Animals being euthanized should experience minimal pain, fear, or other significant stress prior to their death. Carbon dioxide (CO₂) is a frequently used euthanasia agent for small laboratory animals due to its rapid onset of action, safety, and ready availability. The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) has adopted the following guideline to: (1) assist the research community by clarifying the specific procedures relating to the use of CO₂ and (2) promote best practices and ensure that pain and distress are minimized in euthanized laboratory rodents.

The PHS Policy requires that euthanasia be conducted according to the American Veterinarian Medical Association (AVMA) Guidelines for Euthanasia (2013 Edition) (OLAW). The AVMA guidelines set criteria for euthanasia and specify appropriate euthanasia methods and agents based upon the best literature and empirical evidence that minimizes pain and distress to animals during euthanasia.

This guideline concentrates on the following topics regarding the use of CO₂ euthanasia:

- Mechanism of action (CO₂)
- Species
- Administering CO₂
- Confirming death

MECHANISM OF ACTION
CO₂ inhalation causes respiratory acidosis and produces a reversible anesthetic state by rapidly decreasing intracellular pH. Inhaled concentrations of 30% or higher cause deep anesthesia, and death should occur with prolonged exposure. CO₂ has the potential to cause distress via three different mechanisms: (1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of a feeling of “breathlessness” and (3) direct stimulation of ion channels within the amygdala, believed to be associated with the fear response. The discomfort associated with CO₂ is believed to occur starting at approximately a 15% inhaled concentration (AVMA).

Potential pain and distress caused by CO₂ inhalation can be mitigated if the animal loses consciousness before the chamber is at 15% concentration. Therefore, an appropriate gradual displacement of room air with carbon dioxide into the chamber will cause the animal to lose consciousness before the CO₂ is aversive.

A gradual fill rate of 10-30% chamber volume per minute displacement is expected at all rodent euthanasia locations across Penn. (AVMA)

To calculate the flow rate of gas for a 20% displacement per minute:

1. Chamber Volume (in L) = (height in cm) x (width in cm) x (length in cm) / 1000
2. Acceptable flow rate (in L/min) = (Chamber Volume in Liters) X 0.20 / min

SPECIES
Any rodent used for research may be euthanized by CO₂ by following the guidance described below. Examples include mice of the genus Mus, rats of the genus Rattus, in addition to hamsters, gerbils, and other laboratory rodents. Although CO₂ is considered an “acceptable with conditions” form of
CARBON DIOXIDE EUTHANIASIA OF RODENTS

euthanasia for other species (AVMA), the IACUC will require specific justification for CO₂ use in non-
rodents.

ADMINISTERING CO₂

1. All personnel administering CO₂ to rodents must be properly trained (Guide and AVMA). All
Principal Investigators (PI) must assure that their research staff are duly trained and adhere to
animal care and use protocols, policies, and guidelines. Training on the use of the equipment
and appropriate methods of euthanasia is available from the ULAR Training Division.

2. Compressed gas is the only acceptable source of CO₂ for euthanizing rodents. Dry ice, fire
extinguishers and other sources of CO₂ may not be used.

3. Euthanasia chambers should be constructed of clear material (e.g. Plexiglas®) and must be kept
clean to minimize odors that might distress animals subsequently euthanized. Gas must be
delivered in a predictable and controllable fashion, at a low-flow rate of 10-30% volume
displacement per minute (as described above).

4. Laboratory staff must post individual signage at the site of the euthanasia station with clear
instructions as to how to operate the equipment and ensure death of animals (contact the

5. Euthanasia should occur in a procedure room or laboratory, away from other rodent housing.
Satellite housing facilities may not euthanize animals in close proximity to the housing area.

6. When possible, rodents should be euthanized in their home cages (AVMA).

7. The chamber may not be pre-filled with CO₂ prior to placement of animals into the chamber. CO₂
is denser than room air, thus the chamber will need to be “purged” (dumped) between groups
of cages.

8. Because CO₂ first anesthetizes animals and then, only after an adequate exposure time, will
result in death by CO₂ narcosis, rodents must be exposed to the gas until respiration has
ceased, within the euthanasia chamber with CO₂ continuing to flow.

NEONATES
Resistance to hypoxia results in a prolonged time to unconsciousness when CO₂ inhalation is used as a
euthanasia agent in neonatal rodents. The duration of exposure to carbon dioxide varies with the age of
the neonate compared with adult rodents.

Neonates and pups (< 14 days) must go through one complete 10-minute CO₂ exposure cycle to ensure
anesthesia, which must be followed by euthanasia using a physical method (e.g. decapitation, bilateral
thoracotomy).

EMBRYOS AND FETUSES
It is believed that fetuses and embryos are in a state of unconsciousness throughout pregnancy. It is
therefore hypothesized that they cannot consciously experience breathlessness or pain associated with
dying after CO₂ euthanasia of the dam. When fetuses are not required for study, the method chosen
CARBON DIOXIDE EUTHANIASIA OF RODENTS

for euthanasia of a pregnant mother should ensure cerebral anoxia to the fetus and minimally disturb the uterine milieu to minimize fetal arousal (AVMA).

CONFIRMING DEATH
Inhalation of CO\(_2\) produces a reversible anesthetic state, thus animals that are prematurely removed from the chamber prior to death can recover to consciousness (AVMA). Furthermore, death must be confirmed by personnel who have been specifically trained to recognize cessation of vital signs in rodents (Guide). Therefore, all animals being euthanized with CO\(_2\) overdose must also receive a confirmatory method of euthanasia to ensure death.

These confirmatory methods, to be performed after CO\(_2\) overdose, include exsanguination, decapitation, cervical dislocation (adult mice only), bilateral thoracotomy, or at least 50% additional time in the euthanasia chamber filled with 100% CO\(_2\) (adults only; accomplished with a total CO\(_2\) exposure time of 10 minutes at a 20% flow rate, thus animals are euthanized by 7 minutes and maintained for an additional 3 minutes of exposure time in the closed chamber). Death of the animal must be ensured prior to disposal of the rodent carcass.

Failure to confirm death of a euthanized rodent is a significant non-compliance, reportable to the appropriate regulatory and accrediting agencies.

REFERENCES
American Veterinary Medical Association Guidelines for Euthanasia (2013) (AVMA)
Guide for the Care and Use of Laboratory Animals (Guide)
Office of Laboratory Animal Welfare IACUC Guidebook (OLAW)
Public Health Service Policy: Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals (PHS)