



**Duke University & Duke University Medical Center  
Animal Care & Use Program  
Policy**



## **EUTHANASIA**

**PERFORMANCE STANDARD:** When required for scientific data collection or clinical or operational requirements, animals will be humanely killed, as detailed in the approved protocol.

**BACKGROUND:** The NIH Office of Laboratory Animal Welfare (OLAW) issued clarification regarding the use of carbon dioxide for euthanasia of small laboratory animals (July 17, 2002). The Animal Care Unit of the Animal and Plant Health Inspection Service, U.S. Department of Agriculture, has reviewed and has issued concurrence with the guidance provided by OLAW as it relates to USDA-covered species. Public Health Policy (PHS) requires IACUC's to determine that methods of euthanasia proposed in protocols meet criteria outlined in the 2007 AVMA Guidelines on Euthanasia. Unintended recovery of animals after apparent death constitutes serious noncompliance with PHS Policy and serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. Duke is obligated to report to the NIH any instances of animals awaking after intended death.

### **ROLES:**

1. Research and DLAR staff will abide by the position statement below unless documented PRIOR approval for an exemption is provided by the IACUC.
2. Principal investigators (PI) are expected to follow the methods of euthanasia as outlined and approved in their protocols.
3. All individuals who may perform or be expected to perform euthanasia should be familiar with the details in the protocol and clarifications outlined in this policy. Training in the proper methods of euthanasia is available for any research team member by contacting DLAR veterinary staff at 919.681.6792.

**PROTECTIVE POSTURES REQUIRED:** Personnel Protective Equipment (PPE) routinely required for normal animal care or use is required for euthanasia activities.

## **POLICY:**

1. Any method listed in this policy may be used for humane euthanasia, providing the method has been approved on the protocol.
2. **Humane Euthanasia**, by definition of the Duke Animal Care & Use Program, consists of a primary method and a secondary method. Failure to complete both methods results in failed humane euthanasia. Death must be assured by a second form of euthanasia.
3. Chambers must not be overcrowded. A general rule to use is that all animals must be able to place all four of their feet on the cage floor and when they lie down, they must be able to lie on the cage floor and not on top of other animals.
4. Placing animals directly in the CO<sub>2</sub> chamber is not recommended and requires immediate sanitization of the CO<sub>2</sub> chamber after euthanasia. Animals should be in cages.
5. All individuals responsible for administering CO<sub>2</sub> euthanasia must be qualified and trained to perform euthanasia humanely. Documentation of personnel qualifications and training is reflected in the approved protocol.
6. Species must be separated for euthanasia.
7. Placing live animals in with recently deceased animals is prohibited.
8. Disposal of any carcass or parts (all animals) that are not immediately incinerated or otherwise disposed requires:
  - a. Placing the carcass (or parts) in a plastic bag; and
  - b. Labeling the bag with the date of euthanasia, the PIs name, and the person who performed the euthanasia.

### **9. Acceptable Methods of Primary Euthanasia:**

#### **a. Carbon Dioxide (generated by compressed CO<sub>2</sub> cylinders):**

- i. Carbon dioxide is acceptable for euthanasia in appropriate species (generally rodents).
- ii. Compressed CO<sub>2</sub> gas in cylinders is the only acceptable source of carbon dioxide because the inflow to the chamber can be regulated precisely.
- iii. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (eg, antacids) is unacceptable.
- iv. An optimal flow rate should displace at least 20% of the chamber volume per minute.
- v. Loss of consciousness may be induced more rapidly by exposing animals to a CO<sub>2</sub> concentration of 70% or more, by pre-filling the chamber.
- vi. Gas flow should be maintained for at least 1 minute after apparent clinical death. Animals should be left in the chamber for an additional 2-3 minutes (a total of 5 minutes of exposure to CO<sub>2</sub> is usually adequate for euthanasia via CO<sub>2</sub>).

#### **b. Halogenated agents (e.g halothane, enflurane, isoflurane, or other), with or without nitrous oxide:**

- i. Halogenated agents are acceptable for euthanasia of small animals (< 7 kg).
- ii. Halogenated agents may be provided by a closed pre-charged container (bell jar pre-charged with anesthetic) or by a continuous flow vaporizer.
- iii. Animals must never come in direct contact with the halogenated agent.
- iv. An optimal flow rate should displace at least 20% of the chamber volume per minute.

- v. Loss of consciousness should be induced rapidly by exposing animals to the maximum agent concentration possible.
- vi. Gas flow (if using a vaporizer) should be maintained for at least 1 minute after apparent clinical death.
- vii. Animals should be left in the chamber (if using a bell jar) for an additional 2-3 minutes after apparent clinical death.

