Policy #28: Euthanasia of Research and Teaching Animals

A. Definition
Euthanasia is the act of inducing humane death in an animal by methods that induce rapid unconsciousness and death with a minimum of pain or distress.

B. Principle & Purpose:
Animal welfare regulations require that the Institutional Animal Care and Use Committee (IACUC) approve the euthanasia method for research and teaching animals. They also clarify that “unless a deviation is justified for scientific or medical reasons, methods should be consistent with the AVMA Guidelines on Euthanasia”. The purpose of this document is to provide additional clarification and information for methods of euthanasia commonly used in animals at Washington State University. Recommended euthanasia techniques for small mammals, birds, fish, reptiles, and amphibians as well as prenatal and neonatal animals are outlined below.

C. Policy:
In accordance with federal directives, euthanasia of research or teaching animals performed at WSU should be done in a manner consistent with the AVMA Guidelines on Euthanasia. The method utilized must be indicated in the applicable Animal Subjects Approval Form (ASAF) and approved by the IACUC. Any method not listed in the Guidelines as “acceptable” must be adequately described and justified. The 2013 AVMA Guidelines on Euthanasia can be found at https://www.avma.org/KB/Policies/Documents/euthanasia.pdf.

The method of euthanasia of animals intended for human consumption must be compatible with current state and federal food safety regulations, as well as the 2016 AVMA Guidelines for the Humane Slaughter of Animals. These guidelines can be found at https://www.avma.org/KB/Resources/Reference/AnimalWelfare/Documents/Humane-Slaughter-Guidelines.pdf.

D. Training:
All personnel performing animal euthanasia must be trained to safely and adequately perform the euthanasia technique. Many common euthanasia techniques are classified as “acceptable with
conditions” so are considered acceptable only when all the conditions for application of a method are met. The conditions are described in detail within the AVMA guidelines and for methods described within this policy. For physical means of euthanasia, the AVMA guidelines recommend that personnel be initially trained on dead or anesthetized animals to demonstrate proficiency. All Principal Investigators (PI) must assure that their research staff and students are trained in the euthanasia method and all its associated conditions. All training should be documented in writing and training documents should be made available for the IACUC and other regulatory agencies upon request. For supplementary information, assistance, or training in any euthanasia method, please contact Office of the Campus Veterinarian at 509-335-6246 or or.ocv.alert.wsu.edu.

E. Minimizing Pain and Distress:
Pain and distress prior to and during euthanasia should be avoided.

1. Regardless of the technique used, the animal should be carefully handled and/or gently restrained in an appropriate and safe manner prior to euthanasia.

2. Regardless of the technique used, an animal should not be euthanized in the animal housing room/area to minimize stress on the remaining animals. Exceptions may occur given scientific justification (i.e. to prevent spread of infectious disease) or for emergency euthanasia when an animal cannot be readily moved.

3. Chambers used for chemical inhalant euthanasia of multiple animals must not be overcrowded. Overcrowding in this situation is defined as any more than half the housing space normally required for the animals. For example, no more than 10 mice could be placed in a cage that would normally house 5 mice.

4. Animals with a propensity for fighting should not be mixed together for transport to and during euthanasia (i.e. unfamiliar adult male mice, hamsters and other incompatible animals).

F. Confirmation of Death:
Following performance of the euthanasia procedure, it is mandated to verify that death has actually occurred. Verification should be accomplished as outlined below.

Unintended recovery of animals after apparent death from CO₂ or other euthanasia agents constitutes serious noncompliance with the PHS policy. All incidents involving unintended recovery of euthanized animals have to be reported to the Office of Laboratory Animal Welfare at NIH.
1. **Confirming Death in Rodent and Avian Species**
   Rodents and birds, especially neonates, are particularly resistant to euthanasia by overdose of inhaled agents such as CO₂ or even injectable agents; for this reason, the IACUC requires a secondary physical method of euthanasia FOR ALL RODENTS after the animal is profoundly anesthetized and prior to carcass disposal.
   Acceptable SECONDARY physical methods for adult and neonatal rodents and avian species include:
   a. Decapitation
   b. Cardiac perfusion
   c. Removal of vital organs (e.g. heart, lungs, brain)
   d. Opening of the chest cavity to induce pneumothorax
   e. Exsanguination
   f. Cervical dislocation
   NOTE: These procedures may not be performed in conscious animals without specific IACUC approval.

2. **Confirmation of Death in Large Mammalian Species** (dog, cat, swine, rabbit, etc.)
   A combination of criteria is most reliable in confirming death, including lack of pulse, breathing, corneal reflex and response to firm toe pinch, inability to hear respiratory sounds and heartbeat by use of a stethoscope, graying of the mucous membranes, and rigor mortis. None of these signs alone, except for rigor mortis, confirms death.
   a. **Heartbeat**: Thoracic auscultation or direct cardiac palpation is the primary means of confirming death. If there is any question, the thorax should be opened to create a pneumothorax. Lack of electrical activity of the heart as determined by ECG (provided that the leads are correctly connected) may also be utilized to confirm death.
   b. **Corneal reflex**: Touching the cornea of an eye should not elicit a blink reaction or movement of either the eye touched or the consensual eye.
   c. **Pupillary response to light**: Shine a bright light into the eyes of the animal. A constriction (narrowing) of the pupil indicates a neurological response. Upon death, the pupils will become dilated and unresponsive to light. Some drugs and experimental agents (e.g., anticholinergics such as atropine) can prevent pupillary reactivity or otherwise affect this neurological response.
d. **Respiratory pattern:** Profoundly anesthetized animals may exhibit shallow and irregular breathing patterns that may be confused for lack of spontaneous breathing. Thus, lack of spontaneous breathing should not be used as sole criteria for confirming euthanasia.

- **Use of Secondary Methods to Confirm Death in Large Mammals:**
  - Physical Methods: cardiac perfusion, removal of vital organs (e.g. heart, lungs, brain), opening of the chest cavity to induce pneumothorax and exsanguination. These methods may be used after the animal is profoundly anesthetized or stunned and prior to carcass disposal.
  - Potassium Chloride: Administration of potassium chloride for euthanasia in a conscious animal is unacceptable. Intravenous or intracardiac administration in a fully anesthetized animal or unconscious animal may be used to confirm death.

3. **Confirmation of Death in Fetal Mammals**
   a. Embryos and fetuses are in a state of unconsciousness during pregnancy and birth so they do not suffer breathlessness or pain in utero after the death of their dam, whatever the cause.
   b. The fetus is presumed dead if left undisturbed in the uterus for 15 to 20 minutes after the pregnant female has been confirmed dead. See above for confirmation of death in rodents and larger mammals.
   c. Intraperitoneal injection of barbiturates or other anesthetics in a mid-to-late term pregnant female is not recommended because of the likelihood of injecting into the gravid uterus rendering the injection ineffective.

4. **Confirmation of Death in Poikilothermic Vertebrates**
   Additional care must be taken to ensure death following euthanasia in poikilothermic vertebrates such as fish, reptiles and amphibians. Such animals may normally exhibit very low heart rates, and the heart and brain are very tolerant to anoxia; many poikilothermic can voluntarily hold their breath for an hour or more. Death should always be confirmed by physical intervention; secondary methods for poikilothermic vertebrates include the following:
   a. Decapitation followed by pithing or other method of destroying brain tissue
b. Pithing

c. Removal of vital organs (e.g. heart, brain)

d. Rapid freezing with liquid nitrogen

G. Chemical Methods of Euthanasia for Rodents and Birds:

1. **Injectable Anesthetics:**
   Intravenous administration of an overdose of pentobarbital, pentobarbital-based euthanasia solution, ketamine/xylazine, or other injectable anesthetic can be a suitable means of euthanasia. Intraperitoneal administration can be appropriate when venous access is not reasonably achievable. The drug, dose, route of administration and secondary confirmation of euthanasia must be described in the protocol and approved by the IACUC.

2. **Isoflurane or Sevoflurane Inhalation:**
   a. The preferred method of delivering isoflurane or sevoflurane is via precision vaporizer. Under certain circumstances, the open drop method of delivering isoflurane may be appropriate. If using an open-drop system (application of isoflurane to an absorbent material then placed into the chamber), the animal must be separated from the absorbent material by a physical barrier.
   b. A waste gas scavenging system must be in place to eliminate human exposure to inhaled anesthetics.
   c. With any inhalation agent, a secondary physical method of euthanasia is required after the animal is profoundly anesthetized and prior to carcass disposal.

3. **Carbon Dioxide (CO₂) Inhalation:**
   a. Compressed CO₂ gas in cylinders is the only allowable source of carbon dioxide. CO₂ delivery must be monitored to ensure CO₂ does not displace air by more than 10-30% of the chamber volume per minute. CO₂ generated by other methods (e.g., dry ice) is unacceptable.
   b. A secondary physical method of euthanasia is required after the animal is profoundly anesthetized and prior to carcass disposal.
   c. **CO₂ euthanasia of rodents (slow-fill techniques):**
      i. When possible, animals should be euthanized in their home cages
      ii. Do not “pre-fill” the euthanasia chamber with CO₂. Start with room air then fill the chamber with CO₂ at a rate of 10-30% of the chamber volume per minute.
iii. CO₂ first renders the animal anesthetized and, with adequate exposure time, will result in death by CO₂ narcosis. Animals should be left for additional time within the euthanasia chamber, after spontaneous movements have ceased, with CO₂ continuing to flow to ensure death. See chart for estimated CO₂ exposure times.

iv. If the collection of animal tissue is required at or just before the time of death then the animal can be removed from the CO₂ before complete cessation of breathing and euthanized by an appropriate physical method (decapitation of neonatal to adult rodents, cervical dislocation of adults, or other means). The physical method must be applied only when the animal is completely unresponsive to external stimuli (unconscious).

<table>
<thead>
<tr>
<th>Estimated CO₂ Exposure Time for Rodents</th>
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<tbody>
<tr>
<td>AGE &amp; SPECIES</td>
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<tr>
<td>Non-haired rodent pups</td>
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<td>0–6 days</td>
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<td>Hairless rodent pups, eyes closed</td>
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<td>7–13 days</td>
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<tr>
<td>Hairless rodent pups, eyes open, preweaning</td>
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<td>14–20 days</td>
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<tr>
<td>Weanling and adult rodents</td>
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<tr>
<td>21+ days</td>
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</table>

Resistance to hypoxia results in a prolonged time to unconsciousness when CO₂ inhalation is used as a euthanasia agent in neonatal rodents. The duration of exposure to carbon dioxide varies with the age of the neonate compared with adult rodents. **Minimum time in CO₂** (Pritchett et al. 2005, Pritchett-Coming 2009)

d. CO₂ euthanasia of birds (rapid-fill technique):
   i. Adult birds require >40% CO₂ to achieve euthanasia but newly hatched chicks require up to 80-90% CO₂. Birds are not averse to high concentration of CO₂.
   ii. Pre-fill the euthanasia chamber with CO₂. Place the bird in the pre-filled chamber and maintain a high CO₂ flow rate until the animal loses consciousness. The CO₂ flow rate does not have to be precisely monitored with a flow regulator.
   iii. CO₂ first anesthetizes the animal, and then, with adequate exposure time, will result in death by CO₂ narcosis. Animals should be left for additional time.
within the euthanasia chamber, after spontaneous movements have ceased, with CO₂ continuing to flow.

H. Physical Methods of Euthanasia for Rodents and Birds:
The following physical methods of euthanasia for rodents and birds are considered acceptable with conditions by the AVMA. In most circumstances, physical methods of euthanasia must be performed under anesthesia unless an exception is approved by the IACUC. Scientific justification and documentation of experience and/or training of the person(s) who will be performing the procedure must be provided. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency for all physical methods described below.

1. **Cervical Dislocation**
   a. When performed properly, cervical dislocation results in luxation of the cervical vertebrae without crushing of the vertebrae and spinal cord. Manual cervical dislocation can be a humane technique for euthanasia of small birds, poultry, mice, and immature rats weighing less than 200 grams.
   b. Cervical dislocation is difficult to perform properly in neonatal rodents & chicks. In these cases, decapitation may be more humane.

2. **Decapitation**
   a. Decapitation when performed properly is virtually instantaneous and is considered humane.
   b. Guillotines designed for decapitation in adult rodents are commercially available. Guillotines must be kept clean and in good condition with sharp blades. A maintenance log must be maintained for all guillotines used for decapitation. The use of plastic cones to restrain the animal can reduce stress and improve positioning while minimizing the risk of injury to personnel. Please refer to Appendix 1 for the standard operating procedures for guillotine use and maintenance.
   c. Decapitation using scissors or sharp blades is acceptable with conditions for neonatal rats and mice (0-6 days of age) and small birds (<200 grams). Blades must be adequately sharp to cut the cervical spinal column without crushing and is unanesthetized, mice and rats must be ≤6 days of age.
3. **Thoracic Compression**
   Thoracic compression is considered an unacceptable means of euthanizing animals that are not deeply anesthetized or unconscious due to other reasons. It can be appropriate as a secondary method for animals that are insentient or in emergency field situations when there is no other appropriate means of euthanasia available.

I. **Euthanasia of Fish, Reptiles, and Amphibians:**
   Please refer to section F: *Confirmation of Death* above

1. **Chemical Methods**
   a. **Injectable & Immersion Anesthetics:** chemical use may affect human food safety of animal products.
      i. Overdose of pentobarbital via intravenous/intracoelomic/intraperitoneal or lymph space injection. The actual time to death may be prolonged for poikilothermic animals (up to 30 minutes). Intracardiac administration is only acceptable in unconscious animals.
      ii. pH-neutralized Tricaine methanesulfonate (MS-222) immersion may be used to euthanize fish, aquatic and semi-aquatic amphibians. Reptiles and amphibians may be euthanized by intra-coelomic (IC) or lymph sac injection of MS-222. Please refer to the standard operating procedures for use of MS-222: *insert link*
      iii. Benzocaine hydrochloride immersion (≥ 250mg/L & buffered) and topical gel application may also be used to euthanize fish, aquatic and semi-aquatic amphibians.
      iv. Eugenol, isoeugenol and clove oil for fish immersion: fish should be left in solution for a minimum of 10 minutes after cessation of opercular movement. When possible, use products with standardized known concentrations of essential oils.

2. **Inhalation Methods**
   Reptiles and amphibians are capable of breath holding, so time to loss of consciousness and death may be greatly prolonged (several hours). Inhalation methods are considered acceptable with conditions and must be followed by a secondary lethal procedure to insure death.

3. **Physical Methods**
   The following physical methods of euthanasia for reptiles, amphibians and fish are considered acceptable with conditions by the AVMA. In most circumstances,
physical methods of euthanasia must be performed under anesthesia unless an exception is approved by the IACUC. Scientific justification and documentation of experience and/or training of the person(s) who will be performing the procedure must be provided.

a. Hypothermia & Freezing:
   i. Freezing: Freezing is NOT acceptable as a sole method of euthanasia unless animals are sufficiently small (< 4 grams) to permit immediate and irreversible death if placed in liquid N2. Rapid freezing as a secondary confirmation following cessation of respiration after other means of euthanasia is acceptable.
   ii. Hypothermia in ice-chilled water: It is acceptable for zebrafish (D. rerio) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and operculum movements. Zebrafish adults (approx 3.8 cm long) can be rapidly killed (10 to 20 seconds) by immersion in 2° to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes and zebrafish fry for at least 20 minutes following loss of operculum movement. This method does not apply to other fish species.
   iii. Other means of hypothermia or freezing: Placing any conscious animal in a refrigerator, freezer or in direct contact with ice or dry ice for restraint or euthanasia is unacceptable. The formation of ice crystals on the skin and in tissues of a conscious animal will cause pain and distress.

