

EUTHANASIA METHODS
DEFINITIONS, POLICIES AND GUIDELINES

DEFINITIONS:

- Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress.¹

POLICIES AND GUIDELINES

“PHS Policy and USDA Regulations require that an IACUC review and approve the methods of euthanasia which are proposed. These must be consistent with the recommendations of the AVMA Guidelines on Euthanasia or succeeding revised editions, unless there are scientific justifications for alternative methods.” “... criteria used to evaluate the appropriateness of a given method include compatibility with the requirements of the research, reliability, irreversibility, the minimization of distress to animals and persons performing euthanasia, and safety to the latter. The species of animal being used and the qualifications of the investigators are also important considerations. Three categories of methods exist: inhalation and non-inhalation pharmacologic agents, and physical methods.”²

1. Inhalation Agents

- **Carbon Dioxide (CO₂)**

Prolonged carbon dioxide inhalation is an effective and approved method of euthanizing small (less than 800 grams) rodents and small birds when it is done in accordance with the following guidelines. In fact, CO₂ euthanasia has several advantages over other methods of euthanasia. For example, carbon dioxide is a potent central nervous system depressant and thus causes rapid unconsciousness and anesthesia. Carbon dioxide exposure has also been shown to induce analgesia that begins within a few minutes of exposure and lasts for as long as an hour. Carbon dioxide is a relatively inert, inexpensive and easily procured gas that is not very hazardous for exposed humans. Finally, carbon dioxide does not accumulate in or contaminate tissues and has minimal effects on tissue architecture (with the exception of the lungs). Nonetheless, since inhalation of carbon dioxide is known to cause mucosal irritation and thus may cause short-term stress in animals exposed to this gas, a few precautions are warranted.

- a. The current AVMA guidelines (2013) recommend gradually filling of euthanasia chambers at a displacement rate of 10 to 30% of chamber volume per minute. This requires the use of correct equipment to deliver the gas at a pre-defined rate, e.g. an appropriate pressure-reducing regulator and a flow meter or equivalent equipment. The practice of placing animals in a prefilled container of 100% CO₂ is unacceptable.
- b. 100% CO₂ from a compressed gas tank must be used. It is not acceptable to use CO₂ from a dry ice source. The use of CO₂/oxygen mixtures prolongs the time until unconsciousness and death and does not reliably eliminate signs of distress.
- c. CO₂ flow into the chamber should be maintained for at least one minute after animals have stopped breathing, and animals should remain in the chamber for at least one minute after the gas flow has stopped. Regardless of how long the animals are exposed to the CO₂, a secondary physical method (e.g. thoracotomy, decapitation or vital organ removal) must be performed to ensure that the animal is dead.

Note: Rodents under ten days of age are very resistant to the effects of CO₂ and require extended exposure times. Rapid decapitation is recommended for mice and rats under ten days of age. Mice and rats > 10 days of age may euthanasia

be treated as adults.

- **Acceptable Inhalation Agents**

Isoflurane is an acceptable agent with or without pre-medication. As with carbon dioxide, death of the animal must be ensured with a physical method. It is important to minimize potential hazards to personnel by using a fume hood, down-draft table, or other scavenging device.

2. Non-Inhalation Agents

- **Acceptable Non-Inhalation Agents**

Sodium pentobarbital is the most commonly used non-inhalation euthanasia agent. A correct dosage is critical; the euthanasia dose is typically three times the anesthesia dose. Pentobarbital may be delivered intraperitoneally or intravenously in rodents; in larger animals, the agent must be delivered intravenously (or intra-cardiac if the animal already is under anesthesia). A physical method must be used to ensure death.

- **Unacceptable Non-inhalation Agents**

Potassium chloride is unacceptable unless used under anesthesia.

3. Physical Methods

Anesthesia must be used prior to the use of any physical method unless exceptions are approved with proper scientific justification and training.

Physical methods of euthanasia include cervical dislocation, decapitation, exsanguination, and pithing. However, some of these procedures, namely exsanguination, and pithing are not recommended as sole means of euthanasia.

- **Cervical Dislocation**

This method is primarily reserved for euthanasia of mice and neonatal rats.

“Cervical dislocation is acceptable with conditions for mice and rats weighing <200 g. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.”³ Training and documentation by the University Veterinarian in this technique is required.

- **Decapitation**

This method requires scientific justification when used without prior anesthesia. Physical hazard to the investigator must be taken into consideration and it is essential that the equipment be properly maintained with appropriate documentation. Training and documentation by the University Veterinarian in this technique is required.

The American Veterinary Medical Association (AVMA) Guidelines on Euthanasia state that, "The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades." In addition, the IACUC will review guillotine maintenance records during the campus's semimonthly site visits.

- **Physical Methods Of Ensuring Euthanasia**

Following chemical euthanasia, a physical method is required to ensure death. Physical methods include decapitation, thoracotomy; removal of a vital organ, cervical dislocation; and pithing (amphibians).

Footnote 1. Guide for the Care and Use of Laboratory Animals, 8th Edition, page 123

Footnote 2: Institutional Animal Care and Use Committee Guidebook, OLAW and ARENA,
Section C.2.b.

Footnote 3: AVMA Guidelines on Euthanasia, 2013, p. 49