**Euthanasia of Research Animals**

Last Reviewed: May 2, 2024

**I.  Purpose/Scope**  
The purpose of this standard operating procedure (SOP) is to outline the procedures for euthanasia and to ensure compliance with the [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](https://www.umt.edu/research/compliance/iacuc/vetguidelines/euthanasia.php). This procedure applies to all personnel euthanizing animals in research projects sanctioned by The University of Montana Institutional Animal Care and Use Committee (IACUC).

**II.  Policy**  
It is LAR's policy to meet or exceed all federal, state, and local regulations and guidelines and to comply with all institutional policies and procedures as they apply to the use of animals in research. Personnel must attend any applicable training in animal care and use, occupational health and safety, equipment operation, and SOPs before performing activities outlined in this SOP or working under the direct supervision of a trained LAR staff member.

**III. Animal Identification**

It is the responsibility of the person performing euthanasia to verify the identity of the animal(s) to be euthanatized and to ensure that the principal investigator (PI), designated animal user, or the attending veterinarian (AV) has authorized euthanasia. It is imperative to double-check animal identity before performing euthanasia.

**IV. Selection of Specific Agents**  
The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of the protocol. Generally, inhalants (inhalant anesthetics or CO2) or injectable chemical agents (such as a barbiturate) are preferable to physical methods (such as cervical dislocation or decapitation). Euthanasia is to be performed professionally and compassionately by trained personnel. Death should be confirmed by a secondary form of euthanasia such as cervical dislocation, decapitation, or thoracotomy.

**V. Carbon Dioxide (CO2)**  
This method is to be used for rodents under 600 grams:

* Sign in using the clipboard next to the CO2 unit. Fill out completely including PI name, when you started/ended, number of boxes, AUP #, and number of animals
* Place the animal(s) to be euthanatized in the chamber, inside its primary enclosure, if possible
* Fill the euthanasia chamber with CO2 by turning the tank on - the regulator has been preset for low-flow volume appropriate to the chamber. DO NOT readjust the brass knob.
* When no movement or respiration is detected, turn off CO2
* Remove the animal and check for cessation of vital signs
* Use a secondary mechanical means of euthanasia to ensure death (cervical dislocation, decapitation, or thoracotomy)
* When the animal is confirmed dead, place the animal in sealable carcass bags and label it appropriately (e.g., PI name, strain, wild-type, transgenic, contaminated, etc.)



* Discard into LAR trash cans or appropriate carcass freezer ensuring compliance with NIH disposal guidelines. Refer to the chart.
* Wipe the CO2 chamber with Peroxigard spray after use

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| *Wildtype (WT) with no drug* | *Raptor freezer in HSB* |
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| *Transgenic (TG)/TG with drug* | *Label carcass TG and LAR will dispose of it accordingly (i.e. Incineration)—PROHIBITED from disposal into the waste stream* |
| *Contaminated (any drugs used for surgery or injection)* | *Place in designated freezer and LAR will dispose of accordingly* |
| *Chemically injected carcasses* | *Place them in the designated freezer and LAR will have them picked up by risk management for proper disposal.* |
| *BSL-2* | *Autoclave out and then dispose of in LAR trash* |

*NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (NIH GUIDELINES). DEPARTMENT OF HEALTH AND HUMAN SERVICES. National Institute of Health, Apr. 2019, osp.od.nih.gov/wp-content/uploads/NIH\_Guidelines.pdf.  p.128.*

*\*NIH Appendix M. When an animal covered by Appendix M containing recombinant or synthetic nucleic acid molecules or a recombinant or synthetic nucleic acid molecule-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for animals.*

**VI. IP Pentobarbital Euthanasia for Rodents/Animals**  
This method is used for rodents and animals weighing under 600 grams:

* Inject animal with an IP or IV overdose of pentobarbital
* Place the used needle and syringe in a sharps container (do not recap needle)
* When the animal no longer shows any vital signs, use a secondary mechanical means of euthanasia to ensure death (cervical dislocation, decapitation, or creation of pneumothorax) if appropriate for the species
* When the animal is confirmed dead, place the animal in a sealable carcass bag and label it with PI name, strain, gender, etc.
* Discard carcasses into the appropriate LAR freezer thus ensuring compliance with NIH disposal guidelines

**VII. IV Pentobarbital Euthanasia for Animals Over 600 Grams**  
Pentobarbital is used on larger species such as rabbits, sheep, and non-human primates. Each species requires different dose levels to ensure painless death. It is required that trained personnel perform these procedures, under the direct supervision of the Attending Veterinarian (AV).

* Check with AV for proper dosing requirements. Depending on the research protocol, it may be possible to administer a tranquilizer subcutaneously or intramuscularly 10-15 minutes before injection of the euthanasia solution.
* Use the proper needle and syringe size for the species and drug dose volume
* Euthanasia solution must be obtained from the PI. All doses must be signed out and logged in the controlled substance log book by the PI or their research staff (if an authorized user)
* Use an easily identifiable vein for injection
* Place used needle and syringe into a sharps container (do not recap needle)
* When no movement is detected, check for cessation of breathing and heartbeat using a stethoscope
* When the animal is confirmed dead, place the animal in an appropriately sized cadaver bag with the PI’s name, animal strain, gender, ID, and the euthanasia drug used
* Place the bag in the appropriate LAR carcass freezer