POLICY

Animal welfare regulations require that the Attending Veterinarian (AV) shall provide guidance to the Principal Investigator (PI) and staff regarding euthanasia, and that the Institutional Animal Care and Use Committee (IACUC) must approve the method of euthanasia to be utilized for research animals. The method of euthanasia and the criteria used to assess pain and distress in animals must be described in detail in the approved IACUC application. Additionally, in the event that an animal associated with an approved protocol shows evidence of illness or pain requiring emergency care including euthanasia, the AV/designee will exercise professional judgement in the interests and well-being of the animal.

Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort, or distress. The euthanasia method should utilize minimal restraint, be reliable and irreversible, and avoid risk and aversion for personnel. The method must be appropriate for the age and species of the animal, and conform to the most recent AVMA Guidelines on Euthanasia. Any deviations from this policy and/or the AVMA Guidelines must be scientifically justified in writing by the PI and approved by the IACUC. Euthanasia of animals should occur in a procedure room or laboratory, not in an animal housing room.

To ensure a painless death that satisfies research requirements, animals must be euthanized by trained personnel using appropriate technique, equipment, and agents. It is the responsibility of the PI to ensure that personnel performing euthanasia have been properly trained and adhere to IACUC approved protocols and policies. Comparative Medicine offers training to PIs and staff on an as-needed basis.

Following euthanasia, and before disposal, death must be ensured by performing a secondary physical method to ensure that euthanasia is irreversible. Animals found alive after apparent death following euthanasia constitutes serious regulatory non-compliance with the PHS Policy and a serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. Failure to ensure death prior to carcass disposal will result in reporting to oversight and funding agencies, as applicable, and possible sanctions placed on the investigator.

Carcasses are never to be placed into the regular trash. Carcasses are to be securely placed in two leak proof bags. All bags with animal carcasses must be labeled with the following information: IACUC #, Method used to ensure death, Date, and Initials of person disposing of the carcass. The bags are to be placed into a properly labeled dedicated refrigerator, freezer, or cold room that is easily sanitized. Final carcass disposal must be in accordance with federal, state and local regulations.

Animals experimentally treated with hazardous materials must be disposed of according to regulations and guidelines for the agent(s) used.

REQUIREMENTS

The Public Health Service (PHS) Policy in accordance with IV.C. 1. g - Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, unless a deviation is justified for scientific reason in writing by the investigator.

United States Department of Agriculture (USDA) Animal Welfare Act (AWA) 2.31 (d)(5) – Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure or, if appropriate, during the procedure. 2.33 (b)(4) – AV will provide
guidance to principal investigators and other personnel involved in the care and use of animals regarding handling, immobilization, anesthesia, analgesia, tranquilization, and euthanasia.

Office of Laboratory Animal Welfare (OLAW) Guidance NOT-OD-02-062 PHS Policy on Humane Care And Use Of Laboratory Animals Clarification Regarding Use Of Carbon Dioxide For Euthanasia Of Small Laboratory Animals.

American Veterinary Medical Association (AVMA Guidelines for the Euthanasia of Animals), Current - Distress vocalizations, fearful behavior and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, for sensitive species, it is desirable that other animals not be present when individual animal euthanasia is performed.

American Veterinary Medical Association (AVMA Guidelines for the Euthanasia of Animals), Current – Neonatal animals appear to be resistant to hypoxia, and because all inhaled agents ultimately cause hypoxia, neonatal animals take longer to die than adults. Inhaled agents can be used alone in unweaned animals to induce loss of consciousness, but prolonged exposure time or a secondary method may be required to kill the unconscious animal.

The Guide for the Care and Use of Laboratory Animals – Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the current Report of the AVMA Panel on Euthanasia.

ADDITIONAL GUIDANCE


EUTHANASIA OF ADULT RODENTS

1.0 CO₂ Inhalation

1.1 CO₂ inhalation is the most common method of euthanasia used for mice, rats, guinea pigs, gerbils, and hamsters. Compressed gas is the only acceptable source of CO₂ as this allows the inflow of gas to the induction chamber to be controlled.

1.2 Since the anesthetic effects of CO₂ are reversible, animals that are removed prematurely from the chamber prior to death can recover so please follow the procedures below carefully.

