RECOMMENDATIONS FOR THE USE OF CO2
IN RAT AND MOUSE EUTHANASIA

I/II. BACKGROUND:

Although many laboratory rats and mice are euthanized using CO2 according to a variety of methods, there is no definitive guidance on whether and how CO2 can be administered humanely. It is widely recognized that animals should be euthanized with the minimum possible discomfort, pain or distress, for welfare, ethical and legal reasons. However, there are uncertainties relating to the humaneness of some techniques, including the use of CO2. Therefore, the purpose of these guidelines is to describe BRC recommended procedures in the use of CO2 for the euthanasia of rats and mice.

III. REFERENCES:

- The Guide for the Care and Use of Laboratory Animals, National Research Council (1996)
- Hawkins, P. et al. (2006). Newcastle meeting on carbon dioxide euthanasia of laboratory animals, 27th and 28th February, Newcastle upon Tyne, UK.

IV. RECOMMENDED PROCEDURES:

A. General
1. The source of the 100% CO2 must be from a compressed gas cylinder (i.e., dry ice is not acceptable).
2. The use of CO2 euthanasia must be approved in the animal use application (AUA) or under the direction or guidance of the attending veterinarian or his/her designee.
3. It is essential that euthanasia be performed by trained personnel who can perform it in a professional and compassionate manner.
4. Animals should be in a chamber or cage that allows easy visualization at all times during CO2 administration.
5. The animal should have sufficient room to be able to turn around and make normal postural adjustments (if possible, keep animals in their home cage).
6. As detailed below in sections IV.B and IV.C, CO2 flow-rates and cage density guidelines are based upon standard BRC rat and mouse cages (Lab Products® or Allentown®) and an approximate air displacement of 20% of the cage volume per minute.
7. If personnel are using a chamber that differs in size from a standard rodent cage, adjustments in the CO2 flow rates should still reflect the 20% air displacement recommendation.
8. Cages should not be left unattended during gas administration (i.e., continuous monitoring is required until animals are euthanized).

B. Rats
1. Recommended CO2 flow-rates
   a. 4 liters/min (based upon a cage size of 20 L)
2. Cage Density
   a. Pre-weanling and weanling pups (up to 4 weeks of age): no more than 30/cage
   b. Young rats (4-8 weeks old): no more than 10/cage
   c. Adults: no more than 6/cage
C. Mice
1. Recommended CO₂ flow-rates
   a. 1 liters/min (based upon a cage size of 5 L)
2. Cage Density
   a. Pre-weanling and weanling pups (up to 4 weeks of age): no more than 30/cage
   b. Adults (greater than 4 weeks of age): no more than 15/cage

D. To Ensure Euthanasia
   Once animals are unconscious and breathing has stopped, then perform one of the following
   to ensure death:
   1. For mice: perform either a thoracotomy or cervical dislocation.
   2. For rats >200 g.: perform a thoracotomy
   3. For rats <200 g.: perform either a thoracotomy or cervical dislocation.

V. APPROVAL

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