**Instructions: Humane Euthanasia  
of Rodents using Carbon Dioxide (CO2)**

1. Place rodent(s) (> 10 days of age) in a non- precharged chamber. Do not overcrowd: animal(s) must be able to make normal postural adjustments.
2. Introduce CO2 into chamber at a calculated flow rate (30-70% of chamber volume per minute\*) until animal(s) becomes unconscious. After rodent(s) become unconscious, the flow rate can be increased to obtain rapid euthanasia. CO2 flow should be maintained for at least 1 minute after respiratory arrest.
3. **Verify clinical death** via a secondary method of euthanasia (e.g., exsanguination, cervical dislocation) or by palpating to ensure cardiac arrest.
4. Turn off gas flow and close tank valve when finished. Clean chamber thoroughly.

\*For instructions on calculating the correct Flow Rate for your specific chamber: <https://www.rarc.wisc.edu/tools_and_guides/techniques/euthanasia/calculating_co2_flow_rate_for_mice_and_rat_euthanasia.html>

**See the policy** [**UW-4090**](https://policy.wisc.edu/library/UW-4090)**, titled Euthanasia of Rodents using Carbon Dioxide for further information.**

**Instructions: Humane Euthanasia  
of Neonatal Rodents (≤10 days of age)**

For mice, rat and hamster neonates up to and including 10 days of age:

* decapitation, cervical dislocation, or injection with a chemical anesthetic (e.g., pentobarbital 800 mg/kg IP) are acceptable means of euthanasia. Neonates 10 days of age or less are resistant to hypoxia; if CO2 is used, prolonged exposure time is needed to cause loss of consciousness or death. A secondary physical means of euthanasia (decapitation or cervical dislocation) is required when CO2 is used.

For more information: <https://www.rarc.wisc.edu/tools_and_guides/techniques/euthanasia/euthanasia_of_fetal_and_neonate_rodents.html>



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