



Use of Carbon Dioxide for Rodent Euthanasia

Excerpted from the “AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.”

1. Compressed CO₂ gas in cylinders is the only feasible source. Mixtures combining O₂ with CO₂ are not recommended.
 2. Pre-filling euthanasia chambers is *unacceptable*. Euthanasia chambers *must* be fitted with an appropriate pressure-reducing regulator and flow meter (or equivalent equipment) to ensure a displacement rate of 10% to 30% of the chamber volume/minute. CO₂ flow should be maintained for at least 1 minute after respiratory arrest. Instructional signage for each chamber highlighting the flow rate needed is recommended.
 3. Retaining animals within their home cage is preferable. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. Animal density within the chamber should not be greater than standard housing density.
 4. Death must be assured. While death may be confirmed by physical examination or obviated by calibration and validation of the euthanasia chamber and process, assurance via an adjunctive physical method is much preferred.
 5. Immature animals must be exposed to high concentrations of CO₂ for an extended period of time to ensure death.
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Summary Information for Carbon Dioxide (from Appendix 2):

Classification: Respiratory acidosis and produces a reversible anesthetic state followed by hypoxia attributable to depression of vital centers.

Mode of Action: Direct depression of cerebral cortex, subcortical structures, and vital centers; direct depression of heart muscle.

Rapidity: Moderately rapid, depending on protocol (definitions used: immediate = upon application; very rapid = typically within seconds; rapid = typically within a few minutes).

Ease of Performance: Easily with appropriate equipment, closed container, gas source, and once protocol are established.

Safety for Personnel: Minimal hazard with adequate ventilation.

Species Suitability: Most birds and mammals, excluding companion animals.

Efficacy and comments: Effective, but time required may be prolonged in immature and neonatal animals.

Condition: May be used only with those species where aversion or distress can be minimized; gradual fill method must be used; must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank; *an appropriate pressure-reducing regulator and flow meter or equivalent equipment must be used.*