Euthanasia of Research Animals

**Policy:** This policy describes appropriate methods of euthanasia for different species. By default, the IACUC mandates adhering to the most recent AVMA Guidelines for the Euthanasia of Animals (AVMA Guidelines)\(^1\) and/or the euthanasia technique approved in each IACUC Proposal to Use Laboratory Animals in Research and Teaching (“Proposal”). All personnel performing euthanasia must be trained and proficient in the method of euthanasia being performed. RRF veterinary staff offers training on an individualized basis, this can be arranged through the IACUC Office or Attending Veterinarian. Common methods of euthanasia are listed below, and other acceptable methods can be found in the AVMA Guidelines\(^1\).

**Rationale:** Many experimental and teaching Proposals require euthanasia of the animals involved. Euthanasia is defined as the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress\(^2\), or humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death\(^3\). The Guide for the Care and Use of Laboratory Animals\(^2\) states that euthanasia methods should be consistent with the AVMA Guidelines\(^1\) unless a deviation is justified for scientific or medical reasons.

**Procedures, Guidelines, and Exceptions:**

1. Large Animal Euthanasia
   a. Barbiturate overdose (intravenously) to affect
      Death must be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy, exsanguination, vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.
   b. General anesthesia or anesthetic overdose (either injectable or inhaled).
      i. Description of specific anesthetic and dosage must be included in IACUC Proposal.
      ii. Death must be ensured by an adjunctive physical method such as bilateral thoracotomy, exsanguination, vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.
   c. Potassium Chloride administration while anesthetized
      Death must be ensured by an adjunctive physical method such as bilateral thoracotomy, exsanguination, vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.

2. Rodent Euthanasia (Mice & Rats)
   a. Carbon Dioxide (CO\(_2\)) euthanasia of mice and rats is acceptable with conditions.
      i. CO\(_2\) flow rate must be 10-30% chamber volume displacement per minute.
         1. CO\(_2\) euthanasia station-specific directions and flow rates are available at all RRF vivarium euthanasia stations. (see Attachments)
         2. All CO\(_2\) euthanasia stations are required to have flow meters.
ii. Animals must be euthanized in their home cage in a familiar group when possible.
   Cage density must not exceed housing space requirements.
iii. Adult rodent (>21 days of age) CO_2_ exposure must be long enough to ensure cessation of breathing plus an additional 60 seconds.

b. Barbiturate overdose (intraperitoneal) or general anesthesia to effect
   Description of specific drug(s) and dosage must be included in IACUC Proposal

c. Secondary or adjunctive physical methods of euthanasia must be performed on all rodents after CO_2_ euthanasia, barbiturate overdose, or general anesthesia.
   i. Cervical dislocation can be performed in animals <200g.
   ii. Bilateral thoracotomy
   iii. Exanguination or vital organ (brain, heart, lungs, liver, or kidneys) removal
   iv. Decapitation. Equipment for decapitation must be kept clean and in good condition with sharp blades.
   v. Perfusion

d. Physical methods without prior anesthesia are acceptable with conditions when scientifically justified.
   i. Cervical dislocation (animals must be <200g) performed by personnel with demonstrated proficiency
   ii. Decapitation by personnel with demonstrated proficiency using adequately maintained equipment

e. Neonatal mice and rats (<21 days of age)
   i. CO_2_
      1. CO_2_ flow rate must be 10-30% chamber volume displacement per minute.
         a. CO_2_ euthanasia station-specific directions and flow rates are available at all RRF vivarium euthanasia stations. (see Attachments)
         b. All CO_2_ euthanasia stations are required to have flow meters.
   2. Animals must be euthanized in home cage in a familiar group when possible.
   3. Cage density must not exceed housing space requirements.
   4. Neonates must be exposed to CO_2_ until cessation of breathing or until they are nonresponsive to painful stimuli.
   5. Death must be ensured by secondary or adjunctive method after neonates are nonresponsive to painful stimuli.
   ii. Cervical dislocation performed by personnel with demonstrated proficiency
   iii. Decapitation without anesthesia (only in animals <7 days old) with sharp scissors or blades
   iv. Gradual cooling (only in animals <7 days old) using measures to prevent direct contact with ice or precooled surfaces. Death must be ensured by adjunctive method after neonates nonresponsive to painful stimuli.

