

University of Notre Dame Institutional Animal Care and Use Committee Policy for Euthanasia

Definition

The term, euthanasia, is derived from the Greek terms *eu* meaning good and *thanatos* meaning death. Euthanasia therefore is a 'good death' which would be one with minimal pain and distress. The criteria involved in achieving euthanasia include a rapid loss of consciousness, followed by cardiac or respiratory arrest and the loss of brain function. In addition the method should minimize the distress and anxiety experienced by the animal prior to loss of consciousness. The method of euthanasia is determined by the following factors: species, age, weight, health status, skill of personnel, number of animals, and compatibility with the scientific requirements of the study.

Considerations

Euthanasia is to be done in the most humane manner possible and within the scientific limits of the study. The University of Notre Dame adheres to the guidelines for euthanasia described in the American Veterinary Medical Association Guidelines for Euthanasia of Animals: 2013 edition.¹ When performing euthanasia, it is necessary to acknowledge the human-animal bond. Because attachments can occur in the laboratory setting, the euthanasia process should be fully understood, the persons performing euthanasia trained and proficient, and the selected method appropriate for the species. To accommodate individuals having strong feelings concerning euthanasia, the FLSC staff is available for assistance.

Sensitivity and Conduct

1. It is the responsibility of the individual performing euthanasia to assure that the animal intended for euthanasia is the correct animal.
2. Whenever possible, animals should be euthanized out of the sensory range of other animals, particularly conspecifics. **Do Not** euthanize animals in the animal rooms- animals must be removed to one of the procedure rooms.
3. Handle animals gently to minimize stress. Animals must not be restrained in a painful position prior to euthanasia.
4. In animals that are not used to being handled, calming may be accomplished by minimizing visual, auditory, and tactile stimulation.
5. Stress and distress during euthanasia is to be avoided. When using CO₂, animals ideally should be euthanized without disruption of social groups or removal from the home cage when a group is to be euthanized. When possible, inhaled agents should be administered under conditions where animals are most comfortable (for rodents, in the home cage). If animals must be combined, they should be of the same species and compatible cohorts, and, if needed, restrained or separated so that they will not hurt themselves or others. Do not combine cages of males as the addition of strange animals to the cage causes stress due to unfamiliar pheromones and can lead to fighting.
6. All rodent euthanasia will be performed using the Euthanex Euthanasia chambers (large or portable models) unless scientifically justified. The Freimann Life Science Center's SOP for use of the Euthanex Euthanasia Chambers is to be followed for all euthanasia of rodents including mice, rats, hamsters, gerbils, and guinea pigs. This applies to adults and neonates 7 days of age or older.
7. Euthanasia performed with CO₂ followed by cardiac exsanguination will use the Euthanex cage top following the Freimann Life Science Center's SOP.
8. **Training on the use of the Euthanex chamber is required and will be scheduled through the Technical Services and Training Coordinator or the Facility Manager prior to personnel performing CO₂ euthanasia. Assistance is available from FLSC staff upon request.**

Methods

The methods are categorized as acceptable, acceptable with conditions and unacceptable according to the American Veterinary Medical Association Guidelines for Euthanasia of Animals: 2013 edition. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia. Acceptable with conditions methods are those that by the nature of the technique or because of greater potential

for operator or safety hazards might not consistently produce humane death or are methods not well documented in scientific literature. Unacceptable methods are 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. **All persons performing euthanasia must be trained and proficient in the technique(s) employed.**

Acceptable Methods

1. **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. Because these are controlled substances, they must be procured with a DEA license held by the investigator. These preparations can be administered intravenously or intraperitoneally. Do not administer these compounds subcutaneously or intramuscularly.
2. **Carbon Dioxide (CO₂) overdose** - Compressed CO₂ gas in cylinders must be the source of CO₂ because the inflow into the euthanasia chamber can be precisely regulated. The euthanasia chamber is defined as the Euthanex Euthanasia Chamber (large or portable model). 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. Use of the Euthanex Chamber on the preset cycles results in a guaranteed death thus cervical dislocation or bilateral pneumothorax is not necessary. The chamber **must** be disinfected after use. This method can be used on birds and all rodents including neonates. Cervical dislocation or another adjunctive method is required when using any other CO₂ delivery method as allowed by exception on an approved animal protocol.
3. **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. It is recommended that animals be sedated or anesthetized prior to cervical dislocation. 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 chickens, 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. All persons performing cervical dislocation must be trained by or demonstrate technical proficiency to FLSC veterinary staff.
4. **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 such as removal of the heart or exposure of the coelomic cavity.
5. **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 inhalation anesthetic. The vapors are inhaled until respiration ceases and is followed by death. Because the liquid state of most anesthetics is irritating, animals should be prevented from contact with the cotton or gauze in the chamber. Inhalant anesthetic exposure is considered a human health hazard. 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, sevoflurane, enflurane, methoxyflurane, and desflurane, with or without N₂O. Nitrous oxide should not be used alone.
6. **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.

