LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1 – Introduction to the Laboratory Rat

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Rat Module 1 Quiz

Complete the quiz after reading this handout.

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

Score will be available immediately after completion.

Review any incorrect answers once the quiz is graded.

Module 1 Quiz

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The privilege of using animals can be withdrawn for individuals who, by their negligence or deliberate actions, establish non-compliance with CCAC guidelines, McGill Policies and SOPs, and the approved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 environmental enrichment such as wood shavings and hiding tunnels, 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 animals, skin irritations, 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: 5cm^3 or 5% 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 RAT

The common laboratory rat, *Rattus norvegicus,* is an ideal experimental animal for several reasons: abundance of literature published pertaining to them, ease of handling, high fertility rate, short gestation period, low maintenance and disease model for various human disorders and diseases.

6.1. General biology and physiological data

- Most active at night (nocturnal)
- Curious and investigative behaviour
- Poor vision, acute sense of hearing and smell
- Social animals
- Body temperature: 37°c
- Respiratory rate: 75-115 breaths/min
- Heart rate: 260-400 beats/min
- Daily water consumption: 10-12 ml/100 g body weight
- Daily food consumption: 10 g/100 g body weight
- Oestrous cycle: 4-5 days
- Duration of oestrus: 12 hours
- Litter size: 6-12
- Gestation: 20-22 days
- Birth weight: 5 g
- Weaning age: 21 days
- Sexual maturity: 7 weeks
- Breeding duration: 12 - 16 months
- Male adult weight: 450-550 g
- Female adult weight: 250- 300 g
- Life span: 2.5-3.5 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.

7.1.1. Score 1: Rat is emaciated.

- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis.
- Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

7.1.2. Score 2: Rat is under conditioned

- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat.
- Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressur

7.1.3. Score 3: Rat is well-conditioned

- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis.
- Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.

7.1.4. Score 4: Rat is over conditioned

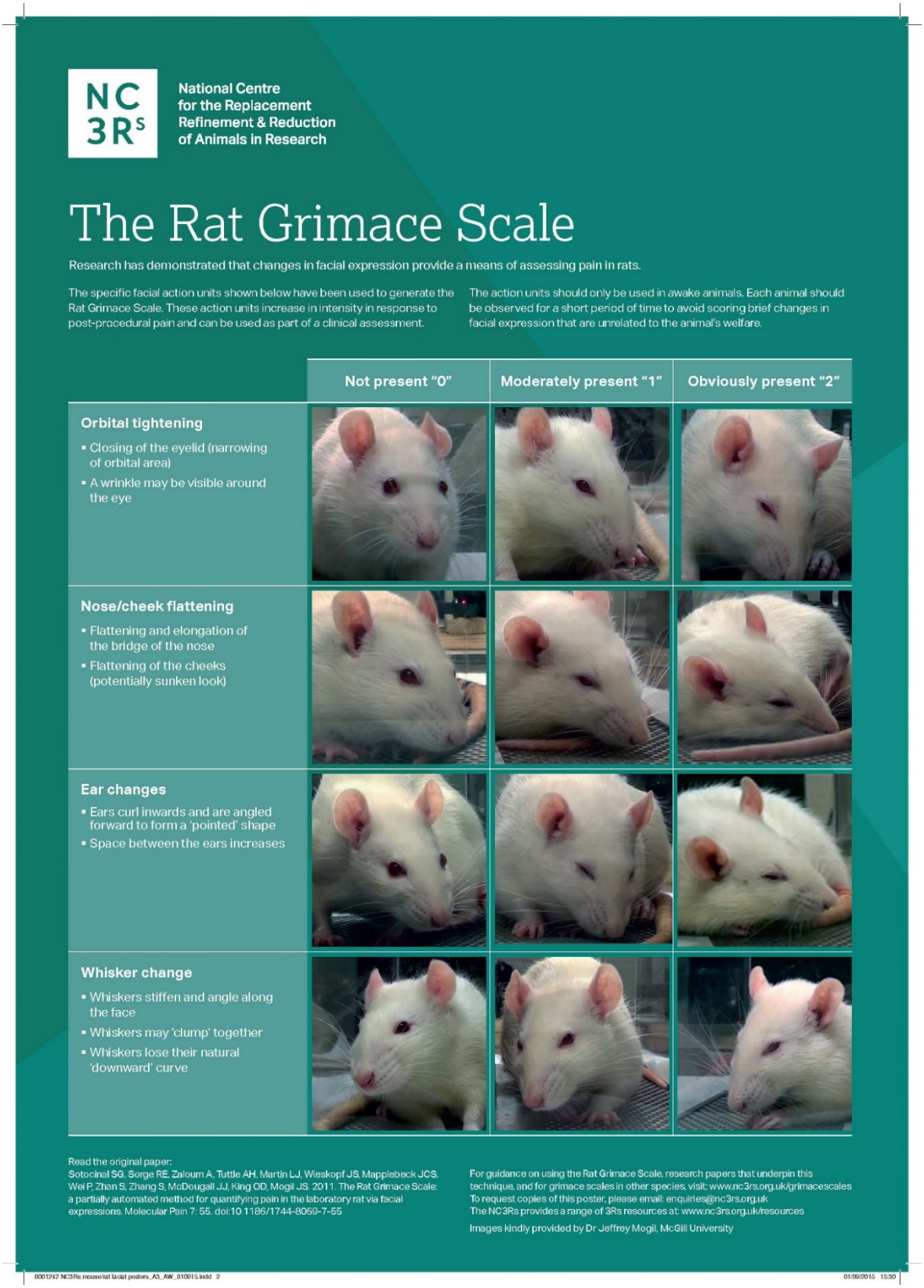
- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis.
- Pins over pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