1.3 Procedures:

A. Euthanasia chambers should be constructed of clear material and must be kept free of debris and excreta.

B. When possible, rodents should be transported and euthanized in their home cages.

C. Regrouping of animals from multiple cages into a single chamber is not recommended to minimize social aggression.

D. The chamber/cage must not be pre-charged with CO₂.

E. Place the animals in the chamber (if using their home cage- place the euthanasia lid on the cage). Do not over-crowd the chamber/cage. All animals must be able to make normal postural adjustments.

F. Gradually introduce CO₂ at a flow rate of 10-20% of the chamber volume per minute. Sudden exposure of conscious animals to CO₂ concentrations of 70% or greater can be distressful and painful.

G. Once all animals are unconscious, the flow rate can be increased to minimize the time to death.

H. Following CO₂ euthanasia, a physical method to ensure death must be performed prior to carcass disposal.
2.0 Anesthetic Inhalation

2.1 The primary action is central nervous system (CNS) depression. The agents are nonflammable and nonexplosive under ordinary environmental conditions. Occupational exposure to inhalant anesthetics constitutes a human health hazard. (e.g. enflurane, isoflurane)

2.2 Since the anesthetic effects of inhalant anesthetics are reversible, animals that are removed prematurely from the chamber prior to death can recover so please follow the procedures below carefully.

2.3 Waste anesthetic gases must be appropriately scavenged to ensure a safe working environment.
   A. A dedicated exhaust system, charcoal canisters, or a vented fume hood must be used to capture waste gases.

2.4 If using an open-drop system (application of isoflurane to an absorbent material then placed into the bottom of the chamber), the animal must be physically separated from the gas by a physical barrier.

2.5 Procedures:
   A. Chambers used for inhalational anesthetics must be of appropriate size as to prevent overcrowding of the chamber.
   B. All animals must be able to make normal postural adjustments.
   C. Loss of consciousness should be induced rapidly by exposing animals to the maximum agent concentration possible.
   D. Once the animal(s) is/are euthanized, a physical method to ensure death must be performed prior to carcass disposal.

3.0 Injectable Anesthetics

3.1 Injectable anesthetics can be effectively used to anesthetize animals prior to performing a physical method of euthanasia.

3.2 Since the anesthetic effects of injectable anesthetics are reversible, animals can recover so please follow the procedures below carefully.

3.3 Procedures:
   A. Administer the anesthetic overdose as described in the approved IACUC protocol and allow sufficient time for the animal to lose consciousness.
   B. Once the animal(s) is/are euthanized, a physical method to ensure death must be performed prior to carcass disposal.

4.0 Cervical dislocation (Under Anesthesia)

4.1 Cervical dislocation is a humane technique when performed by individuals with a high degree of technical proficiency.

4.2 Cervical dislocation is limited to rodents weighing < 200 grams.

4.3 Cervical dislocation in unanesthetized rodents is permitted only if its use is scientifically justified.

5.0 Decapitation (Under Anesthesia)

5.1 Decapitation when performed properly is nearly instantaneous and is considered humane.

5.2 Guillotines that are designed for decapitation in adult rodents are commercially available. Equipment must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals is recommended.
as it reduces distress from handling, minimizes the possibility of personnel injury, and improves positioning of the animal in the guillotine.

5.3 Decapitation in unanesthetized rodents is permitted only if its use is scientifically justified.
5.4 Refer to the Policy for Guillotine Use and Maintenance for further information.

6.0 Other Acceptable Methods Of Euthanasia In Rodents
   6.1 Microwave Irradiation: microwave irradiation in a specially designed and approved apparatus.
   6.2 IV administration of KCl under anesthesia.
   6.3 IV or IP administration of a barbituric acid derivative.

EUTHANASIA OF RODENT FETUSES
1.0 Mouse, Rat, and Hamster up to 14 days gestation; Guinea Pigs up to 35 days gestation
   1.1 Neural development during this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetuses at this stage of development.

2.0 Mouse, Rat, and Hamster Fetuses over 15 days gestation; Guinea Pigs over 35 days gestation through birth
   2.1 The neural development during this time supports that pain may be perceived. Methods to euthanize include injection of anesthetics or decapitation with sharp surgical scissors or scalpels.
   2.2 If chemical fixation of the whole fetus is required, fetuses must be anesthetized by hypothermia or by injection with an anesthetic prior to immersion in fixative.

