Title: Euthanasia of Teaching and Research Animals
Policy Number: 019
Policy Intent: The purpose of this policy is to describe approved methods for animal euthanasia, while avoiding or minimizing animal discomfort, distress, and pain.

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1. Introduction

Performing euthanasia correctly is an ethical imperative. Proper euthanasia is quick, minimizes pain/distress and reliably results in death. Practical issues such as degree of technical difficulty, time required to perform the procedure, readily available equipment/resources to perform the procedure, as well as aesthetics and human emotion must be considered. Standardized guidelines for humane euthanasia are detailed in the 2020 AVMA Guidelines on Euthanasia and available HERE.

2. Policy

A. The term euthanasia means “good death”. Therefore, euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness. The IACUC endorses the AVMA guidelines as the recognized authority with respect to animal euthanasia. The IACUC also recognizes that certain research parameters dictate that alternative techniques may need to be evaluated by, and approved by the committee.
B. Humane Killing: the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia. These acknowledgements are not intended to condone a lower standard for the humane termination of wildlife. The best methods possible under the circumstances must be applied, and new technology and methods demonstrated to be superior to previously used methods must be embraced.

3. Responsibility

A. University veterinarians oversee all aspects of animal health, and are assisted by Animal Care Services (ACS) technicians and staff.
B. Facility Managers ensure implementation of all methods and procedures.
C. Research staff are required to follow these guidelines.
D. Animals should never be euthanized in animal housing rooms or in the direct presence of conspecifics. Special circumstances such as during quarantine and/or exposure to infectious agents or aquatic organisms (e.g. fish and amphibians) euthanized by MS222 where multiple individuals may be placed in the same immersion bath may be exceptions.

4. **Acceptable Procedures, Definitions and Terms**
   This is not intended to be a comprehensive list, but rather a list of procedures, definitions and terms meant to describe those techniques.
   A. Exsanguination: With an animal under deep anesthesia, withdrawing the maximal volume of blood via cardiac puncture, abdominal aorta puncture, or bilateral transection of the cervical vasculature. Exsanguination without anesthesia or loss of consciousness is unacceptable.
   B. Gaseous Carbon Dioxide (CO_2): Must be supplied using a compressed gas tank. As gas displacement rate is critical to the humane application of CO_2, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized is necessary. Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 30% to 70% of the chamber volume/min is recommended for rodents. Whenever gradual displacement methods are used, CO_2 flow shall be maintained for at least 1 minute after respiratory arrest. The use of dry ice as a source of CO_2 for euthanasia is not permitted.
   C. Inhalant Anesthesia (Deep or Overdose): Anesthetic agent(s) delivered as a volatile gas to the respiratory tract to induce anesthesia. Personnel should minimize their exposure to these agents as some are considered chemical hazards. In the animal facilities, these agents should only be used in a system with an active gas scavenging device or ducted biosafety cabinet. Note: This method is not approved as a sole means of euthanasia. In the case of field research, a vaporizer and scavenging device is not practical. In this instance, the “bell jar” technique is appropriate. The open drop method (“bell jar”) utilizes a small container with an isoflurane soaked cotton ball or gauze contained within an object in which the animal cannot come into physical contact with the liquid anesthetic (e.g. tea ball infuser). This technique should only be performed in the outside air to prevent release of volatile agents into a closed space. Because anesthesia machines are available to researchers on campus, PI’s that wish to utilize the open drop method (“bell jar”) in lab settings must obtain IACUC approval. If the open drop method is used in a laboratory, the procedure must occur in a fume hood.
   D. Injectable Anesthesia (Overdose): Chemical agent(s) administered by injection with a needle and syringe to induce anesthesia. Common routes of injection include, but are not limited to, intraperitoneal (IP), intramuscular (IM) or intravenous (IV). Injectable anesthetics are easy to administer, require minimal equipment, and avoid safety concerns associated with inhalants, but may require a DEA license.
   E. Physical Euthanasia: Individuals who perform physical euthanasia on unanesthetized animals must first be trained and proficient as determined by IACUC approved designees. Physical euthanasia on unanesthetized animals, irrespective of age, can only be done if the procedure is described in the approved AUP.
      1) Cervical Dislocation: cervical dislocation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, its use is scientifically justified, and it is described in an approved AUP. Manual cervical dislocation is a humane method of euthanasia when limited to rodents and birds weighing less than 200 grams. Personnel using cervical dislocation must be adequately trained, demonstrate their technical proficiency, and must consistently apply this method humanely and effectively.

