

 <p>Institutional Animal Care and Use Committee Guiding Principles and Procedures</p>	<p>Effective: 8/24/2017</p> <p>Revised: 1/21/2021</p>
<p>Title: Euthanasia</p>	<p>Page 1 of 8</p>

## Guiding Principle

The use of animals in research and teaching is a privilege granted to institutions, investigators, staff, and students that commit to meeting high ethical and regulatory standards. Surgery being done for research purposes should therefore meet the requirements of the Guide, USDA Policy, and this guiding principle.

Appropriate euthanasia, death with minimal pain and distress, involves rapid loss of consciousness followed by cardiac or respiratory arrest and the loss of brain function. The method of euthanasia is determined by species, age, weight, health status, skill of personnel, number of animals, and compatibility with the scientific requirements of the study.

“Personnel who perform euthanasia must demonstrate proficiency in the use of the technique in a closely supervised environment. Each facility or institution where euthanasia is performed (whether a clinic, laboratory, or other setting) is responsible for training its personnel adequately to ensure the facility or institution operates in compliance with federal, state, and local laws. Furthermore, experience in the humane restraint of the species of animal to be euthanized is important and should be expected, to ensure that animal pain and distress are minimized.” (AVMA Guidelines for the Euthanasia of Animals: 2020 Edition)

This document establishes policies and provides guidelines for the performance of euthanasia on animal species maintained at North Dakota State University.

## Requirements

**AVMA Guidelines for the Euthanasia of Animals:** 2020 Edition, January 17, 2020

**The Guide, Eighth Edition, page 123,** “Standardized methods of euthanasia that are predictable and controllable should be developed and approved by the Attending Veterinarian (AV) and IACUC. Euthanasia should be carried out in a manner that avoids animal distress.”

**Guide for the Care and Use of Agricultural Animals in Research and Teaching, Third Edition, January 2010, page 14,** “Protocols for euthanasia should follow current guidelines established by the American Veterinary Medical Association (AVMA; [www.avma.org](http://www.avma.org)) and copies of the protocols should be made available to all personnel who euthanize animals.”

**Animal Welfare Act** in accordance with (9CFR Ch. 1), Part 2-Subpart C, 2.31 (C)(xi), “Methods of euthanasia used must be in accordance with the definition of the term set forth in 9CFR Part 1, 1.1 of this subchapter unless deviation is justified for scientific reasons, in writing, by the investigator.

**The Public Health Service (PHS) Policy** in accordance with IV.C.1.g, page 14 “Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, unless deviation is justified for scientific reasons in writing by the investigator.”

**Animal Care Inspection Guide 2015, Policy #3 Veterinary Care, Euthanasia,** “The method of euthanasia should be consistent with the AVMA Guidelines on Euthanasia, the American Association of Zoo Veterinarians (AAZV), Guidelines for Euthanasia of Nondomestic Animals, or the European Commission Working Party documents.....Also note that in accordance with the “Expired Medical Materials” section of this policy, the use of expired euthanasia drugs is considered inadequate veterinary care.”

### **Responsibilities:**

All personnel conducting euthanasia will be trained. Training in specific requirements and methods of euthanasia must be obtained through the AV Office. The procedures should minimize animal pain and distress. The AV has overall responsibility for providing guidance to investigators and animal care personnel regarding recommended and approved methods of euthanasia. The IACUC must review and approve all methods of euthanasia proposed as a component of an animal use protocol. The AVMA Guidelines for the Euthanasia of Animals: 2020 Edition establishes the acceptable methods of euthanasia. Technicians who report that the euthanasia methods process is a major source of personal stress should not be required to administer euthanasia.

### **General Guidelines:**

Animals being euthanized should not experience pain, fear, or other significant stress prior to their death. In some instances this may require that the animals be rendered unconscious through some other painless method prior to euthanasia. Animals should not be euthanized in the presence of other animals, particularly animals of the same species.

In all cases, death must be ensured. This determination may be made by auscultation for cessation of both heartbeat and respiration by a trained individual in large animals, or by utilizing a secondary means of death (decapitation, pneumothorax, etc.) following euthanasia with an inhalant agent (anesthetic overdose or CO<sub>2</sub>) in rodent species.

Prior to euthanizing an animal as a component of an animal use protocol, study personnel must ensure that the method of euthanasia used is the same as that described in the approved animal use protocol.

Animals should be appropriately disposed of after euthanasia. Animals should be taken to the veterinary diagnostic laboratory for disposal or properly composted.

