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Guidelines for Euthanasia of Research and Teaching Animals

Purpose
To provide guidance regarding humane euthanasia of animals used in research or teaching.

Background
According to the Guide for the Care and Use of Laboratory Animals [1], PHS Policy on the Humane Care and Use of Laboratory Animals [2], and the Animal Welfare Regulations, methods of euthanasia should be consistent with the American Veterinary Medical Association Guidelines on Euthanasia (AVMA Guidelines), unless a deviation is justified for scientific or medical reasons. In addition, methods must be specified and approved in Animal Care and Use Protocols. Methods are chosen to minimize pain and distress to the animals as well as meet with the needs of the research or teaching protocol.

Policy Statement
The method of euthanasia must be an acceptable method as outlined in the AVMA Guidelines; deviations from the AVMA Guidelines require IACUC approval prior to being implemented. If unique circumstances arise that require deviation from these guidelines the Investigators must consult with Campus Veterinary Services (530-752-0514).

Chemical methods of euthanasia (CO$_2$ chamber, inhalant anesthetics, Sodium Pentobarbital) should be followed with a physical method (cervical dislocation, bilateral thoracotomy, or exsanguination) to ensure death unless the effectiveness of the chemical method (e.g., specific chamber and process) has been appropriately validated.

Personnel performing euthanasia must be trained, knowledgeable and proficient in the chosen techniques and training must be documented. Personnel using physical methods of euthanasia must be well trained and demonstrate proficiency for each type of physical method performed to ensure euthanasia is conducted appropriately.

Ideally euthanasia should be performed in procedure rooms or laboratory space away from other animals. Death must be confirmed prior to bagging the animal for disposal.

Euthanasia of Mouse and Rat Fetuses and Neonates
Mice and rats older than 14 days should be euthanized following the guidelines for adult rodents.

1. Rodent fetuses along with other mammals are unconscious in utero and hypoxia does not evoke a response. Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized, if fetuses are not harvested.
   - If fetuses are harvested:
     1. Fetuses up to 14 days of gestation:
     2. Euthanasia of the dam or removal of the fetus results in rapid fetal death since they cannot survive outside of the uterus.
     3. Fetuses from 15 days of gestation to birth:
     4. Decapitation with surgical scissors, hypothermia, or cervical dislocation are acceptable physical methods. An injection of a chemical anesthetic overdose is an acceptable chemical method.

2. Neonates:
   - Neonates up to 14 days of age:
     1. Injection of an overdose of chemical anesthetics should be used whenever possible.
     2. Decapitation and cervical dislocation are acceptable methods of euthanasia with appropriate training and demonstrated proficiency.
     3. Hypothermia may be used as a method of euthanasia of altricial neonates provided they are less than 5 days old and they are not placed directly on the frozen surface (i.e., place them in a latex bag or cloth).
     4. Altricial neonates less than 5 days of age do not have sufficient nervous system development to perceive pain and may be quickly euthanized by rapidly freezing in liquid N₂.

Neonatal rodents are resistant to the effects of CO₂, an adjunctive method (e.g., cervical dislocation or decapitation) should be performed after a neonate is nonresponsive to painful stimuli. The use of CO₂ in neonates is highly discouraged.

Other Euthanasia Methods

1. Carbon dioxide:
   A CO₂ chamber is the most common method of euthanasia for small rodents. The chamber must allow viewing of the animal during euthanasia. Proper technique must be followed to ensure a humane death, as CO₂ may have noxious properties that can cause unnecessary pain and suffering.
   1. Euthanasia in the home cage is recommended. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. Do not overcrowd the chamber; all animals in the chamber must be able to make normal postural adjustments.
   2. The flow rate for CO₂ euthanasia systems should displace 10% to 30% of the chamber or cage volume/min. The higher range is recommended and should be continued at least one minute after respiratory arrest.
   3. Prefilled chambers are unacceptable.
   4. An appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized are absolutely necessary.
5. Remove the animal from the chamber and confirm the absence of respiration. It is important to verify that an animal is dead after exposure to CO$_2$. Death may be confirmed by physical examination, ensured by an adjunctive physical method, or obviated by calibration and validation of the euthanasia chamber and process.

6. Clean the chamber with disinfectant to remove all urine, feces, and fir.

7. **CO$_2$ generated from dry ice is NOT an acceptable method of euthanasia.**

2. Potassium Chloride (KCl) saturated solution:
   Personnel performing this technique must be trained and knowledgeable in anesthetic techniques, and be competent in assessing the level of unconsciousness that is required for administration of potassium chloride solution IV. Administration of potassium chloride solution IV requires animal to be in a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli.

3. Cervical dislocation of rodents, small rabbits, poultry and other birds:
   1. The use of cervical dislocation for euthanasia is limited to small birds, poultry, mice, immature rats (<200 g), and rabbits (<1 kg).
   2. Cervical dislocation has generally been used for small birds (<220 g) when no other method is available, but the procedure has been performed on birds as large as 2.3 kg (5.1 lb).
   3. Cervical dislocation should only be performed by well-trained personnel who have demonstrated proficiency in the technique.

4. Decapitation of rodents, small rabbits, poultry and other birds:
   1. Decapitation is acceptable with conditions for mice and rats. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.
   2. Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.
   3. Decapitation should only be performed by well-trained personnel who have demonstrated proficiency in the technique.

5. Double Pithing of frogs and amphibians:
   Pithing may be used as a second-step euthanasia method to ensure death in an animal that has been rendered unconscious by other means. Pithing of frogs and amphibians may be used in the animal is anesthetized.

6. Tricaine Methanesulfonate (MS222) in amphibians and fish:
   The solution must be buffered with sodium bicarbonate resulting in a pH between 7.0 - 7.5. Due to species differences in response to MS222, a secondary method of euthanasia is recommended in some finfish and amphibians to ensure death.

**NOTE:** Please refer to the *AVMA Guidelines for Euthanasia of Animals: 2013* for information on additional euthanasia methods

## Animal Disposal
After death has been ensured, place the animal carcass in a disposable waterproof bag. Seal the bag and place the bagged carcass in one of the barrels found inside the cold rooms or freezers designated for animal
Animals that are radioactive must be disposed according to the procedures stated in the Principal Investigator's Radioactive Use Authorization. Animals that are infectious must be disposed according to the procedures stated in the Principal Investigator's Biological Use Authorization.

Procedure: IACUC-37  
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**Contact**

IACUC-Institutional Animal Care and Use Committee  
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More information  

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