

1. DEFINITION

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.

2. POLICY

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 Application to Use Animals in Research or Teaching. Additionally, the protocol must include contact information for all members of the research group, so that Veterinary Services can always reach someone with authority to deal with sick or injured animals.

The Principle Investigator (PI) is responsible for assuring the committee that each member of the study team is prepared for and familiar with an established course of action to alleviate pain or distress in the event that an animal must be euthanized. At least one member of the study team must be available locally at all times to euthanize any animal exhibiting symptoms of pain or distress. PIs are ultimately responsible for the euthanasia of all animals purchased or bred under their approved protocols. The Attending Veterinarian should be notified at this point, unless immediate euthanasia is required to relieve acute animal suffering.

The method by which death will be confirmed in euthanized animals must be described in the approved protocol. Since rodents can be particularly resistant to euthanasia by standard methods, IACUC policy requires that an approved secondary physical method of euthanasia be employed prior to carcass disposal in ALL rodent species.

3. 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.

4. 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.

Acceptable secondary physical methods for adult and neonatal rodents include:

- a. Decapitation
- b. Cardiac perfusion
- c. Removal of vital organs (e.g. heart, lungs, brain)
- d. Opening of the chest cavity to induce pneumothorax
- e. Cutting the major blood vessels to induce exsanguination (e.g. aorta, vena cava)
- f. 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. Formal training in cervical dislocation is recommended – contact the Attending Veterinarian for details.

These procedures may not be performed in conscious animals without specific IACUC approval.

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 by the IACUC to the Office of Laboratory Animal Welfare at NIH.

5. CONFIRMATION OF DEATH IN NON-RODENT MAMMALIAN SPECIES

- a. **Heartbeat:** must be assessed for five minutes or more. The best assessment is through direct palpation of either the pulse in the carotid or femoral artery or direct cardiac palpation. If there is any question, the thorax should be opened, the heart exposed and viewed directly or palpated to confirm lack of activity. Arterial pulse of smaller species may be difficult to palpate, so direct inspection of cardiac mechanical activity is necessary. Lack of electrical activity of the heart as determined by ECG (provided that the leads are correctly connected) may also be utilized to confirm death.
- b. **Pupillary response to light:** Shine a bright light into the eyes of the animal. A constriction (narrowing) of the pupil indicates a neurological response. Upon

death, the pupils will become dilated and unresponsive to light. Some drugs and experimental agents (e.g., anticholinergics such as atropine) can prevent pupillary reactivity or otherwise affect this neurological response.

- c. **Respiratory pattern:** Profoundly anesthetized animals may exhibit shallow and irregular breathing patterns that may be confused for lack of spontaneous breathing. Thus, lack of spontaneous breathing should not be used as sole criteria for confirming euthanasia.

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 placement of the head in liquid nitrogen.

7. AGENTS AND METHODS OF EUTHANASIA BY SPECIES (*from Appendix I from AVMA Guidelines on Euthanasia 2013*)

Species	Acceptable* (refer to Appendix 1 for details)	Conditionally acceptable† (refer to Appendix 1 for details)
Amphibians	As appropriate by species— Injected barbiturates, dissociative agents and anesthetics as specified, topical buffered tricaine methanesulfonate or benzocaine hydrochloride	As appropriate by species—Inhaled anesthetics as specified, CO ₂ , penetrating captive bolt or firearm, manually applied blunt force trauma to the head, rapid freezing
Birds	Intravenous barbiturates; anesthetic overdose	Inhaled anesthetics, CO ₂ , CO, N ₂ , Ar, cervical dislocation (small birds and poultry), decapitation (small birds); manual blunt force trauma, electrocution, gunshot, captive bolt (for poultry)
Fish	Immersion in buffered benzocaine or benzocaine hydrochloride, isoflurane, sevoflurane, quinaldine sulfate, buffered tricaine methanesulfonate, 2-phenoxyethanol, injected pentobarbital, rapid chilling (appropriate Zebrafish/research setting)	Eugenol, isoeugenol, clove oil, CO ₂ -saturated water (aquarium-fish facilities/fisheries), decapitation/cervical transection/manually applied blunt force trauma followed by pithing, rapid chilling followed by adjunctive method (aquarium-fish facilities), maceration (research setting)

Rabbits	Intravenous barbiturates	Inhaled anesthetic overdose, CO ₂ , cervical dislocation (as anatomically appropriate), penetrating captive bolt
Reptiles	As appropriate by species—Injected barbiturates, dissociative agents and anesthetics as specified	As appropriate by species—Inhaled anesthetics as specified, CO ₂ , penetrating captive bolt or firearm, manually applied blunt force trauma to the head, rapid freezing for animals < 4 g
Rodents and other small mammals	Injected barbiturates and barbiturate combinations, dissociative agent combinations	Inhaled anesthetics, CO ₂ , CO, tribromoethanol, ethanol, cervical dislocation, decapitation, focused beam microwave irradiation

*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.

The following additional guidelines must be followed when using CO₂ as a euthanasia agent:

- a. CO₂ must be delivered from compressed gas canister only, i.e., not from dry ice.
- b. High concentrations of CO₂ may be distressful to some species. Accordingly, pre-filling the chamber is recommended only under circumstances in which such use has not been shown to cause distress.
- c. Chambers used for CO₂ euthanasia (either the rodents' home cage or the euthanasia chamber itself) must not be overcrowded. Overcrowding in this situation is defined as less than one half the normal housing space normally required for the animals.
- d. Cages used to transport animals between the housing area and procedure room/euthanasia chamber must not be overcrowded.
- e. Male mice from different cages should not be mixed in transport cages or the euthanasia chamber to prevent distress and/or fighting.

Notes and References:

1. 2013 Edition, [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.](#)

8. WHO TO CONTACT FOR HELP

For more detailed information and training in acceptable euthanasia methods, please contact the SHSU IACUC Attending Veterinarian (for the name of the Attending Veterinarian, please see the IACUC Contact page on the Research and Sponsored Programs webpage).

APPROVED: _____ <signed>
Dana L. Gibson, President

DATE: _____ 7/9/14

CERTIFICATION STATEMENT

This academic policy statement (APS) has been approved by the reviewer(s) listed below and represents SHSU's Division of Academic Affairs' policy from the date of this document until superseded.

Original:	February 12, 2014	Review Cycle:	February 1, ENY*
Reviewer(s):	Council of Academic Deans Academic Affairs Council	Review Date:	February 1, 2016

Approved:	_____ <signed> Jaimie L. Hebert Provost and Vice President for Academic Affairs	Date:	_____ 7/17/14
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*ENY = Even Numbered Year