



Noteworthy Extrapolated Laboratory Animal Excerpts from the (2020) AVMA Euthanasia Guidelines

As condensed by Milton April, DVM, ACLAM

While not a regulatory body, the American Veterinary Medical Association (AVMA) offers guidance to those who may apply these **Guidelines** as part of regulatory structures designed to protect the welfare of laboratory animals used for human purposes. By creating and maintaining these **Guidelines**, the AVMA hopes to ensure that when a veterinarian or other professional intentionally kills an animal under his or her charge, it is done with respect for the interests of the animal and that the process is as humane as possible, that is to say, it minimizes pain and distress to the animal, and death occurs as rapidly as possible.

The **Guidelines** set criteria for euthanasia, specify appropriate euthanasia methods and agents, and are intended to assist veterinarians in their exercise of professional judgment. The **Guidelines** acknowledge that euthanasia is a process involving more than just what happens to an animal at the time of its death. Apart from delineating appropriate methods and agents, these **Guidelines** also recognize the importance of considering and applying appropriate pre-euthanasia (eg, sedation) and animal handling practices, as well as attention to disposal of animals' remains.

In evaluating methods of euthanasia, the AVMA panel of experts considered the following criteria:

- (1) Ability to induce loss of consciousness and death with a minimum of pain and distress;
- (2) Time required to induce loss of consciousness; (3) reliability; (4) safety of personnel; (5) irreversibility;
- (6) compatibility with intended animal use and purpose; (7) emotional effect on observers or operators;
- (8) compatibility with subsequent evaluation, examination, or use of tissue; (9) drug availability and human abuse potential; (10) compatibility with species, age, and health status; (11) ability to maintain equipment in proper working order; (12) safety for predators or scavengers should the animal's remains be consumed;
- (13) legal and regulatory requirements; and (14) environmental impacts of the methods of disposal or final disposition of animal remains.

Personnel who perform euthanasia must demonstrate proficiency in the use of the technique in a closely supervised environment. Each facility or institution where euthanasia is performed (whether a clinic, laboratory, or other setting) is responsible for training its personnel adequately to ensure the facility or institution operates in compliance with federal, state, and local laws.

Furthermore, experience in the humane restraint of the species of animal to be euthanized is important and should be expected to ensure that animal's pain and distress are minimized. Training and experience should include familiarity with the normal behavior of the species being euthanized. Also, an appreciation of how handling and restraint affect animal behavior and understanding of the mechanism by which the selected technique induces loss of consciousness and death. Euthanasia should only be attempted when the necessary drugs, supplies and appropriate equipment are available to ensure a smooth and gentle procedure. Selection of the most appropriate method of euthanasia in any given situation depends on the species and number of animals involved, available means of animal restraint, skill of personnel, and other considerations.

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The following contingencies are common to all inhaled euthanasia agents:

- (1) Time to unconsciousness with inhaled agents is dependent on the displacement rate, container volume, and concentration. An understanding of the principles governing delivery of gases into enclosed spaces is necessary for appropriate application of both prefill and gradual displacement methods.
- (2) Loss of consciousness will be more rapid if animals are initially exposed to a higher concentration of the agent. However, for many agents and species, forced exposure to high concentrations can be aversive and distressing, such that gradual exposure may be the most pragmatic and humane option.
- (3) Inhaled agents must be supplied in purified form without contaminants or adulterants, typically from a commercially supplied source, cylinder, or tank, such that an effective displacement rate and/or concentration can be readily quantified. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate.
- (4) The equipment used to deliver and maintain inhaled agents must be in good working order and in compliance with recommended state and federal regulations. Leaky or faulty equipment may lead to slow, distressful death and may be harmful to other laboratory animals or to operating personnel.
- (5) Neonatal animals appear to be resistant to hypoxia, and because all inhaled agents ultimately cause hypoxia, neonatal animals take longer to die than adults. Inhaled agents can be used alone in unweaned animals to induce loss of consciousness, but prolonged exposure time or a secondary method may be required to kill the unconscious animal.

NOTE:

Due to resistant respiratory hypoxia in neonatal and immature animals (e.g., rodents, rabbits, guinea pigs, birds, poults, piglets), high CO₂ concentrations combined with extended exposure times may be required to ensure death. High CO₂ concentrations (> 70%) and extended exposure times (> 5 minutes) are required for effective euthanasia of newly hatched chickens. On the day of birth, rats and mice exposed to 100% CO₂ may require exposure times of 35 and 50 minutes, respectively, to ensure death. Rodents with altricial young, such as mice and rats, must be differentiated from rodents with precocial young, such as guinea pigs. Precocial young should be treated as adults. By 10 days of age for rats and mice, exposure times of at least 5 minutes are sufficient to ensure death. Rodent fetuses along with other mammals are unconscious in utero, thus hypoxia does not evoke a response. Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized.