### **Methods:**

According to the American Veterinary Medical Association Guidelines for Euthanasia of Animals: 2020 edition, the methods of euthanasia are categorized as:

- Acceptable methods -those that consistently produce a humane death when used as the sole means of euthanasia.
- Acceptable with conditions -those that by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in scientific literature, and;
- Unacceptable - those techniques that are deemed inhumane under any conditions or pose a substantial risk to the person applying the technique.

There are also adjunctive methods, which are methods that cannot be used as the sole method of euthanasia, but can be used in conjunction with other methods to produce a humane death.

### **Acceptable Methods (AVMA Panel 2020)**

*The following descriptions are not inclusive, but represent the primary methods of euthanasia used at NDSU.*

*Barbiturate overdose* - Overdosing with a commercial injectable euthanasia solution or injectable sodium pentobarbital (100 mg/kg) can be used to euthanize all animal species. It is the preferred method of euthanasia for dogs, cats, small animals and horses. The recommended dosage for euthanasia is found in the product label information. These substances can be administered intravenously or intraperitoneally. **Do not** administer these substances subcutaneously or intramuscularly.

Because these are controlled substances, they must be procured with a DEA license held by an individual at the physical location where the substance is stored and used; detailed records of use must be kept. Additional information regarding the use of controlled substances can be found in the NDSU Pharmaceutical Management of Controlled and Non-Controlled Drugs Guiding Principle.

*Carbon Dioxide (CO<sub>2</sub>) overdose* - Compressed CO<sub>2</sub> gas in cylinders must be the source of CO<sub>2</sub> because the inflow (flowmeter needed) into the euthanasia chamber must be precisely regulated. The chamber is preset to deliver CO<sub>2</sub> at a rate of 30%-70% displacement of room air per minute but NOT pre-filled. Once animals are placed in the chamber, the cycle is started and when completed the animals can be removed. This method can be used on birds and all rodents including neonates.

*Inhalant Anesthetic overdose* - Inhaled anesthetics can be administered as part of a 2-step process where animals are first rendered unconscious through inhaled anesthetic agent (isoflurane preferred) exposure followed by a secondary method. All inhalation anesthetics must be used under a fume hood or with approved gas scavenging equipment. If done under a fume hood, the animal(s) are placed in a closed chamber containing cotton or gauze soaked with an appropriate amount of anesthetic. The vapors are inhaled until respiration ceases and is followed by death. The liquid state of most anesthetics is irritating; therefore animals should not be allowed to come in contact with the anesthetic soaked cotton or gauze.

*Cervical Dislocation* - This procedure is only for use in avian species and rodents under 200 grams body weight. This method is humane when applied by individuals with a demonstrated high degree of technical proficiency.

For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.

For poultry and other birds, the legs of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious. This method is best conducted on sedated or anesthetized animals. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.

*MS-222 (Tricaine methanesulfonate) overdose* - Fish and some amphibians and reptiles can be overdosed by immersion in a solution of MS-222.. Animals should be placed in the solution until immobilized and then death assured by an adjunctive method. Immersion of

fish in solutions of MS-222 for 30 minutes following loss of rhythmic opercular movement is sufficient for euthanasia of most fish.

Benzocaine hydrochloride (gel and solutions) – Benzocaine base, a compound similar to MS 222 are acceptable agents for euthanasia for fish and amphibians.

*Clove Oil* - Clove oil, isoeugenol, and eugenol are acceptable agents of euthanasia for finfish. It is recommended that, whenever possible, products with standardized, known concentrations of essential oils be used so that accurate dosing can occur. These agents are not acceptable means of euthanasia for animals intended for consumption.

*Captive Bolt* - Penetrating captive bolt applies concussion and trauma to the cerebral hemisphere and brainstem. This method is preferred for ruminants and swine. Effective use results in immediate collapse, tetanic spasm, hind limb movement and loss of corneal reflex. Captive bolt devices must be used by trained individuals in appropriately restrained animals. Proper maintenance and cleaning are essential to use of the device.

Nonpenetrating captive bolt does not penetrate the brain and only stuns the animal. It should not be used as the sole means of euthanasia and should be followed by an appropriate adjunctive method.

#### **Acceptable Adjunctive Methods:**

*Exsanguination* - Death can be assured by the removal of a large volume of blood. This technique is never performed on a conscious animal. Animals may be exsanguinated to obtain blood products, but only as an adjunctive method (following sedation, anesthesia, or captive bolt).

*Pneumothorax* - To create a pneumothorax on an anesthetized or unconscious animal, a cut is made bilaterally through the chest wall using scissors or a scalpel blade or the diaphragm can be lacerated.

