

University of Louisville  
Institutional Animal Care and Use Committee  
*Policies and Procedures*

## 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)<sup>1</sup> 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. Comparative Medicine Research Unit (CMRU) 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<sup>1</sup>.

**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<sup>2</sup>, 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<sup>3</sup>. *The Guide for the Care and Use of Laboratory Animals*<sup>2</sup> states that euthanasia methods should be consistent with the AVMA Guidelines<sup>1</sup> 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<sub>2</sub>) euthanasia of mice and rats is acceptable with conditions.
    - i. CO<sub>2</sub> flow rate must be 30-70% chamber volume displacement per minute.
      1. CO<sub>2</sub> euthanasia station-specific directions and flow rates are available at all CMRU vivarium euthanasia stations. (see Attachments)
      2. All CO<sub>2</sub> euthanasia stations are required to have flow meters.

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- 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>
    - 1. CO<sub>2</sub> flow rate must be 30-70% chamber volume displacement per minute.
      - a. CO<sub>2</sub> euthanasia station-specific directions and flow rates are available at all CMRU vivarium euthanasia stations. (see Attachments)
      - b. All CO<sub>2</sub> 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<sub>2</sub> 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 or cessation of breathing.
  - 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. Zebrafish Euthanasia

- a. Immersion of adults in tricaine methanesulfonate, buffered, at least 250-500 mg/L. Assurance of death will be performed by cessation of opercular movement for a minimum of 30 minutes or a secondary method of euthanasia. Immersion of zebrafish <14 dpf in MS 222 alone is not effective for euthanasia.
- b. Immersion in benzocaine hydrochloride, buffered, at least 250 mg/L (solution). Assurance of death will be performed by cessation of opercular movement for a minimum of 30 minutes or a secondary method of euthanasia.

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- c. Immersion in ethanol, at 10-30 mL per zebrafish of 95% ethanol per liter. Assurance of death must be performed by cessation of opercular movement for a minimum of 30 minutes or a secondary method of euthanasia.
  - d. Rapid chilling (2-4°C) until loss of orientation and cessation of opercular movements; animals must not be in direct contact with ice. Assurance of death must be performed by cessation of opercular movement for a minimum of 10 minutes (adults) or 20 minutes (zebrafish fry 4-7 dpf). This procedure may be combined with immersion in dilute sodium hypochlorite or calcium hypochlorite at 500mg/L solution for embryos and larvae. Rapid chilling has been shown to be unreliable for embryos <3dpf.
  - e. Immersion in diluted sodium or calcium hypochlorite solution is acceptable for embryos up to 7 days of age.
    - i. 6.15% sodium hypochlorite, add to the culture system water at  $\geq 1$  part bleach to 5 parts system water.
    - ii. 8.25% sodium hypochlorite, add to the culture system water at  $\geq 1$  part bleach to 7 parts system water.
  - f. Secondary methods of euthanasia
    - i. Pithing
    - ii. Exsanguination
    - iii. Freezing
    - iv. Decapitation followed by pithing
    - v. Cervical transection followed by pithing
    - vi. Maceration
    - vii. Dilute sodium or calcium hypochlorite solution at 500mg/L (following MS 222 or rapid chilling of embryos or larvae)
  - g. Other acceptable methods for euthanasia of zebrafish are available and can be found in the AVMA Guidelines<sup>1</sup>.
4. 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 CMRU veterinary staff is encouraged when determining euthanasia methods for any species.

### **References:**

1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.  
(<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>)
2. National Research Council, *Guide for the Care and Use of Laboratory Animals*, 8<sup>th</sup> Ed., National Academy Press, Revised 2011.
3. Animal Welfare Act, 7 U.S.C. § 2131-2159 (2015).  
(<https://www.govinfo.gov/content/pkg/USCODE-2015-title7/html/USCODE-2015-title7-chap54.htm>)

### **Attachments:** Guidelines for the Humane Euthanasia of Rodents (by CMRU Facility)

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## Appendix I

Agents and methods of euthanasia by species.

Species	Acceptable	Acceptable with conditions (for adjunctive methods, see text)
Aquatic invertebrates	S6.3: Immersion in anesthetic solution (magnesium salts, clove oil, eugenol, ethanol)	S6.3: Adjunctive methods (second step) include 70% alcohol and neutral-buffered 10% formalin, pithing, freezing, boiling
Amphibians	S7.3: As appropriate by species—Injected barbiturates, dissociative agents and anesthetics as specified, topical or injected buffered MS 222 or topical benzocaine hydrochloride	S7.3: As appropriate by species—Inhaled anesthetics as specified, CO <sub>2</sub> , PCB or firearm, manually applied blunt force trauma to the head, rapid freezing of small (< 4 g [0.1 oz]) individuals where immediate death occurs
Avians (See also Poultry)	S5: IV barbiturates	S5: Inhaled anesthetics, CO <sub>2</sub> , CO, N <sub>2</sub> , Ar, cervical dislocation (small birds and poultry), decapitation (small birds) S7.6: Gunshot (free-ranging birds)
Cats	S1: IV barbiturates, injected anesthetic overdose Tributame, T-61	S1: Barbiturates (alternate routes of administration), inhaled anesthetic overdose, CO,* CO <sub>2</sub> ,* gunshot*
Cattle	S3.2: IV barbiturates	S3.2: Gunshot, PCB
Dogs	S1: IV barbiturates, injected anesthetic overdose Tributame, T-61	S1: Barbiturates (alternate routes of administration), inhaled anesthetic overdose, CO,* CO <sub>2</sub> ,* gunshot,* PCB*
Fish	S6.2: Immersion in buffered benzocaine or benzocaine hydrochloride, isoflurane, sevoflurane, quinaldine sulfate, buffered MS 222, 2-phenoxyethanol, injected pentobarbital, rapid chilling (appropriate species), ethanol	S6.2: Eugenol, isoeugenol, clove oil, CO <sub>2</sub> -saturated water, decapitation/cervical transection/manually applied blunt force trauma followed by pithing or exsanguination, maceration (research setting), captive bolt (large fish)
Equids	S4: IV barbiturates	S4: PCB, gunshot
Marine mammals	S7.5 (captive): Injected barbiturates S7.7 (free ranging): Injected barbiturates or anesthetic overdose	S7.5 (captive): Inhaled anesthetics S7.7 (free ranging): Gunshot, manually applied blunt force trauma, implosive decerebration
Nonhuman primates	S2.3, S7.4: Injected barbiturates or anesthetic overdose	S2.3, S7.4 (as appropriate by species): Inhaled anesthetic, CO, CO <sub>2</sub>
Poultry	S3.4: Injected barbiturates and anesthetic overdose	S3.4: CO <sub>2</sub> , CO, N <sub>2</sub> , Ar, low-atmospheric-pressure stunning, cervical dislocation (as anatomically appropriate), decapitation, manual blunt force trauma, electrocution, gunshot, captive bolt
Rabbits	S2.4: IV barbiturates	S2.4: Inhaled anesthetic overdose, CO <sub>2</sub> , cervical dislocation (as anatomically appropriate), PCB, NPCB
Reptiles	S7.3: As appropriate by species—Injected barbiturates/MS 222, dissociative agents with adjunctive method and anesthetics as specified	S7.3: As appropriate by species—Inhaled anesthetics as specified, CO <sub>2</sub> , PCB or firearm, manually applied blunt force trauma, rapid freezing for animals < 4 g where immediate death occurs, spinal cord severance/destruction of brain (crocodilians)
Rodents	S2.2: Injected barbiturates and barbiturate combinations, dissociative agent combinations	S2.2: Inhaled anesthetics, CO <sub>2</sub> , CO, tribromoethanol, ethanol, cervical dislocation, decapitation, focused beam microwave irradiation
Small ruminants	S3.2: Injected barbiturates	S3.2: CO <sub>2</sub> (goat kids), Gunshot, PCB, NPCB (goat kids)
Swine	S3.3: Injected barbiturates	S3.3: CO <sub>2</sub> , CO, NO, N <sub>2</sub> , Ar, gunshot, electrocution, PCB, NPCB (piglets), manually applied blunt force trauma

\*Not recommended for routine use.