

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1 – Introduction to the Laboratory Mouse

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1. ANIMALS USED IN RESEARCH

At McGill, the use of animals is subjected to scientific/pedagogical merit and ethical review to ensure that animals are used only when necessary and under humane and appropriate conditions. McGill University is committed 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 for receiving federal Tri-Agency and other research funds.

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The privilege of using animals can be withdrawn for individuals who, by their negligence or deliberate actions, establish non-compliance with CCAC guidelines, McGill Policies and SOPs, and the approved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 comply with CCAC and McGill University policies and guidelines; are peer-reviewed for scientific or pedagogical merit; are approved by the local Facility Animal Care Committee (FACC) before animals are purchased and used; and are performed in a facility which ensures the safety of the staff and students while maintaining the health and welfare of animals through high standards of animal care and facility management.

AUPs also contain detailed information on:

- All research, teaching, testing and husbandry procedures including euthanasia and the use of potentially hazardous agents.
- The numbers of animals to be used in a given year including alternatives for replacement and reduction of animal use.
- Anticipated signs of morbidity and monitoring frequency.
- Endpoints, which are clear criteria set to prevent or relieve unnecessary pain or distress to a research animal.

McGill University has created over 100 [Standard Operating Procedures](#) (SOPs) that provide a detailed description of commonly used procedures including analgesia, anesthesia, surgical and experimental procedures, euthanasia, etc. SOPs establish best practices and offer investigators an alternative to writing detailed procedures in their AUP.

The [Quality Assistance Program](#) (also known as the 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.

1.2. 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 or using and 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 [Policy for Animal Welfare and Compliance Concerns](#) describes participants' responsibilities. Failure to adhere to McGill policies, procedures and guidelines may result in access to the animal facility being revoked.

2. OCCUPATIONAL HEALTH PROGRAM

The Occupational Health Program (OHP) for Animal Related Activities addresses the health risks which 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.

Consult the [Allergy Prevention factsheet](#) for a list of symptoms and tips on preventing allergies.

3. HUSBANDRY AND VETERINARY CARE

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.

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,. The 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 if you have any questions concerning animal health and wellbeing.

3.1. Veterinary Care program

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

4. 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.

4.1. 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: 19-21 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: 7-9 months
- Male adult weight: 25-40 g
- Female adult weight: 20-40 g
- Life span: 1.5-3.0 years

4.2. 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.
- 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.



4.3. 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.



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National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening <ul style="list-style-type: none"> • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye 			
Nose bulge <ul style="list-style-type: none"> • Bulging on the bridge of the nose • Vertical wrinkles on the side of the nose 			
Cheek bulge <ul style="list-style-type: none"> • Bulging of the cheeks 			
Ear position <ul style="list-style-type: none"> • Ears rotate outwards and/or backwards, away from the face • Ears may fold to form a 'pointed' shape • Space between the ears increases 			
Whisker change <ul style="list-style-type: none"> • Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' • Whiskers may clump together • Whiskers lose their natural 'downward' curve 			

Read the original paper:
Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingram J, Kissien-Ross T, LaCroix-Fralich ML, Matsumiya L, Sorge RE, Sotocinal SG, Takaki JM, Wornat D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6): 447-449. doi:10.1038/nmeth.1455


For guidance on using the Mouse Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimacescales. To request copies of this poster, please email enquiries@nc3rs.org.uk. The NC3Rs provides a range of 3Rs resources at: www.nc3rs.org.uk/resources. Images kindly provided by Dr. Jeffrey Mogil, McGill University.


4.4. Cage density

We adhere 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 to 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.





How many animals in a standard mouse cage?
Combien d'animaux par cage à souris standard?


5 adults
5 adultes

OR
OU

 ♀ +  + 1 large litter (8+ pups)
1 grosse portée (8+ souriceaux) +  ♂

OR
OU


 ♀ +  ♀ +  ♂


With 1 small litter (6-8 pups)
Avec 1 petite portée (6 à 8 souriceaux)

With 1 small litter (6-8 pups)
Avec 1 petite portée (6 à 8 souriceaux)

* pups must be removed when they reach 21 days of age
* les souriceaux de 21 jours doivent être séparés

OR
OU

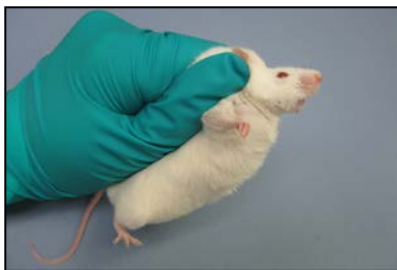

Up to 8 juveniles (3-6 weeks of age)
Jusqu'à 8 juvéniles (âgés de 3-6 semaines)

 Your Veterinarian
Votre Vétérinaire

5. HANDLING AND RESTRAINT

5.1. 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. Never hold a mouse by the tip of the tail.
- Whenever possible, consider using alternative methods to avoid handling mice by their tail to reduce handling stress, e.g., using tubes to scoop mice to transfer from one location to another.
- 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.



