

## Standard Operating Procedure

SOP Number: **02-18-5818**

Service: **Research**

Operating Section: **IACUC**

Unit: **CMF**

Title: **Guidelines for Euthanasia of Rodents Using Carbon Dioxide**

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### Purpose:

To establish guidelines for euthanasia of rodents using carbon dioxide.

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### Procedure:

Rodents must be euthanized by trained personnel using appropriate techniques, equipment and agents. This is necessary to ensure a painless death that satisfies federal requirements. Death should be induced as painlessly and quickly as possible. Upon completion of the procedure, death must be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting an animal's fixed and dilated pupils. Euthanasia should not be performed in the animal room. The euthanasia method must be appropriate to the species, approved in the animal study proposal and conform to the most recent AVMA Guidelines for the Euthanasia of Animals: 2020 Edition<sup>1</sup>.

CO<sub>2</sub> inhalation is a common method of euthanasia used for mice, rats and hamsters. A few important aspects of this procedure are:

- 1) Unless scientifically justified and approved by the IACUC, euthanasia cannot be performed in the animal room, but instead must be performed in an investigator laboratory (under a hood) or in the CMF CO<sub>2</sub> stations.
- 2) Animals must be transported to be euthanized in a manner that avoids overcrowding, physical trauma, and fighting. In general, males from different cages or incompatible females should not be placed in the same cage during transport. If fighting occurs, animals must be separated immediately.
- 3) The euthanasia chamber must allow ready visibility of the animals. Do not overcrowd the chamber; all animals in the chamber must be able to make normal postural adjustments. Only one species at a time is allowed to be placed into a chamber. Euthanasia must always be done in cohorts (live animals should not be placed in the chamber with dead animals). Chambers must be kept clean to minimize odors that might distress animals prior to euthanasia.
- 4) Compressed CO<sub>2</sub> gas in cylinders, with a flow meter, is the only approved source of carbon dioxide as it allows the inflow of gas to be controlled. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide at the rate of 30-70% of the chamber volume per minute so as to optimize reduction in distress. Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful. Follow instructions located on the euthanasia chambers for filling each particular chamber (varies by chamber volume). After the animals become unconscious, the flow rate can be increased to minimize the time to death.

- 5) Expected time to unconsciousness is usually within 2 to 3 minutes<sup>2</sup>. Observe each rodent for lack of respiration and faded eye color. Maintain CO<sub>2</sub> flow for a minimum of 1 minute after respiration ceases. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO<sub>2</sub>. If unconsciousness has not yet occurred within 2 to 3 minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO<sub>2</sub> supply, and/or leaks. Appropriate CO<sub>2</sub> concentrations and exposure times will prevent unintended recovery.
- 6) It is important to confirm that an animal is dead after removing it from the chamber. To ensure death it is recommended that CO<sub>2</sub> use be followed by a secondary euthanasia method. For mice, secondary euthanasia methods include cervical dislocation, exsanguinations, thoracotomy, decapitation or anesthetic overdose. For rats, secondary methods include all of the above, except that cervical dislocation can only be performed on rats weighing less than 200 grams.
- 7) Neonatal animals (up to 10 days of age) are resistant to the effects of CO<sub>2</sub>, therefore, alternative methods are recommended. Carbon dioxide may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (i.e. decapitation using sharp blades).

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](https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf)
2. Neil L, Weary DM. Behavioral Responses of the Rats to Gradual-fill Carbon Dioxide Euthanasia and Reduced Oxygen Concentrations. Applied Animal Behavior Science 100 (2006) 295-308.

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**APPROVALS**

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