1. **Operator requirement**
   1.1. Operators must be properly trained and validated to be competent before conducting the euthanasia procedures.

2. **Arrangement for rodent euthanasia to be done by APCF staff**
   2.1. Animals are euthanized in their home cage. Aggressive animals must be isolated in another clean cage.
   2.2. A properly filled *Euthanasia Card* is inserted into the cage card holders to indicate the euthanasia request by the users.
   2.3. Cages are transferred to the waiting rack outside Room 7215 procedure room for the arrangement of euthanasia by designated APCF staff.
   2.4. Make sure feeds and water is available before the animals are euthanized.
   2.5. Unless for biocontainment consideration in which case animal must be immediately euthanized, individually ventilated cages are placed on the waiting rack with their covers removed to ensure animals have sufficient ventilation while waiting for euthanasia.

3. **Euthanasia by CO₂ exposure (routine)**
   3.1. Animals must be euthanized in their home cage.
   3.2. Exemptions to 3.1 may be approved by the Director of APCF (Interim).
   3.3. Cages containing rodents to be euthanized are transferred into the CO₂ chamber without prefilled with CO₂.
   3.4. Cover the chamber and open valves between the CO₂ supply and the chamber.
   3.5. Open the exhaust valve slightly, according to a visual instruction posted on site, without turning on the exhaust pump.
   3.6. Adjust the flow regulator to the value marked by designated line on the flowmeter to achieve a chamber filling rate at 25% of chamber volume per minute.
   3.7. Let the animals be exposed to CO₂ for 5 min to 10min.
   3.8. Keep monitoring if the mice older than 10 days of age loss conscious in 2 minutes and cease breathing in 5 minutes.
3.9. If the above phenomena are not observed, the operator will seek help from an APCF technician. APCF technicians will inspect any leakage or short of CO2 service for immediate fixing.

3.10. If the CO2 service cannot be fixed immediately, the animals must be euthanized by cervical dislocation or by decapitation immediately.

3.11. Close the air inlet valve after the time required for CO2 exposure and turn on the air exhaust system/valve for 1 min to remove CO2 in the chamber.

3.12. For rodents older than 10 days of age, one of the following method to assure animal death are conducted on each animal:
   3.12.1. By a physical procedure, i.e. cervical dislocation (mice only) or decapitation.
   3.12.2. By in injection of Dorminal into the thoracic cavity (mice: 0.5ml/animal; rat: 1ml/animal).

3.13. For pups younger than and at 10 days of age (which are resistant to hypoxia), the gas exhaust step is omitted after CO2 Exposure. The treated pups are quickly removed from the chamber (in their nest if possible), with minimal stimulation, to a designated clean container set at room temperature and immediately placed into a designated location in the freezer, locating next to the chamber. They MUST never be placed in direct contact with any cold surface.

3.14. Animals under hypothermia treatment are left in the freezer for one hour or more before being wrapped for disposal.

3.15. Wrap the carcasses in a designated plastic bag, fill in the log sheet and put the carcasses in one of the designated freezers.

4. Euthanasia by CO2 exposure (for rats exclusively for teaching purpose)
   4.1. Let the animals be exposed to CO2 for 5 min without opening the exhaust valve nor the exhaust pump.
   4.2. Stop the CO2 gas supply and let the chamber stand for an additional 10 mins and observe whether the lips, tails and feet turn blue via the chamber to confirm death.
   4.3. Run the air exhaust for 1 min and remove the animals from the chamber.

5. References
   5.1. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition