

San Francisco State University

Institutional Animal Care and Use Committee Euthanasia Guidelines

Purpose: These guidelines have been developed to provide minimum standards **for euthanasia of commonly used laboratory animals** and assure that all SFSU procedures involving euthanasia of experimental animals are in compliance with federal animal welfare regulations and follow standards described in the American Veterinary Medical Association (AVMA) Euthanasia Guidelines and the *Guide for Care and Use of Laboratory Animals (Eighth edition)*. It is the responsibility of the investigator to ensure the use of techniques and procedures which result in the least pain and distress to the animal while adequately addressing the needs of the experimental design. **All euthanasia methods must be reviewed and approved by the IACUC prior to their implementation.** Exceptions to these guidelines are possible if justified for the experiment and reviewed and approved by the IACUC.

Training: It is the responsibility of the investigator to assure that all individuals performing euthanasia are adequately trained to do so. Competency for performing all methods of euthanasia without supervision by a previously certified individual must be certified by the veterinarian or Animal Facility staff. Additional competency for performing euthanasia by decapitation or cervical dislocation without anesthesia must be certified by the veterinarian.

Euthanasia of Rodents

Methods: Anesthetic Overdose

- a. Chemical euthanasia with an overdose of intravenous pentobarbital is the euthanasia method preferred by the AVMA for mammals with practical vascular access (e.g. rabbits).
- b. Intraperitoneal pentobarbital or an overdose of inhaled anesthetic (e.g. isoflurane) is acceptable for smaller mammals/reptiles/amphibians when intravenous access is not practical.
- c. Chemical euthanasia must be followed by a physical procedure that assures death (e.g. bilateral thoracotomy, cervical dislocation, pithing, or organ removal).

Methods: Carbon Dioxide Inhalation

- a. Exposure to CO₂ gas in an enclosed chamber is an acceptable form of euthanasia for small mammals with conditions as outlined below.
- b. Compressed CO₂ in cylinders is the only allowable source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods, such as from dry ice, is unacceptable. The CO₂ flow rate into the chamber should displace 10-30% of the chamber volume per minute.
- c. Animals should be placed in the chamber or container and the lid replaced or closed. The entire home cage of animals should be used without introduction of new animals, whenever possible. Rodents must never be "collected" in a group cage with unfamiliar rodents prior to euthanasia.
- d. Unconsciousness will occur within 30 seconds but animals should be left in the container with the gas flowing for at least an additional 60 seconds. Neonates should be exposed for 5 minutes. Never leave a euthanasia chamber with flowing gas unattended.
- e. Prior to carcass disposal, a second physical means of euthanasia must be performing to assure death: cervical dislocation (rodents < 200 g), bilateral thoracotomy (rodents > 200 g), decapitation (neonates) or organ removal. The physical euthanasia method may be performed as soon as the animals lose their righting reflex.

Methods: Cervical Dislocation/Decapitation with Guillotine

- a. The IACUC recommends anesthesia for animals undergoing cervical dislocation/decapitation.
- b. When properly performed, cervical dislocation may be used for euthanasia of mice and other small rodents and rats weighing less than 200 grams.
- c. When properly performed, decapitation may be used for euthanasia of mice and rats.
- d. The following conditions must be met if cervical dislocation/decapitation without pre-anesthesia is performed:
 1. The AUP must document the scientific justification for this request and provide documentation of experience and/or training of the person(s) who will be performing the procedure. Veterinary certification will be required.
 2. If a guillotine is used for decapitation, “the equipment used for decapitation should be maintained in good working order and serviced on a regular schedule to maintain sharpness of blades.” (AVMA Guidelines) Guillotines in use must be sharpened at least annually. If equipment has been out of service it must be sharpened prior to re-commissioning.
 3. Guillotine use and maintenance must be logged and records must be available for review by Animal Facility staff and the IACUC.

Euthanasia of Neonatal Rodents and Fetuses

- a. Neonates: Euthanasia of rodent neonates is the same procedure as for adults described in the above methods except that AUP approval is not required for decapitation without anesthesia. Neonates of altricial rodents (mice, rats, hamsters) are relatively resistant to the effects of CO₂ and other inhalants and may require longer exposure times. A secondary physical method (cervical dislocation, decapitation) is required.
- b. Fetuses:
 - For mouse, rat and hamster fetuses up to 15 days and guinea pig fetuses up to 35 days of gestation - euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to blood loss and non-viability, thus no additional method of euthanasia is required.
 - For mouse, rat and hamster fetuses >15 days and guinea pig fetuses >35 days of gestation - after general anesthesia or euthanasia of the mother, decapitation or cervical dislocation of the fetus must be performed.

