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

2. **Arrangement of rodent euthanasia to be done by APCF staff**  
2.1. Whenever possible, animals are euthanized in their home cage. Aggressive animals must be isolated in another clean cage.  
2.2. A properly filled *Euthanasia Request Card* is inserted into the cage cardholders to indicate the euthanasia request by the users.  
2.3. Individually ventilated cages MUST be kept in connection to the ventilated rack, in a cage slot within those allocated to the respective user group.  
2.4. The animals to be euthanized must be housed with the same housing quality as if no euthanasia is planned.  
2.5. No regrouping of animals are allowed unless in an emergency situation approved by the facility manager.

3. **Mouse Euthanasia by CO₂ inhalation using Automatic Chamber System**  
3.1. Cages may be held in the IVC rack in the Necropsy Room temporarily until a scheduled time for euthanizing the animals. Food and water must be available during the waiting time.  
3.2. Pre-operation check  
   a. Gas source is connected.  
   b. The main supply of CO₂ is turned on.  
   c. Turn on the regulator heater.  
   d. The valve for the air exhaust is opened.  
   e. Set up the system for using one chamber or two chambers.  
3.3. Loading the system
a. Cage tops are removed. Animal escape is ensured by covering the opened half of the cage with the porous plastic lid.
b. Put the cages into the chamber.
c. Close and lock the chamber door.

3.4. Running the cycle
   a. Only APCF validated programs are used. (Validated programs are listed on the equipment)
   b. Start the euthanasia program by pressing Start System on the control box.
   c. The system will automatically lock the chamber doors, charge the chamber(s) with CO₂, shut off the gas supply, holding in dwell time and eventually exhaust the gas.

3.5. Death confirmation: refer to section 5. Death Confirmation

3.6. After the cycle and handling all carcasses, turn off the regulator heater in the gas cylinder cabinet.

4. Mouse Euthanasia by CO₂ exposure in Automatic Prodigy (cage level system)
4.1. Mice are euthanized as soon as the cage are removed from the animal room to decrease stress.

4.2. Pre-operation system setup
   a. Check gas is connected to the Prodigy system
   b. Check the gas hose is connected to the output of the Prodigy and the Euthanex Lid
   c. Check the gas regulator is set for a pressure output at 20 PSI.
   d. Switch on the Prodigy unit by pressing the On switch at the back. The unit will boot and come to the home screen.
   e. Insert the gas hose into the cage via the valve originally for water bottle.

4.3. Running the cycle
   a. Select the cage type and the number of cages on the control unit.
   b. The system will automatically charge the cage(s) with CO₂, shut off the gas supply and hold in dwell cycle.
   c. The cage will then be opened to confirm the dead of the animals as described in section 5.

5. Death confirmation
5.1. For rodents older than 10 days of age. No death confirmation is required for validated programs. If required, do one of the following procedures.
   a. By a physical procedure, i.e. cervical dislocation (mice only) or decapitation.
   b. By injecting 0.5ml of Dorminal (i.e. 240mg/ml pentobarbital) peritoneally.

5.2. For pups younger than and at 10 days of age (which are resistant to hypoxia),
   a. the 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. They MUST never be placed in direct contact with any cold surface.
   b. Animals under hypothermia treatment are left in the freezer for one hour or more before being wrapped for disposal.
6. Carcass Disposal
   6.1. Wrap the carcasses in designated plastic bags, fill in the log sheet and put the carcasses into the freezer.
   6.2. Carcasses are disposed as Clinical Waste Group 2 by a licensed contractor. Prior to the collection of the carcasses by the contractor, the carcasses are bagged with the appropriate yellow plastic bags and kept in the designated freezers.

7. Contingency
   7.1. If loss of consciousness and cease breathing at the expected time are not observed, the operator will seek help from an APCF technician. APCF technicians will inspect any leakage or short of CO₂ service.
   7.2. If the CO₂ service cannot be fixed immediately, the animals must be euthanized by cervical dislocation or by decapitation immediately.

8. References