7.1.5. Score 5: Rat is obese

- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis.
- Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

6.3. The Rat Grimace Scale (Sotocinal et al. 2011)

The rat grimace scale is a standardized behavioral coding system that demonstrates facial expressions which can be used to assess pain in the laboratory rat. Animals should be evaluated by quietly observing them without moving the home cage.



NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

The Rat Grimace Scale

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

The specific facial action units shown below have been used to generate the Rat 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 ▪ Closing of the eyelid (narrowing of orbital area) ▪ A wrinkle may be visible around the eye			
Nose/cheek flattening ▪ Flattening and elongation of the bridge of the nose ▪ Flattening of the cheeks (potentially sunken look)			
Ear changes ▪ Ears curl inwards and are angled forward to form a 'pointed' shape ▪ Space between the ears increases			
Whisker change ▪ Whiskers stiffen and angle along the face ▪ Whiskers may 'clump' together ▪ Whiskers lose their natural 'downward' curve			

Read the original paper:
Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JCS, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Molecular Pain 7: 55. doi:10.1186/1744-8069-7-55

For guidance on using the Rat 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

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6.4. Cage density

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

6.5. Cage density, standard shoebox cages

6.6. Cage density, double-decker cages

McGill

How many animals in a ventilated double-decker rat cage?
Combien d'animaux par cage à rat ventilée double?

8 rats < 200g each
8 rats < 200g chaque

OR
OU

6 adults < 300g each
6 adultes < 300g chaque

OR
OU

4 adults > 300g each
4 adultes > 300g chaque

OR
OU

2 females with litter
2 femelles avec portée

Your Veterinarian
Votre Vétérinaire

7. HANDLING AND RESTRAINT

7.2. How to pick up a rat

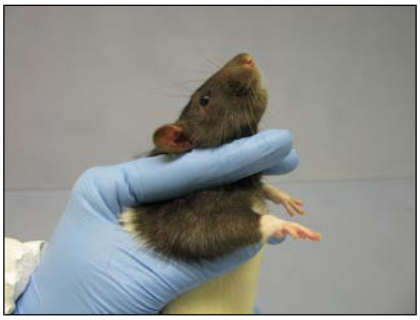
- Gentle handling will minimize stress. The stress of handling may affect physiological parameters and behavior, and therefore have a significant impact on scientific outcomes.
- Avoid lifting rats by their tail to reduce handling stress. Their tail skin is fragile and can easily strip from the underlying tissue.
- Rats should not be scruffed by the skin on the back of the neck.
- Before opening the cage, observe the animals within. Nervous or young animals can jump out very quickly and escape.

7.2. Manual restraint

- To allow for procedures or health assessments, animals may need to be manually restrained.
- Restrain should be gently yet secure. When adequately restrained, the rat remains calm and does not struggle.

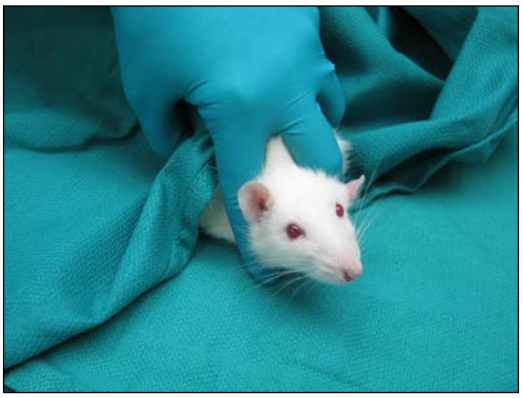
7.2.1. “V” grip

- With your non-dominant hand, slide your index and middle finger along both sides of the head as far as possible and grasp the head with your knuckles resting on the jaw bones.
- Place your thumb and remaining fingers under both forelimbs to grasp the thorax.
- If possible, support the lower body with your free hand or rest the rat on your chest (or on your legs if sitting). This is especially important for larger or gestating animals.



7.2.2. Towel

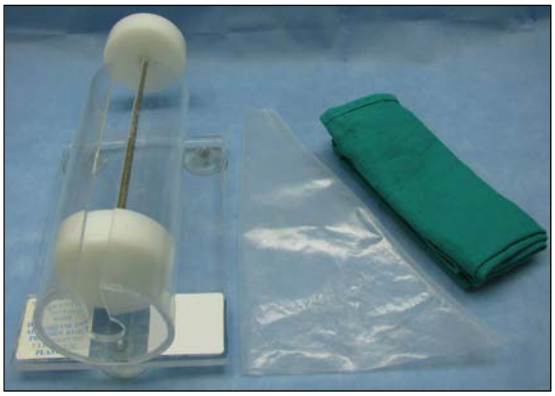
- Wrapping a towel around the animal will help rats feel secure and is calming, particularly if the eyes of the rats are covered.
- This method has the additional advantage of controlling the hind limbs and preventing potential scratches to the handler.



View the Handling & Restraint Instructional Video

7.3. Restraint devices

- Using a restraint device can calm the rats by helping them feel secure.
- 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.

