



## Additional Information for Investigators and Staff Regarding Rodent Euthanasia Via Carbon Dioxide Overdose

### What is the reason for changes related to the use of CO<sub>2</sub> for rodent euthanasia?

Accredited biomedical research institutions must comply with the *Guidelines for the Euthanasia of Animals* put forth by the American Veterinary Medical Association (AVMA); these were most recently revised in 2013. The goal of the AVMA revisions regarding procedures for euthanasia using CO<sub>2</sub> is to minimize any associated pain and distress.

The primary issue is that gaseous CO<sub>2</sub> is converted to carbonic acid when it contacts the moist mucous membranes of the nose, eye, trachea, and lungs, resulting in pain. At >40% CO<sub>2</sub>, nasal pain and cardiovascular changes indicative of pain develop. Additionally, previous standard practice used a 'pre-filled chamber' methodology, but current research indicates that animals placed in prefilled chambers likely experience a period of distress prior to becoming unconscious. The new guidelines require that chambers be gradually filled with CO<sub>2</sub>, and that a flow rate of 10-30% be used. The primary goal is to create unconsciousness prior to reaching CO<sub>2</sub> concentrations that might induce pain or distress.

### How can I calculate the flow rate to ensure 20% displacement per minute and ensure that I am meeting the AVMA Guidelines?

1. Chamber Volume (in L) =  $\frac{(\text{height in cm}) \times (\text{width in cm}) \times (\text{length in cm})}{1000}$
2. Acceptable flow rate (in L/min) = (Chamber Volume in Liters) X 0.20 / min

### If I am consolidating rodents, how many animals can be placed into a cage and still meet the AVMA Guidelines?

For mice, consolidate within the animal room according to the following:

- a. For a #1 (6x10x5 box) you may have a maximum of 10 mice per box.
- b. For a #3 (8x17x5 box) you may have a maximum of 25 mice per box.
- c. Regardless, rodents should have ample room to move and assume normal posture.
- d. Upon completion of mouse consolidation, **immediately** (under 10 minutes) take rodents to the designated euthanasia room to be euthanized.

Stress in mice may be reduced by euthanasia in the home cage. Other rodent species should not be combined and must be euthanized in their home cage, unless alternatives have been reviewed and approved by the IACUC.

### Where can I purchase a flow meter for my laboratory CO<sub>2</sub> euthanasia station?

- Cryogenic Gases (uses shortcode):
  - Flow meter by Western Medica (FME802)  
and connectors (M24-45P and M1-320-12FM or M1- 320-12FMH)
- Fisher Scientific (uses shortcode):
  - Various models (search for CO<sub>2</sub> euthanasia)



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### At what age can neonates be effectively and humanely euthanized with CO<sub>2</sub>?

CO<sub>2</sub> is acceptable for euthanasia in mice, rats, hamsters, and gerbils but ONLY when rodents are 10 days of age or greater, as neonatal rodents are resistant to hypoxia and may take up to 50 minutes or more to die from CO<sub>2</sub> exposure (which is not acceptable).

### Is CO<sub>2</sub> an acceptable method of euthanizing embryos and fetuses?

It is believed that fetuses and embryos are in a state of unconsciousness throughout pregnancy. It is therefore hypothesized that they cannot consciously experience breathlessness or pain associated with dying after CO<sub>2</sub> euthanasia of the dam. (AVMA).

### Why must I confirm death following CO<sub>2</sub>?

Inhalation of CO<sub>2</sub> produces a reversible anesthetic state. Thus, rodents that are prematurely removed from the chamber prior to death can recover to consciousness (AVMA). Furthermore, death must be confirmed by personnel who have been specifically trained to recognize cessation of vital signs in rodents (Guide). Therefore, all rodents being euthanized with CO<sub>2</sub> overdose must also receive a secondary method of euthanasia to ensure death.

### What are acceptable secondary methods to ensure death?

Secondary methods include exsanguination, decapitation, and bilateral pneumothorax (making an incision into both sides of the chest). Death of the animal must be ensured prior to disposal of the carcass.

### Definitions:

1. **Euthanasia:** ending the life of an animal in a way that minimizes or eliminates pain and distress. Some methods of euthanasia require that a secondary physical method follow the primary method.
2. **Secondary Methods of Euthanasia** consist of a physical disruption of tissues or organs and is a method to ensure euthanasia. Acceptable secondary methods include bilateral pneumothorax by making an incision into both sides of the chest, exsanguination, or decapitation.
3. **Euthanasia Chambers** that are used for exposure to euthanasia-inducing gases may be either the animal's home cage (**preferred**) or a chamber specifically designed for the purpose of euthanasia. The Euthanasia Chamber must allow unobstructed viewing of the animal during the euthanasia activity.