EUTHANASIA OF RODENT NEONATES
1.0 Mouse, Rat, and Hamster Neonates up to 10 days of age
   1.1 Acceptable methods include CO₂ inhalation, inhalant anesthetic agents, injection of chemical anesthetics or euthanizing agents, or anesthesia followed by decapitation with sharp surgical scissors or scalpels.
      A. Resistance to hypoxia results in a prolonged exposure time to unconsciousness when CO₂ or inhaled anesthetics is used.
   1.2 The use of CO₂, inhalant anesthetic agents, injectable chemical anesthetics or euthanizing agents requires a physical method to ensure death prior to carcass disposal. Cervical dislocation is an AVMA approved method of euthanasia for neonatal rodents. However, due to the small size of altricial (hairless) neonates it can be difficult to perform aesthetically. Therefore, the IACUC and the Attending Veterinarian require decapitation instead of cervical dislocation as a more aesthetic method of euthanasia for neonatal rats, hamsters and mice up to 10 days of age. If there are specific experimental reasons that cervical dislocation must be used in neonates before eleven days of age, the reasons must be listed in the protocol and reviewed and approved by the IACUC.
   1.3 Additional methods include immersion in, or perfusion with chemical fixatives or immersion in liquid nitrogen. These methods should only be performed if preceded by anesthesia.
   1.4 Anesthesia in neonatal rodents may be induced by inhalant or injectable anesthetics. Prolonged exposure to inhalant anesthetics may be necessary. Alternatively, hypothermia may be used to induce anesthesia in pups 6 days of age or less.
**2.0 Guinea Pig Neonates**

2.1 Follow guidelines for adult rodents.

**EUTHANASIA OF NON-RODENT MAMMALS**

1.0 Intravenous injection of a barbituric acid derivative is the preferred method of euthanasia of non-rodent mammals. Intraperitoneal injection may be used in situations when an intravenous injection would be distressful or even dangerous. Intracardiac injection must only be used if the animal is unconscious, or anesthetized.

2.0 **Once the animal(s) is/are euthanized, a physical method to ensure death must be performed prior to carcass disposal.** Common methods approved by the IACUC include bilateral thoracotomy, exsanguinations, and removal of vital organs.

**EUTHANASIA OF REPTILES/AMPHIBIANS/FISH**

1.0 Ectothermic vertebrates require special consideration because these animals may normally exhibit very low heart rates, are very tolerant to hypoxia, and can hold their breath for long periods of time. Absence of a heartbeat and/or breathing will not necessarily provide confirmation of death. **Once the animal is/are euthanized a physical method to ensure death must be performed prior to carcass disposal.** Common methods approved by the IACUC to ensure death include pithing and decapitation, removal of organs, and exsanguination.

2.0 Use of MS 222:

2.1 The solution must be buffered with sodium bicarbonate to a pH between 7.0-7.5.

2.2 Stock solutions should be protected from light and refrigerated or frozen if possible. The solution must be replaced monthly and any time a brown color is observed.

2.3 Fish: Fish should be left in this solution for at least 10 minutes following cessation of opercular movement. Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills.

2.4 Amphibians: Amphibians should be left in this solution for at least 10 minutes following cessation of movement. MS 222 may also be injected into lymph spaces and or the coelomic cavity with the exception of Xenopus laevis. By itself, intracoelomic injection of MS 222 is not considered to be an acceptable method of euthanasia for X. laevis.

**PHYSICAL METHODS COMMONLY APPROVED BY THE IACUC TO ENSURE DEATH PRIOR TO CARCASS DISPOSAL:**

1.0 Cervical dislocation (rats must be < 200g)

2.0 Decapitation (required for neonatal rats, hamsters and mice < 10 days old)

3.0 Bilateral Thoracotomy

4.0 Exsanguination

4.1 Animals may be exsanguinated to obtain blood products, but only when they are anesthetized.

5.0 Removal of Vital Organs

5.1 Animals may require vital organs to be collected, but only when they are anesthetized.
6.0 Pithing (Fish and Amphibians Only)

CARCASS DISPOSAL:

1.0 Animal users within the UNMC facilities are to follow all applicable UNMC carcass disposal standard operating procedures.

2.0 Animal users within the UNO facilities are to follow all applicable UNO carcass disposal standard operating procedures.

3.0 All carcasses are to be placed in body bags and labeled with the following information: IACUC #, method used to ensure death, date and initials of person disposing of the carcass.

LINKS TO RELATED FORMS, RECORD LOGS, AND SOPS

UNMC Carcass Disposal Standard Operating Procedure – Contact Comparative Medicine

UNO Carcass Disposal Standard Operating Procedure – Contact UNO Animal Care Coordinator