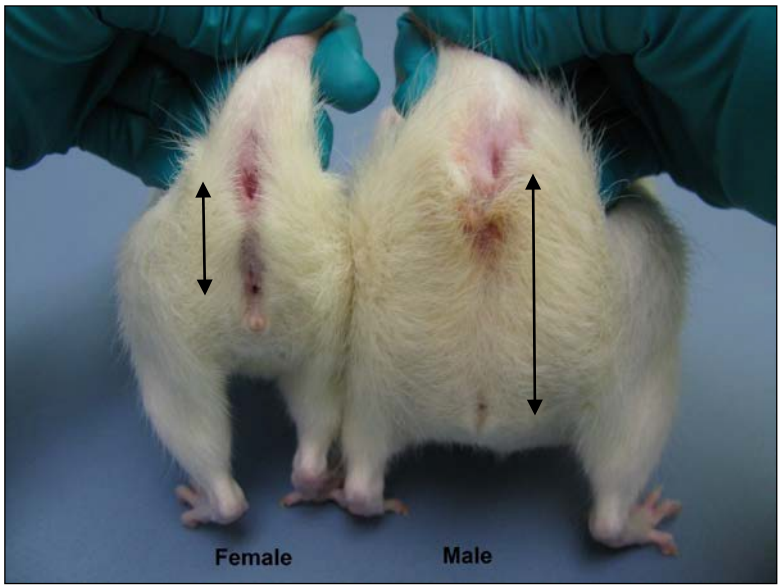


7.4. Animal-related injuries

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



9. IDENTIFICATION

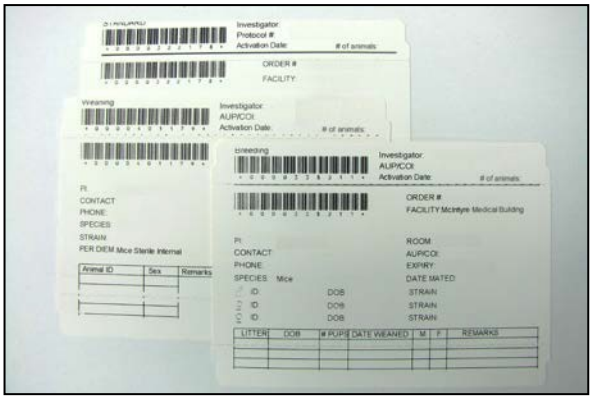
9.1. Selecting an identification method

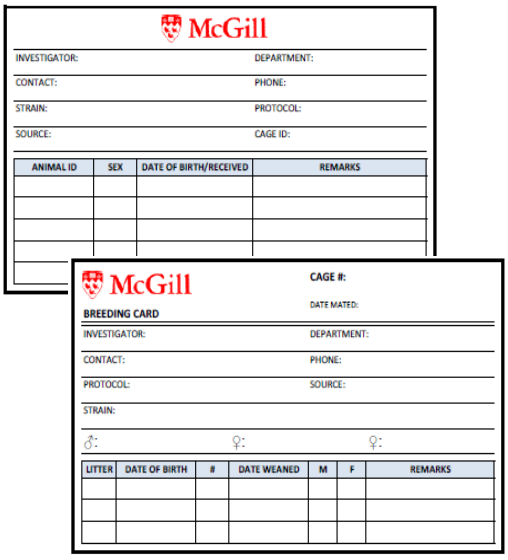
The selection of identification methods should take into consideration:

- 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 animals 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



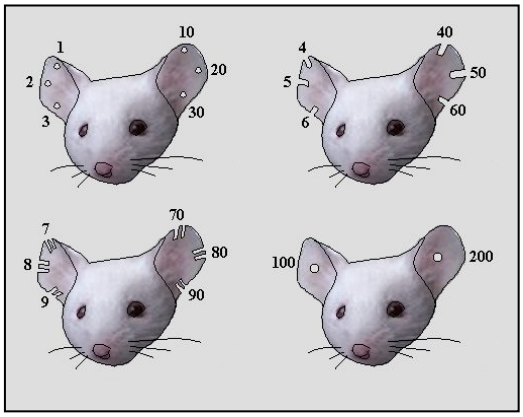


9.3. Temporary marking

- 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.

9.4. Ear punching/notching

View the rat ear punching instructional video



- 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

View the rat ear tag application instructional video

- 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 animals.
- 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 rats 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 rats less than 21 days of age:
  - General anesthesia is recommended but not required.
  - Gently, but securely, restrain rat (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 rat tails.
  - Place tissue sample into an identified collection tube.
  - Check for bleeding before returning rat 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 rats 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

- 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

Rat can be euthanized in a variety of acceptable, effective and humane methods. Euthanasia 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. CO_2 asphyxiation under isoflurane anesthesia

View the isoflurane/CO_2 euthanasia instructional video

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

11.1.2. CO_2 asphyxiation

- CO_2 alone should not be used where other methods are practical for the experiment and the species.
- In order to minimize stress animals should be euthanized in their home cage (do not pool rats 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 rat cage (12" x 9" x6"): 5.25 LPM
- Cages of different dimensions: a gradual-fill rate of less than 40% and greater than 30% of the chamber volume per minute should be used.
- 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 or opacity in eyes.
- A physical method of euthanasia, such as pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

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 heart beat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as 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 O_2 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 isoflurane, CO_2 or injectable 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 otherwise described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

11.2.1. Cervical dislocation

- Cervical dislocation, as a primary or secondary method of euthanasia, is not to be used on rats weighing over 200g.
- 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.

  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.

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.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

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. CO_2 asphyxiation under isoflurane anesthesia:

- Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations and exposure to CO_2, therefore, alternative methods are recommended.
- Isoflurane/CO_2 may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. 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.

11.3.2. 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.

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).

  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 rats 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.

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 over 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. CO_2 asphyxiation

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

- 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 CO_2 (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 CO_2.

RODENT EUTHANASIA

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

12. REFERENCES

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

McGill Standard Operating Procedures (SOP)

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

1.2. 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

Photographing/filming guidelines

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

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 Animal Health Technicians in preparing this handout.

