

**Guidelines for Euthanasia of Rodents
Using Carbon Dioxide (CO₂)
Revised by UCAR 2/15/23**

CO₂ inhalation is considered an acceptable method of euthanasia for small rodents (e.g., mice, rats, gerbils, hamsters, guinea pigs and degus) when properly administered. The AVMA Guidelines for the Euthanasia of Animals: 2020 Edition¹ describes that certain critical factors must be met to use CO₂ inhalation for euthanasia: “Carbon dioxide and CO₂ gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank.... As gas displacement rate is critical to the humane application of CO₂, an appropriate pressure-reducing regulator and flow meter, or equivalent equipment, with demonstrated capability for generating the recommended displacement rates for the size of the container being utilized is absolutely necessary.”

The euthanasia method must be appropriate to the research goals, species and age of the animal, approved in the animal care and use protocol and must be consistent with the current AVMA Guidelines on Euthanasia.

UCAR guidelines for the Use of CO₂ for rodent euthanasia include the following:

1. All euthanasia chambers must be transparent so that all animals can be observed during the process.
2. A CO₂ euthanasia SOP must be developed and posted for each location where CO₂ euthanasia is performed. The SOP must meet UCAR guidelines.
3. Use compressed CO₂ gas cylinders fitted with a pressure-reducing regulator and an attached calibrated flow meter. Do not pre-charge the chamber; prefilled chambers are unacceptable per the AVMA Guidelines due to the potential for significant pain.
4. Place the animal(s) in the chamber and introduce CO₂ at a rate of 30% to 70% of the chamber volume per minute. This will produce rapid unconsciousness with minimal distress in animals (AVMA 2020, pg. 31).
 - a. ***Small Mouse Cage (7½" wide x 11½" deep x 5" high): 2.0 - 5.0 liters per minute.***
 - b. ***Large Mouse Cage (10½" wide x 19" deep x 6" high): 6.0 – 14.0 liters per minute.***
 - c. ***Rat Cage (10½" wide x 19" deep x 8" high): 8.0 – 18.0 liters per minute.***

Once the rodent appears unconscious (i.e., recumbent, absence of purposeful movement) you may increase the flow rate of CO₂.

5. Neonatal animals (up to 10 days of age) are resistant to the hypoxia-inducing

effects of CO₂. Please refer to the UCAR Guidelines for the [Euthanasia of Fetuses, Neonates and Embryos](#).

6. Animals should be euthanized in their home cage whenever possible. Do not overcrowd chambers/cages. Do not combine species. Rodents from different cages should not be combined prior to euthanasia even if totaling less than 5 due to potential for distress and fighting.

In general, there should be no more animals in euthanasia cages than allowed per the Mouse Cage Density and Rat Cage Density policies:

- a. Standard mouse cage – 5 mice
 - b. Large mouse cage – 10 mice
 - c. Standard rat cage – 5 rats
7. If home cages cannot be used, the CO₂ euthanasia chamber must be emptied and cleaned between each use to remove debris, pheromones and accumulated CO₂.
 8. Rodents older than ten days must be left in the CO₂ environment for at least 1 minute after respiratory arrest. Observe the rodents for cessation of breathing and heartbeat. Other signs of death are faded eye color, fully dilated pupils and relaxation of anal/urinary sphincters, which may result in rodent urinating and defecating. **Never leave rodents unattended during euthanasia.**
 9. Upon completion of the procedure, death must be confirmed by performing a secondary physical method (i.e., decapitation, perfusion with a histological fixative via the major blood vessels, thoracotomy, complete severing of the spine just below the base of the skull using a dorsal approach and/or cervical dislocation for animals under 200g). After verifying death, place animal carcass in a bag, tie the bag securely and attach the completed Euthanasia Tag (yellow) prior to putting carcass bags in morgue coolers or freezers.

In the Vivarium, you may use a SMARTBOX Station or CO₂ euthanasia station, which consists of a gas tank, a regulator and flow meter which is connected to a lid that will fit on a mouse or a rat home cage. Directions and settings are provided in each room.

Also located in the vivarium are Euthanex Euthanasia Equipment. The equipment is operated by the Vivarium personnel. **You will not receive carcasses after euthanasia.** The instructions for leaving cages of rodents for euthanasia are:

- a. Cages must be labeled with **Investigator's name and UCAR number**,
- b. Do not leave sick, injured or fighting animals,
- c. Do not leave unweaned/nursing pups without their dam,
- d. Do not leave rodents that have been exposed to hazards

Do not combine cages of mice.

IMPORTANT: Unintended recovery of rodents after apparent death from CO₂ (e.g., in a necropsy cooler, morgue cooler, morgue freezer) is a serious noncompliance issue. This is why it is imperative that death is verified by a secondary method. Unintended recoveries will be reported to NIH's Office of Laboratory Animal Welfare and could result in loss of your privileges to work with animals. For more information refer to <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>.

References

1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf
2. OLAW FAQ F 1. Is the use of carbon dioxide as an acceptable euthanasia agent? <https://olaw.nih.gov/guidance/faqs>