Carbon dioxide (CO₂), when used properly, is classified by the 2000 Report of the American Veterinary Medical Association 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; and (3) inexpensive, nonflammable, non-explosive, and poses minimal hazard to personnel when used with properly designed equipment.

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

It is not desirable to prefill (pre-charge) the euthanasia chamber 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 20% of the chamber volume per minute) to complete the process. 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 2007 Report of the AVMA Panel on Euthanasia, "Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. CO₂ generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable." 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).

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