*Pithing* - pithing is used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species, such as frogs, with anatomic features that facilitate easy access to the CNS, pithing may be used as a sole means of euthanasia, but an anesthetic overdose is more suitable. Pithing in ruminants is performed by inserting a pithing rod or tool through the entry site produced in the skull by a captive bolt device. The operator manipulates the pithing tool to substantially destroy both brainstem and spinal cord tissue. This method should not be used in ruminants intended for food because of possible contamination of the meat with specified risk materials

#### **NDSU Species Recommended Methods of Euthanasia**

*Below are preferred methods of euthanasia. Additional AVMA approved methods of euthanasia would also be acceptable. Methods of euthanasia will be reviewed and approved by the IACUC on a protocol by protocol basis.*

**Cat** - Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)

**Dog** - Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)

**Goat** - Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) or penetrating captive bolt followed by adjunct method,

**Sheep** - Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) or penetrating captive bolt followed by adjunct method

**Dairy and Beef Cattle** - Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) or penetrating captive bolt followed by adjunct method

**Horse** - Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)

**Swine** –

- Adult sows/boars, grower/finisher pigs: Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) or penetrating captive bolt followed by adjunct method;
- Nursery Pigs: (70 lb or Lighter) nonpenetrating captive bolt or anesthetic overdose.
- Suckling Pigs: nonpenetrating captive bolt; anesthetic overdose; or blunt force trauma.

**Mouse** - Pentobarbital or pentobarbital containing euthanasia solution (150 mg/kg IP) or Carbon Dioxide

**Rat** - Pentobarbital or pentobarbital containing euthanasia solution (150 mg/kg IP) or Carbon Dioxide

**Amphibians** - Tricaine methane sulfonate (MS-222), 1-3% solution buffered with sodium bicarbonate or sodium phosphate to a pH of 7.0-7.5. Whole body cooling followed by freezing may be appropriate in some species, and will be considered on a case by case basis after discussion with the AV, PI and expert consultants. In cases where physiologically active tissues are required, pithing and decapitation may be used per AVMA guidelines. Inhaled anesthetics such as isoflurane are acceptable, however, death must be verified by a second lethal procedure.

**Reptiles:** Acceptable methods include sodium pentobarbital (60 to 100 mg/kg IV, intracoelomically or in lymphatic spaces; and buffered MS 222 in the coelomic cavity. Whole body cooling followed by freezing may be appropriate in some species, and will be considered on a case by case basis after discussion with the AV, PI and expert

consultant(s). Inhaled anesthetics such as isoflurane are acceptable, however, death must be verified by a second lethal procedure.

**Avian** – Carbon Dioxide. Methods of euthanasia (i.e. thoracic compression) in field studies may be allowed when performed by trained individuals

**Gunshot** – *use of firearms is dependent upon location and the training of the individual performing euthanasia*

### **Controlled Substance Use**

Controlled substances must be purchased, stored, and utilized according to the [U.S. Department of Justice Drug Enforcement Administration Regulations](#).

A list of controlled substances can be found at:

[https://www.deadiversion.usdoj.gov/schedules/orangebook/c\\_cs\\_alpha.pdf](https://www.deadiversion.usdoj.gov/schedules/orangebook/c_cs_alpha.pdf)

Commonly used controlled substances used for euthanasia purposes include

- Pentobarbital-Schedule II
- Pentobarbital containing euthanasia solutions (e.g. Euthasol)-Schedule III

### **Logs:**

Euthanasia logs are required for all NDSU animal facilities. Templates are provided by the IACUC Office. The templates can be found on the IACUC Forms page as well as the NDSU IACUC Guidelines page. Study and/or facility specific templates can be created but must include the information listed on the IACUC Office templates.

### **References:**

Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 8th ed., 2011.

<https://www.aalac.org/resources/theguide.cfm>

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, January 17, 2020

<https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx>

Report of the ACLAM Task Force on Rodent Euthanasia

[https://www.aclam.org/Content/files/files/Public/Active/report\\_rodent\\_euth.pdf](https://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf)

Guide for the care and use of agricultural animals in research and teaching

[https://www.aalac.org/about/Ag\\_Guide\\_3rd\\_ed.pdf](https://www.aalac.org/about/Ag_Guide_3rd_ed.pdf)

Drug Enforcement Administration Diversion Control Division

<https://www.deadiversion.usdoj.gov/index.html>

Shine, R., Lesku, J. A., Lillywhite, H.B., (2019). Assessment of the cooling-then-freezing method for euthanasia of amphibians and reptiles. *Journal of the American Veterinary Medical Association*, 255(1), 48-50.