2) Decapitation: decapitation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, its use is scientifically justified, and it is described in an approved AUP. When performed properly this technique is nearly instantaneous and is considered humane. Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available. Sharp scissors can be used to decapitate neonatal rodents. Check guillotine and scissor blades frequently to ensure sharpness. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to minimize stress from handling, minimize the chance of injury to personnel, and improves positioning of the animal in the guillotine. (2020 AVMA Guidelines for Euthanasia)
3) Cardiac puncture is prohibited on conscious animals. Animals must be deeply anesthetized for either exsanguination or injection of euthanasia solution into the heart. Intracardiac injection of pentobarbital in unconscious animals as the second step euthanasia procedure is acceptable. (2020 AVMA Guidelines for Euthanasia)
4) Penetrating Captive bolt: A penetrating captive bolt works by concussion and trauma to the brain. It causes immediate unconsciousness and destruction of brain tissue as a result of penetration of the discharged bolt. Because placement and positioning of the projectile is critical, some degree of restraint is required for proper use of this device. A rope halter is sufficient to restrain the head for ensuring proper placement of the penetrating captive bolt. In addition, only trained personnel are permitted to perform captive bolt euthanasia.
    i. Second shot. Although one well-placed shot from a penetrating captive bolt is usually sufficient to cause immediate loss of consciousness with little likelihood of return to consciousness, one should always be prepared to deliver a second or even a third shot if necessary.
    ii.
    iii. Death must be assured through a lack of a corneal reflex or auscultation.

F. Secondary physical method to ensure death: must be performed in rodent and rabbits and some fish/amphibians in order to confirm that animals are dead, one of the following secondary physical methods must be performed on animals that have been anesthetized with approved agents:
1) cervical dislocation;
2) decapitation;
3) thoracotomy [open the chest cavity using sharp scissors or scalpel]; or
4) collection of vital organs.
5) exsanguination
6) immersion in liquid nitrogen
7) lose dose ethanol (finfish, tadpoles)

G. Thoracic Compression: Thoracic compression is an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient due to other reasons but may be appropriate as a secondary method for an unconscious animal. Thoracic compression must be justified and approved in an AUP.

5. Conditionally Acceptable Procedures, Definitions and Terms

A. Firearms: use will follow established TTU OP's, will require qualification and training, and will be restricted to areas where discharge of firearms is lawful.
   1) While Penetrating Captive Bolt (PCB) will be the preferred and standard method of euthanasia in domestic farm species, certain circumstances may necessitate the use of firearms for the safety of employees or other herd animals.
      i. The use will be restricted to direct consultation with and approval of the ACS veterinary staff
      ii. The use should never occur in the presence of classes or the public
   2) Qualification shall be accomplished by proof of firearms handling and use training such as hunter safety courses, licensed concealed carry certification or military training.
   3) Personnel will additionally be trained in the proper
      i. Selection of an appropriate firearm and bullet with sufficient velocity, energy and size to pass through the skull (enter the brain), and cause massive brain destruction.
      ii. Placement of projectile.
      iii. Recognition of signs of death to ensure proper euthanasia/humane killing.
   4) Second shot. Although one well-placed bullet is usually sufficient to cause immediate loss of consciousness with little likelihood of return to consciousness, one should always be prepared to deliver a second or even a third shot if necessary.
      i. Death must be assured via a lack of corneal reflex or auscultation.
   5) For wildlife, permission of landowner and all necessary provincial and federal permits are obtained. Gunshot targeted to the heart (chest) or the neck (vertebrae) presents challenges for accurate placement, but may be the best option for free-ranging or other settings where close approach is not possible or where the head must be preserved for disease testing (rabies, CWD or other suspected neurological disease) or museum specimens. Gunshot to the chest or neck may not result in rapid death and may be considered humane killing rather than euthanasia (AVMA Guidelines for the Euthanasia of Animals:2020). One should always be prepared to deliver a second or even a third shot if necessary. Death must be assured via lack of corneal reflex or auscultation.

6. Euthanasia of sick or injured animals

A. Sick or injured animals that cannot be successfully treated or relieved of pain and distress should be euthanized promptly.
B. Properly trained research personnel are responsible for euthanizing sick, injured or moribund animals as soon as these conditions are recognized.
   1) Research personnel must contact unit or ACS staff immediately if assistance is required for euthanasia.
C. To investigate unexpected illnesses, research personnel may contact ACS to arrange for euthanasia and necropsy of the animals.
D. ACS veterinarians have the authority to euthanize moribund animals, animals experiencing more than momentary or slight pain and/or distress, and non-study related causes leading to uncontrolled pain or distress.

E. If an ACS veterinarian is unable to contact research personnel regarding the care or treatment of a moribund animal, ACS veterinarians or designated representatives are authorized to euthanize the animal.
F. Appropriate emergency contact numbers for veterinarians and all research personnel should be posted in the animal facilities.

7. Verification of Death

A. Verification of death is required prior to disposal of a body by:
   1) Observing for the absence of movement.
   2) Observing for the absence of respiratory and heartbeat activity for at least 3 minutes.
   3) Check for lack of corneal and toe pinch reflex.

8. References

- American Veterinary Medical Association (2020) AVMA Guidelines on Euthanasia. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
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- NIH (2002) Guidelines for the Euthanasia of Rodent Feti and Neonates. (Complete document HERE)
- Guidelines of the American Society of Mammologists for the use of wild mammals in research and education http://www.mammalsociety.org/committees/animal-care-and-use#tab3
- TTU Operating Policies and Procedures