Euthanasia of Non-mammalian Vertebrates including Birds, Reptiles, Amphibians and Fish

The same principles for euthanasia of small mammals apply to birds and ectotherms: a physical method must follow chemical euthanasia and physical euthanasia without anesthesia must be justified in the AUP and approved by IACUC.

Common Methods

1. Chemical Methods
 - a. An overdose of pentobarbital can be used to euthanize ectotherms and birds via intracoelomic/intraperitoneal injection. The actual time to death may be very prolonged for ectotherms.
 - b. pH-neutralized Tricaine methanesulfonate (MS-222) administered in water may be used to euthanize fish, aquatic and semi-aquatic amphibians. Reptiles may be euthanized by intra-coelomic (IC) injection of 250-500 mg/kg pH-neutralized 1% MS-222, followed by 0.5-1 ml 50% unbuffered MS-222 (Conroy et al, 2009).

- c. Benzocaine administered in water may also be used to euthanize fish, aquatic and semi-aquatic amphibians. A minimum 0.03% solution of benzocaine is recommended for euthanasia.

2. Physical Methods

- a. Freezing following cessation of respiration after chemical euthanasia is acceptable. Freezing is NOT allowed as the sole method of euthanasia. Lowering the ambient temperature may facilitate handling; however, there is no evidence that it raises the pain threshold in ectotherms. Formation of ice crystals on the skin and in the tissues of an animal may cause pain and distress.
- b. Decapitation: the central nervous system of reptiles is extremely tolerant to anoxia. Therefore, methods of euthanasia that induce unconsciousness by interruption of blood supply to the head, e.g., decapitation, cervical dislocation, and exsanguination, are inappropriate for reptiles when used alone. These methods can only be used on small reptiles that are already unconscious by a chemical agent or concussion or when followed immediately with double-pithing, freezing in liquid nitrogen or dry ice.
- c. Double-pithing can only be carried out on unconscious animals and performed by trained personnel.

Birds and Reptiles: Hatchlings, Embryos and Eggs

1. Hatchlings

- a. Avian and reptile embryos that hatch, either intentionally or unintentionally, are live vertebrate animals and are regulated by PHS Policy. For this reason, any project in which birds or reptiles may hatch must include a contingency plan for humane euthanasia of hatchlings in the MAUP.
- b. Acceptable methods of euthanasia of avian or reptile hatchlings include overdose of CO₂ or anesthetic agents. A secondary physical method is required to ensure death, for example decapitation, cervical dislocation (birds) or decapitation/pithing (reptiles).

2. Embryos & Eggs

- a. For embryos/eggs >50% gestation, use methods appropriate for hatched birds or reptiles (see above).
- b. For embryos/eggs <50% gestation, prior to neural tube formation (Close et al, AAZV guidelines), destroy the viability of the egg via one of the following methods: shaking, puncturing, freezing (<4C for 4 hours) or coating eggs with oil.

Amphibians and Fish: Larvae and Embryos

1. Larvae: For the purposes of these guidelines, the larval stage of amphibians and fish begins at the same time as hatching and independent feeding. Acceptable methods of euthanasia for adult amphibians and fish apply to the larvae of these species (ie, anesthetic agent followed by secondary physical method).
2. Embryos: For the purposes of these guidelines, amphibians and fish are embryos prior to the larval stage (i.e., up to hatching and independent feeding), and therefore have yet to develop pain systems (AHAW Panel Report). Embryos of these species are disposed of depending on their experimental treatment and transgenic status, but care should be taken to recognize the variation among these species of the stage at which independent feeding begins.

References

Aspects of the biology and welfare of animals used for experimental and other scientific purposes, 2005. Animal Health and Welfare (AHAW) Panel, European Food Safety Authority Journal Annex, 292:1-136.

AVMA Guidelines on Euthanasia, American Veterinary Medical Association, June 2007, 2011. The AVMA Guidelines may be downloaded from:

<https://ebusiness.avma.org/EBusiness50/ProductCatalog/product.aspx?ID=85>

Guidelines for Euthanasia of Nondomestic Animals, American Association of Zoo Veterinarians, 2006.

Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002.

Recommendations for euthanasia of experimental animals: Part 2, Working Party Report, 1997. Close, B et al, Laboratory Animals 31: 1-32.

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