**Standard Operating Procedure**

**Euthanasia of Rodents**

**Purpose**

The purpose of this document is to establish uniform procedures for the proper methods to euthanize animals while minimizing discomfort, distress, and pain. This SOP contains direction developed solely to provide internal guidance to employees

**Scope**

This SOP applies to all staff members.

**Personnel Qualifications/Responsibilities/Authority**

1. The VP has authority in directing and establishing this SOP.

2. The Director is responsible for maintaining this SOP.

3. The Manager is responsible for the implementation, enforcing and training of SOP material to all associated staff members.

4. All staff members associated with the vivarium operation are responsible for familiarizing themselves with this SOP prior to the execution of their duties.

**Definitions**

**Euthanasia:** or literally, “good death” – is the act of terminating a life while minimizing or eliminating pain or distress. According to the AVMA Guidelines for the Euthanasia of Animals (2020 ed.) the primary objective regarding euthanasia of an animal is “(1) their

humane disposition to induce death in a manner that is in accord with an animal’s interest and/or because it is a matter of welfare, and (2) the use of humane techniques to induce the most rapid and painless and distress-free death possible.” The chosen method of euthanasia should result in “the rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, a loss of brain function.”

**AVMA**: American Veterinary Medical Association

**Exsanguination**: Severe loss of blood due to internal or external hemorrhage

**Equipment and Materials**

● Compressed CO2 gas cylinder/ house gas equipped with an EZ-Systems euthanex Prodigy unit attached.

● Disposal or carcass bag

● Euthanasia chamber with Euthanex lid

● Any tools required to perform secondary method of euthanasia

● Proper PPE

**Step by Step Procedure**

1. If using CO2 delivered by a gas cylinder, confirm that the tank has enough gas available.

2. Collect cages of animals intended for euthanasia and delivery them to the procedure room where euthanasia is permitted

1. Do not euthanize animals in animal holding rooms or in sight of other animals unless is special approved circumstances such as quarantine or exposure to infectious agents

2. Perform euthanasia in a biosafety cabinet when euthanizing BSL2 cages

3. Euthanasia of more than one animal should always be performed in cohorts of live animals (live animals must never be placed in a chamber with dead animals)

3. Use animal home cage as euthanasia cage when possible. If the home cage is not possible, use an appropriately sized cage, and freshly sanitized chamber. Minimum floor space requirements as listed in the “Guide” must be met for all rodents at all times.

4. Place Euthanex lid on top of the euthanasia chamber such that holes in the lid are over the cage to allow displacement of air. If using home cage, remove the filter lid prior to placement of the Euthanex lid.

5. Turn on CO2

6. The Euthanex Smart Box Prodigy System will be used when performing euthanasia of rodents. Turn the system on by depressing the power switch on the back of the Smart Box.

7. The Prodigy system will be programmed by the Manager or designee, with cage dimensions and the flow rate will be calculated by the system automatically. CO2 occurs at a displacement rate of **30-70%** chamber volume per minute (approximately 1-3L per minute) to avoid delivering the gas at a high velocity causing pain and distress to the animals.

This system allows for up to three cages of animals to be euthanized concurrently. The touch screen display shows the cycle, cycle time, and total time remaining.

1. Follow the onscreen prompts to begin the process.

1. Press mouse cage

2. Press the number of cages (1-3)

3. Press start

2. The system will “charge” - slowly begin filling the cage with CO2 for five minutes.

3. Prefilling the CO2 chamber is not accepted - as sudden exposure to high concentrations of CO2 may be distressing to some species

8. Allow CO2 to flow at the desired rate, gas concentration may be increased as loss of consciousness is observed and flow should be maintained 1 minute after apparent clinical death (i.e.., cessation of cardiovascular and respiratory movement)

1. Animals are not to be left unattended during euthanasia procedures

9. Once complete, observe the animals for any movements and/or breathing (some animals will take longer than others to expire). Once the animals are unconscious and have stopped breathing you will perform an approved secondary form of euthanasia such as: cervical dislocation, decapitation or thoracotomy and as dictated by the client’s protocol or IACUC.

10. Turn off CO2

11. Chambers and lids must be sanitized between animals or groups of animals

**Neonates:**

12. The use of sharp scissors for decapitation is recommended for euthanasia of neonatal rodents up to 7 days of age.

13. Neonates: (up until approximately 10 days) are resistant to CO2 euthanasia due to their inherent resistance to hypoxia and may require prolonged exposure time to any type of inhalant. Consequently, CO2 alone should **not** be used alone as a sole means of euthanizing neonates. CO2 may be used to induce narcosis but must be followed with another acceptable method of euthanasia (e.g., decapitation, cervical dislocation, or thoracotomy) to ensure death.

14. Place animal(s) in an appropriately sized labeled leak-proof plastic carcass bag and close/seal the bag for placement in the carcass freezer until disposal.

15. Clean area after use, and disinfect any equipment, tools and tables.

16. In case personnel should find live animals bagged for disposal (death verification was not correctly followed) then this matter must be brought to the immediate attention of the ACF manager, principal investigator and Attending Veterinarian.

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**References**

1. *Guide for the Care and Use of Laboratory Animals*; 8th Edition; Institute of Laboratory Animal Resources; U.S. Department of Health and Human Services; National Institutes of Health Publication No 85-23, Revised 2011.

2. *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition;* American Veterinary Medical Association (AVMA); Version 2020.0.1

3. “ALAT Training Manual”, American Association for Lab Animal Science 4. https://euthanex.com