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**UC Davis
Institutional Animal Care and Use Committee (IACUC)**

Title: Guidelines for Euthanasia of Research and Teaching Animals

I. Purpose

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

II. Background

According to the *Guide for the Care and Use of Laboratory Animals*, *PHS Policy on the Humane Care and Use of Laboratory Animals*, and the *Animal Welfare Regulations*, methods of euthanasia should be consistent with the American Veterinary Medical Association Guidelines for Euthanasia of Animals (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 the needs of the research or teaching protocol.

III. 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 implementation. If unique circumstances arise that require deviation from these guidelines the Principal Investigator must consult with Campus Veterinary Services (530-752-0514).

Chemical methods of euthanasia (CO₂ chamber, inhalant anesthetics, Sodium Pentobarbital) must be confirmed by a secondary physical method and/or through direct assessment (e.g. thoracic auscultation, ECG, observation.). Regardless of which method of euthanasia is performed, personnel must ensure that death has occurred. Assurance of death must include ascertaining the absence of heartbeat and respiration.

Personnel performing euthanasia must be trained, knowledgeable, and proficient in the chosen techniques, trained on how to ensure that animals are no longer viable, and

training must be documented. Personnel using physical methods of euthanasia must be well trained and have demonstrated proficiency for each type of physical method used to ensure euthanasia is conducted appropriately.

Individuals who need to perform cervical dislocation and/or decapitation without anesthesia in select adult species (see V.C., below), for research or teaching, must be certified to perform these techniques. Certification must be verified by an approved Designated Trainer. Individuals can obtain assistance in identifying an approved Designated Trainer by contacting Campus Veterinary Services or the IACUC Office. Designated Trainers will provide training and assessment of the participant's skill level and verify the individual's proficiency in use of the technique(s). Once an individual has been certified as proficient in a particular technique they may be listed as certified to perform the technique on an approved Animal Care and Use Protocol.

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

NOTE: Please refer to the [AVMA Guidelines for Euthanasia of Animals: 2020](#) for information on other species and additional euthanasia methods not covered in this policy.

IV. **Euthanasia of Mice and Rat Fetuses and Neonates**

A. Fetuses:

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 collected.

1. If fetuses are collected:

a. Fetuses up to 14 days of gestation:

Euthanasia of the dam or removal of the fetus results in rapid fetal death since they cannot survive outside of the uterus.

b. Fetuses from 15 days of gestation to birth:

An injection of a chemical anesthetic overdose is an acceptable chemical method. Decapitation with surgical scissors, hypothermia, or cervical dislocation are acceptable physical methods.

B. Neonates*:

1. Neonates less than 5 days of age:

Altricial neonates less than 5 days of age do not have sufficient central nervous system development to perceive pain and may be quickly euthanized by rapidly freezing in liquid nitrogen (N₂).

2. Neonates up to 10 days of age:

- a. Hypothermia may be used as a method of euthanasia of altricial neonates up to 10 days old and provided they are **not** placed directly on a frozen surface (i.e., place in a latex glove, plastic bag or on a cloth).
- b. Decapitation using scissors or a sharp blade is acceptable for altricial neonates.

3. Neonates 10-14 days of age:

- a. Injection of an overdose of chemical anesthetic should be used whenever possible.
- b. Cervical dislocation is an acceptable method of euthanasia with appropriate training and demonstrated proficiency.
- c. Decapitation is an acceptable method of euthanasia with documented training. Depending on the developmental stage of animals, this technique may require the use of a guillotine.

Neonatal rodents are resistant to the effects of CO₂, thus an adjunctive method (e.g., cervical dislocation or decapitation) should be performed after a neonate is nonresponsive to painful stimuli. The use of CO₂ as a sole method of euthanasia in neonates is strongly discouraged and must be justified and approved in an IACUC protocol.

Mice and rats older than 14 days should be euthanized following the guidelines for adult rodents.

*Precocious neonates (i.e. guinea pigs) should be treated as adults.

V. **Other Euthanasia Methods**

A. Carbon dioxide (CO₂):

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 has noxious properties that can cause unnecessary pain and distress.

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. If combining animals from multiple cages for euthanasia, euthanasia must be performed immediately to prevent animals from fighting.

2. The flow rate for CO₂ euthanasia systems should displace 30% to 70% 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 utilized are required.
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 CO₂. Death must be confirmed by a secondary physical method (i.e., bilateral thoracotomy, cervical dislocation, decapitation) or through direct assessment (e.g. thoracic auscultation, ECG, observation).
6. Clean the chamber with disinfectant to remove all urine, feces, and fur.
7. ***CO₂ generated from dry ice is NOT an acceptable method of euthanasia.***

B. 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 intravenously (IV). Administration of potassium chloride solution IV requires animals to be at a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli.