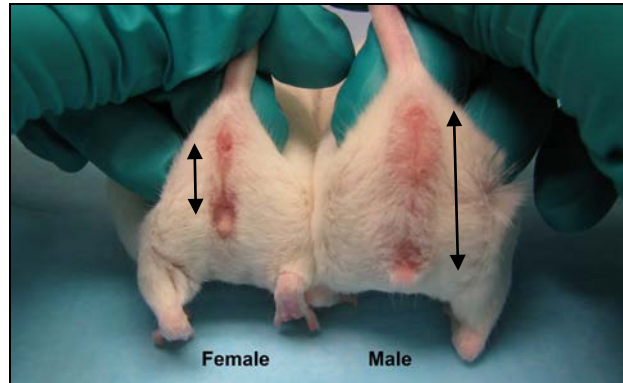
5.2. Restraint devices

- 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.



6. SEX DETERMINATION

- Sexing of mice is based upon 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. Compare animals of similar age.



7. IDENTIFICATION

7.1. 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.

INVESTIGATOR:	DEPARTMENT:		
CONTACT:	PHONE:		
STRAIN:	PROTOCOL:		
SOURCE:	CAGE ID:		
ANIMAL ID	SEX	DATE OF BIRTH/RECEIVED	REMARKS

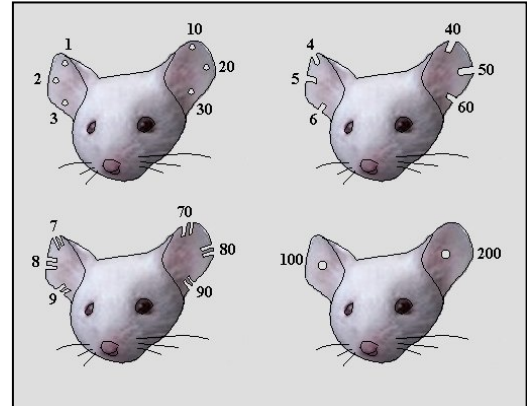
		CAGE #:				
BREEDING CARD		DATE MATED:				
INVESTIGATOR:	DEPARTMENT:					
CONTACT:	PHONE:					
PROTOCOL:	SOURCE:					
STRAIN:						
♂:	♀:	♀:				
LITTER	DATE OF BIRTH	#	DATE WEANED	M	F	REMARKS

7.2. 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.

7.3. Ear punching/notching

- This method cannot be used 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.
- 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.



7.4. 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 1/3) 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.

7.5. 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.

7.6. 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.

8. TISSUE SAMPLING FOR GENOTYPING

8.1. 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.

8.2. Skin swabbing

- 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.
- Properly identify samples to match animal identifications.

8.3. Buccal epithelial cell

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

8.4. 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.

8.5. 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.

8.5.1. 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.

8.5.2. 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.

9. 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.

It is the responsibility of the research staff to monitor animals according to the frequency indicated in the Animal Use Protocol. The frequency of monitoring should be increased as health status declines or as the endpoints are approaching.

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.

General intervention points include:

- 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).

10. EUTHANASIA

Mice can be euthanized in a variety of acceptable, effective and humane methods; these methods can be either chemical or physical. Only the approved euthanasia method described in the Animal Use Protocol can be used.

10.1. Adult rodents - Chemical methods

10.1.1. 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.

10.1.2. 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.

10.1.3. 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.

10.1.4. 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.

10.2. 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. 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).

10.2.1. Cervical dislocation

- 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.

10.2.2. 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.

10.2.3. 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.
- 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.

10.3. 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:

10.3.1. 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.

10.3.2. CO₂ 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.

10.3.3. 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.

10.3.4. 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).

10.3.5. 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.

10.4. Gestating rodents

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

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

10.4.1. CO₂ asphyxiation under isoflurane anesthesia




- CO₂ asphyxiation under isoflurane anesthesia of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.

10.4.2. CO₂ asphyxiation

- CO₂ asphyxiation of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.

10.4.3. 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
 <ul style="list-style-type: none"> 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
 <ul style="list-style-type: none"> 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.							
 <ul style="list-style-type: none"> 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

11. REFERENCES

11.1. 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
Comparative Pathology	comparative.pathology@mcgill.ca

11.2. McGill Standard Operating Procedures (SOP)

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

11.3. 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.
- Link to theory course: <http://animalcare.mcgill.ca/>
- Email: animalcare@mcgill.ca

11.4. Photographing/filming guidelines

- McGill Social Media Guidelines: <https://www.mcgill.ca/newsroom/socialmedia/guidelines>
- UACC Guidelines for photography/filming of animals for research purposes: http://www.mcgill.ca/research/files/research/guidelines_for_photography_-_nov_2015.pdf

11.5. Useful links

- Canadian Council on Animal Care: www.ccac.ca
- McGill Quality Assistance Program: <http://www.mcgill.ca/research/researchers/compliance/animal/qualityassistance>
- McGill Policy on the Study and Care of Animals: https://www.mcgill.ca/secretariat/files/secretariat/animals_policy_on_the_study_and_care_of.pdf
- Animals in Research and Teaching: <https://www.mcgill.ca/research/researchers/compliance/animal>
- McGill Occupational Health Program: <http://www.mcgill.ca/research/researchers/compliance/animal/occupational>

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