Standard Euthanasia Guidelines – Recommended Best Practices

General Considerations:

- Euthanasia techniques must be consistent with the AVMA Guidelines on Euthanasia, June 2007. Methods are chosen to minimize animal pain and distress consistent with needs of the research protocol.

- 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 2007 are expectable. Do not euthanize animals in a room with other animals present.

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 If Carbon Dioxide For Euthanasia Of Small Laboratory Animals.

American Veterinary Medical Association (AVMA Guidelines on Euthanasia), 2007 – 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 2007 or later editions).*

<table>
<thead>
<tr>
<th>Agents and Methods of Euthanasia by Species</th>
<th>Acceptable</th>
<th>Conditionally Acceptable</th>
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</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
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<tr>
<td>Rodents and other small mammals</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>Rabbits</td>
<td>Barbiturates, inhalant anesthetics, CO2, CO, potassium chloride in conjunction with general anesthesia</td>
<td>N2, Ar, cervical dislocation (&lt; 1 kg), decapitation, penetrating captive bolt</td>
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<tr>
<td>Fish</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>
</tr>
<tr>
<td>Amphibians</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>
</tr>
<tr>
<td>Reptiles</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>
</tr>
</tbody>
</table>

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 Appendix 2, 3, and 4, for further information.
Euthanasia of Adult Rodents and Rabbits:

**CO2 Inhalation**
CO2 inhalation is the most common method of euthanasia used for rodents and rabbits. 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: 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.

References:
The University of Nebraska Medical Center – Animal Care and Use Program Policies and Procedures
The University of Wisconsin-Milwaukee – Euthanasia Procedures
The University of California – San Francisco – IACUC Standard Euthanasia Guidelines for Rodents
The University of California – San Diego – IACUC Policy and Guidelines for Euthanasia
The University of Georgia – IACUC Policy of Rodent Euthanasia Using CO₂
The AVMA Panel on Euthanasia - 2007