University of Notre Dame
Institutional Animal Care and Use Committee
Standard Operating Procedure 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 2007 American Veterinary Medical Association Guidelines on Euthanasia.[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. Overcrowding of the CO_2 chamber is to be avoided. The FLSC limit of animals in the translucent chambers are as follows:
   - 8 adult mice or 12 immature mice
   - 3 adult rats or 5 weanlings
   - 1 guinea pig
   - 1 chicken
   - 2 gerbils
   - 1 hamster
   - Adult is defined as 8 weeks of age or older
   - Immature/ Weanling is defined as 2 to 7 weeks of age
   - The FLSC limit of animals per cage in the opaque chambers are as follows:
   - No more than double the maximum limit of animals permitted for housing in the cage:
   - 10 mice per JAG cage, 8 mice per clear shoe box cage
   - No more than 2 females with litters per cage.
   - Do not combine cages of mature males - to avoid fighting.
6. **Training and/or assistance is available from FLSC staff upon request.**

Methods

The methods are categorized as acceptable and conditionally acceptable and unacceptable according to the 2000 Report of the American Veterinary Medical Association Panel on Euthanasia. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia. Conditionally acceptable 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 through FLSC. These preparations can be administered intravenously or intraperitoneally. Do not administer these compounds subcutaneously or intramuscularly.
2. Carbon Dioxide (CO_2)overdose - Compressed CO_2 gas in cylinders must be the source of CO_2 because the inflow into the euthanasia chamber can be precisely regulated. The euthanasia chamber is defined as 1) A translucent plastic 8 liter container & lid with a pre-drilled hole on the side of the container near the top or 2) An opaque plastic container with a lid designed to accommodate either a small shoebox mouse cage or a standard AW rat cage, having a pre-drilled hole near the middle of the lid. The chamber should be slowly filled but **NOT** pre-filled. Do not overcrowd the animals in the chamber. Once placed in the chamber, the animal(s) should quickly succumb. If the animal(s) continue to move additional CO_2 should be added slowly. The chamber must be disinfected after use. This method can be used on birds and all rodents (except neonates). Cervical dislocation or an adjunctive method is required after respiratory arrest.
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. In lieu of demonstrated technical competence, animals must 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. The animal should immediately become unresponsive, although some involuntary muscle activity may persist. For chickens, the animal is firmly grasped around the body with one hand and the head with the other. The neck is extended and cervical dislocation performed by quickly twisting the head. This method is best conducted on sedated or anesthetized animals.
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 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 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: halothane, enflurane, isoflurane, sevoflurane, desflurane.

Conditionally Acceptable Methods

1. 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 rodents to reduce the stress that accompanies the restraint in conscious animals. This technique can also be performed on amphibians. Again, sedation or anesthesia must be used 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.

Adjunctive Methods

When using techniques other than cervical dislocation, an adjunct method must be included to assure death and prevent resuscitation. Acceptable 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. The heart can also be cut or removed to ensure death.
4. 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.

Other less commonly used methods may be acceptable if approved by the IACUC.

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. The carcass is placed in a plastic carcass bag and sealed with tape.
3. The bags are placed in the carcass freezer in a red biohazard bag.
4. Do not place experimental animal carcasses in the wire baskets.
5. Disinfect the euthanasia chamber and instruments using Nolvasan® Surgical Scrub. Rinse well and allow to air dry.
6. Clean work surface with the spray disinfectant provided in the procedure rooms.

Methods for Euthanasia

Species	Acceptable	Conditionally Acceptable	Adjunctive
Amphibians	injectable barbiturate, MS-222, benzocaine	decapitation with pithing	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 2007 American Veterinary Medical AssociationGuidelines on Euthanasia is on file in FLSC and can be found on the FLSC web page (www.nd.edu/~ndflsc/SOPDownloads.html) .**

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