**Acceptable Methods of Euthanasia**

**Purpose:**

Euthanasia is the act of inducing humane death in an animal with rapid unconsciousness and death with minimum pain, discomfort, or distress. The term euthanasia is derived from the Greek terms meaning “good death”. The term is used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress. The purpose of this policy is to outline acceptable methods of euthanasia for laboratory animals at the **INSTITUTION**.

**Scope:**

This policy applies to all researchers and animal care staff at **INSTITUTION** who are performing euthanasia of animal subjects as part of an IACUC approved protocol.

1. **Policy:**
   1. The methods utilized to euthanize any animal on an IACUC-approved protocol must comply with the [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.](https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf)
   2. All individuals responsible for euthanasia of research animals must be qualified and trained to perform euthanasia humanely, per the IACUC training policy. As different euthanasia methods require different techniques, each euthanasia method requires a specific training. The Researcher is responsible for ensuring appropriate documentation. Documentation of personnel training may be reviewed by the IACUC.
   3. Investigators will perform euthanasia following approved IACUC standard operating procedures.
   4. Methods not covered in approved IACUC standard operating procedures need to be approved by the IACUC. The Researcher should confer with the attending or clinical veterinarian prior to submitting the protocol to the IACUC for any such method. The IACUC must grant an exemption to any deviation from approved policies and procedures.
   5. The euthanasia method must be appropriate to the research goals, species and age of the animal, and approved in the animal study proposal.
   6. In this policy, we establish that, for the purposes of euthanasia, animals will be considered altricial until and including the age of 10 days after birth.
   7. The techniques should minimize stress and anxiety experienced by the animal prior to unconsciousness. Stress can be further minimized by technical proficiency and humane handling of the animals to be euthanized.
2. **IACUC Approved Acceptable Chemical Methods**
   1. **Pentobarbital and Phenytoin (Euthasol)**
      1. Chemical euthanasia with an overdose of intravenous pentobarbital is the preferred euthanasia method for mammals with practical vascular access (e.g., rabbits).
      2. Intraperitoneal pentobarbital is acceptable for smaller mammals when intravenous access is not practical.
      3. Pentobarbital-based euthanasia solution (Euthasol: 390mg Pentobarbital/50mg Phenytoin /ml) can be used for immediate euthanasia.
   2. **Carbon Dioxide (CO2) Inhalation**
      1. Compressed CO2 gas in cylinders is the only allowable source of carbon dioxide because the inflow to the chamber can be regulated precisely. CO2 delivery must be monitored to ensure CO2 does not displace air by more or less than 30-70% of the chamber volume per minute. Prefilled CO2 chambers must not be used.
      2. CO2 generated by other methods (e.g., dry ice) is unacceptable.
      3. To ensure compliance with the AVMA CO2 guidelines; the use of the Euthanex Smartbox Automated Euthanasia chambers is recommended. See the ***Euthanasia of Rodents SOP*.**
      4. Euthanasia must be confirmed by a secondary method (see below).
      5. CO2 is **NOT** an appropriate method of euthanasia for neonates under 10 days of age.
   3. **Isoflurane Inhalation Overdose**
      1. The required method of delivering isoflurane is via precision vaporizer..
      2. Euthanasia must be confirmed; a secondary method of euthanasia is required (see below).
3. **IACUC Approved Acceptable Physical Methods**

Physical methods of euthanasia can be used as secondary methods of euthanasia or, following careful training and assessment, as a primary method. Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately.

* 1. **Cervical Dislocation**
     1. When properly performed by trained personnel, manual cervical dislocation is a humane technique for euthanasia of mice, other small rodents, and rats weighing less than 200 grams.
     2. This should only be used as a secondary method until proficiency is demonstrated.
  2. **Decapitation**
     1. Decapitation is the preferred method for altricial neonates.
     2. Decapitation when performed properly is virtually instantaneous and is considered humane. This method must be justified by the experimental design and approved by the IACUC.
     3. Decapitation without pre‐sedation is acceptable for fetal and altricial mice and rats.
     4. Unless excised, fetuses inside a pregnant dam euthanized via CO2 do not need to be decapitated, per AVMA guidelines.
     5. Guillotines that are designed for decapitation of rodents are commercially available. It is critical to follow manufacturer guidelines to ensure equipment used is appropriate for the size of animals being euthanized. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.
     6. Sharp scissors may be used for smaller animals, such as neonates and mice given that the scissors are large and sturdy enough to ensure that decapitation occurs rapidly and in one swift motion.
     7. The action should be smooth with no perceptible binding or resistance, and the blade must be rust-free, sharp, and decapitate with minimal force. The IACUC recommends the following technique to assess the sharpness of the blade: the blade is sharp enough that it will cut a thick rubber band, without dragging it between the blades and sticking.
     8. After use, the entire guillotine or surgical scissors must be rinsed with water and scrubbed to remove gross contamination. The unit(s) should then be thoroughly disinfected.
     9. The frequency of guillotine and/or surgical scissor sharpening will depend on the animal species involved and volume of use, however, a minimum of every 12 months is required. Alternatively, surgical scissors for decapitation could be replaced every 12 months.
     10. All laboratories using a guillotine or surgical scissors for decapitation must maintain a log to record dates of use and sharpening.
  3. **Exsanguination**
     1. With an animal under deep anesthesia, withdrawing the maximal volume of blood via cardiac puncture, abdominal aorta puncture, or bilateral transection of the cervical vasculature.

1. **Confirmation of Euthanasia** 
   1. For all euthanasia methods, verification of complete euthanasia is required by veterinary, technical, and investigative staff. Use the following methods to confirm death of the animal:

* Secondary methods: cervical dislocation, decapitation, or removal of major organs, or exsanguination secondary to CO2 euthanasia.
* Visual inspection to assure no signs of respiration.
* Ensure that the heart is not beating by feeling the chest between your thumb and forefinger.
* Ensure that there is no blink reflex by touching the eyeball.
* If there is a heartbeat or blink reflex, repeat the euthanasia process as described above.

1. **Responsibilities:** 
   1. All individuals to whom this policy applies are responsible for becoming familiar with and following this policy. Researchers are responsible for promoting the understanding of this policy and for taking appropriate steps to help ensure compliance with it.
2. **Review of Policy:**
   1. This policy will be periodically reviewed and updated to ensure alignment with evolving ethical standards, best practices, and institutional and regulatory requirements.