**c. Cervical Dislocation (under anesthesia):**

- i. In the absence of tissue procurement, cervical dislocation with anesthesia requires a physical secondary method.
- ii. Cervical dislocation with anesthesia is acceptable euthanasia of poultry, other small birds, and rabbits weighing < 1 kg.
- iii. The effectiveness of dislocation must be verified by separation of cervical tissues. When the spinal cord is severed, a 2-4 mm space will be palpable between the occipital condyles and the first cervical vertebra. Occasionally, however, the dislocation occurs between thoracic vertebrae.
- iv. Check closely to confirm respiratory arrest, and when possible verify, by palpation, that there is no heart beat.
- v. A secondary method of euthanasia is required when performing cervical dislocation under anesthesia.

**d. Decapitation (with anesthesia):**

- i. This technique is conditionally acceptable, and it should be used in research settings when its use is required by the experimental design and approved by the Institutional Animal Care and Use Committee.
- ii. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.
- iii. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

**e. MS 222 (aquatic species):**

- i. Available as tricaine methane sulfonate (TMS), MS 222 can be used for the euthanasia of amphibians and fish.
- ii. Tricaine is a benzoic acid derivative and generally should be buffered with sodium bicarbonate.
- iii. A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution. The stock solution should be stored in a dark brown bottle, and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed.
- iv. For euthanasia, a concentration  $\geq 250$  mg/L is recommended:

- v. Use of 222:
  - 1. 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. 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 pleuroperitoneal cavities.
- vi. Death must be assured by a second form of euthanasia. The IACUC recommends the following methods as secondary methods to ensure euthanasia of aquatics:
  - 1. Pithing (spinal column)
  - 2. Decapitation
  - 3. Removal of multiple organs for tissue procurement
  - 4. Exsanguination

**f. Barbiturates:**

- i. The advantages of using barbiturates for euthanasia in small animals far outweigh the disadvantages. Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia of dogs, cats, other small animals, and horses. 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 heavily sedated, unconscious, or anesthetized.
- ii. The pharmacologic properties and recommended use of combination products that combine sodium pentobarbital with lidocaine or phenytoin are interchangeable with those of pure barbituric acid derivatives. Sodium pentobarbital can be administered intravenously, intraabdominally, or intrapleuroperitoneally in most ectothermic animals, depending on anatomic features.
- iii. Subcutaneous lymph spaces may also be used in frogs and toads. Time to effect may be variable, with death occurring in up to 30 minutes. Barbiturates other than pentobarbital can cause pain on injection.
- iv. A secondary method of euthanasia is required to assure death.

**10. Conditionally Accepted Methods:**

**a. Cervical Dislocation (without anesthesia):**

- i. Manual cervical dislocation can be a humane technique for euthanasia of poultry, other small birds, mice, rats weighing < 200 g, and rabbits weighing < 1 kg when performed by individuals with a demonstrated high degree of technical proficiency.
- ii. The need for technical competency is greater in heavy rats and rabbits, in which the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult.
- iii. This technique should be used only when scientifically justified by the user and approved by the Institutional Animal Care and Use Committee.
- iv. A secondary method of euthanasia is required to assure death.

- b. Decapitation (without anesthesia):**
- i. This technique should be used only when required by the experimental design and approved by the Institutional Animal Care and Use Committee.
  - ii. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.
  - iii. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
- c. Exsanguination (with anesthesia):**
- iv. Because anxiety is associated with extreme hypovolemia, exsanguination alone must not be used as a sole means of euthanasia.
  - v. Animals may be exsanguinated to obtain blood products, but only when they are sedated, stunned, or anesthetized.
- d. Ether:**
- vi. Ether, due to the human safety concerns with its use, is strongly discouraged as a method of euthanasia.
  - vii. Ether should only be used in carefully controlled situations in compliance with state and federal occupational health and safety regulations, and only when scientifically justified by the investigator and approved by the IACUC.
- e. Chloral hydrate:**
- viii. This agent is conditionally acceptable for euthanasia of large animals only when administered intravenously, and only after sedation.
  - ix. Chloral hydrate is not acceptable for dogs, cats, and other small animals because the side effects may be severe, reactions can be aesthetically objectionable, and other products are better choices.
- f. Potassium Chloride:**
- x. Administration of potassium chloride intravenously requires animals to be in a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli.
  - xi. Saturated potassium chloride solutions are effective in causing cardiac arrest following rapid intracardiac or intravenous injection.
- g. Nitrogen and Argon:**
- xii. These gases can be distressful to some species. Therefore, this technique is conditionally acceptable only if O<sub>2</sub> concentrations <2% are achieved rapidly, and animals are heavily sedated or anesthetized.
  - xiii. With heavy sedation or anesthesia, it should be recognized that death may be delayed.
  - xiv. Although N<sub>2</sub> and Ar are effective, other methods of euthanasia are preferable.

