



Animal Facility SOP 17.3

Euthanasia SOP– Use of alternatives to CO2 Euthanasia

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I. Purpose and Definition

Describes acceptable methods for euthanasia.

Euthanasia is the act of inducing painless death. Euthanasia methods should provide rapid unconsciousness followed by cardiac or respiratory arrest. Movement does not necessarily indicate consciousness.

Euthanasia methods must be in compliance with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (2013 or current edition) unless specifically justified on scientific grounds and approved by the IACUC.

II. Scope

Applies to investigators, their technical staff, and husbandry staff and any others who euthanize rodents.

iii. Materials and Equipment

- Carbon dioxide chamber and regulator
- Cervical dislocator
- Scissors
- Anesthesia machine with isoflurane supply
- Various anesthetic drugs - depending on protocol
- Guillotine

IV. Procedures

1. Physical Methods

Agent	Dose (milligrams per kilogram)	Route	Comments
Cervical dislocation	N/A	N/A	Suitable only in mice and rats weighing less than 200 grams; acceptable with prior sedation or anesthesia; training in this procedure is required before use;

			exceptions must be approved by IACUC
Decapitation	N/A	N/A	Required for neonatal rodents following sedation or anesthesia (sharp scissors may be used); allowed for mice following sedation or anesthesia; if unable to sedate or anesthetize because chemical residue-free tissues are required, the head should be immediately frozen in liquid nitrogen or dry ice subsequent to severing; exceptions must be approved by IACUC.
Exsanguination	N/A	N/A	Acceptable under anesthesia

2. Inhalant Anesthetics (including carbon dioxide)

Acceptable methods for euthanasia include placing animals directly into a closed, non-pprecharged chamber and gradually filling the chamber with CO₂ at a displacement rate of 10-30% (using a pressure reducing regulator and flow meter) or by placing anesthetized/unconscious animals in a chamber prefilled with CO₂. Carbon dioxide flow should be maintained at least 1 minute after respiratory arrest.

Gaseous euthanasia

Agent	Dose (milligrams per kilogram)	Route	Comments
Inhalant – Carbon dioxide 70-100%	N/A	Inhalation	Safe, inexpensive and effective; tissue changes may be seen; time required to produce death may be prolonged in immature and neonatal animals.
Inhalant - Isoflurane	5% until cessation of respiration for > 1 minute	Inhalation	Must be vented to outside the facility (via chemical fume hood or Class II biological safety cabinet with outside ducted exhaust (B1/B2)) or captured in a carbon canister. Use a non-rebreathing system; nonflammable and nonexplosive; euthanasia easily performed in closed container; may produce changes in parenchymal organs.

All deaths are to be verified; a secondary method must be performed to ensure death (e.g., pharmacologic agent, exsanguination, decapitation, or thoracotomy). In the case of neonatal mice, follow up with a secondary **physical** method (decapitation) is required.

3. Injectable Chemical Agents

The most common chemical agent used to euthanize rodents is sodium pentobarbital (sometimes known as Euthasol). This controlled drug may be delivered either intravenously (IV) or intraperitoneally (IP). In rodents, IP delivery is much more practical.

Sodium pentobarbital should be dosed at ≥ 100 mg/kg. Please check the bottle for concentration information.

4. Terminal Perfusion

Terminal vital perfusion may be performed if described thoroughly in an animal use protocol approved by the MSU IACUC.

Animal Incident Form

https://docs.google.com/document/d/1Q9c8KZEBE-wVPVvcyPiilagFvpbuh7dfR_YBmop4szc/edit?usp=sharing