POLICY ON EUTHANASIA, CONFIRMATION OF DEATH, AND CARCASS DISPOSAL

CRITICAL COMPLIANCE CONCEPTS

1. The method of euthanasia used must comply with the 2013 Guidelines for the Euthanasia of Animals and this guidance policy of the IACUC.
2. Euthanasia of rodents with CO₂ must be at a controlled rate of 10-30% per min of their container volume.
3. Death must be confirmed by a second lethal procedure (small animals) or cardiac monitoring in large animals.
4. Carcasses must be disposed of appropriately. Specific disposal practices are required for hazardous carcasses.

Euthanasia is the act of killing animals using humane methods with no or only momentary pain and distress. Euthanasia causes rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, the loss of brain function and death. Euthanasia is often necessary at specific scientific time points. At other times euthanasia is performed when a research animal has lived out its useful lifespan (such as aged breeding mouse). Current regulations require that many animal use protocols provide criteria for humane endpoints when euthanasia is to relieve unrelenting pain or distress. Criteria for humane endpoints delineated in approved animal protocols must trigger euthanasia by either members of the investigative team and/or veterinary staff.

Regulations: The Guide for the Care and Use of Laboratory Animals \(^1\) and the Animal Welfare Act (CFR 9 as amended) require that animals are euthanized using the methods detailed in the 2013 AVMA Guidelines for the Euthanasia of Animals \(^2\) (http://www.avma.org/KB/Policies/Documents/euthanasia.pdf). The particular method chosen must be described in the animal use protocol and approved by the IACUC. Any deviation from the methods of euthanasia described in the 2013 AVMA Guidelines for the Euthanasia of Animals \(^2\) must be scientifically justified and be approved by the IACUC.

Methods: Euthanasia must be performed in a professional and compassionate manner by trained personnel using methods appropriate for the species. Euthanasia must avoid inducing distress in the subject and other animals in the area. The selection of a specific drug and/or method for euthanasia depends upon the animal species and the objectives of the protocol. Vocalization and/or the release of pheromones from animals in distress may induce distress in other animals that are present; therefore, euthanasia should not be performed in the immediate presence of other animals.
1. **Overdose by inhalant or injectable chemical agents.** Generally, inhaled or injected chemical agents (e.g. gas anesthetics, carbon dioxide, barbiturates) are preferable to physical methods (e.g. cervical dislocation, decapitation, and guillotine). Physical euthanasia methods are often performed after euthanasia by chemical methods to confirm death (see below).

   a. **Carbon dioxide** (CO\(_2\)) can only be used to euthanize small rodents (mice, rats, hamsters, gerbils, guinea pigs, chipmunks, squirrels), insectivores (musk shrews), and birds. The University of Virginia does not permit the use of CO\(_2\) for euthanasia of dogs, cats, swine, rabbits, mink, or ferrets. IACUC approval is required to use carbon dioxide to euthanize any non-rodent species.

   CO\(_2\) exposure by gradually increasing ambient concentration in a controlled manner is less likely to cause pain or distress prior to onset of unconsciousness; a CO\(_2\) displacement rate of 10% to 30% of the chamber volume/min is recommended. CO\(_2\) flow should be maintained for at least one minute after respiratory arrest.\(^2\) Our standard rat cages at UVa have an internal volume of 26.15 L, so a CO\(_2\) flow rate of 8.7L/min is the maximum rate (30%) at which they are to be filled to euthanize rats and to euthanize mice when the mouse cage without filter top is placed inside a rat cage. The maximum number of animals euthanized in a mouse box is limited: ≤10 adult mice, ≤15 pre-weanling mice per mouse cage; no more than 2 rats (200g-450g) or one rat >450g per rat cage.

   b. An **overdose of inhalational anesthesia**, such as isoflurane or sevoflurane can only be used to euthanize small rodents and rabbits. Animals need to be exposed for at least three minutes after respiratory arrest to ensure death. If liquid inhalation anesthetics (e.g. isoflurane) are used in a bell jar or other enclosure, animals must not come in physical contact with the anesthetic agent. The waste anesthetic gas must be appropriately scavenged to prevent personnel respiratory exposure to trace anesthetic gases (i.e., performed in a certified chemical fume hood).

   c. **Injectable anesthetics or barbiturates** act quickly to render animals unconscious. Consult with the veterinary staff for the most appropriate drug and dose for the species being euthanized. Barbiturates (euthanasia solution) and some anesthetics are controlled drugs and must be documented accordingly (refer to ACUC Policy on the Use of Laboratory Mixtures or Dilutions of Anesthetics or Analgesics). Only pharmaceutical grade barbiturates can be used for euthanasia unless IACUC approval is received for the use of chemical grade barbiturates. Death must be confirmed by creating a pneumothorax, or using a stethoscope to listen to the heart and confirm it is not beating for at least one minute.

   d. Carbon monoxide or potassium chloride cannot be used as the sole agent of euthanasia for any species.

2. **Physical methods** might have to be used if chemical agents can potentially confound experimental results. The use of physical methods must be scientifically justified and
approved by the IACUC in the animal use protocol.

a. **Cervical dislocation** of small rodents <200g must be performed under anesthesia. Cervical dislocation without anesthesia must be approved in the animal use protocol and must be performed by trained personnel. Cervical dislocation is not permitted in rodents larger than 200gm, even to confirm death.

b. **Decapitation** of small anesthetized rodents must be performed by trained personnel. Specialized guillotines or scissors can be used (refer to the ACUC Policy and Procedures for Euthanasia by Decapitation). Decapitation without anesthesia must be scientifically justified and approved by the IACUC in the animal use protocol.

