WVU IACUC Guidelines:
Euthanasia Guidelines

Purpose

The purpose of these guidelines is to describe acceptable methods for the euthanasia of animals used in research and teaching at West Virginia University (WVU). All animal euthanasia ***must*** be performed by properly trained personnel.

Background

The *Guide for the Care and Use of Laboratory Animals* (the *Guide*, NRC 2011) states: "Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the *AVMA Guidelines on Euthanasia"* (p. 123)

The *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* states: "Euthanasia methods are classified in the Guidelines as acceptable, acceptable with conditions, and unacceptable. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia. Methods acceptable with conditions are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met." (p.9)

Definitions

- **Euthanasia:** A method of killing that minimizes pain, distress, and anxiety experienced by the animal prior to loss of consciousness and causes rapid loss of consciousness followed by cardiac or respiratory arrest and death.
- **Humane Killing:** Killing performed in a manner that minimizes animal distress but may not meet the requirements of euthanasia due to situational constraints.
- **Adjunctive Method:** A method of assuring death that may be used after an animal has been made unconscious. Adjunctive method can also be referred to as a "secondary method of euthanasia", "confirmatory method" or "verification of death."
- **Acceptable:** A method considered to reliably meet the requirements of euthanasia.
- **Acceptable with conditions:** A method considered to reliably meet the requirements of euthanasia when specific conditions are met.
- **Unacceptable:** A method that does not meet the requirements of euthanasia.

Guidelines

- Any animal euthanized on a WVU IACUC-approved animal use protocol requires a method of verifying euthanasia. Both the primary and secondary/verification methods of euthanasia ***must*** be described in the IACUC-approved animal use protocol.
- When selecting a euthanasia method, consideration should be given to minimizing pain and fear experienced by the animal.

- All euthanasia procedures ***must*** be continuously monitored by the person(s) performing the procedure, until confirmation of death.
- It is good practice to euthanize animals out of the sensory range of other animals in order to minimize distress to those nearby.
- Animals may be euthanized by perfusion or exsanguination as a primary method of euthanasia if they are fully anesthetized (deep surgical plane) prior to initiation of procedure. A surgical plane of anesthesia ***must*** be attained in the animal before any incisions are made and maintained until the heart stops.
- Animal carcasses and tissues ***must*** be appropriately disposed of after euthanasia.
- Contact veterinary staff and/or facility manager for species- or area-specific euthanasia SOPs that may be available in your area.
- Listed below are some commonly used and accepted methods of euthanasia for different species. This list is not inclusive. Please see the current "AVMA Guidelines on Euthanasia" for further information.

Considerations when training or demonstrating euthanasia

- Training should include information on the methods of euthanasia that personnel are expected to utilize and should provide information regarding how the chosen technique induces loss of consciousness and death.
- It is suggested that animals that have already been euthanized by other means or are deeply anesthetized be used when training personnel to perform physical methods.
- Personnel also need to be trained on techniques used to verify death.
- Proficiency should be confirmed prior to allowing individuals to perform the procedure without observation.
- The chosen method of euthanasia should also take into account the effects of the method on personnel, in some situations (e.g.- animal care staff who cared for the animals, students, and those from other backgrounds/cultures).

Species	Acceptable	Acceptable with Conditions	Secondary Methods/ Verification of Euthanasia
Rodents	Injectable barbiturate overdose	**CO_2 inhalation overdose**[1]**:** Chamber displacement rate of 30-70% per minute. **Inhaled anesthetic overdose**[3]**:** Prolonged exposure time may be needed. **Cervical dislocation**[2]**:** Can be performed in mice and rats <200g. **Decapitation**[2]**:** Rodent guillotines ***must*** be kept clean, in good condition, with sharp blades.	• Cervical dislocation • Bilateral thoracotomy • Decapitation • Vital tissue harvest (inclusive of heart, lungs, and/or brain) • Continued exposure to CO_2 for at least 15 minutes after respiratory arrest

Rodent Fetuses and Neonates	Euthanasia of dam and mammalian fetuses Injectable barbiturate overdose	**Inhaled anesthetic overdose**[3]**:** Prolonged exposure time required. **Cervical dislocation**[2] **Decapitation**[2]	• Cervical dislocation • Decapitation
Rabbits	Injectable barbiturate overdose	**Inhaled anesthetic overdose**[3]**:** Provide sedative before removing from home cage. **CO_2 inhalation overdose**[1]**:** Chamber displacement rate of 50-60% per minute. Premedication with sedative may reduce aversive response. **Cervical dislocation**[2]**:** Performed by individuals with a high degree of technical proficiency.	• Bilateral thoracotomy • Sternotomy • Vital tissue harvest (inclusive of heart, lungs, and/or brain) • Delivery of potassium chloride • Exsanguination
Laboratory Fish	**Rapid Chilling (zebrafish):** 2-4°C until loss of orientation and cessation of opercular movements. Adults exposed for 10 minutes (minimum) after cessation of opercular movements; Zebrafish fry (4-14 days after fertilization (dpf)) exposed 20 minutes (minimum) after cessation of opercular movements. Rapid chilling is unreliable in embryos < 3 days dpf. **Immersion in diluted sodium or calcium hypochlorite solution:** Embryos < 7 dpf.	**MS222 (Tricaine methanesulfate)**: Immersion in MS222 for 30 minutes following loss of rhythmic opercular movements. Secondary method should be performed. Not effective for zebrafish eggs, embryos, or larvae (<14 days)	As part of a 2-step method following MS222 overdose: • Decapitation • Pithing • Exsanguination • Freezing Immersion in diluted sodium or calcium hypochlorite solution should also be used secondary to Rapid Chilling or MS222 for zebrafish embryos < 3 dpf.
Bovine	Injectable barbiturate overdose	**PCB or NPCB**[4] -Penetrating captive bolt or non-penetrating captive bolt: NPCB should be limited to calves. Both ***MUST*** include a secondary method.	Under deep surgical anesthetic plane or after rendering unconscious by appropriate means:

Bovine (cont.)			• Potassium Chloride administration IV (rapidly) • Exsanguination • Pithing **Exsanguination:** transect carotid arteries and jugular veins close to thoracic inlet. Use a pointed, very sharp knife with rigid blade at least 6 inches long.
Small Ruminants (sheep and goats)	Injectable barbiturate overdose	**PCB or NPCB**[4] -Penetrating captive bolt or non-penetrating captive bolt: NPCB should be limited to neonatal/young animals. ***MUST*** include a secondary method.	Under deep surgical anesthetic plane or after rendering unconscious by appropriate means: • Potassium Chloride administration IV (rapidly) • Exsanguination (transect carotid arteries & jugular veins close to thoracic inlet; use pointed, very sharp knife with rigid blade at least 6 inches long) • Pithing
Swine	Injectable barbiturate overdose	**PCB**[4] – Penetrating captive bolt: requires restraint of animal, all pigs should be observed for confirmation that animal is rendered insensible.	Under deep surgical anesthetic plane or after rendering unconscious by appropriate means: • Exsanguination (transect carotid arteries & jugular veins close to thoracic inlet; use pointed, very sharp knife with rigid blade at least 6 inches long) • Pithing
Poultry	Injectable barbiturate overdose	**Inhaled Gases:** verification of death ***must*** occur. Gases supplied via commercially available cylinder or tank. Gas-dispensing system has ability to maintain necessary gas concentration. **Carbon Dioxide:** No flow rate requirement for poultry. CO_2 concentrations may	Under deep surgical anesthetic plane or after rendering unconscious by appropriate means: • Potassium Chloride administration IV or intracardiac • Exsanguination (biosecurity precautions should be observed)

Poultry (cont.)		need to be higher for younger animals (75% or higher for 5 minutes of day hatch chicks). **Cervical Dislocation:** ***Must*** result in luxation of cervical vertebrae without primary crushing of the vertebrae and spinal cord. Separation of cervical vertebrae should be verified by palpation. **Decapitation:** ***Must*** be performed by competent personnel. Performed with sharp instrument. **Captive Bolt[4] (penetrating or nonpenetrating)**: The captive bolt device ***must*** be appropriately designed and configured for species of bird and size of animal, provide sufficient impact and be properly applied.	
Avian	IV administration of barbiturate +/- inhalant anesthesia. **Injection euthanasia agent (under anesthesia)** intracelomic, Intracardiac or intraosseous (not femur or humerus) routes can be used. If intracelomic route used avoid injection into air sacs.	**Inhaled anesthetics:** High concentrations of inhaled gas. **Carbon Dioxide:** >40% concentrations	Under deep surgical anesthetic plane or after rendering unconscious by appropriate means: • Potassium Chloride administration IV or intracardiac • Exsanguination • Thoracic compression
FinFish (non-laboratory species)	Step 1: Immersion in anesthetic solutions render animal unconscious, followed by a secondary method.	**Decapitation** **Cervical Transection** followed by pithing.	Under deep surgical anesthetic plane: • Decapitation • Pithing
Free-ranging wildlife	Overdose of injectable anesthetic agents (including barbiturates)	**Gunshot**: Bullet placement is to the head (targeted to destroy brain tissue)	Under deep anesthesia or unconsciousness: • Potassium chloride administration IV or intracardiac • Exsanguination

[1] CO_2 source ***must*** be a compressed gas cylinder. Animals should NOT be combined from different cages. Animals should be euthanized in their home cage, if possible. Observe cage density guidelines. Continue CO_2 until one minute after breathing ceases.

[2] See IACUC #11-003 "Euthanasia by Decapitation or Cervical Dislocation without Anesthesia" for additional information when utilizing these methods as a primary or adjunctive method.

[3] See IACUC #11-005 "Calibration of Vaporizers for Inhalation Anesthesia in Animals" for additional information when utilizing anesthetic vaporizers. Adjust anesthetic flow rate or concentration to 5% or greater. Continue exposure until one minute after breathing ceases. "DROP JAR METHOD"- animal ***must not*** have direct contact with the anesthetic agent and this method ***must*** be performed in a fume hood or hard-ducted biosafety cabinet.

[4] PCB or NPCB use ***must*** be done by a properly trained individual.

References

1. AVMA Guidelines for the Euthanasia of Animals: 2020 edition.
2. Management of Animal Care and Use Programs in Research, Education, and Testing. 2nd edition. Weichbrod RH, Thompson GAH, Norton JN, editors. 2018. Chapter 35 – Euthanasia https://www.ncbi.nlm.nih.gov/books/NBK500441/