EUTHANASIA INSTRUCTIONS

1. Euthanasia must be performed by trained personnel using appropriate technique, equipment, and agents.

2. DO NOT PRE-CHARGE CHAMBER WITH CO_2.

3. Place animals, preferably in the home cage, into the euthanasia chamber. Remove the home cage lid and any items that may block view of the animal(s), then close the euthanasia chamber lid.
   - Do not exceed 10 mice or 3 rats in the chamber.
   - Do not stack home cages on top of one another.
   - Do not leave animals unattended in euthanasia chamber.

4. Verify hose attachment to the euthanasia chamber lid. Turn the CO2 flow on fully and confirm that the CO2 regulator is adjusted to 55-60 CFH.

5. Expected time to unconsciousness and respiratory arrest is 2-4 minutes. If unconsciousness does not occur within 3 minutes, check:
   - CO2 regulator is adjusted to 55 – 60 CFH.
   - Absence of CO2 supply or leaks in system.
   - Report issues with euthanasia chamber to DLAR Veterinary Services.

6. Observe each rodent to confirm that respiration has stopped. Maintain CO_2 flow and monitor the animal(s) for at least 1 minute after respiratory arrest.

7. Remove carcasses and perform the secondary method of euthanasia approved in your IACUC protocol (*ex. bilateral thoracotomy, cervical dislocation, decapitation*).

8. Remove carcasses and spray the interior and exterior of the euthanasia chamber with disinfectant at the end of each use or between groups of animals if home cage is not used. Wait 5 minutes before wiping the chamber dry.

9. Dump the CO_2 from the chamber between cohorts of animals.

   Note: Neonatal animals (up to 14 days of age) are resistant to the effects of CO_2; therefore, alternative methods of euthanasia <u>MUST</u> be performed. CO_2 may be used for narcosis if approved on your IACUC protocol and must be followed by another method of euthanasia as specified and approved in the protocol.