

# IACUC Policy on Euthanasia of Laboratory Animals

The term euthanasia is derived from the Greek terms “eu,” meaning “good,” and “thanatos,” meaning “death”. A “good death” would be one that occurs without pain or distress. For the purpose of this policy, euthanasia is the act of inducing humane death in an animal. The Institutional Animal Care and Use Committee (IACUC) uses the 2020 AVMA Guidelines for the Euthanasia of Animals as its guide in reviewing methods of euthanasia in Animal Use Proposals.

Euthanasia techniques should result in rapid unconsciousness followed by cardiac or respiratory arrest and ultimate loss of brain function. In addition, the technique should minimize any stress and anxiety experienced by the animal prior to unconsciousness. Stress may be minimized by technical proficiency and humane handling of the animals to be euthanized.

Personnel performing euthanasia procedures must be adequately trained in the specific method to be used.

Deviations from the recommendations of the AVMA must be fully justified in the proposal.

## Methods Commonly in Use:

1. **Inhalant Anesthetics:** Halothane, isoflurane, sevoflurane, or desflurane, with or without nitrous oxide (N<sub>2</sub>O), are acceptable with conditions for euthanasia of laboratory rodents. Nitrous oxide should not be used alone for euthanasia. These agents may be useful in cases where physical restraint is difficult or impractical. When used as a sole euthanasia agent delivered via vaporizer or anesthetic chamber (open-drop technique), animals may need to be exposed for prolonged time periods to ensure death. Death may be confirmed by physical examination, ensured by adjunctive physical method, or obviated by validation of euthanasia chambers and process.
2. **Carbon Dioxide:** Carbon dioxide, with or without premedication with inhaled anesthetics, is acceptable with conditions for euthanasia of small rodents. Compressed CO<sub>2</sub> gas in cylinders is the only acceptable source of CO<sub>2</sub> because gas inflow to the chamber can be precisely regulated. An optimal flow rate for CO<sub>2</sub> euthanasia systems should displace 30% to 70% of the chamber or cage volume/min. Prefilled chambers are unacceptable. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. It is important to verify that an animal is dead after exposure to CO<sub>2</sub>. Death must be confirmed by physical examination and can be ensured by a secondary physical method.
3. **Barbituric Acid Derivatives:** Injectable barbiturates act quickly and smoothly to render rodents unconscious. If there is vascular access, IV administration is preferred. The IP route is, however, more practical. The euthanasia dose is typically three times the anesthetic dose. Pentobarbital is the most commonly used barbiturate for laboratory rodents because of its long shelf life and rapidity of action. **Potassium chloride:** Potassium chloride may be used intravenously to stop the heart in deeply anesthetized animals. It may be used only in combination with existing deep anesthesia.

4. **Cervical Dislocation:** Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated high degree of technical proficiency. Cervical dislocation without anesthesia will be approved only when approved by the IACUC. Before using the technique of cervical dislocation, it should be practiced on deeply anesthetized rodents until the operator is competent. Restrain the rodent in a normal standing position on a firm, flat surface and grasp the base of the tail with one hand. Place a stick-type pen, a rod-shaped piece of sealed wood or metal, or the thumb and first finger of the other hand against the back of the neck at the base of the skull. To produce the dislocation, quickly push forward and down with the hand or object restraining the head while pulling backward at a 30-degree angle from the table with the hand holding the tail. Performing the procedure on a surface that the animal can grip may make it easier to gain access to the base of the skull because rodents often stretch themselves forward when held by the tail. The effectiveness of dislocation can be verified by separation of cervical tissues. When the spinal cord is severed, a 2-4 mm space will be palpable between the occipital condyles and the first cervical vertebra. Occasionally, however, the dislocation occurs between thoracic vertebrae. Check closely to confirm respiratory arrest, and when possible verify, by palpation, that there is no heartbeat.
5. **Decapitation:** Decapitation is used in laboratory settings because it yields tissues uncontaminated by chemical agents. Specialized rodent guillotines are available and must be kept clean and in good condition with sharp blades. Decapitation is acceptable with conditions for mice and rats. Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so. Personnel performing this technique should recognize the inherent danger of the guillotine and take adequate precautions to prevent personal injury. The IACUC requires anesthesia prior to decapitation unless there is an approved justification in the IACUC protocol. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades and proper alignment and contact between blades (this can be checked by cutting a piece of folded paper). Rodents acclimated to being handled are calmer, less stressed, and facilitate the process.