

University of California, Berkeley
Office of Laboratory Animal Care
Euthanasia Guidelines

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The Office of Laboratory Animal Care (OLAC) and the Animal Care and Use Committee (ACUC) use the 2007 American Veterinary Medical Association's (AVMA) Guidelines on Euthanasia as University guidelines. Call the ACUC office at 642-8855 for a copy of this report or download it from www.acuc.berkeley.edu.

Additional clarification and information is provided below for methods of euthanasia commonly used in small mammals, birds and ectotherms.

Please Note: Individuals who receive ACUC approval for rodent euthanasia must receive approval of their technical competence from OLAC staff before performing the procedure without supervision.

Cervical Dislocation

1. When properly performed, manual cervical dislocation is a humane technique for euthanasia of poultry, small birds, mice, rats weighing less than 200 grams, and rabbits weighing less than one kilogram.
2. Although the AVMA Guidelines on Euthanasia recommends sedation or light anesthesia for animals undergoing cervical dislocation, it is recognized that this practice is sometimes inconsistent with the need to obtain data from animals that are free from the effects of exogenous drugs.
3. If cervical dislocation *without pre-sedation* is proposed, the Animal Use Protocol (AUP) must give the scientific justification for this choice and provide documentation of experience and/or training of the person(s) who will be performing the procedure.

Carbon Dioxide Inhalation

1. Carbon dioxide provides rapid depressant and anesthetic effects.
2. Carbon dioxide is nonflammable, non-explosive and poses minimal hazards to personnel when used correctly with properly designed and maintained equipment.
3. Carbon dioxide asphyxiation is a humane technique for euthanasia of rats and mice.
4. Neonates are resistant to the effects of CO₂ and special attention must be taken when euthanizing neonates.

Materials:

- CO₂ tanks and euthanasia chambers are provided for use in the animal facilities at no charge.
- An impervious container with a lid or a double-strength plastic bag.

Methods:

1. Compressed CO₂ gas in cylinders is the only recommended 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.

2. Place animals in the chamber or container and replace the lid or close the opening. The entire cage of animals should be placed in the chamber, without introduction of new animals, whenever possible. Rodents must never be “collected” in a group cage with unfamiliar rodents prior to euthanasia.
3. Turn on the CO₂ to as high flow rate as possible (should optimally displace 20% of the chamber volume per minute).
4. Unconsciousness will occur within 30 seconds, but animals should be left in the container with the gas flowing for an additional 60 seconds. Neonates should be exposed for 5 minutes. Never leave a euthanasia chamber with flowing gas unattended.
5. Prior to carcass disposal, verify animal death by performing a second physical means of euthanasia, e.g., cervical dislocation or bilateral thoracotomy or decapitation (neonates).

Euthanasia of ectotherms

The same principles for euthanasia of rodents apply to ectotherms;

1. A physical method must follow chemical euthanasia, and
2. Physical euthanasia without anesthesia must be justified and approved in the AUP.

Chemical Methods:

1. Pentobarbital
Ectotherms can be euthanized by injecting an overdose of pentobarbital by the intracoelomic route. The actual time to death may be very prolonged and it is critical that a physical method follow.
2. Tricaine methanesulfonate (MS-222), Benzocaine
These agents are administered in water and used primarily for fish, aquatic and semi-aquatic amphibians.
 - Unbuffered MS-222 is acidic and considered to be irritating to the skin of fish and amphibians. A 0.1% (1 g/L) solution of buffered tricaine methanesulfonate may be made by adding 2 g of tricaine methanesulfonate powder to 2 liters of distilled water to which 50 ml of 0.5 M sodium phosphate has been added. This will result in a solution having a pH between 7.0 and 7.4.
 - A minimum 0.03% solution of benzocaine is recommended for euthanasia.

Physical Methods:

1. Freezing
Freezing following cessation of respiration after chemical euthanasia is acceptable. Freezing is NOT allowed as a sole manner 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.
2. 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.
3. Double Pithing
Can only be carried out on unconscious animals and performed by trained personnel.

For information, assistance or certification/training in any euthanasia technique, contact the ACUC office at 642-8855 or OLAC at 642-9232.

References:

Amphibian Medicine and Captive Husbandry, Wright and Whitaker, Krieger Publishing Company, Malabar, Florida, 2001, 115-119.

Report of the ACLAM task force on rodent euthanasia, Toth et al., October 2005.

AVMA Guidelines on Euthanasia, American Veterinary Medical Association, June 2007.