Acceptable with Conditions Method

Decapitation - This method can be used for rodents and allows for the recovery of tissues and body fluids that are chemically uncontaminated as well as anatomically undamaged brain tissue. Decapitation without sedation or anesthetic must be scientifically justified. Plastic restraint cones are recommended when decapitating adult rodents to reduce the stress that accompanies the restraint in conscious animals. Decapitation of adults requires the use of specially designed equipment (guillotines) that must be maintained to ensure that the sharpness of the blades. Decapitation of neonates 6 days of age or younger is permitted using scissors. The operator must be trained by FLSC veterinary staff in the use of the guillotine or scissors and the handling and restraint of the animals when performing any decapitation.

Adjunctive Methods

When using techniques other than cervical dislocation or the Euthanex Chamber, an adjunct method must be included to assure death and prevent resuscitation. Approved adjunctive methods are listed below.

1. **Cervical Dislocation** - See as described under Approved Methods.
2. **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 when they are sedated or anesthetized.
3. **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 bilaterally. The heart can also be cut or removed to ensure death.
4. **Double Pithing** - This method is only to be used in amphibians. The animal must be anesthetized or unconscious. Pithing is the insertion of a needle, sharp probe or #11 scalpel blade into the brain. Double pithing involves the severing of the spinal cord at the base of the skull. A needle, sharp probe or #11 scalpel blades can be used dependent on the size of the animal. Other less commonly used methods may be acceptable if approved by the IACUC.
5. **Decapitation** - This technique when performed on amphibians must be preceded by sedation or anesthesia. The sedation must take effect prior to the procedure and pithing performed afterwards. 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.

Unacceptable Methods

There are several techniques that have been identified as unacceptable in the American Veterinary Medical Association Guidelines for Euthanasia of Animals: 2013 edition. They are listed below:

1. **Thoracic Compression** –Thoracic (cardiopulmonary, cardiac) compression is a method that has been used by biologists to terminate the lives of wild, small mammals and birds mainly under field conditions when other methods are not available. Based on current knowledge of avian physiology and euthanasia, thoracic compression can result in significant levels of pain and distress before animals become unconscious, thus lacking key humane considerations that can be addressed by other methods. Thoracic compression is an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient due to other reasons.
2. **Slow chilling or freezing** of unanesthetized animals, including placing finfish into a freezer without prior anesthesia, is also an unacceptable method.
3. **Metomidate** – Metomidate has been used for euthanasia of some finfish species, its listing in the Index of Legally Marketed Unapproved New Animal Drugs for Minor Species by the FDA (with a specified use for sedation/anesthesia) means that its extralabel use for euthanasia is currently illegal.
4. **Chloral hydrate and α chloralose** – Are not acceptable euthanasia agents because the associated adverse effects may be severe, reactions can be aesthetically objectionable, and other products are better choices.
5. **Oral Route** – The oral route is generally unacceptable as a sole means of euthanasia with any agent, but may be an appropriate way to deliver sedatives prior to administration of parenteral euthanasia agents.
6. **Topical Route** - Currently there are no topical euthanasia agents that are US FDA approved for any species.
7. **Automotive Exhaust** – Carbon Monoxide from automotive exhaust is not acceptable as a means of euthanasia in any circumstance.

Disposal

Prompt disposal of animal carcasses and tissues is required. There are several sizes of bags available in the procedure rooms of FLSC. All carcasses to be autoclaved will need to be placed in a small autoclavable biohazard bag, which are located in room 464 or on the bottom shelf in room 467. Should the animal carcass need to be autoclaved prior to disposal, FLSC staff should be notified.

1. Select a bag appropriate for the size or number of animals.
2. All vertebrate animals must be placed in the carcass freezer for disposal. Do not place vertebrate animals in trash receptacles!
3. The carcass is placed in a plastic carcass bag and sealed with tape or knotted in the case of large bags.
4. Do not include any paper or other materials in the carcass bag.
5. The bags are placed in the carcass freezer in a red biohazard bag.
6. Do not place experimental animal carcasses in the wire baskets.
7. Disinfect the euthanasia chamber and instruments using Nolvasan® Surgical Scrub. Rinse well and allow to air dry.
8. Clean work surface with the spray disinfectant provided in the procedure rooms.

Table of Methods for Euthanasia

Species	Acceptable	Acceptable with Conditions	Adjunctive
Amphibians	injectable barbiturate, MS-222, benzocaine		Pithing, decapitation with pithing
Birds	injectable barbiturate, CO ₂ , cervical dislocation		exsanguination, cervical dislocation
Fish	injectable barbiturate, MS-222, benzocaine	decapitation with pithing	pithing
Nonhuman Primates	injectable barbiturate	inhalation anesthetics, CO ₂	
Rabbits	injectable barbiturate, inhalation anesthetics	cervical dislocation(<1kg) decapitation, exsanguination with sedation/anesthetic	pneumothorax, exsanguination
Rodents	injectable barbiturate, inhalation anesthetics, CO ₂ , cervical dislocation (rats <200gm)	decapitation	pneumothorax, exsanguination, cervical dislocation

1. The American Veterinary Medical Association Guidelines for Animal Euthanasia: 2013 Edition is on file in FLSC and can be found on the FLSC web page (www.nd.edu/~ndflsc/SOPDownloads.html) .