LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

Introduction to the Laboratory Hamster

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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The privilege of using animals can be withdrawn for individuals who, by their negligence or deliberate actions, establish non-compliance with CCAC guidelines, McGill Policies and SOPs, and the approved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 Guidelines 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 hamster, excessive scratching or eye infections. A member of the Veterinary Care staff will contact you if they find a veterinary case.

4. THE LABORATORY HAMSTER

The Syrian or golden hamster, *Mesocricetus auratus*, is the most commonly used hamster in biomedical research. The hamster can be ideal as a model for infectious diseases due to its similar immune responses to humans. Due to its unique cheek pouches, the hamster can also be used in tumor and toxicology studies.

4.1. General biology and physiological data

- Most active at night (nocturnal)
- Curious and investigative behavior
- Tendency to hoard or be aggressive – benefit from extra enrichment (wheels, nesting material)
- Poor vision, acute sense of hearing (startles easily) and smell
- Solitary animals usually, females or males may require separation if aggressive
- Body temperature: 37-39°C
- Respiratory rate: 50-135 breaths/minute
- Heart rate: 325-560 beats/minute
- Daily water consumption: 8-10 ml per 100g BW
- Daily food consumption: 8-12 g per 100g BW
- Oestrous cycle length: 4-5 days; no post-partum estrus
- Average litter size: 4-12
- Gestation period: 15-22 days; breeders must be separated before birth to avoid aggressivity.
- Average birth weight: 2.0 g
- Weaning age: 21-28 days
- Sexual maturity: 4-6 weeks; but should not be bred until 10 weeks to avoid stillborn pups.
- Reproductive span: 11-18 months
- Male adult weight: 80-150 g
- Female adult weight: 90-160 g
- Life span: 2.0-3.0 years
4.2 Hamster Biological features

Cheek pouches:
Hamster’s cheek pouches extend from the head to the neck area and are used to carry and store food or nesting material. A female also uses her cheek pouches to carry or conceal her young. Often, persons unfamiliar with this feature will report that the hamster has swellings or lesions in the throat area when they have observed a hamster with full pouches.

Flank glands:
Sebaceous scent glands are located on the dorsal flank (hip region) along the spine. These are present in both sexes, although they are more prominent in males. When the hair is removed, these glands are visible as dark areas of the skin. These glands are stimulated by androgens. The secretions are used to mark territory and are involved in sexual behavior.

Urine:
It is normal to notice milky urine from your hamsters.

Pseudo-hibernation:
Hamsters do not undergo a true hibernation; they remain sensitive to touch and so are readily aroused from their hibernation state. Under conditions of shortened day length, cool temperatures (8°C, 48°F), less light, and social isolation, hamsters hoard food and enter into pseudo-hibernation. During this state, the hamster has a decreased body temperature, low respiration rate, and a low heart rate. The animal will arouse every two to three days with short periods of normal alertness and activity.

Stomach:
Hamster stomachs are different from other rodents as they are divided into two compartments - the non-glandular forestomach (red arrow) and the glandular stomach (blue arrow). The tissue of the forestomach is similar to the gut tissue of ruminants, so it may play a similar role in microbial fermentation of cellulose.
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. There is however no published BC scoring system for the hamsters, but comparisons with the mouse or rat BC scoring system can be done.

4.3. The Grimace Scale

The grimace scale is a standardized behavioral coding system that demonstrates facial expressions which can be used to assess pain in the multiple laboratory animals. There is however no published grimace scale for the hamsters, but comparisons with the mouse or rat grimace scale can be done.

4.4. Cage density and Environmental Enrichment

We adhere to national and international guidelines that determine how many hamsters can live in a standard cage. Overcrowding cages is detrimental to the wellbeing of the hamsters.

Per single, standard rat cage, you can house:

- **Up 2 adult hamsters** (over 4 weeks old). Hamsters must be of the same sex if not a breeding cage.
- 1 breeding male and 1 breeding female
- 1 breeding female with one litter
- Do not exceed 400g (total weight of all hamsters) per cage.

Each cage should have enrichment:

- **Nesting material** (Crinkly paper, cotton squares, nesting sheets, and/or other substrate)
- 1 shelter (PVC tube, cellulose based shelter)
- 1 running wheel
- **Chewing devices** (wood blocks, nylon bone)
5. HANDLING AND RESTRAINT

5.1. Manual restraint

- Proper handling of hamsters is quite different from handling mice and rats due to differences in anatomy.
- Do not startle the animal, and let it know of your presence prior to handling, especially if the hamster is sleeping (they startle easily).
- The easiest and most common technique used for routine handling is to scoop the animal either with cupped hands or with a tube.
- First method: grasp the hamster around the head and shoulders:
  - As hamsters have an abundance of loose skin along the head/shoulders, continue closing your hand until you feel the bunching of the skin but without squeezing its body.
  - At this point, the skin over the chest and abdomen should be taut, however, the animal should be able to breathe without any difficulty.

- Second method: place your first and second fingers on either side of the head, grasping the head with your knuckles and positioning the forelegs of the hamster between your adjacent fingers.
6. SEX DETERMINATION

- Sexing of hamsters is based upon ano-genital distance.
- Males have a greater distance between the anus and urogenital opening than females. 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.
- In addition, only females have nipples.

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.
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 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.

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 ½) so that it rests against the hamster and does not bend the ear, catch on the cage or cause the hamster 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. Micro-tattooing
- It is recommended to use local or general anesthesia for the procedure.
- Use a micro-tattooer or small needle to inject tattoo ink in the toe pads.
- 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. 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 (AUP). The frequency of monitoring should be increased as health status declines or as the endpoints are approaching.

Humane interventions are clearly defined in the 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. Intervention points must be selected to 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 controls 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: 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, diagnostic tests.
9. EUTHANASIA

Hamsters 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.

9.1. Adult rodents - Chemical methods

9.1.1. 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 two hamster or one litter per cage (do not pool hamsters 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.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

9.1.2. 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 two hamster or one litter per cage (do not pool hamsters 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 rat cage (12” x 9” x6”): 5.25 LPM

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.

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.

### 9.1.3. Barbiturate or injectable anesthetic overdose

- Inject pentobarbital at a dose of 120mg/kg 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.

### 9.1.4. 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 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.
9.2. 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).

9.2.1. Cervical dislocation
- Cervical dislocation performed on live animals requires specialized training.
- Technique is difficult to do in the hamster due to the absence of a tail.

9.2.2. Pneumothorax
- Preferred physical method for hamsters.
- 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.

9.2.3. 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 hamster 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.

9.3. For neonatal or gestational hamsters, refer to SOP 301: Rodent Euthanasia
- May require specialized training.
# 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</td>
<td>CO₂ ASPHYXIATION</td>
</tr>
<tr>
<td>Adult rodent</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Gestating rodent (under 17 days gestation)</td>
<td>YES*</td>
<td>YES*</td>
</tr>
<tr>
<td>Gestating rodent (over 17 days gestation)</td>
<td>YES*</td>
<td>YES*</td>
</tr>
<tr>
<td>Pups less than 10 days old</td>
<td>Only as Narcosis</td>
<td>Only as Narcosis</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.
10 POST-MORTEM BLOOD COLLECTION

10.1 Cardiac puncture procedure

- Terminal procedure.
- This procedure can only be done under anesthesia or less than a minute after euthanasia. If done under anesthesia, pneumothorax is necessary to confirm death of the animal.
- Prepare a 3cc syringe with a 23G 1½” needle.
- Place the hamster in dorsal recumbency.
- Palpate the xiphoid process between the last two ribs at the tip of the sternum.
- Insert the tip of the needle between the left side of the xiphoid process and the last rib.
- Once you puncture the skin, gently pull back on the plunger to create a minimal amount of negative pressure within the syringe and maintain it.
- Penetrate the thoracic cavity slowly while directing your needle toward the heart at an angle of approximately 40-45 degrees. Note: The heart is slightly left of the midline.
- When a small quantity of blood flows into the hub of the needle, stabilize your needle and continue to pull back on the plunger slowly. The blood should flow into the syringe at a steady rate. Note: If the blood flow stops, you change the angle of the needle slightly, rotate it or make very small movements to alter the needle placement.
11. REFERENCES

11.1 Animal Resource Division of McGill University Health Centre Research Institute

Veterinary Care/ Drug Purchasing and Technical Services Requests: VetGlen@muhc.mcgill.ca; VetMGH@muhc.mcgill.ca
Workshop and Training: ARD.training@muhc.mcgill.ca
Equipment Rental/Material: iLab
Imports, Transfers and Quarantine: ARD.transfers@muhc.mcgill.ca
Quality Assistance: ARD.qualityassistance@muhc.mcgill.ca
Animal Use Protocol Questions: FACC.admin@muhc.mcgill.ca

11.2 ARD RI-MUHC Standard Operating Procedures

https://researchportal.muhc.mcgill.ca

11.3 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

11.4 McGill Standard Operating Procedures

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

The UACC would like to acknowledge the invaluable help of the Comparative Medicine and Animal Resources Centre and the Animal Resource Division of the McGill University Health Center Research Institute Animal Health Technicians in preparing this handout.