Guidelines for the Euthanasia of Rodents, Rodent Fetuses, and Neonates Using: CO2, Decapitation, and/or Cervical Dislocation Methods

The recommended methods for rodent euthanasia are anesthetic overdose or CO2/O2 asphyxiation. In accordance with the AVMA Guidelines on Euthanasia 2007, anesthesia must be administered prior to euthanasia by a physical method unless evidence is provided that such agents would interfere with the experimental design. The IACUC will approve the use of the physical methods described below without prior anesthesia only when adequate scientific justification is provided. Euthanasia procedures other than those listed below must be detailed in the animal protocol form and must be approved by the IACUC prior to implementation.

Rodents must be euthanized by trained personnel using appropriate technique, equipment, and agents. This is necessary to ensure a painless death that satisfies research requirements. Death should be induced as painlessly and quickly as possible. Upon completion of the procedure, death must be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting animal’s fixed and dilated pupils. Euthanasia should not be performed in the animal room.

C02: CO2 inhalation is a common method of euthanasia. Some important aspects of this procedure are:

- The euthanasia chamber should allow for visibility of the animals. Do not over crowd the chamber: all animals in the chamber must be able to make normal postural movements.
- Compressed CO2 gas in cylinders is the only recommended source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 20-30% O2 to 80-70% CO2, to optimize reduction in distress. After the animals are unconscious, shut off the O2 and the flow rate of the CO2 can be increased to minimize the time to death.
- Animals should be left in the chamber until death has been ensured.
- Neonatal animals (up to 14 days of age) are resistant to the effects of CO2, therefore an alternative method is recommended. CO2 may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation).
- If death cannot be ensured following CO2 exposure, another approved method of euthanasia must be added while the animal is under CO2 narcosis to assure death.

Decapitation: Personnel responsible for performing euthanasia by decapitation must be properly trained and proficient in carrying out the technique. There is an inherent danger in the use of the guillotine and personnel should take adequate safety precautions. Recommendations of the AVMA Guidelines on Euthanasia 2007, states “Equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of the blades.”

Guidelines for Maintaining Guillotine:

- After each use, the unit should be wiped clean of any biological fluids and properly disinfected.
- Using safety precautions carefully wash the unit in soap and water and thoroughly towel dry.
- Add a few drops of light machine oil (3 in 1) should be applied to the blade surfaces and blade channels, and then run the blades together several times to spread the oil evenly over all moving surfaces.
- Blades should be replaced or sharpened regularly depending upon frequency of use.
- The following technique is recommended to assess the sharpness of a guillotine: A sharp guillotine is sharp enough if it will cut a piece of paper, without dragging it between the blades, sticking or tearing.

(Note: If scissors are used for neonate decapitation they too should be maintained in good working order, sharpened on a regular basis and assessed for sharpness.)
**Cervical dislocation**: Personnel responsible for performing euthanasia by cervical dislocation must be properly trained and proficient in carrying out the technique. Consistent with the AVMA Guidelines on Euthanasia 2007, cervical dislocation may not be performed on rodents weighing greater than 200g since the large physical mass in the cervical region makes manual cervical dislocation physically more difficult. Decapitation should be used if a physical method is required for rodents weighing more than 200g.

The AVMA Guidelines on Euthanasia 2007 stipulates that personnel responsible for performing euthanasia by decapitation or cervical dislocation must be properly trained and proficient in carrying out these techniques. Therefore, personnel who will perform decapitation or cervical dislocation without prior anesthesia must contact the Animal Vivarium Manager to receive training in these techniques prior to performing euthanasia.

**Fetuses**:  
- Fetuses up to 14 days in gestation: Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetuses at this stage of development.

- Fetuses 15 days in gestation to birth: The literature on the development of pain pathways suggests the possibility of pain perception at this time. Whereas fetuses at this age are resistant to inhalant anesthetics including CO2, euthanasia may be induced by the skillful injection of chemical anesthetics. The physical methods of decapitation with surgical scissors or cervical dislocation are acceptable methods of euthanasia. Rapid freezing, without prior anesthesia, as a sole means of euthanasia is not allowed. Animals must be anesthetized prior to freezing. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus, by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g. pentobarbital. CMU’s attending veterinarian must be consulted for consideration of fetal sensitivity to specific anesthetic agents. When fetuses are not required for the study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetuses as well.

**Neonates**:  
- Up to 14 days of age: Acceptable methods for the euthanasia of neonatal mice and rats include: injection of a chemical anesthetics, (e.g. pentobarbital), decapitation, or cervical dislocation, or cervical dislocation. These animals are sensitive to inhalant anesthetics; i.e. halothane or Isoflurane (used with appropriate safety factors). Immersion in liquid nitrogen may be used only if preceded by anesthesia. Additionally, anesthesia should precede immersion or perfusion with chemical fixatives. Anesthesia maybe induced by inhalant or injectable anesthetics, consult with the institutes veterinarian for appropriate agents, dosages and routes of administration. Hypothermia maybe used to induce anesthesia in pups six days of age or less, when adequately justified.

- Older than 14 days: Follow the guidelines for adult rodents.

With all forms of euthanasia, the person performing the procedures must be fully trained and proficient.
References: