

 <p>Institutional Animal Care and Use Committee Guiding Principles and Procedures</p>	<p>Effective: 8/24/2017</p> <p>Revised:</p>
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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.

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: 2013 Edition, February 2013

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) 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: 2013 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 CO₂) 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: 2013 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 2013)

Barbiturate overdose - Overdosing with a commercial injectable euthanasia solution or injectable sodium pentobarbital (100 mg/kg) can be used to euthanize all animal species. 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 (CO₂) overdose - Compressed CO₂ gas in cylinders must be the source of CO₂ because the inflow (flowmeter needed) into the euthanasia chamber must be precisely regulated. The chamber is preset to deliver CO₂ at a rate of 10% - 30% 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.

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. To perform on rodents, place a closed scissor or other similar object firmly across the animal's neck at the base of the skull and

quickly perform cervical dislocation by grasping the tail near the base and sharply pulling away from the body while applying pressure in a downward direction on the scissor. The animal should immediately become unresponsive, although some involuntary muscle activity may persist. 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.

MS-222 (Tricaine) or Benzocaine overdose - Fish and amphibians can be overdosed by immersion in a solution of MS-222 at a concentration of 1-3 gm/liter of tap water buffered to pH 7.0 – 8.0 with sodium bicarbonate (1 – 4 g/L) or Benzocaine hydrochloride at a concentration >250 mg/liter of tap water. Animals should be placed in the solution until immobilized and then death assured by an adjunctive method.

Inhalant Anesthetic overdose - This method is recommended for neonates but must be followed by an adjunctive method. 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. All inhalation anesthetics must be used under a fume hood or with approved gas scavenging equipment. Inhalation anesthetics for euthanasia are listed in order of preference: isoflurane, halothane, sevoflurane, enflurane, methoxyflurane, and desflurane, with or without N₂O.

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. 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.

Decapitation - This technique when performed on amphibians or rodents must be preceded by sedation or anesthesia. The sedation must take effect prior to the procedure.

Decapitation requires the use of specially designed equipment (guillotines) that must be maintained to ensure that the sharpness of the blades. The operator must be trained in the use of the guillotine and the handling and restraint of the animals when performing this technique.

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 - This method is only to be used in amphibians. The animal must be anesthetized or unconscious. Pithing involves the severing of the spinal cord at the base of the skull. A needle, sharp probe or #11 scalpel blade can be used dependent on the size of the animal.

NDSU Species Recommended Methods of Euthanasia

Below are recommended 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

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

Euthanasia of Rodents using Carbon Dioxide:

https://oacu.oir.nih.gov/sites/default/files/uploads/ arac-guidelines/rodent_euthanasia_pup.pdf

Euthanasia of Rodent fetuses and neonate:

https://oacu.oir.nih.gov/sites/default/files/uploads/ arac-guidelines/rodent_euthanasia_pup.pdf

Rat - Pentobarbital or pentobarbital containing euthanasia solution (100 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

Avian – Carbon Dioxide

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

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.aaalac.org/resources/theguide.cfm>

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition, February 2013

<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

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

https://www.aaalac.org/about/Ag_Guide_3rd_ed.pdf

Drug Enforcement Administration Diversion Control Division

<https://www.dea.gov/diversion/index.html>