

**University of Colorado at Boulder
Office of Research Integrity
Institutional Animal Care and Use Committee
SOP # 19
Title: Carbon Dioxide Euthanasia**

PURPOSE

The purpose of this SOP is to standardize the practices of carbon dioxide euthanasia across the CU Boulder campuses, and ensure that all personnel in the animal program are adhering to the recommendations for carbon dioxide euthanasia in accordance with the [AVMA Guidelines for the Euthanasia of Animals: 2013 Edition](#) as required by the PHS Policy and USDA Policy 3 on Veterinary Care.

DEFINITIONS

Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress.

BACKGROUND

Carbon dioxide is a common method of euthanasia for rodents. Carbon dioxide produces a reversible anesthetic state in animals by rapidly decreasing intracellular pH. Carbon dioxide can cause pain and distress in animals and humans by several different mechanisms: "(1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of so-called air hunger and a feeling of breathlessness, and (3) direct stimulation of ion channels within the amygdala associated with the fear response" (AVMA 2013). Because of the potential for causing pain in animals if carbon dioxide is not administered properly, it is important to know the recommendations of the AVMA Guidelines 2013 for this method of euthanasia, and continue to put those into practice.

The only acceptable source of carbon dioxide for animal research is commercially supplied cylinders or tanks of compressed gas. Gas displacement rate within a chamber has been determined to be critical to the humane application of carbon dioxide through the use of a pressure-reducing regulator and flow meter. Pre-filling a cage or container with carbon dioxide and then directly placing animals inside is an unacceptable method of euthanasia, as high concentrations of carbon dioxide exposure (<35% initially) have been shown to cause pain and distress in rodents and other animals. This is why it is important to gradually fill a container with carbon dioxide, so that animals become unconscious prior to death.

(AVMA Guidelines for the Euthanasia of Animals 2013 Edition; Duke University's Policy on Euthanasia V. 23 Aug 2012)

CARBON DIOXIDE SOURCE

1. All individuals responsible for administering carbon dioxide euthanasia must be qualified and trained to perform this method of euthanasia humanely.
2. Carbon dioxide and carbon dioxide gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. Compressed carbon dioxide gas in cylinders is the only acceptable source of carbon dioxide.

IMPORTANT CONSIDERATIONS FOR CO₂ EUTHANASIA

1. Species must be separated for euthanasia (you cannot euthanize rats and mice within the same chamber)
2. Preferably euthanasia should occur in an animal's home cage, as this is less stressful than being euthanized in a brand new or foreign cage with unfamiliar animals. Do not combine cages of different cage mates.
3. Carbon dioxide euthanasia is not appropriate for immature animals such as rodent neonates, as they are extremely resistant to carbon dioxide and will not go unconscious rapidly.

PROCEDURE FOR HUMANE CARBON DIOXIDE EUTHANASIA

1. Place the animals in the home cage (or chamber) before initiating the flow of carbon dioxide.
2. Note: Since carbon dioxide is heavier than air, the entry of the administration tube should be close to the top or on the top of the chamber used for euthanasia.
3. Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 10% to 30% of the chamber volume/min is recommended. Slowly fill the enclosure with carbon dioxide to displace 30% of the chamber volume per minute. Flow meters which effectively regulate the displacement rate should be obtained. The formula to calculate the flow rate of any size chamber is:
 1. Volume (in L) = (height in cm) x (width in cm) x (length in cm) divided by 1000
Maximum acceptable flow rate = (Cage Volume in Liters) X 0.3
Therefore:
$$\frac{\text{height (cm)} \times \text{width (cm)} \times \text{length (cm)}}{1000 \text{ cm}^3 \text{ per L}} = \text{liters} \times .30 = \text{maximum flow rate/minute}$$
 2. Expose the animals to carbon dioxide until complete cessation of breathing is observed, plus an additional minute at a minimum.

CONFIRMATION OF DEATH

For the use of carbon dioxide for euthanasia of rodents, cessation of breathing must be followed by a secondary method of euthanasia to ensure death. Approved secondary physical methods of euthanasia after carbon dioxide are:

- i. Decapitation using very sharp scissors or guillotine
- ii. Cardiac perfusion
- iii. Removal of vital organs (e.g. heart, lungs, brain)
- iv. Bilateral Thoracotomy
- v. Cutting the major blood vessels to induce exsanguination (e.g. aorta, vena cava)
- vi. Cervical dislocation on adult rodents weighing less than 200 grams. Cervical dislocation is an inappropriate method to use on rats larger than 200 grams; and on neonates at any time prior to 21 days of age.

NOTE: Carbon dioxide and a secondary method of euthanasia must always be listed on an approved IACUC protocol prior to use with vertebrate animals.

REFERENCES

- AVMA Guidelines for the Euthanasia of Animals 2013 Edition
- Duke University & Duke University Medical Center Animal Care and Use Program Policy on Euthanasia Version Approved by the IACUC 27 June 2013
[http://vetmed.duhs.duke.edu/PDF/Policies/Animal%20Use%20Policies/policy_on_euthanasia - mice & rats.pdf](http://vetmed.duhs.duke.edu/PDF/Policies/Animal%20Use%20Policies/policy_on_euthanasia_-_mice_&_rats.pdf)