



## GUIDELINES FOR EUTHANASIA OF LABORATORY ANIMALS

The objective of euthanasia of laboratory animals is to provide a swift and painless death to the animal while ensuring the safety of laboratory personnel. Some general considerations for choosing a euthanasia method include the method's ability to induce quick loss of consciousness and death without inducing pain or distress, reliability and irreversibility of the method, safety of personnel, and compatibility with desired experimental outcomes.

General modes of action of euthanasia methods differ (e.g., hypoxia, central nervous system depression, physical disruption of brain activity) and, dependent on the animal species, some methods may be more acceptable than others. For a detailed and thorough description of different methods, doses and species acceptability, refer to the 2020 version of the AVMA Guidelines for Euthanasia of Animals (<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>)

Acceptable methods recommended by the AVMA do not require scientific justification in the IACUC Animal Use Protocol. Conditionally acceptable methods are not preferred and require justification in the IACUC Animal Use Protocol. Any methods not listed below are unacceptable. However, use of these methods may be required in unusual circumstances; strong scientific justification must be provided in the IACUC Animal Use Protocol, and a veterinary consultation must be included.

The table below summarizes the acceptable and conditionally acceptable methods of euthanasia for species currently within the BRF. For guidelines on euthanasia methods for species not listed, contact the BRF veterinarians. **In keeping with federal guidelines, all drugs administered to any live vertebrate animals for euthanasia (as well as anesthesia) must be used before their expiration date.**

	<u>Acceptable</u>	<u>Conditionally acceptable</u>
<b>Rodents</b>	barbiturates <sup>1</sup> , carbon dioxide*, inhalant anesthetics, KCl in  conjunction with general anesthetic	cervical dislocation <sup>2</sup> decapitation <sup>3</sup>
<b>Swine</b>	barbiturates <sup>1</sup> , KCl in conjunction with general anesthesia	inhalant anesthetics, carbon dioxide
<b>Amphibian</b>	barbiturates <sup>1</sup> , Tricaine methane	decapitation, pithing <sup>4</sup>

Sulfonate (MS 222), benzocaine  
hydrochloride

<sup>1</sup>sodium pentobarbital for euthanasia should be given at dosages 4-5 times higher than the anesthetic dose

<sup>2</sup>cervical dislocations for adult mice or rats <200 gms

<sup>3</sup>To use decapitation on non-anesthetized animals, personnel must be properly trained in this technique; the need to use this method must be scientifically justified in an approved protocol; guillotines and scissors must be sharp and maintained on a regular basis. See additional information below.

<sup>4</sup>only on anesthetized animals

## Special Considerations:

**I. Inhalant Agents: A secondary, physical method of ensuring death is required** (for example, cervical dislocation, decapitation, opening the thoracic cavity).

**Isoflurane** with or without nitrous oxide is acceptable for animals < 7 kg. Nitrous oxide must not be used alone. Appropriate scavenging is required.

**Carbon dioxide\*\*.** Only compressed carbon dioxide gas supplied in cylinders may be used. The system must utilize an appropriate pressure-reducing regulator and flow meter for a gradual chamber fill; the chamber may not be pre-filled.

*\*\*\*AVMA guidelines on CO2 use in rodents have changed in 2020. See updated IACUC Guidelines on Use of Carbon Dioxide for Euthanasia of Rodents for additional information.*

## **II. Non-inhalant Pharmaceutical Agents:**

### **Barbiturates**

*Pentobarbital* – 200 mg/kg IV is sufficient for euthanasia of most lab animal species. Intraperitoneal injection may be used in situations where this approach would cause less stress than IV injection.

**Potassium chloride** – 1-2 mmol/kg IV or intracardiac delivery. This method is NOT acceptable in unanesthetized animals. However, when used after general anesthesia is obtained, it is acceptable for most species.

*NOTE: A secondary, physical method of assuring death is always recommended. For example, decapitation, cervical dislocation, or opening of the thorax should be considered. This ensures that no animal assumed dead will “revive” in a freezer or other disposal location.*

### **III. Physical methods:**

**Cervical dislocation** – This is a humane technique for mice and for rats (<200g) *when performed by trained personnel.*

**Decapitation** – This technique is acceptable if performed correctly on anesthetized animals by trained individuals under the details of an approved IACUC protocol. Individuals performing decapitations must be well-versed in the correct use and maintenance of guillotines to ensure proper function, humane euthanasia, and operator safety.

If this method is used without anesthesia, proper training must be documented, and scientific justification must be provided in a protocol for IACUC approval. The use of plastic restraint sleeves such as Decapicones® is recommended to ensure operator safety and efficient euthanasia.

Neonatal rodents less than 10 days of age may be decapitated with dedicated sharp scissors without prior anesthesia. *See IACUC Guideline on Euthanasia of Rodent Neonates or Fetuses for additional information.*

Guillotines should be appropriate for the species and must be rust-free, clean, and sharp. The IACUC recommends that each guillotine (or decapitation scissor) is assessed for sharpness prior to use as follows: a guillotine (or scissors) is sharp enough to use if it will cut a thick rubber band without dragging it between the blades and sticking. After use, the guillotine (or scissors) should be rinsed in cold water to remove residual blood or tissues and gross contamination. It should then be thoroughly disinfected. After cleaning a guillotine or scissors, a 95% alcohol rinse will reduce the need to hand dry the equipment.

Periodic oiling and sharpening of guillotine or scissor blades should be done by a professional service. Frequency depends on the animal species involved and volume of use, but general guidelines are that sharpening should be done after 100 hours of use, when a guillotine fails the sharpness test described above, or annually, whichever comes first. The PI is responsible for arranging for guillotine maintenance and blade sharpening. The BRF services their guillotines annually and PIs can contact the BRF to arrange maintenance of their guillotine at the same time. Documentation of guillotine maintenance and blade sharpening must be evident on the unit via a sticker indicating the date of last servicing/ sharpening.

**Exsanguination** – is NOT accepted as the sole means of euthanasia. It should be used only when animals are previously rendered unconscious with a general anesthetic.

#### **IV. Other Important Considerations:**

In addition to ensuring a more humane death, good euthanasia practices will reduce the possibility of adverse physiologic changes in other animals (such as a release of hormones and alterations in brain chemistry), which could affect research results.

- Whenever possible, animals should not be present during the euthanasia of other animals, especially of their own species.
- Many species, including rodents, react adversely to the smell of blood, and animals should never be decapitated in the presence of other animals. Ideally, the person performing decapitation should change gloves and clean the guillotine of blood between animals.
- Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal can cause anxiety, apprehension, and stress in other animals that can be avoided.