPRIVATION</strong></th>
<th>When water is removed for any period of time.</th>
</tr>
</thead>
</table>

- Water deprivation and its duration must be described in the approved protocol.
- The duration of deprivation that will achieve scientific objectives should be indicated.
- Water deprivation must be indicated by adding one piece of food coloring to each bottle (when not using colored water bottles).
- If the food/water level is critically low and you cannot be reached, Veterinary Care staff will be notified. Regular food or water may be administered at the discretion of the veterinarian.

<table>
<thead>
<tr>
<th><strong>KEEP CARCASSES</strong></th>
<th><strong>POST-PROCEDURE MONITORING</strong></th>
<th>When animals are anesthetized or undergo procedures such as surgeries or irradiation.</th>
</tr>
</thead>
</table>

- Please refrigerate any dead animals found and contact:
  - **NAME**
  - **EMAIL**
  - **PHONE**

<table>
<thead>
<tr>
<th><strong>POST-PROCEDURE CART</strong></th>
<th><strong>DAY 1</strong></th>
<th><strong>DAY 2</strong></th>
<th><strong>DAY 3</strong></th>
<th><strong>DAY 4</strong></th>
<th><strong>DAY 5</strong></th>
<th><strong>DAY 5.5</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>0°C</td>
<td>0°C</td>
<td>0°C</td>
<td>0°C</td>
<td>0°C</td>
<td>0°C</td>
</tr>
<tr>
<td>Core temperature</td>
<td>38°C</td>
<td>38°C</td>
<td>38°C</td>
<td>38°C</td>
<td>38°C</td>
<td>38°C</td>
</tr>
<tr>
<td>Heart rate</td>
<td>60 bpm</td>
<td>60 bpm</td>
<td>60 bpm</td>
<td>60 bpm</td>
<td>60 bpm</td>
<td>60 bpm</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>20 rpm</td>
<td>20 rpm</td>
<td>20 rpm</td>
<td>20 rpm</td>
<td>20 rpm</td>
<td>20 rpm</td>
</tr>
<tr>
<td>Cannulation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Neoadjuvant therapy</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Postoperative day</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**WARNING**: shaded areas are optional.
Mouse Module 1 Quiz

You must score 20/25 to successfully complete the training.

Score will be available immediately after completion.

Don’t forget to review any incorrect answers once the quiz is graded.

Start the Quiz