**SOP Number:** C-011  
**Last revised:** 15Nov2017  
**SOP Title:** Rodent Euthanasia By Carbon Dioxide Gas in 7H facility  
**Purpose:** To minimize distress and pain on rodents during euthanasia with carbon dioxide gas  
**Responsibility:** Technicians, Laboratory Assistants/Attendants and Animal Users  
**Related SOP:** NA  
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**Approved by:** Anthony James (D/APCF)

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 Card* is inserted into the cage card holders 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.

3. **Euthanasia by CO₂ exposure**  
   3.1. With the whole IVC cage covered by the filter top, insert the CO₂ supplying tubing into the cage valves for water bottle nozzle.  
   3.2. Open the CO₂ supplying valve.  
   3.3. Adjust the CO₂ flow to 1.5 - 2 LPM (DO not exceed 2 LPM) which was calculated to achieve a filling rate at 10% to 30% per minute for all available cage sizes.  
   3.4. Let the animals be exposed to CO₂ for 5 min.  
   3.5. Keep monitoring if the mice older than 10 days of age loss conscious in 2 minutes and cease breathing in 5 minutes.  
   3.6. 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.  
   3.7. If the CO₂ service cannot be fixed immediately, the animals must be euthanized by cervical dislocation or by decapitation immediately.  
   3.8. Close the air inlet valve after the time required for CO₂ exposure.
3.9. For rodents older than 10 days of age, one of the following methods to assure animal death are conducted on each animal:
   3.9.1. By a physical procedure, i.e. cervical dislocation (mice only) or decapitation.
   3.9.2. By injection of Dorminal into the thoracic cavity (mice: 0.5ml/animal; rat: 1ml/animal).

3.10. For pups younger than and at 10 days of age (which are resistant to hypoxia), 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, locating next to the chamber. They MUST never be placed in direct contact with any cold surface.

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

3.12. Wrap the carcasses in a designated plastic bag, fill in the log sheet and put the carcasses in the freezer outside 7H facility.

4. References