Carbon dioxide (CO₂), when used properly, is classified by the American Veterinary Medical Association (AVMA) Panel on Euthanasia as a safe method of euthanasia for many small laboratory animals. CO₂ has many advantages including: (1) rapid depressant, analgesic, and anesthetic effects; (2) easy availability in compressed gas cylinders; (3) inexpensive, nonflammable, non-explosive, and poses minimal hazard to personnel when used with properly designed equipment; (4) does not result in accumulation of toxic tissue residues in animals from which food is produced.

Euthanasia must be performed by trained personnel using appropriate technique, equipment, and agents.

Although CO₂ is generally considered an acceptable form of euthanasia for small laboratory animals when properly administered, its acceptability is predicated on the following:

The euthanasia chamber should not be prefilled (pre-charged) with CO₂ since high concentrations (>70%) can cause nasal irritation, discomfort, and excitability. Rather, the animals should first be placed into the chamber, followed by the addition of CO₂ at a low flow rate (e.g., a rate sufficient to displace approximately 30% of the chamber volume per minute) to complete the process. The CO₂-flow rate must be measured with use of a flow meter, and the rate of ~30% chamber volume/minute determined for each individual euthanasia chamber. Rapid gas flows should be avoided since excessive noises ("winds") can develop and induce excitement and distress in the animals. Gas flow should be maintained for at least 1 minute after apparent clinical death (e.g., at least one minute after the animal has quit breathing). It is important to confirm that an animal is dead after removing it from the chamber. Unintended recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times or by other means that are appropriate to the species being studied. Examples of other means used to assure death after CO₂ euthanasia include decapitation, cervical dislocation and thoracotomy.

According to the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, "As gas displacement rate is critical to the humane application of CO₂, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized is absolutely necessary." Only one species at a time should be placed into a chamber, and the chamber must not be overcrowded. When placed into the chamber, all animals must have floor space. Euthanasia should always be done in cohorts (live animals should not be placed in the chamber with dead animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.

Neonates: Since the time period for euthanasia is substantially prolonged in neonatal animals due to their inherent resistance to hypoxia, CO₂ narcosis must be followed by a physical means of euthanasia after the animals lose consciousness to ensure irreversibility of the procedure (e.g., decapitation, cervical dislocation, or thoracotomy).