

**University of Colorado at Boulder, Office of Research Integrity
Institutional Animal Care and Use Committee
SOP #6**

Title: Euthanasia

1. PURPOSE

Describe the procedures for euthanasia.

2. DEFINITIONS

Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress.

3. BACKGROUND

Animal welfare regulations require that the Institutional Animal Care and Use Committee (IACUC) approve the euthanasia method for research animals. The proposed method of euthanasia and the criteria used to assess pain and distress in animals must be described in detail in the IACUC *protocol*. The Principle Investigator is responsible for assuring the committee that each member of the study team is prepared and familiar with an established course of action in the event that an animal must be euthanized to alleviate pain or distress. Additionally, the protocol must include contact information for all members of the research group, so that someone with authority to deal with sick or injured animals can always be reached. Animal care staff will only euthanize animals after contacting laboratory staff, unless immediate euthanasia is required to relieve acute animal suffering.

IACUC policy also requires that an approved secondary physical method of euthanasia be employed prior to carcass disposal in ALL species.

4. PROCEDURES

- A. All methods of euthanasia must be listed on a protocol and approved by the IACUC in advance.
- B. Final disposition of an animal must be listed in the protocol. Animals are often euthanized at the end of a procedure as listed in the protocol.
- C. Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly killed at the end of the procedure or, if appropriate, during the procedure¹. Humane endpoints must be indicated in the protocol if animals are expected to be moribund during certain experimental procedures.
- D. Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, 2007 [formerly the 2000 Report of the AVMA Panel on Euthanasia], unless a deviation is justified for scientific reasons in writing by the investigator and approved by the IACUC².
- E. Death must be confirmed by one of the methods consistent with the AVMA Guidelines on Euthanasia. See below for more guidance. A secondary method of euthanasia and confirmation of death must be listed in the approved protocol. Rodents must receive a secondary physical method of euthanasia as listed in the approved protocol.

¹ PHS Policy IV, C, 1, c

² PHS Policy IV, C, 1, g

NOTE: Unintended recovery of animals after apparent death from CO₂ or other euthanasia agents constitutes serious noncompliance. All incidents involving unintended recovery of euthanized animals are reported to the Office of Laboratory Animal Welfare at NIH.

5. Confirming Death in Rodent Species

Rodents, especially neonates, are particularly resistant to euthanasia by overdose of inhaled agents such as CO₂ or even injectable agents; for this reason, the IACUC requires a **secondary physical method of euthanasia FOR ALL RODENTS** after the animal is profoundly anesthetized, prior to carcass disposal. Inadequate exposure time to **CO₂** may result in animals that appear dead but can awaken from deep anesthesia.

One of the following procedures **MUST** be followed to **assure death**. This should match the description in your IACUC approved protocol.

- Decapitation
- Cardiac perfusion
- Removal of vital organs (e.g. heart, lungs, brain)
- Opening of the chest cavity to induce pneumothorax
- Cutting the major blood vessels to induce exsanguination (e.g. aorta, vena cava)
- Cervical dislocation may only be used in adult rodents, as it can be difficult to perform in neonates and thus is not appropriate for use in animals prior to weaning.

6. Confirmation of Death in Ectothermic Vertebrates

Additional care must be taken to ensure death following euthanasia in ectothermic vertebrates such as fish, reptiles and amphibians. Such animals may normally exhibit very low heart rates, and the heart and brain are very tolerant to hypoxia; many ectotherms can voluntarily hold their breath for an hour or more. Absence of heart rate and/or breathing will not necessarily provide confirmation of death in these animals; secondary methods for ectothermic vertebrates should always include either removal of the heart or decapitation followed by pithing the brain or placement of the head in liquid nitrogen.

7. Ensuring Humane Euthanasia of Laboratory Animals

The table below summarizes the American Veterinary Medical Association's (AVMA) recommendations on humane euthanasia methods. Personnel must be adequately trained in performing the approved techniques **and** in confirming death. A profoundly anesthetized or severely ill animal can appear dead upon cursory examination; one cannot rely solely on imprecise measures such as lack of movement and lack of visible breathing to declare an animal dead.

Agents and Methods of Euthanasia by Species
from Appendix I from AVMA Guidelines on Euthanasia 2007, pages 28-29
<http://grants.nih.gov/grants/olaw/Euthanasia2007.pdf>

Species	Acceptable* (refer to Appendix 2 and text for details)	Conditionally acceptable† (refer to Appendix 3 and text for details)
Amphibians	Barbiturates, inhalant anesthetics (in appropriate species), CO ₂ , CO, tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, double pithing	Penetrating captive bolt, gunshot, stunning and decapitation, decapitation and pithing
Birds	Barbiturates, inhalant anesthetics, CO ₂ , CO, gunshot (free-ranging only)	N ₂ , Ar, cervical dislocation, decapitation, thoracic compression (small, free-ranging only), maceration (chicks, poults, and piped eggs only)
Cats	Barbiturates, inhalant anesthetics, CO ₂ , CO, potassium chloride in conjunction with general anesthesia	N ₂ , Ar
Dogs	Barbiturates, inhalant anesthetics, CO ₂ , CO, potassium chloride in conjunction with general anesthesia	N ₂ , Ar, penetrating captive bolt, electrocution
Fish	Barbiturates, inhalant anesthetics, CO ₂ , tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, 2-phenoxyethanol	Decapitation and pithing, stunning and decapitation/pithing
Nonhuman primates	Barbiturates	Inhalant anesthetics, CO ₂ , CO, N ₂ , Ar
Rabbits	Barbiturates, inhalant anesthetics, CO ₂ , CO, potassium chloride in conjunction with general anesthesia	N ₂ , Ar, cervical dislocation (< 1 kg), decapitation, penetrating captive bolt
Reptiles	Barbiturates, inhalant anesthetics (in appropriate species), CO ₂ (in appropriate species)	Penetrating captive bolt, gunshot, decapitation and pithing, stunning and decapitation, head immediately placed in liquid nitrogen
Rodents and other small mammals	Barbiturates, inhalant anesthetics, CO ₂ , CO, potassium chloride in conjunction with general anesthesia, microwave irradiation	Methoxyflurane, ether, N ₂ , Ar, cervical dislocation (rats < 200 g), decapitation
Swine	Barbiturates, CO ₂ , potassium chloride in conjunction with general anesthesia, penetrating captive bolt	Inhalant anesthetics, CO, chloral hydrate (IV, after sedation), gunshot, electrocution, blow to the head (< 3 weeks of age)
Free-ranging wildlife	Barbiturates IV or IP, inhalant anesthetics, potassium chloride in conjunction with general anesthesia	CO ₂ , CO, N ₂ , Ar, penetrating captive bolt, gunshot, kill traps (scientifically tested)

*Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia.

†Conditionally acceptable methods are those that by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature. Conditionally acceptable euthanasia methods may only be performed when scientifically justified and approved by the IACUC.

Notes and References:

1. June 2007 [AVMA Guidelines on Euthanasia](#)
2. [PHS Policy on Humane Care and Use of Laboratory Animals Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals, July 17, 2002.](#)

Who to Contact for Help:

For more detailed information and training in acceptable euthanasia methods, please contact the Institutional Veterinarian at 303-492-3411 or the IACUC office at 303-492-8187.