C. Cervical dislocation (without anesthesia) of rodents, small rabbits, chickens, and other birds:

1. Cervical dislocation is acceptable for mice and rats <200 g.
2. The use of cervical dislocation for euthanasia is limited to small birds (<200 g), chickens, mice, immature rats (< 200 g), and rabbits (< 1 kg).
3. All users approved to perform cervical dislocation (without anesthesia) as the method of euthanasia must be certified by one of the approved campus trainers. *Personnel will be trained using at least two anesthetized/euthanized animals and must then demonstrate proficiency on two live, conscious animals to receive certification.*

D. Decapitation (without anesthesia) of rodents, small rabbits, poultry, and other birds:

1. Decapitation is acceptable for mice and rats.

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 it is effective.
3. All users approved to perform decapitation (without anesthesia) as the method of euthanasia must be certified by one of the approved campus trainers. *Personnel will be trained using at least two anesthetized/euthanized animals and must then demonstrate proficiency on two live, conscious animals to be receive certification.*

E. Euthanasia of amphibians, reptiles and fish:

1. Pithing of amphibians:

Pithing (destroying both the brainstem and spinal cord tissue) can be used as a second-step euthanasia method in unconscious animals when performed by properly trained individuals.

2. Decapitation of amphibians and reptiles:

After an animal has been anesthetized decapitation must be followed by pithing or another method to destroy brain tissue. Decapitation can only be used as part of a 3-step euthanasia process (anesthesia, decapitation, pithing).

3. Buffered 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.

- a. Amphibians MS222 dose = 500 mg/L (immersion for at least one hour). If immersion time is less a secondary euthanasia method must be used.

NOTE: Overdose of MS222 in frogs must be followed by pithing with or without decapitation.

- b. Finfish MS222 dose = 500 mg/L (immersion for at least 30 minutes). If immersion time is less a secondary euthanasia method must be used.

4. Rapid chilling (hypothermic shock) in finfish:

Rapid chilling is acceptable for small-bodied (3.8-cm-long or smaller) tropical and subtropical stenothermic finfish, for which the lower lethal temperature range is above 4°C.

Because it is often difficult to confirm that an amphibian or reptile is dead, the application of two or more euthanasia procedures is recommended.

VI. **Equipment Maintenance**

In accordance with the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition “The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.”

- A. Decapitation may be accomplished by use of a commercial guillotine, dedicated scissors or razor/scalpel blades.
1. Scissors, razors, or scalpel blades may only be used for neonatal rodents (altricial neonates less than 10 days of age) and small amphibians/fish.
 2. Dedicated scissors must be clean, in good condition, sharp, and move freely.
 3. Razors and scalpel blades must be new.
 4. Guillotine:
 - a. Guillotines used to perform decapitation must be maintained in good working condition, serviced on a regular basis to ensure sharpness of blades, and cleaned after each use.
 - b. Before each use of a guillotine, it should be checked for rust, lack of visible nicks or other damage to the cutting edges, and cleanliness. The operator should ensure that the action is smooth with no perceptible binding or resistance.
 - c. A record certifying maintenance/sharpening of a guillotine must be maintained. The IACUC recommends sharpening at least annually, however the species involved and the number of animals will dictate how often the blades need to be sharpened.
 - d. Professional blade sharpening services may be used.
 - e. If lubrication of the guillotine is necessary, the use of a Teflon or Silicone containing compound is recommended.
 - f. Old guillotine blades that are no longer serviceable must be discarded in the sharps container.
 - g. The responsibility for sharpening the guillotine rests with the Principal Investigator.
 - h. Guillotines and their maintenance records will be inspected as part of the IACUC semi-annual inspections.
 - i. The IACUC requires that all individuals using guillotines be trained and certified on proper use. Training must be documented.

VII. **Animal Carcass Disposal**

After death has been ensured, place the carcass in a disposable waterproof bag. Seal the bag and place the bagged carcass in one of the barrels found inside one of the cold rooms or freezers designated for animal disposal.

Carcasses that are radioactive must be disposed of according to the procedures stated in the Principal Investigator's Radioactive Use Authorization. Carcasses that are infectious must be disposed of according to the procedures stated in the Principal Investigator's Biological Use Authorization. Carcasses that have chemical contamination must be disposed of according to procedure established during review of the protocol by the Chemical Hygiene Officer.

VIII. **References:**

1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>
2. Guide for the Care and Use of Laboratory Animals, Eight Edition
<https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth>