

MANUAL DE EUTANÁSIA DO BIOTÉRIO DA UNIFAL-MG

Rat can be euthanized in a variety of acceptable, effective and humane methods. Euthanasia methods can be either chemical or physical.

1 - Adult rodents - Chemical methods

1.1 - CO₂ asphyxiation: In order to minimize stress animals should be euthanized in their home cage. There should be a maximum of: 4 adult rats weighing up to 300 g; 3 adult rats weighing up to 400 g; 2 adult rats weighing up to 500 g; 1 female with 1 litter (pups over 10 days old). Place the appropriate sized lid on the animal cage with grid removed.

Connect the regulator hose to lid fitting.

Do not pre-charge the chamber.

Plug in the heater unit if necessary and available (e.g. if euthanizing many cages)

Open the CO₂ tank valve.

Set the regulator to the appropriate setting:

- Standard mouse cage (7.25" x 11.5" x 5"): 2 LPM (Litres per minute)
- Standard rat cage (12" x 9" x 6"): 5.25 LPM
- Cages of different dimensions:

Measure the cages width, length and height and multiply them to determine the volume in cubic inches. Then divide this by 61 to convert into liters and multiply by 20% to determine flow rate.

height x width x length X 20% = flow rate (LPM)

Once the animals become unconscious, the flow rate can be increased to minimize the time of death. Please note that the time required for euthanasia can be several minutes. Maintain the CO₂ flow until the animal has stopped breathing. Close the valve on the tank. Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.

To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change in eyes.

Following euthanasia by CO₂, physical euthanasia such as cervical dislocation is strongly recommended to ensure death.

1.2 - Barbiturate or injectable anesthetic overdose

Inject three times the anesthetic dose intra-venously or intra-peritoneally.

Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.

To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change in eyes.

A physical method of euthanasia, such as cervical dislocation, is recommended on your animals before disposal to ensure that they have been correctly euthanized.

1.3 - Overdose of inhalant anesthetic

Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanized.

The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate amount of the anesthetic. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Procedures should be conducted in a chemical fume hood to prevent inhalation of the anesthetic by personnel.

The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter through a nose cone.

Sufficient air or O₂ must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a large container, there will be sufficient O₂ in the chamber to prevent hypoxemia.

To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change in eyes.

Following euthanasia by CO₂, physical euthanasia such as cervical dislocation is strongly recommended to ensure death.

2 - Adult Rodents - Physical methods

Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed.

Anesthesia or sedation is necessary prior to physical methods of euthanasia, unless described in the Animal Use

Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

2.1 - Cervical dislocation

For mice and rats under 200g, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a narrow, blunt instrument such as the dull edge of a scissor blade, acrylic ruler or cage card holder is pressed at the base of the skull is pressed at the base of the skull.

With the other hand, the base of the tail or the hind limbs is quickly pulled, causing separation of the cervical vertebrae from the skull.

2.2 - Decapitation

Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.

The use of a plastic conical restraining bag is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

2.3 - Exsanguination

Animals may be exsanguinated to obtain blood products, but only when they are deeply anesthetized or recently euthanized by CO₂ asphyxiation.

Collect blood from the heart.

To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change in eyes.

A physical method of euthanasia, such as cervical dislocation or pneumothorax is recommended on your animals before disposal to ensure that they have been correctly euthanized.

2.4 - Pneumothorax

A pneumothorax can be performed after euthanasia by CO₂ asphyxiation or by barbiturate or injectable anesthetic overdose.

With sharp scissors, the diaphragm is lacerated causing the lungs to collapse therefore insuring the death of the animal before disposing of it.

3 - Neonatal Rodents

Rodents over 10 days old or older can be euthanized by the same procedures as adult rodents.

Rodents under 10 days old must be euthanized by one of the following methods:

3.1. CO₂ asphyxiation

3.2. Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended.

3.3. CO₂ may be used for narcosis of neonatal animals but it must be followed by another method of euthanasia (e.g. decapitation using sharp blades).

3.4. Keeping neonates warm during CO₂ exposure for narcosis may decrease the time to death.

3.5. Barbiturate overdose

- 3.6. Inject 3 times the anesthetic dose IP.
- 3.7. May be followed by a physical method of euthanasia (e.g. decapitation using sharp blades).
- 3.8. Overdose of inhalant anesthetic followed by decapitation
- 3.9. Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations, therefore, alternative methods are recommended.
- 3.10. Inhalant anesthetics may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).
- 3.11. Decapitation
- 3.12. Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.

4 - Gestating Rodents

Gestating rodents with fetuses under 17 days old can be euthanized by the same procedures as adult rodents.




Gestating rodents with fetuses over 17 days must be euthanized by one of the following methods:

- 4.1. CO₂ asphyxiation of the mother, followed by decapitation or barbiturate overdose (IP) of the fetuses.
- 4.2. Overdose of injectable anesthetics to the mother.

Unacceptable Euthanasia Techniques for Rodents

- Decompression
- Asphyxiation
- Air embolism
- Rapid freezing
- Carbon monoxide
- Methoxyflurane
- Ether
- Nitrogen
- Nitrous oxide
- Chloroform
- Chloral hydrate
- Poisons (strychnine, cyanide)
- Household products and solvents (acetone, alcohol)

MOUSE EUTHANASIA

Method of Euthanasia	CO2 Asphyxiation	Barbiturate or Injectable Anesthetic Overdose	Inhalant Anesthetic Overdose	Exsanguination	Cervical Dislocation	Decapitation
 Adult mouse and gestating mouse (under 17 days gestation)	YES	YES	YES	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia
 Gestating mouse (more than 17 days gestation)	YES	YES decapitation of pups not required	YES	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia
Decapitation of pups required after euthanasia of mom						
 Pups under 10 days old	Only as Narcosis	YES	Only as Narcosis	NO	NO	YES

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