

**Guidelines for Euthanasia of Rodents  
Using Carbon Dioxide (CO<sub>2</sub>)  
Revised by UCAR 2/15/23**

1

describes that certain critical factors must be met to use CO<sub>2</sub> inhalation for euthanasia: “Carbon dioxide and CO<sub>2</sub> 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 CO<sub>2</sub>, 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.”

effects of CO<sub>2</sub>. 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

**Do not combine cages of mice.**

**IMPORTANT:** Unintended recovery of rodents after apparent death from CO<sub>2</sub> (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. 2013. <http://www.avma.org/CE/AVMA-2013-001211files>