

# IACUC

## STANDARD OPERATING PROCEDURE (SOP)

### CO<sub>2</sub> EUTHANASIA

University of Rhode Island  
Office of Research Integrity

#### BACKGROUND

This procedure outlines the proper methods of using carbon dioxide to euthanize rodent species, while avoiding or minimizing discomfort, distress, and pain. For a complete discussion of the conditions and techniques available for rodent euthanasia refer to the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*.

#### PROCEDURES

The following conditions must be met when performing CO<sub>2</sub> euthanasia:

- Compressed CO<sub>2</sub> in gas cylinders is the only acceptable source of CO<sub>2</sub> for euthanasia. CO<sub>2</sub> generated by other methods such as from dry ice, fire extinguishers, or chemical means is unacceptable.
- Animals are placed into chambers so that they have sufficient floor space and are not overcrowded. Minimum space requirements as listed in the *Guide for the Care and Use of Laboratory Animals, Eighth Edition* must be met for all rodents until time of euthanasia.
- Euthanasia of more than one animal at a time should always be performed in cohorts of live animals (i.e., live animals must not be placed in the chamber with dead animals).

To reduce stress, rodents should be euthanized in their home cage whenever possible. This can be accomplished by delivering CO<sub>2</sub>:

- a. Via a Euthanex® lid over the home cage after removing the filter and wire tops.
- b. Via the air supply port of an IVC cage.

Alternatively, animal(s) are placed in a clean microisolator box with a fitted lid.

CO<sub>2</sub> delivery to the microisolator is accomplished by turning the CO<sub>2</sub> cylinder valve and flow meter on so that animal(s) are **slowly** exposed to increasing levels of CO<sub>2</sub>. An optimal flow rate for CO<sub>2</sub> euthanasia systems should displace 10% to 30% of the chamber or cage volume/min. **Prefilled chambers are unacceptable.**

Implementation Date: February 19 <sup>th</sup> , 2014	Reviewed/Revised Date: April 30 <sup>th</sup> , 2014	IACUC Chair: T. Bradley
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**FLOWMETER CALCULATION INSTRUCTIONS**

(For Tank Chambers of varying size)

The flow meter allows accurate filling of the chamber volume at any given flow rate of CO<sub>2</sub> per minute. However, percent flow rate must first be converted to liters/minute (L/min) and will depend on the size of the chamber. To make the conversion from 20% flow rate per minute to L/Min, the following calculation is used:

1. Determine the volume of the euthanasia chamber in liters by measuring its length, width, and height in centimeters (cm).
2. Multiply these dimensions together, (length x width x height), to obtain the volume in cubic centimeters (cm<sup>3</sup>)
3. Convert volume in cm<sup>3</sup> to volume in liters (1 cm<sup>3</sup> = 0.001 liters so multiply volume in cm<sup>3</sup> by 0.001 to get volume in liters)
4. Multiply volume of cage in liters by 20% flow per minute.
5. If the flow meter is calibrated for air, divide by 0.812, the conversion factor for CO<sub>2</sub>.

Flow Rates for Standard Cages

Species	Cage Type	Flow Rate (L/min)
Mice	Static (Small Shoebox)	2
	Static (Large Shoebox)	6
	IVC (M.I.C.E.)	1.5
	IVC (Allentown)	1.6
Rats	Static	8

**ADDITIONAL CONSIDERATIONS**

- Gas flow may be increased as loss of consciousness is observed and flow should be maintained for at least one minute after apparent clinical death (e.g., cessation of cardiovascular and respiratory movements).
- Turn off CO<sub>2</sub> flow meter and cylinder valve.
- Chambers/lids must be cleaned/dried between animals or groups of animals to minimize odors that might distress animals prior to euthanasia.

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- Animals must not be euthanatized in animal housing rooms except during special circumstances such as during quarantine and/or exposure to infectious agents
- Death must be verified after euthanasia and prior to disposal. Unintended recovery must be prevented by the use of appropriate CO<sub>2</sub> concentrations and exposure times, or by other means such as a secondary physical method. The recommended secondary physical methods are:
  1. Decapitation
  2. Perfusion of a histological fixative via the major blood vessels
  3. Pneumothorax by opening the thorax
  4. Complete severing of the spine just below the base of the skull using a dorsal approach
  5. Cervical dislocation for animals under 200g.

**It is important that you understand that unintended recovery of animals after apparent death from CO<sub>2</sub> (e.g., in a necropsy cooler, morgue cooler) is a serious noncompliance issue. It will be reported to OLAW and could result in loss of privileges or funding.**

#### REFERENCES

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

<https://www.avma.org/kb/policies/documents/euthanasia.pdf>

Guide for the Care and Use of Laboratory Animals, 8<sup>th</sup> Edition (2011)

<http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf>

Public Health Service Policy on Humane Care and Use of Laboratory Animals (2002)

<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>

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