Comparative Medicine
Animal Resources Centre

Contact aht.arc@mcgill.ca to obtain special cards

FRONT	BACK	WHEN TO USE
SPECIAL FOOD START DATE: END DATE: TYPE OF FOOD: CONTACT NAME: TELEPHONE: CELL PHONE: EMAIL: Indicate day food is changed or replenished ☐ Monday ☐ Tuesday ☐ Wednesday ☐ Thursday ☐ Friday ☐ Saturday ☐ Sunday If you cannot be reached and food/water level is critically low, veterinary care staff will be notified. (SEE REVERSE)	**SPECIAL FOOD/WATER** ✓ 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 sterile in barrier facilities ✓ Water bottle must be replaced at least once a week. ✓ Special water should be tinted by adding one drop of food coloring to each bottle (when not using a colored water bottle) ✓ When food/water levels are low, we will inform you by email or phone. ✓ You may leave spare food/water in a designated area ✓ 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.	When feeding a diet different than the standard chow.
SPECIAL WATER START DATE: END DATE: TYPE OF WATER: CONTACT NAME: TELEPHONE: CELL PHONE: EMAIL: Indicate day water is changed or replenished ☐ Monday ☐ Tuesday ☐ Wednesday ☐ Thursday ☐ Friday ☐ Saturday ☐ Sunday If you cannot be reached and food/water level is critically low, veterinary care staff will be notified. (SEE REVERSE)	**SPECIAL FOOD/WATER** ✓ 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 sterile in barrier facilities ✓ Water bottle must be replaced at least once a week. ✓ Special water should be tinted by adding one drop of food coloring to each bottle (when not using a colored water bottle) ✓ When food/water levels are low, we will inform you by email or phone. ✓ You may leave spare food/water in a designated area ✓ 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.	When administering treated or medicated water.
FOOD DEPRIVATION START DATE: START TIME: END DATE: END TIME: CONTACT NAME: TELEPHONE: CELL PHONE: EMAIL: Veterinary care staff will be notified if food has not been given after the end date/time or if animals are in poor condition. (SEE REVERSE)	**FOOD DEPRIVATION** ✓ Food deprivation and its duration must be described in the approved protocol. ✓ The shortest period of deprivation that will achieve scientific objectives should be utilized. ✓ Research staff are responsible for cages being food/water deprived. ✓ All the information on the front of this card must be completed. ✓ Animals must have access to water during the period of food deprivation. ✓ Animals must be monitored at least once daily for general condition. ✓ Weight loss should not exceed 20% of baseline bodyweight and Body Condition score should be below 2. ✓ After the deprivation period, animals must have free access to food, or be euthanized.	When food is removed for any period of time.
WATER DEPRIVATION START DATE: START TIME: END DATE: END TIME: CONTACT NAME: TELEPHONE: CELL PHONE: EMAIL: Veterinary care staff will be notified if water has not been given after the end date/time or if animals are in poor condition. (SEE REVERSE)	**WATER DEPRIVATION** ✓ Water deprivation and its duration must be described in the approved protocol. ✓ The shortest period of deprivation that will achieve scientific objectives should be utilized. ✓ Water deprivation must never exceed 48 hours. ✓ Research staff are responsible for cages being food/water deprived. ✓ All the information on the front of this card must be completed. ✓ Animals must be monitored at least once daily for general condition. ✓ Weight loss should not exceed 20% of baseline bodyweight and Body Condition score should not be below 2. ✓ After the deprivation period, animals must have free access to water, or be euthanized.	When water is removed for any period of time.
POST-PROCEDURE MONITORING McGill PROCEDURE: DATE: PERFORMED BY: ANESTHETIC USED: ☐ Ketamine/Xylazine/Acepromazine ☐ Isoflurane ☐ Other: ANALGESIC USED: ☐ Buprenorphine (SC or IP, every 8 hours) mice: 0.1mg/kg - rats: 0.05mg/kg Dose: ☐ Carprofen (SC, every 24 hours) mice: 20mg/kg - rats: 5-10mg/kg Dose: ☐ Other: Dose: ☐ Lidocaine/bupivacaine (local analgesic) HEAT SOURCE USED: ☐ During procedure ☐ During recovery ☐ RECOVERY (Animal must be monitored until full recovery) Time:	**POST-PROCEDURE CARE** shaded areas are optional	When animals are anesthetized or undergo procedures such as surgeries or irradiation.
KEEP CARCASSES Please refrigerate any dead animals found and contact: NAME EMAIL PHONE	-	When you need to retrieve carcasses and be notified promptly of dead animals found.

Annex 1 - SPECIAL CARDS

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 7-15
Buprenorphine	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	
Carprofen	☐	☐	☐	☐	☐	
SC saline or LRS 0.2-0.5ml/10g BW	☐	☐	☐	☐	☐	
Wet food	☐	☐	☐	☐	☐	
Time of monitoring						
Initials						
Remove sutures						☐

Comparative Medicine
Animal Resources Centre

Rat 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