b. Decapitation & Pithing:
   i. Decapitation by guillotine or shears should only be performed as part of a 3-step euthanasia protocol (anesthesia, decapitation and pithing). The central nervous system of poikilotherms is extremely tolerant to anoxia so methods of euthanasia that induce unconsciousness by interruption of blood supply to the head are inappropriate when used alone. Decapitation can only be used on anesthetized animals and when followed immediately with pithing.
   ii. Pithing is destruction of the brain, brainstem & spinal cord by insertion of a bolt, cane or rod into the brain cavity. It can be used as a second-step
euthanasia method in unconscious animals or immediately after
decapitation.

iii. Captive bolt or gunshot to the brain may be appropriate for large finfish
and reptiles when conducted by properly trained personnel.

J. Avian eggs:
1. If eggs are at <50% of the incubation period
   a. CO$_2$ for >20 minutes
   b. Cooling (<4°C for 4 hours)
   c. Freezing
   d. Maceration
   e. Adding (oiling eggs)
2. If eggs are at >50% of incubation period
   a. CO$_2$ for >20 minutes
   b. Anesthetic overdose
   c. Decapitation of the embryo

K. Mass depopulation and humane killing in emergency situations:
1. Mass depopulation refers to methods by which large numbers of animals must be destroyed
   quickly and efficiently with as much consideration given to the welfare of the animals as
   practicable, but where the circumstances and tasks facing those doing the depopulation are
   understood to be extenuating. The AVMA is drafting new guidelines for mass depopulation but
   the guidelines are not yet published.

2. In the event of an emergency requiring mass animal depopulation for reasons of animal
   welfare and/or public health, the WSU animal care community including veterinary, research
   and animal care personnel will attempt to follow this policy and the 2013 AVMA guidelines for
   euthanasia. If circumstances of an emergency occurs such that following the euthanasia
   guidelines are unsafe and/or unachievable, methods of mass depopulation may be utilized.
   These include but are not limited to:
   a. Mass delivery of CO$_2$ and/or gaseous anesthetics for rodents
   b. Gunshot or captive bolt for large animals
Reference:

WSU IACUC Policy #28: 8.24.17
Appendix 1:

Guidelines for Guillotine Maintenance and Use

Purpose:
To standardize the procedures for guillotine maintenance and use.

Review/Revisions:
The IACUC will review and revise this guidance as needed.

Personnel:
Personnel performing decapitation must be properly trained before performing decapitation. Office of the Campus Veterinary can assist in training for individuals performing decapitation of non-anesthetized animals.

Use:
Guillotines should be clean, in good condition, sharp and move freely. The action should be smooth with no perceptible binding or resistance, and the blade must be rust-free, sharp, and decapitate with minimal force. Lubrication may not be needed, but if it is, the use of a Teflon® or Silicone® containing compound is recommended over petroleum based compounds. Petroleum compounds will dry out or leave deposits that hinder operation. Any deficiencies must be repaired prior to use.
Disposable plastic containers (such as Decapicones® or Rodent Restraint Cones®) are recommended to improve positioning, minimize distress and increase personnel safety.
After use, guillotines should be rinsed, cleaned, rinsed with 70% alcohol, and thoroughly dried.

Maintenance:
Blades should be sharpened periodically. The frequency of sharpening depends on frequency of unit use and the species euthanized.
Blades that are no longer serviceable must be placed in a sharps container for disposal.

Inspection:
Personnel utilizing guillotines must keep a log of use and maintenance. Guillotines and their maintenance logs will be inspected as part of the IACUC semi-annual inspections.
Guillotines should be rinsed, cleaned, disinfected, rinsed with 70% alcohol and thoroughly dried after each use. Blades should be sharpened periodically based on frequency of use and species euthanized.

Date(s) Sent for Maintenance:_______________________________________________

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<tr>
<th>Date</th>
<th>Initials</th>
<th>Species Euthanized</th>
<th># of Animals</th>
<th>Cleaned (√)</th>
<th>Blade Sharpening (√)</th>
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