Guidelines and Policy for Standard Euthanasia
Guidelines – Recommended Best Practices for Laboratory Animals

General Considerations:

- Euthanasia techniques must be consistent with the AVMA Guidelines on Euthanasia, June 2013. Methods are chosen to minimize animal pain and distress consistent with needs of the research protocol.

- **Euthanasia** – the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress.

- The method of euthanasia must be specified in the approved Institutional Animal Care and Use (IACUC) protocol. Any chemical euthanasia method must be followed by a physical method from which the animal cannot recover such as decapitation, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a major organ.

- Use of anesthetic for euthanasia must be an overdose, not an anesthetic dose. Regardless of amount of chemical administered, animal must be completely non-responsive to noxious stimuli (hind paw pinch) before any physical means are applied. Many of the chemical euthanasia drugs are controlled substances and must be maintained according to DEA regulations.

- Physical methods of euthanasia such as decapitation or cervical dislocation of anaesthetized animals require demonstration of competence, and may be approved with proper justification in the IACUC protocol.

- Only trained personnel should perform euthanasia. It is the responsibility of the Principal Investigator to assure that personnel performing euthanasia have been trained to perform the procedure used. The ARC offers training on CO₂ euthanasia during the Animal Care and Use Training session.

- The techniques listed below are suggested common methods for euthanasia of animals. Other methods outlined in the AVMA Panel on Euthanasia, June 2013 are expectable. Do not euthanize animals in a room with other animals present.

- **NOTE:** Unintended recovery of animals after apparent death from CO₂ or other euthanasia agents constitutes serious regulatory noncompliance. All incidents involving unintended recovery of euthanized animals are reported to the Office of Laboratory Animal Welfare at NIH.
Requirements:
The Public Health Service (PHS) Policy in accordance with IV.C. 1.g – Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, unless a deviation is justified for scientific reason in writing by the investigator.

United States Department of Agriculture AWA USDA 2.31 (d)(5) – Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure, or if appropriate, during the procedure. 2.33 (b)(4) – AV will provide guidance to principal investigators and other personnel involved in the care and use of animals regarding handling, immobilization, anesthesia, analgesia, tranquilization, and euthanasia;

Office of Laboratory Animal Welfare (OLAW) – Guidance NOT-OD-02-062 PHS Policy on Humane Care And Use Of Laboratory Animals Clarification Regarding Use Of Carbon Dioxide For Euthanasia Of Small Laboratory Animals.

American Veterinary Medical Association (AVMA Guidelines on Euthanasia), 2013 – Distress vocalizations, fearful behavior and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, for sensitive species, it is desirable that other animals not be present when individual animal euthanasia is performed.
**The Guide for the Care and Use of Laboratory Animals** – The guide recommends on pg. 123; *unless a deviation is justified for scientific or medial reasons, methods should be consistent with the AVMA Guidelines on Euthanasia (AVMA 2013 or later editions).*

### Agents and Methods of Euthanasia by Species
*(from Appendix 1 on the AVMA Guidelines 2013)*

<table>
<thead>
<tr>
<th>Species</th>
<th>Acceptable</th>
<th>Conditionally Acceptable</th>
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<tbody>
<tr>
<td><strong>Rodents and other small mammals</strong></td>
<td>Barbiturates, inhalant anesthetics, CO2, CO, potassium chloride in conjunction with general anesthesia.</td>
<td>Methoxyflurane, ether, N2, Ar, cervical dislocation (rats &lt; 200 g), decapitation</td>
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<tr>
<td><strong>Fish</strong></td>
<td>Barbiturates, inhalant anesthetics, CO2, tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, 2- phenoxyethanol</td>
<td>Decapitation and pithing, stunning and decapitation/pithing</td>
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<tr>
<td><strong>Amphibians</strong></td>
<td>Barbiturates, inhalant anesthetics (in appropriate species), CO2, CO, tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, double pithing</td>
<td>Penetrating captive bolt, gunshot, stunning and decapitation, decapitation and pithing</td>
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<tr>
<td><strong>Reptiles</strong></td>
<td>Barbiturates, inhalant anesthetics (in appropriate species), CO2 (in appropriate species)</td>
<td>Penetrating captive bolt, gunshot, decapitation and pithing, stunning and decapitation</td>
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**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 error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature.