*Neonatal mice and rats* <5 days of age must be euthanized by decapitation. The use of isoflurane or CO$_2$ is not recommended unless there is at least ten minutes of exposure to cause cessation of respiration.

**Hypothermia and thoracic compression of small wildlife** are not approved methods of euthanasia for any species.

**Euthanasia of Fish, Amphibians, and Reptiles:** Methods for euthanizing these families of animals are provided in the following policies:
- Policy on Anesthesia, Analgesia, Surgery, and Euthanasia of Amphibians
- Policy on Anesthesia, Analgesia, Surgery, and Euthanasia of Fish
- Policy on Anesthesia, Analgesia, Surgery, and Euthanasia of Reptiles

**Confirmation of Death:** After performing **euthanasia by any method, death must be guaranteed.** In rodents, performing a second method of euthanasia is the preferred manner (cervical dislocation in rodents <200gm body weight, decapitation, creation of pneumothorax on both sides of the chest, exsanguination, etc.). In larger animals (rabbit, dog, swine, ferret, etc.), death can be confirmed by cardiac arrest documented by an electrocardiogram or use of a stethoscope to confirm the heart does not beat for at least one minute, decapitation, or creating a bilateral pneumothorax. **For rodents euthanized in animal biosafety level 3 (ABSL3) with CO$_2$ directly injected into a gasketed clamped cage confirmation of death is not required if the rodents remain in the CO$_2$ flooded cage for a minimum of five minutes after cessation of breathing.** This exemption from confirmation of death in ABSL3 is to preclude unnecessary exposure of research personnel to BSL3 pathogens that would occur when performing a secondary physical method of euthanasia.

Investigators may request personnel in the Center for Comparative Medicine euthanize their laboratory animals by submitting a signed “Request for Euthanasia” form (available at https://www.virginia.edu/vpr/ccm/links.html). The form must be submitted at least 48 hours in advance of the euthanasia to be performed. This service is not provided on evenings, weekends or holidays. For this reason, it is imperative that all lab personnel handling animals on weekends/holidays be adequately trained to perform euthanasia in case the need arises.
The Office of Environmental Health and Safety has specific recommendations on laboratory animal carcass disposal which can be found at https://vprgsecure.web.virginia.edu/cold_fusion/help/waste/help_01.cfm

Carcass Disposal: Once an animal is euthanized, and death is confirmed, it is the responsibility of the individual(s) performing the procedure(s) to place the carcass in an appropriately colored opaque bag (see below). Appropriately bagged animal carcasses must be placed in a vivarium carcass storage area: cold room, refrigerator or freezer. Consult the vivarium supervisor if you are uncertain where carcasses are to be placed. Radioactive animal carcasses must be stored in a commissioned area and research personnel must contact Radiation Safety personnel (see below) for pick up.

1. **Non-biohazardous**: Non-biohazardous animal carcasses and tissues (e.g., body parts, blood, or materials containing animal blood) must be placed into opaque bags and put in the appropriate vivarium cold room, freezer or refrigerator designated for carcass disposal. Consult the vivarium supervisor if you are uncertain where carcasses are to be placed. Rodents are placed in small black bags; large animals are placed in three mil thick green bags. Bags for disposal can be obtained from vivaria supervisors.

2. **Biohazardous**: Large animal carcasses and tissues generated from ABSL2 experiments must be placed into a red plastic bag; rodents can be placed in a small opaque black bag marked with the biohazard symbol and returned to the appropriate vivarium cold room, refrigerator or freezer. Consult the vivarium supervisor if you are uncertain where carcasses are to be placed. ABSL2 rodent carcasses must be put in a large red tote or receptacle lined with a red bag denoting biohazard carcasses. Bags for disposal may be obtained from vivaria supervisors. All infectious materials from animal use areas are transported in leak-proof, covered containers and must be surface disinfected before moving the material. In ABSL3 areas, carcasses must be disposed of in accordance with Select Agent inventory procedures that require stringent accounting for each animal on study (outlined in ABSL3 standard operating procedures).

3. **Radioactive**: Radioactive carcasses must be disposed of in accordance with Radiation Safety policy. Radioactive small (rodent) laboratory animal carcasses must be placed in opaque yellow plastic bags clearly labeled on the outside with “Radioactive” warning tape. The laboratory should call the Radiation Safety Office (982-4919) for pickup. Radioactive large animal carcasses must be placed in special radioactive waste yellow bags and boxes provided to the investigator by Radiation Safety, and picked up directly by Radiation Safety personnel. Radioactive carcasses must never to be transferred to vivaria cold rooms or freezers unless the rooms or freezers have been commissioned by Radiation Safety for this purpose.

4. **Chemical**: Animal carcasses laden with certain hazardous chemicals must be disposed of in opaque plastic bags and placed into waste containers located in the vivarium cold room or freezer unless the chemical’s presence requires special handling as described in the PI's protocol. Consult the Chemical Safety Officer in the Office of Environmental Health and Safety with any questions (982-4911).
If the research staff would like (non-radioactive) animals that die unexpectedly to be held for necropsy, tissue collection, etc., they should contact the appropriate vivarium supervisor. These animals will be placed in the appropriate opaque bag, put inside the vivarium cold room and labeled “DO NOT DISCARD” with the investigator’s name, phone number, and date. These will be retained for one week for the lab to obtain before permanent disposal.

References:

2 *AVMA Guidelines for the Euthanasia of Animals (Formerly the AVMA Guidelines for Euthanasia)*, June 2013. ([https://www.avma.org/KB/Policies/Documents/euthanasia.pdf](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)).