



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>) The table below summarizes some of the acceptable and conditionally acceptable methods of euthanasia for species currently within the BRF. *Note that in keeping with federal guidelines, all drugs administered to any live vertebrate animals for euthanasia (as well as anesthesia) must be used prior to their expiration date.*

	<u>Acceptable</u>	<u>Conditionally acceptable</u>
Rodents	barbiturates ¹ , carbon dioxide*, inhalant anesthetics, KCl in conjunction with general anesthetic	cervical dislocation ² decapitation ³
Swine	barbiturates ¹ , KCl in conjunction with general anesthesia	inhalant anesthetics, carbon dioxide
Amphibians	barbiturates ¹ , Tricaine methane sulfonate(MS 222), benzocaine hydrochloride	decapitation, pithing ⁴

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

²cervical dislocations for mice or rats <200 gms

³To use decapitation on non-anesthetized animals, personnel must be properly trained in this technique; the need to use this method must be scientifically justified; guillotines and scissors must be sharp and maintained on a regular basis.

⁴only on anesthetized animals

I. Inhalant Agents:

- Isoflurane* with or without nitrous oxide is acceptable for animals < 7 kg. Nitrous oxide should not be used alone.

*Appropriate scavenging is required

- Carbon dioxide**

1. 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; chamber may not be pre-filled.
2. **A secondary, physical method of ensuring death is required** (for example, cervical dislocation, decapitation, opening the thoracic cavity).

***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. When used after general anesthesia is obtained, this agent is acceptable for most species.

NOTE: A secondary, physical method of assuring death is always recommended. For example, decapitation, cervical dislocation or opening the thorax should be considered. This is to ensure 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 should be performed on anesthetized animals by trained personnel. If this method is used without anesthesia, proper training must be documented and scientific justification provided in an approved IACUC protocol. Guillotines should be appropriate for the species and must be sharp (scheduled maintenance and inspections of the equipment is needed).

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

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.

Acceptable methods recommended by the AVMA and cited above do not require scientific justification in the IACUC Animal Use Protocol. Conditionally acceptable methods are not the preferred methods and require justification in the IACUC Animal Use Protocol. Any methods not listed above are unacceptable and the use of any unacceptable method is strongly discouraged. However, there may be unusual circumstances requiring the use of these methods; strong scientific justification should be provided in the IACUC Animal Use Protocol and a veterinary consult must be included.

IV. Other Important Considerations:

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. 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.