Refer to the [AVMA Guidelines for Euthanasia](https://www.avma.org) Appendix 2, 3, and 4, for further information.
Common Methods of Euthanasia

- **Inhalation of anesthesia gas** – acceptable with conditions for rodents and other small animals (<7kg). Typically used as part of a two-step process with a secondary physical method of euthanasia such as decapitation or cervical dislocation.

- **Inhalation of CO₂** – acceptable with conditions, including the special considerations listed below.

- **Immersion agents** – e.g. MS-222. Acceptable for aquatic species, usually in connection with a secondary physical method.

- **Cervical Dislocation** – acceptable for mice and immature rats. Requires training and should be performed under anesthesia unless specifically approved by the IACUC.

- **Decapitation** – acceptable for rodents. Requires training; anesthesia recommended unless approved by the IACUC. Guillotines must be sharpened and adjusted frequently to ensure proper performance.

- **Injectable barbiturate agents** – acceptable for most species.

Euthanasia of Adult Rodents:

**CO₂ Inhalation**

CO₂ inhalation is the most common method of euthanasia used for rodents. Compressed gas is the only acceptable source of CO₂ as this allows the inflow of gas to the induction chamber to be controlled. Since the anesthetic effects of CO₂ are reversible, animal that are removed prematurely from the chamber prior to death can recover. Thus, the IACUC requires either a physical method to ensure death following CO₂ or prolonged exposure (more than 10 minutes) in the CO₂ chamber followed with a paw/tail pinch test. See the posted ARC SOP on CO₂ euthanasia for more information.

**Anesthetic Inhalation**

The primary action is central nervous system (CNS) depression. The agents are nonflammable and nonexplosive under ordinary environmental conditions. Occupational exposure to inhalant anesthetics constitutes a human health hazard (e.g isoflurane) and procedures should be done either under a hood (with dedicated exhaust system) or with an anesthetic gas extractor. Since the anesthetic effects of inhalant anesthetics are reversible, animal that are removed prematurely from the chamber prior to death can recover. Thus, the IACUC requires a physical method to ensure death following euthanasia by inhalant anesthetics.
Injectable Anesthetics
Injectable anesthetics can be effectively used to anesthetize animals prior to performing a physical method of euthanasia. Since the anesthetic effects of injectable anesthetics are reversible, animals can recover. Thus, the IACUC requires a physical method to ensure death following euthanasia by injectable anesthetics prior to carcass disposal. Administer the anesthetic overdose as described in the approved IACUC protocol and allow sufficient time for the animal to lose consciousness. Following the anesthetic euthanasia, a physical method to ensure death must be performed prior to carcass disposal.

Cervical dislocation (Under Anesthesia)
Cervical dislocation is a humane technique when performed by individuals with a high degree of technical proficiency. Cervical dislocation is limited to rodents weighing less than 200 grams. Cervical dislocation in unanesthetized rodents is permitted only if its use is scientifically justified.

Decapitation (Under Anesthesia)
Decapitation when performed properly is nearly instantaneous and is considered humane. Guillotines that are designed for decapitation in adult rodents are commercially available. Equipment must 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 is recommended as it reduces distress from handling, minimizes the possibility of personnel injury, and improves positioning of the animal in the guillotine. Decapitation in unanesthetized rodents is permitted only if its use is scientifically justified.

Euthanasia of Rodent Fetuses:

Mouse and Rat Fetuses up to 14 days gestation
Neural development during this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetus at this stage of development.

Mouse and Rat Fetuses over 15 days gestation
The neural development during this time supports that pain may be perceived. Methods to euthanize include injection of anesthetics, decapitation with sharp surgical scissors or scalpels, or cervical dislocation.
Euthanasia of Rodent Neonates

Mouse and Rat Neonates up to 10 days of age
Acceptable methods include inhaled agents, injection of anesthetics, decapitation with sharp surgical scissors or scalpels, or cervical dislocation. Resistance to hypoxia results in a prolonged time to unconsciousness when CO2 is used. Prolonged exposure to inhalant anesthetics may be necessary. The use of CO2, inhalant anesthetic agents, or chemical anesthetics requires a physical method to ensure death (i.e. followed with decapitation with sharp surgical scissors or scalpels, or cervical dislocation).

Euthanasia of Reptiles/Amphibians/Fish

Ectothermic vertebrates require special consideration because these animals may normally exhibit very low heart rates, are very tolerant to hypoxia, and can hold their breath for long periods of time. Absence of a heartbeat and/or breathing will not necessarily provide confirmation of death. Therefore, once the animal is euthanized following the primary method it is necessary to perform a physical method of ensuring death. Recommended methods to ensure death include pithing and decapitation, removal of organs, and exsanguination.

Use of MS 222:
Fish: Fish should be left in this solution for at least 10 minutes following cessation of opercular movement. Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills.

Amphibians and Aquatic Species: Amphibians should be left in this solution for at least 10 minutes following cessation of movement. MS 222 may also be injected into lymph spaces and pleuroperitoneal cavities.
1. Injectable anesthetic overdose: Nembutal or Euthasol® 120 mg/kg intra-coelomic or intra-cardiac (fish, frogs, toads, salamanders) or in dorsal lymph sac (frogs and toads). Death results in 15-30 minutes.
2. Percutaneous anesthetic overdose: Immersion in 6.5% MS222 for at least 30 minutes (aquatic species only)
3. Physical method: Immersion in ice water for immobilization and decapitation using guillotine followed by immediate double pithing. (aquatic species only)

Confirmation of death: Amphibians may have reflexive movements after death. To verify death – check for respiration and reflexes like righting reflex and horizontal flotation. In case of doubt, use mechanical methods described in (3).
Reptiles (Lizard/Turtle Euthanasia):

1. Injectable anesthetic overdose: Nembutal or Euthasol® 150-200 mg/kg intra-coelomic or intra-cardiac.

   **Confirmation of death:** reptiles may have reflexive movements and heartbeat after death. To verify death – check for respiration and reflexes like righting reflex, reflex withdrawal to noxious stimuli. Remove a vital organ (decapitate or open thorax and remove heart).

2. **Lizard euthanasia (two stage procedure)**
   
   **Stage 1:** Inject intracoelomically a volume of the buffered 1% MS222 solution containing 250-500 mg/kg of MS-222

   **Stage 2:** After loss of righting reflex inject 0.1 to 1.0 ml (depending on size of lizard) into the heart.

   Secondary physical euthanasia, while not typically necessary, may be performed through removal of heart.

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**Confirmation of Death in Euthanized Animals**

All research personnel must receive adequate and appropriate training in all methods of euthanasia employed in the laboratory; they must also be trained to evaluate vital signs in the species used to confirm death in the animals. A profoundly anesthetized or severely ill animal can appear dead upon first examination; measurements such as lack of movement or visible lack of breathing are not precise enough to declare that a euthanized animals is dead.

**Special Considerations for Rodents**

As mentioned above rodents, especially neonates, are particularly resistant to euthanasia by overdose agents such as CO₂ or even injectable agents; for this reason, the IACUC requires either a.) A secondary physical method of euthanasia FOR ALL RODENTS or b.) Prolonged exposure at least 50% additional time in the euthanasia cage/chamber filled with 30% CO₂ adult rodents only. Marquette University IACUC confirmatory methods to be performed after CO₂ overdose include:

- Exsanguination
- Decapitation
- Cervical dislocation (adult mice only)
- Bilateral thoracotomy
Special Considerations and Confirmation of Death in Ectothermic Vertebrates

Additional care must be taken to ensure death following euthanasia in ectothermic 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 hypoxia; many ectotherms can voluntarily hold their breath for an hour or more. Absence of heart rate and/or breathing will not necessarily provide confirmation of death in these animals; secondary methods for ectothermic vertebrates should include either removal of the heart or decapitation followed by pithing.