PROPER STEPS FOR CO₂ EUTHANASIA OF RODENTS USING A CUSTOM FLOW METER

EUTHANASIA SHOULD ONLY BE PERFORMED BY APPROPRIATELY TRAINED STAFF

Calculating acceptable flow rate for new equipment or changing chambers (different sized chambers require different flow rates).

\[
\text{Chamber volume (L)} = \frac{\text{(height in cm) x (width in cm) x (length in cm)}}{1000}
\]

\[
\text{Acceptable flow rate (L/min)} = \frac{\text{(Chamber volume in Liters) x 0.20}}{\text{minute}}
\]

Label flow meter at the acceptable flow rate with secure tape, permanent marker, etc.

1. Check equipment before starting the procedure – ensure there is sufficient tank pressure (at least 250 psi) and the flow meter is functional.

2. Place the mice or rats into the euthanasia chamber. Do not mix cages of animals. Close the chamber.

3. Open the valve to release CO₂. Set flow to the acceptable flow rate as indicated by the label.

4. Rodents must remain in the chamber until respiration has ceased. It may take 5 minutes or more as it will be about that long to approach 100% CO₂ in the chamber.

5. Confirm death of all animal(s) as per the approved IACUC protocol.
   - Cessation of breathing should be accompanied by at least 3 extra minutes at 100% CO₂, after breathing stops.
   - A physical method (cervical dislocation, decapitation, or exsanguination) is preferred.

6. Close the valve to stop the delivery of CO₂.

7. Properly dispose of all deceased animals. Confirm death before disposing of the carcasses.

8. If additional animals will be euthanized, you must remove the CO₂ in the chamber before placing new animals in the chamber.
   - CO₂ is heavier than room air and will remain in the undisturbed chamber.
   - To purge the chamber of existing gas, simply turn the empty chamber upside down.

9. Spray euthanasia chamber with disinfectant (e.g. Clidox®) and wipe clean after the last group of rodents is euthanized, or between groups if the chamber is soiled.

**Animals must NEVER be left unattended in the euthanasia chamber. Lab personnel must remain in the vicinity of the chamber at all times.**