3. Other species
   a. Appendix 1 briefly lists acceptable methods of euthanasia for a variety of species.
      i. Euthanasia method(s) must be listed and approved in the IACUC Proposal.
      ii. Consultation with RRF veterinary staff is encouraged when determining euthanasia methods for any species.
References:
   (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)

Attachments: Guidelines for the Humane Euthanasia of Rodents (by RRF Facility)
### Appendix 1

Agents and methods of euthanasia by species.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Acceptable</th>
<th>Acceptable With Conditions (for Adjunctive Methods, see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquatic invertebrates</td>
<td>S6.3: Immersion in anesthetic solution (magnesium salts, clove oil, eugenol, ethanol)</td>
<td>S6.3: Adjunctive methods (second step) include 70% alcohol and neutral-buffered 10% formalin, pithing, freezing, boiling</td>
</tr>
<tr>
<td>Amphibians</td>
<td>S7.3: As appropriate by species—Injected barbiturates, dissociative agents and anesthetics as specified, topical buffered tricaine methanesulfonate or benzocaine hydrochloride</td>
<td>S7.3: As appropriate by species—Inhaled anesthetics as specified, CO₂, penetrating captive bolt or firearm, manually applied blunt force trauma to the head, rapid freezing</td>
</tr>
<tr>
<td>Avians (See also Poultry)</td>
<td>S5: Intravenous barbiturates</td>
<td>S5: Inhaled anesthetics, CO₂, CO, N₂, Ar, cervical dislocation (small birds and poultry), decapitation (small birds)</td>
</tr>
<tr>
<td>Cats</td>
<td>S1: Intravenous barbiturates, injected anesthetic overdose, Tributane, T-61</td>
<td>S1: Barbiturates (alternate routes of administration), inhaled anesthetic overdose, CO, CO₂, gunshot*</td>
</tr>
<tr>
<td>Cattle</td>
<td>S3.2: Intravenous barbiturates</td>
<td>S3.2: Gunshot, penetrating captive bolt</td>
</tr>
<tr>
<td>Dogs</td>
<td>S1: Intravenous barbiturates, injected anesthetic overdose, Tributane, T-61</td>
<td>S1: Barbiturates (alternate routes of administration), inhaled anesthetic overdose, CO, CO₂, gunshot*</td>
</tr>
<tr>
<td>Finfish</td>
<td>S6.2: Immersion in buffered benzocaine or benzocaine hydrochloride, isoflurane, sevoflurane, quinaldine sulfate, buffered tricaine methanesulfonate, 2-phenoxyethanol, injected pentobarbital, rapid chilling (appropriate zebrafish/research setting)</td>
<td>S6.2: Eugenol, isoeugenol, clove oil, CO₂-saturated water (aquarium-fish facilities/fisheries), decapitation/cervical transection/manually applied blunt force trauma followed by pithing, rapid chilling followed by adjunctive method (aquarium-fish facilities), maceration (research setting)</td>
</tr>
<tr>
<td>Equids</td>
<td>S4: Intravenous barbiturates</td>
<td>S4: Penetrating captive bolt, gunshot</td>
</tr>
<tr>
<td>Marine mammals</td>
<td>S7.5 (captive): Injected barbiturates S7.7 (free ranging): Injected barbiturates or anesthetic overdose</td>
<td>S7.5 (captive): Inhaled anesthetics S7.7 (free ranging): Gunshot, manually applied blunt force trauma, implosive decerebration</td>
</tr>
<tr>
<td>Nonhuman primates</td>
<td>S2.3, S7.4: Injected barbiturates or anesthetic overdose</td>
<td>S2.3, S7.4 (as appropriate by species): Inhaled anesthetic, CO, CO₂</td>
</tr>
<tr>
<td>Poultry</td>
<td>S3.4: Injected barbiturates and anesthetic overdose</td>
<td>S3.4: CO₂, CO, N₂, Ar, cervical dislocation (as anatomically appropriate), decapitation, manual blunt force trauma, electrocution, gunshot, captive bolt</td>
</tr>
<tr>
<td>Rabbits</td>
<td>S2.4: Intravenous barbiturates</td>
<td>S2.4: Inhaled anesthetic overdose, CO₂, cervical dislocation (as anatomically appropriate), penetrating captive bolt</td>
</tr>
<tr>
<td>Reptiles</td>
<td>S7.3: As appropriate by species—Injected barbiturates, dissociative agents and anesthetics as specified</td>
<td>S7.3: As appropriate by species—Inhaled anesthetics as specified, CO₂, penetrating captive bolt or firearm, manually applied blunt force trauma to the head, rapid freezing for animals &lt; 4 g</td>
</tr>
<tr>
<td>Rodents</td>
<td>S2.2: Injected barbiturates and barbiturate combinations, dissociative agent combinations</td>
<td>S2.2: Inhaled anesthetics, CO₂, CO₂, tribromoethanol, ethanol, cervical dislocation, decapitation, focused beam microwave irradiation</td>
</tr>
<tr>
<td>Small ruminants</td>
<td>S3.2: Injected barbiturates</td>
<td>S3.2: Gunshot, penetrating captive bolt</td>
</tr>
<tr>
<td>Swine</td>
<td>S3.3: Injected barbiturates</td>
<td>S3.3: CO₂, CO, N₂, Ar, gunshot, electrocution, nonpenetrating captive bolt, manually applied blunt force trauma</td>
</tr>
</tbody>
</table>

*Not recommended for routine use.*