## 11. Unacceptable Methods:

- a. **Carbon Dioxide by generation of CO<sub>2</sub> using dry ice** is prohibited by virtue of NIH/PHS policy.
- b. **Barbiturates in combination with neuromuscular agents** as a means of primary euthanasia, is prohibited due to the inability to assess sentience under neuromuscular blockade.
- c. **Nitrous Oxide:** Nitrous oxide (N<sub>2</sub>O) may be used with other inhalants to speed the onset of anesthesia, but alone it does not induce anesthesia in animals, even at 100% concentration. When used by itself, N<sub>2</sub>O produces hypoxemia before respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness.
- d. **Strychnine, Nicotine, Caffeine, Magnesium sulfate, Potassium chloride, Cleaning agents, Solvents, Disinfectants** and other toxins or salts, and all neuromuscular blocking agents are unacceptable.
- e. **Hypothermia** is not an appropriate method of euthanasia.
- f. **Rapid freezing** as a sole means of euthanasia is not humane.
- g. **Avertin** as a sole agent of euthanasia is not acceptable. Avertin may be used as anesthesia for other methods of euthanasia (e.g. cervical dislocation, exsanguination, etc.).
- h. **Ketamine (with Xylazine or Acepromazine)** is not acceptable as a sole agent of euthanasia. These agents may be used as anesthesia for other methods of euthanasia (e.g. cervical dislocation, exsanguination, etc.). The high doses of ketamine required for euthanasia have caused convulsions prior to death.

## 12. Approved Secondary Methods of Euthanasia:

- a. **CO<sub>2</sub> Euthanasia:** The IACUC approves the following methods as secondary methods to ensure euthanasia, when the methods are specified as part of an IACUC approved protocol:
  - i. Bilateral Thoracotomy;
  - ii. Collection of tissues sufficient to assure the animal will not recover; or
  - iii. Exsanguination;Cervical dislocation IS NOT an approved method for secondary euthanasia.
- b. **Other methods of euthanasia:** The IACUC recommends that all methods of primary euthanasia will be followed by a physical secondary method. If no method is specified in the protocol, then bilateral thoracotomy is IACUC approved as a program standard for a secondary method of euthanasia.

13. **Procedures Involving Brain Dead Animals:** Modeling of human clinical events for animal based research will at times produce conditions not addressed by the AVMA Panel on Euthanasia (e.g. brain death through increased intra-cranial pressure, decerebration, etc). The Duke IACUC establishes this position for such circumstances, where animals may be without cerebral function but is being maintained to assure other organ function.

- a. **General guidance:** The Duke IACUC expects continued insentience in the research patient, that insentience be documented for the duration of the experiment, and that at no time is the animal preparation left unattended.
- b. **Definition of brain death:** Brain death is defined as (1) irreversible cessation of circulatory and respiratory functions, or (2) irreversible cessation of all functions of the brain.
- c. **Criteria for assessing brain sentience and function:**
  - i. A clinical neurological examination is the standard for determination of brain death. Specific neurologic parameters vary by species. The selected parameters and will be determined with veterinary consultation as part of the animal use protocol review and approval process. Considerations will be given to the following assessment options:
    1. Absence of motor responses
    2. Absence of pupillary responses to light
    3. Absence of corneal reflexes
    4. Absence of caloric responses
    5. Absence of gag reflex
    6. Absence of coughing in response to tracheal suctioning
    7. Absence of sucking and rooting reflexes
    8. Absence of respiratory drive
    9. Absence of electrical activity (electrical activity absent at levels greater than 2uV)
    10. Absence of signs of cerebral arterial pulsations (Doppler or CT)
    11. Absence of cerebral blood flow (cerebral angiography or nuclear imaging)
  - ii. Two confirmatory tests at least 10 minutes apart are necessary to determine brain death. Determination of brain death is required prior to initiation of USDA category 'D' procedures.
- d. **Monitoring of Brain Death Experimental Subjects:**
  - i. At no time during the study will the animal preparation be left unattended.
  - ii. Experimentation on a brain dead animal involving mechanical ventilation or circulatory assistance (to maintain other organ function), requires continued monitoring using 5 or more assessment tools (may use those defined above or other assessment tools determined in consultation with a veterinarian.).
  - iii. Monitoring must be accomplished at least every 15 minutes.
  - iv. Monitoring must continue from initial anesthesia, through the 'brain death period' and until a terminal method of causing irreversible cessation of circulatory and respiratory function has occurred.
  - v. Monitoring records must be included as part of the animal's medical records (USDA covered species) or the research records (for non-USDA covered species).