

Policy on Rodent Euthanasia via Euthanex Lid Administered Carbon Dioxide

Date Reviewed: 6/18/2019

Carbon dioxide (CO₂) is a frequently used euthanasia agent for small laboratory animals due to its rapid onset of action, safety, and ready availability. However, if not administered properly, CO₂ inhalation has the potential to cause pain and distress on respiratory and ocular membranes.

- 1. All personnel administering CO₂ to rodents must be properly trained in the procedure. Training on the use of the equipment and appropriate methods of euthanasia is available from the vivarium manager.
- 2. Compressed gas is the only acceptable source of CO₂ for euthanizing rodents. Other sources such as dry ice must not be used.
- 3. All CO₂ stations must have a regulator and flow meter installed in order to control the flow rate. The regulator is automatically set to match either mouse or rat cages. The regulator is set to provide a rate of 20% volume displacement per minute.
- 4. Alternative euthanasia chambers must be constructed of clear material (e.g. Plexiglas® or polycarbonate) and must be kept clean to minimize odors that might distress animals subsequently euthanized. The CO2 flowmeter must be set to provide a rate of 10-30% volume displacement per minute.
- 5. Individual signage must be posted at the site of each euthanasia station with step-bystep instructions as to how to operate the equipment and ensure death of animals using a secondary method of euthanasia.
- 6. In some cases, adult and juvenile mice may be crowded together from separate cages IMMEDIATELY PRIOR (less than 5 minutes) to administration of CO2 gas. Animals from separate cages that have been crowded together may not be left unattended at any time. Stress may also be reduced by euthanizing rodents in their home cage.
- 7. The chamber must not be pre-filled with CO₂ prior to placement of animals into the chamber, and must be "purged" (dumped) between euthanasia sessions.
- 8. Death must be confirmed by inspection, a secondary method of euthanasia must be used to ensure death if there is any sign of life such as toe pinch, blink reflex, palpable heartbeat, or respiratory attempts. These secondary methods include exsanguination, decapitation, and creation of a bilateral pneumothorax.
- 9. <u>DO NOT USE CO</u>₂ for euthanasia of any rodent younger than 10 days of age as neonatal rodents are resistant to hypoxia. CO₂ may be used for sedation prior to decapitation. (Note: The Euthanex machine uses CO2 and compensates for



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- neonatal resistance to hypoxia by maintaining a 100% CO2 atmosphere for one hour)
- 10. Embryos and fetuses: CO for euthanasia of a pregnant mother is an acceptable method to cause death of the embryos and fetuses, when fetuses are not required for study.
- 11. Deviations from this policy require scientific justification and approval by the IACUC.