- (6) Rapid gas flows can produce noise or cold drafts leading to animal fright and escape behaviors. If high flows are required, equipment should be designed to minimize noise, regulate temperature, and gas streams blowing directly on the animals.

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- (7) When possible, inhaled agents should be administered under conditions where animals are most comfortable (e.g., for rodents, in the home cage; for pigs, in small groups). If animals need to be combined, they should be of the same species and compatible cohorts, and if needed, be restrained or separated so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean so as to minimize odors that might cause distress in animals subsequently euthanized.
- (8) Because some inhaled agents may be lighter or heavier than air, layering or loss of agent may permit animals to avoid exposure. Mixing can be maximized by ensuring incoming gas or vapor flow rates are sufficient. Chambers and containers should be leak free as possible.
- (9) Death must be verified following administration of inhaled agents. This can be done either by examination of individual animals or by adherence to validated exposure processes proven to result in death.

NOTE:

CO₂ gas displacement rate is critical to the humane application of inhaled methods, such that an appropriate pressure-reducing regulator and flow meter combination or equivalent equipment with demonstrated capability for generating the recommended displacement rate for the size container being utilized is absolutely necessary when compressed gases are used for euthanasia.

CO₂ is commercially supplied in cylinders under high pressure and unique in that it is supplied as a liquefied gas under high pressure. By reducing high pressure at the cylinder valve, gas flow is made constant to the flow meter as cylinder pressure decreases during use.

With CO₂, the regulator also acts to prevent high gas flow rates that can lead to delivery of freezing gas and dry ice snow to the animals, as well as regulating icing and cylinder freezing. It is recommended that commercial gas warmers be added to CO₂ cylinder gas tank valves.

Gas Anesthesia prior to Euthanasia with CO₂:

As an opinion from the Euthanex Corporation, in our experience with testing applications, indications are that it is not necessary to use inhaled anesthetics for rodents with the Euthanex CO₂ euthanasia apparatus. It is also our respective opinion, when using CO₂ for rabbit euthanasia, it is less an aversive procedure if anesthesia or tranquilization is administered through the state of narcosis to final euthanasia.

Recommended euthanasia methods of rabbits depend on the facilities and conditions in which the animals are housed and maintained. In a controlled setting such as a biomedical research facility, researchers routinely have access to restraining devices for sedation or tranquilization. In all situations, the animals should be handled and euthanized in a manner least distressful to them. (ref. page 63)

NOTE:

A displacement rate from 30% to 70% of the chamber volume/min is recommended. Whenever gradual displacement methods are used, CO₂ flow should be maintained for at least 1 minute after respiratory arrest. If animals need to be combined, they should be of the same species in similar conditions and, if needed, restrained so that they will not injure themselves or harm other animals.

Oxygen administered together with CO₂ appears to provide little advantage and is not recommended for euthanasia.

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It is imperative that immature animals must be exposed to higher concentrations of CO₂ for an extended period of time to ensure death.

The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO₂, is unacceptable. A 2-step process, where animals are first rendered unconscious and then immersed into 100% CO₂, is recommended when gradual displacement methods are not or cannot be used.

Carbon dioxide and CO₂ gas mixtures should be supplied in a precisely regulated and purified forms, typically from trusted commercially supplied cylinders or tanks. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate.

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.

In addition to humane outcomes, an important consideration in the choice of method for euthanasia of laboratory animals is the research objectives for the animals being euthanized. (ref. pages 60-63)

General Considerations when using CO₂ for Euthanasia:

Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized. Carbon dioxide may cause pain due to the formation of carbonic acid when it contacts moisture on the respiratory and ocular membranes. Thus, a gradual fill method of CO₂ is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness.

In humans and rats, most nociceptors begin to respond at CO₂ concentrations of approximately 40%. Humans report discomfort begins at 30% to 50% CO₂, and intensifies to overt pain with higher concentrations CO₂ concentrations. Typically, 25% to 50% CO₂ does not offer agony in rodents, suggesting gradual displacement methods are the manner of choice and less likely to produce pain and discomfort prior to unconsciousness. Inhaled CO₂ is known to induce a reflex apnea and changes in heart rate, but if initiated correctly does not initiate transfer of harmful body substances into the soft tissues or blood.

With mild increases in inspired CO₂, increased ventilation results in a reduction or elimination of air hunger, but there are limits to this compensatory mechanism such that air hunger may reoccur during spontaneous breathing with moderate hypercarbia and hypoxemia. Adding O₂ to CO₂ may or may not preclude signs of distress. Supplemental O₂ will, however, prolong time to hypoxemic death and may delay onset of unconsciousness. There appears to be no advantage to combining O₂ with CO₂ for euthanasia.

Distress during CO₂ exposure has also been examined by means of behavioral assessment and aversion testing. Variability in behavioral responses to CO₂ has been reported for rats and mice, pigs, and poultry. While signs of distress have been reported in some studies, other researchers have not consistently observed these effects. This may be due to variations in methods of CO₂ exposure or types of behaviors assessed, as well as animal strain variability.

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As a general rule, a gentle death that takes longer, but is preferable to a rapid more distressing death. Gradual-fill CO₂ exposure causes aversion in rodents beginning at approximately a 15% concentration and lasting to onset of unconsciousness. If an appropriate gradual displacement rate is used, animals will lose consciousness before CO₂ concentrations become painful. A 20%/min gradual displacement produces unconsciousness in approximately 100 seconds at a CO₂ concentration of 30%. A slower 10%/min displacement increases time to onset of unconsciousness to 160 seconds at a CO₂ concentration of 21%. As with other inhaled agents, time to unconsciousness with CO₂ is dependent on the displacement rate, container volume, and concentration used.

In rats, unconsciousness is induced in approximately 30 seconds with 80% to 100% CO₂; and 40 to 50 seconds with 70% CO₂. Similarly, a rapidly increasing concentration (flow rate > 50% of the chamber volume per minute) induces unconsciousness in only 26 to 48 seconds.

In contrast to other species, a large proportion of chickens and turkeys will enter a chamber containing moderate concentrations of CO₂ (60%) to gain access to food or social contact. Following incapacitation and prior to loss of consciousness, birds may show behaviors such as open-beak breathing and head-shaking. Birds exposed to high concentrations of inhaled anesthetic gases, including CO₂, lose consciousness rapidly and thus maybe the preferred method of euthanasia.

It appears that birds are more willing than rodents to tolerate CO₂ at higher concentrations which are sufficient to induce loss of bearing, and rapid loss of consciousness following shortly there afterward. Euthanasia via exposure to CO₂ has been described for individual birds and small groups, and its application to euthanasia of chickens, turkeys, and ducks has been studied extensively, resulting in information about times to collapse, unconsciousness and death, loss of somatosensory evoked potentials, and changes in EEG. Leghorn chicks 7 days of age collapsed in 12 seconds after exposure to 97% CO₂. A two-minute exposure to 90% CO₂ was sufficient to kill day-old chicks exposed in batches. Broilers/layers chickens 5 weeks of age collapsed an average of 17-20 seconds after placed in an adequate euthanasia chest filled with 60% CO₂.

Neonatal birds may be more acclimated to CO₂ concentrations because of the unhatched bird's recent environment and thus require higher CO₂ concentrations (80-90%) for longer period of time for euthanasia. Embryonated chicken eggs may be destroyed by prolonged exposure to high concentrations of CO₂ for 20 plus minutes. Carbon dioxide does not result in accumulation of toxic tissue residues in animals from which food is produced. (ref. pages 78, 80-82)

The Euthanex Corporation endorses these primary recommendations:

Advantages in using CO₂:

Carbon dioxide is inexpensive, nonflammable, and non-explosive and poses minimal hazard to personnel when used with properly designed equipment. The rapid depressant, analgesic, and anesthetic effects of CO₂ are well established. Carbon dioxide is readily available in compressed gas cylinders or tanks and quality commercial vendors are available throughout the country.

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Accommodations in using CO2:

Differences in response to CO2 inhalation exist between and within species, strains, and breeds, making broad generalizations for CO2 use challenging.

CO2 administered by prefill or high gradual displacement methods, can be aversive to some species, therefore potential exists to cause discomfort and distress.

CO2 is heavier than air, therefore incomplete or improper filling (layering of chamber gas) may permit animals raise their heads above the effective concentrations to avoid proper exposure.

Neonatal, immature animals, and some aquatic and burrowing species may have extraordinary and unexpected tolerance for CO2.

CO2 induction for loss of consciousness and euthanasia at variable concentrations may produce diverse postmortem lesions.

Considerations when using CO2:

CO2 with or without premedication of inhaled anesthetics, is acceptable for euthanasia of common laboratory rodents.

Compressed CO2 gas in cylinders is the recommended source of CO2 because gas inflow to the chamber can be precisely regulated.

An optimal flow rate for CO2 euthanasia systems should displace 30% to 70% of the chamber or cage volume per minute.

CO2 euthanasia with prefilled chambers is not acceptable unless the animals have been pre-anesthetized or otherwise rendered unconscious.

If euthanasia with CO2 cannot be conducted in the home cage, chambers should be emptied and cleaned between uses.

Death may be confirmed by physical examination, ensured by adjunctive physical methods, or by validation of automated chamber process.

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