Guidelines for Rats and Mice Euthanasia Procedures for Investigative Personnel

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1. Purpose
To establish acceptable methods of euthanasia for investigative personnel to perform. The current procedure is composed of two parts; a primary method followed by a secondary method to ensure death and/or prevent revival.

2. Responsibility
1. Investigative Personnel

3. Definitions
1. **Euthanasia**: Term used to describe the ending the life of an individual animal in a way that minimizes or eliminates pain and distress.
2. **Euthanasia Chamber**: Used for exposure to euthanasia-inducing gases and may be either the animal’s home cage or a chamber specifically designed for the purpose of euthanasia. The euthanasia chamber or euthanasia chamber lid must be transparent and allow an unobstructed view of the animal during the euthanasia process.

4. Procedures
1. **General Procedures**
   1. Methods of euthanasia must be in accordance with an IACUC approved animal use protocol.
   2. Deviations from this Guideline require scientific justification and approval by the IACUC.
   3. Methods described in this document are the most common forms of euthanasia. Contact Training Core for training on other euthanasia methods, ULAM-trainingcore@umich.edu.

2. **Animal Consolidation Procedure for Inhalant Euthanasia**
   1. Consolidate mice within the animal room.
      1. Maximum of 10 mice in a standard mouse shoe box cage.
   2. Provide ample space for animals to move and assume normal postures.
   3. Upon consolidation, animals are euthanized immediately (maximum of 10 minutes from the time the consolidation process begins).
   4. Follow standard housing densities when consolidating other rodents (e.g. rats) for euthanasia.

3. **Primary Euthanasia Method**
   1. Employ euthanasia methods according to the IACUC animal use protocol.
   2. Calibrate the CO₂ stations to the optimal flow rate, if rate is not currently calibrated (see appendix C).
   3. Ensure the euthanasia chamber is NOT pre-filled with carbon dioxide (CO₂) prior to placement of animals into the chamber.
   4. Observe and monitor animals continuously until death.
   5. It may take 5 minutes or more for animals to stop breathing during euthanasia.
      1. The most common method of adult and adolescent rodent (> 10 days old) euthanasia is exposure to CO₂ in compressed gas form (dry ice is not acceptable).
      2. Euthanize pregnant rodents with CO₂. This method causes death to embryos and fetuses.
      4. Neonates (pinkies), without hair and <10 days old, are typically euthanized by decapitation. Inhalation anesthetics or CO₂ exposure may be used as an induction method prior to decapitation.
1. CO₂ euthanasia is not as effective in neonates.
2. If CO₂ euthanasia is employed with neonates, a secondary method must be performed.
3. Clean or disinfect the euthanasia chambers, between groups of animals and after the euthanasia process is complete.
4. Follow the CO₂ Step by Step Euthanasia Procedures posted within the euthanasia room to euthanize animals.

4. **Secondary Euthanasia Method**
   1. Procedures must be employed to ensure death so that the animal cannot revive.
   2. Secondary methods of euthanasia include the following: bilateral pneumothorax, decapitation, exsanguination or, removal of a vital organ.
   3. The most commonly used secondary method is bilateral pneumothorax.
      1. Using a scalpel blade, an incision is made in both sides of the rodent's chest cavity or through the diaphragm.

5. **Carcass Disposal**
   1. Place animal carcasses in an opaque plastic bag or glove.
   2. Seal bag or glove tightly.
   3. Label the bag or glove with the following information:
      1. Date
      2. Principal Investigator (PI)
   4. Place bag or glove in walk-in cooler or refrigerator.
   5. Carcasses are disposed of after 72 hours.

5. **Related Documents**
   1. UM Methods of Euthanasia by Species
   2. IACUC Statement on Confirmation of Euthanasia via Rigor Mortis ([No link to documents new location](#))

6. **Appendices**
   1. Appendix A: CO₂ Step by Step Rodent Euthanasia Instructions
   2. Appendix B: CO₂ Step by Step Rodent Euthanasia Instructions (Brehm / BSRB)
   3. Appendix C: Calculate CO₂ Flow Rate
   4. Appendix D: CO₂ Euthanasia Station Ordering Information