Noteworthy Excerpts from the (2013) AVMA Euthanasia Guidelines

While not a regulatory body, the American Veterinary Medical Association (AVMA) also hopes to offer guidance to those who may apply these *Guidelines* as part of regulatory structures designed to protect the welfare of 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 (i.e., that it minimizes pain and distress to the animal, and death occurs as rapidly as possible.)

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 requirements; and
(14) Environmental impacts of the methods of disposal or 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, an appreciation of how handling and restraint affect that behavior, and an 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. Information in the scientific literature and available from practical experience focuses primarily on domesticated animals, but the same general considerations should be applied to all species.

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 or vapors 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 high 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 state and federal regulations. Leaky or faulty equipment may lead to slow, distressful death and may be hazardous to other animals and to personnel.

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(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 CO_2 concentrations combined with extended exposure times may be required to ensure death.
High CO_2 concentrations (> 60%) 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% CO_2 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 and gas streams blowing directly on the animals.

(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 as 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:
CO_2 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. CO_2 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 CO_2, 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 regulator icing and cylinder freezing. Recommend that commercial gas warmers be added to CO_2 cylinder gas tank valves.

Gas Anesthesia prior to Euthanasia with CO_2:
As an extrapolative opinion from the Euthanex Corporation, in our experience with testing applications, indications are that it is not necessary to use inhaled anesthetics with the Euthanex CO2 euthanasia apparatus. It is our respective opinion, that CO_2 when used as recommended for rodents and rabbits, it is less aversive through narcosis and anesthesia to euthanasia. Although inhaled anesthetics are routinely used to produce general anesthesia in animals, these agents may be aversive and distressful under certain conditions. Flecknell et.al., reported violent struggling accompanied by apnea and bradycardia in rabbits administered isoflurane, halothane, and sevoflurane by mask or induction chamber, and concluded these agents were aversive and should be avoided whenever possible. Leach et.al., found inhaled anesthetic vapors to be associated with some degree of aversion in laboratory rodents, with increasing aversion noted as concentration increased; halothane was least aversive for rats, while halothane and enflurane were least aversive for mice. Makowska and Weary also reported halothane and isoflurane to be aversive to male Wistar rats.

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[Flecknell PA, Roughan JV, Hedenqvist P. Induction of anaesthesia with sevoflurane and isoflurane in the rabbit. *Lab Anim* 1999;33:41–46.
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Makowska LJ, Weary DM. Rat aversion to induction with inhaled anaesthetics. *Appl Anim Behav Sci* 2009;119:229–235.]

NOTE:
A displacement rate from 10% to 30% of the chamber volume/min is recommended. Whenever gradual displacement methods are used, CO_2 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 and, if needed, restrained so that they will not hurt themselves or others. Immature animals must be exposed to higher concentrations of CO_2 for an extended period of time to ensure death. Oxygen administered together with CO_2 appears to provide little advantage and is not recommended for euthanasia. The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO_2, is unacceptable.

A 2-step process, where animals are first rendered unconscious and then immersed into 100% CO_2, is preferred when gradual displacement methods cannot be used. Immersion of poultry in lesser concentrations is acceptable with conditions as it does not appear to be distressing. Carbon dioxide and CO_2 gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. 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 CO_2, 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.

General Considerations when using CO_2 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 CO_2 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 CO_2 concentrations of approximately 40%. Humans report discomfort begins at 30% to 50% CO_2, and intensifies to overt pain with higher concentrations. Inhaled irritants are known to induce a reflex apnea and heart rate reduction, and these responses are thought to reduce transfer of harmful substances into the body. In rats, 100% CO_2 elicits apnea and bradycardia, but CO_2 at concentrations of 10%, 25%, and 50% does not, suggesting gradual displacement methods are less likely to produce pain prior to unconsciousness in rodents.

With mild increases in inspired CO_2, 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 O_2 to CO_2 may or may not preclude signs of distress. Supplemental O_2 will, however, prolong time to hypoxemic death and may delay onset of unconsciousness. There appears to be no advantage to combining O_2 with CO_2 for euthanasia.

Distress during CO_2 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 gas exposure and types of behaviors assessed, as well as strain variability.

As a general rule, a gentle death that takes longer, but is preferable to a rapid more distressing death. Gradual-fill CO_2 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 CO_2 concentrations become painful. A 20%/min gradual displacement produces unconsciousness in approximately100 seconds at a CO_2 concentration of 30%. A slower 10%/min displacement increases time to onset of unconsciousness to 160 seconds at a CO_2 concentration of 21%. As with other inhaled agents, time to unconsciousness with CO_2 is dependent on the displacement rate, container volume, and concentration used. In rats, unconsciousness is induced in approximately 30 seconds with 80% to 100% CO_2; and 40 to 50

seconds with 70% CO_2. 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 CO_2 (60%) to gain access to food or social contact. Following incapacitation and prior to loss of consciousness, birds in these studies show behaviors such as open-beak breathing and head-shaking; these behaviors, however, may not be associated with distress because birds do not withdraw from CO_2 when these behaviors occur. Thus, it appears that birds are more willing than rodents to tolerate CO_2 at concentrations that are sufficient to induce loss of posture, and that loss of consciousness follows shortly afterwards.

Euthanasia via exposure to CO_2 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% CO_2. Two minute exposure to 90% CO_2 was sufficient to kill day-old chicks exposed in batches. Broilers 5 weeks of age collapsed an average of 17 seconds after entering a tunnel filled with 60% CO_2.

The Euthanex Corporation endorses these primary recommendations:

Advantages in using CO_2:

- The rapid depressant, analgesic, and anesthetic effects of CO_2 are well established.
- Carbon dioxide is readily available in compressed gas cylinders. Commercial vendors are available throughout the country.
- Carbon dioxide is inexpensive, nonflammable, and non-explosive and poses minimal hazard to personnel when used with properly designed equipment.
- Carbon dioxide does not result in accumulation of toxic tissue residues in animals from which food is produced.

Accommodations in using CO_2:

- Differences in response to CO_2 inhalation exist between and within species, strains, and breeds, making broad generalizations difficult.
- CO_2 administered by prefill or high gradual displacement methods, can be aversive to some species, therefore potential exists to cause distress.
- CO_2 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 exposure.
- Neonatal, immature animals, and some aquatic and burrowing species may have extraordinary and unexpected tolerance for CO_2.
- Induction loss of consciousness at concentrations < 80% may produce postmortem pulmonary and upper respiratory tract lesions.

Considerations when using CO_2:

- Carbon dioxide, with or without premedication with inhaled anesthetics, is acceptable for euthanasia of common laboratory rodents.
- Compressed CO_2 gas in cylinders is the recommended source of CO_2 because gas inflow to the chamber can be precisely regulated.
- An optimal flow rate for CO_2 euthanasia systems should displace 10% to 30% of the chamber or cage volume per minute.
- CO_2 euthanasia with prefilled chambers is not acceptable unless the animals have been pre-anesthetized or otherwise rendered unconscious.
- If euthanasia with CO_2 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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