

Office of the Attending Veterinarian Guidelines for Euthanasia of Research Animals

The Guidelines that follow describe approaches which facilitate compliance with the IACUC Policy on Euthanasia of Vertebrate Animals (Policy 103). The Attending Veterinarian has overall responsibility for providing guidance to investigators and animal care personnel regarding recommended and approved methods of euthanasia. The Institutional Animal Care and Use Committee (IACUC) must review and approve all methods of euthanasia proposed as a component of an animal use protocol. It is the responsibility of the Principal Investigator to ensure that personnel are properly trained and proficient in the method of euthanasia to be used.

Animals should not be euthanized in the presence of other animals, particularly animals of the same species (conspecifics).

Prior to euthanizing an animal as a component of an animal use protocol, personnel must ensure that the method of euthanasia to be employed is the same as that described in the approved animal use protocol.

In all cases, death must be ensured. This determination may be made by auscultation for cessation of both heartbeat and respiration by a qualified individual in larger animals or by utilizing an unequivocal secondary means of ensuring death (decapitation, opening thoracic cavity, etc.) following euthanasia with an inhalant agent (anesthetic overdose or CO₂).

Cadavers must be appropriately disposed after euthanasia. In the case of rodents or other small laboratory animals, the cadavers should be placed in leak resistant plastic bags, clearly marked with first and last name of PI and/or the protocol number and placed in the appropriate refrigerator for disposal.

Recommended Methods of Euthanasia:

There are a number of acceptable euthanasia agents and methods with the final specific agent and method of choice dependent upon the experimental design, the species involved, the familiarity of the individual with the specific agent or method, and the availability of the agent or method. The following brief list represents the agents recommended for use in the listed species where no specific experimental requirement exists.

Species	Recommended Method of Euthanasia
Cat	Pentobarbital or pentobarbital containing euthanasia solution ² (100 mg/kg IV)
Dog	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Goat	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Sheep	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Swine	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)

Rabbit	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) w/wo prior tranquilization or anesthesia
Primate	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Hamster	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO ₂
Mouse	Pentobarbital or pentobarbital containing euthanasia solution (150 mg/kg IP), CO ₂
Rat	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO ₂
Guinea Pig	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO ₂
Amphibians	Tricaine methane sulfonate (MS-222) 1-3% solution buffered with sodium bicarbonate or sodium phosphate to a pH of 7.0-7.5
Avians	CO ₂ , Argon

¹ Pentobarbital is a CII controlled drug

² Pentobarbital containing euthanasia solutions are generally CIII controlled drugs

Euthanasia of Rodents using Carbon Dioxide:

The euthanasia method must be appropriate to the species, approved in the animal use protocol and conform to the most current edition of the *AVMA Guidelines for the Euthanasia of Animals*. CO₂ inhalation is the most common method of euthanasia used for mice, rats, guinea pigs, and hamsters.

A few important aspects of this procedure are:

1. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments. Animals from different cages should not be mixed together for euthanasia, since they could fight prior to death.
2. Compressed CO₂ gas in cylinders is the only approved source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled.
3. The *AVMA Guidelines for the Euthanasia of Animals* concludes that “the practice of immersion, where conscious rodents are placed directly into a container prefilled with 100% CO₂, is unacceptable”.

It is preferable that animals are euthanized in their home cage to minimize stress. If this is not possible, the euthanasia chamber must be emptied and cleaned between uses. CO₂ must be supplied from either gas cylinders or building CO₂ gas distribution systems equipped with an appropriate pressure reducing regulator and

flow meter combination (or equivalent) to permit precise regulation of gas flow to the chamber. **The CO₂ flow should be set to displace 30-70% of the chamber volume per minute:**

Flow Rates for 50% Volume Displacement per Minute

Cage Type	Cage Size (W x L x H)	Flow Rate
Mouse w/lid	7" x 11" x 8"	5.0 l/min
Rat w/lid	9.25" x 17.75" x 10.5"	14.1 l/min

After the animals become unconscious, the flow rate can be increased to minimize the time to death.

4. Animals should be left in the container for at least 1 minute after respiratory arrest. Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and the use of a secondary means to ensure death. The secondary methods may include decapitation, cervical dislocation, thoracotomy, etc.
5. Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended⁷. Carbon dioxide may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Keeping neonates warm during CO₂ exposure may decrease the time to death⁵.

The following statement is recommended for inclusion in an Animal Use Protocol for any species where CO₂ euthanasia is to be used:

“Animals will be euthanized by gradual (30-70%/minute) displacement of chamber air with compressed CO₂ delivered through a precision flowmeter. Following [unconsciousness/death] the animals will be subject to [cervical dislocation/decapitation/thoracotomy] as a secondary means to ensure death.”

Euthanasia of Rodents using Physical Methods:

The euthanasia of rodents using physical methods (decapitation, cervical dislocation, etc.) must be approved in the animal use protocol and conform to the most recent edition of the *AVMA Guidelines for the Euthanasia of Animals*.

A few important aspects of this procedure are:

1. These methods may be aesthetically displeasing, however in certain conditions, they may be the most appropriate choice.
2. Personnel using physical methods of euthanasia must be well trained on the method performed to ensure euthanasia is conducted appropriately.

3. Guillotines for adult rodent decapitation are commercially available. For euthanasia of neonatal rodents, sharp scissors/blades can be used for decapitation. Care must be exercised to prevent injuries when using sharp instruments. It is recommended that blades be tested periodically for sharpness on suitable materials such as polyethylene tubing. A sharp blade will cut the test material cleanly with minimal force without dragging it between the blades or showing signs of sticking.
4. As part of the policy (IACUC Policy 103) that is relevant to these guidelines, the equipment used to perform decapitation must be maintained in good working order. Guillotines must be serviced on a yearly basis to ensure sharpness and functionality of the instrument. Service records must be maintained.
5. The use of plastic cones to restrain animals is recommended as they appear to reduce stress, minimize the chance of injury to personnel, and improve positioning of the animal.

Details on the euthanasia of other animals including immature rodents, farm animals, etc. are found in the most recent edition of the AVMA Guidelines for the Euthanasia of Animals. Please consult that document or the Office of the Attending Veterinarian.