

DIVISION OF LABORATORY ANIMAL HEALTH
CO2 EUTHANASIA GUIDELINES

Euthanasia chamber: When possible, euthanasia should be conducted in cohorts in their home cage to minimize animal distress and anxiety. If home cage euthanasia cannot be performed, the process must minimize pain and distress resulting from exposure to the vocalization and odors of anxious animals. The chamber and stainless-steel euthanasia lid should be cleaned and gloves changed between each use. Live animals should not be placed in the chamber with dead animals.

Euthanasia chamber floor space: Chamber or home cage must not be overcrowded. All animals must have sufficient floor space and must be able to make normal postural adjustments.

Euthanasia chamber/home cages: Euthanasia chamber/home cage should allow ready visibility of all animals in the chamber/home cage.

Species: Only one species at a time should be placed into a chamber. Species should not be mixed (rats are predators to mice).

CO2-flow rate: 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 (rate sufficient to displace approximately 30% of the chamber volume per minute) to complete the process. CO₂ flow rate must be measured with use of a flowmeter. Turn the flow meter to 1.5 for mouse box or 4.0 for rat box. Gas flow should be maintained for at least 1 minute after apparent clinical death (at least one minute after the animal has quit breathing). Death must be confirmed by a secondary method (cervical dislocation, decapitation, thoracotomy). Neonates must be euthanized by physical means following CO₂ narcosis.

Euthanasia chamber flushing: Residual CO₂ gas in euthanasia chamber must be flushed out between euthanasia sessions.

Euthanasia site: Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents or under BSL-2 conditions.

Personnel euthanizing animals: Rodents must be euthanized by trained personnel using appropriate techniques, equipment and agents.