Carbon dioxide for euthanasia and anesthesia:  
Concerns regarding pain and distress?

Introduction

Carbon dioxide (CO₂) is commonly used for euthanasia and anesthesia of laboratory rodents. It is a common euthanasia agent apparently because of its ease of use, its relative safety, and because it can be used to euthanize large numbers of animals in a short time span. In large institutions and those with significant rodent breeding programs (increasingly common as more and more scientists use genetically modified strains), there are occasions when large numbers of rodents are euthanized in a short time (Kline, 1963) and an appropriate gas agent is often the best way to approach such a challenge. However, CO₂ is not a physiologically inert compound and the published evidence on whether or not its administration causes pain or distress in animals is sufficiently mixed as to raise questions about its routine use. This paper reviews this published evidence and includes information on the effects of CO₂ in both humans and animals. Alternatives to the use of CO₂ as a sole agent for euthanasia are also suggested.

Animal welfare should be the main factor taken into consideration when choosing an appropriate method of euthanasia. The least stressful procedure should be utilized whenever possible - the term “euthanasia” being derived from a Greek term meaning “good death.” Most importantly, proper and thorough hands-on training plays a substantial role in animal welfare when euthanasia, or whatever procedure is chosen, is conducted. It is the responsibility of the Institutional Animal Care and Use Committee to make sure that proper training in euthanasia is conducted and that such training is adequately documented.

The current standards for euthanasia in the U.S. are set out in the 2000 Report of the AVMA Panel on Euthanasia. The report, written by the American Veterinary Medical Association (AVMA), indicates that a “good death” is one “that occurs without pain and distress” and “the technique should minimize any stress and anxiety experienced by the animal prior to unconsciousness.” The report further states, “[e]uthanasia techniques should result in rapid unconsciousness followed by cardiac or respiratory arrest and ultimate loss of brain function.” However, there is no clear guidance on what might be considered “rapid”—seconds, tens of seconds, minutes? Nonetheless, one can obtain some idea of what might be acceptable by the discussion over decapitation. The 1986 and 1993 AVMA reports on euthanasia cautioned against the use of decapitation because one study of rats found that the decapitated head continued to produce EEG traces for an average of 13.6 seconds after decapitation (range of 5.6 to 29.5 seconds). Therefore, we will proceed in this paper on the assumption that any euthanasia procedure that causes distress for 13.6 seconds or more should be used, if at all, with circumspection and caution.
There is a body of evidence in the published literature that CO₂, at least under some circumstances, causes pain and distress for longer than ten seconds. Therefore, its use as an appropriate stand-alone option for anesthesia or euthanasia needs to, at the very least, be reassessed. The use of any anesthetic or other technique for routine euthanasia should not become taken for granted. As new data come available, all routine methods should be subjected to periodic scrutiny – as occurs when the AVMA periodically revises its guidelines.

Despite the evidence from the published literature of the problems associated with the use of CO₂, The HSUS has received reports from concerned laboratory animal veterinarians that CO₂ can be used humanely when done properly by well-trained personnel. Therefore, the validity of the peer-reviewed literature on CO₂ is under question. This raises an important question—if the published literature is not valid, how are best practices and techniques to be determined and disseminated? The latest AVMA report is one possible source of guidance but it is not that helpful on the question of CO₂ use as a laboratory euthanasia agent. Its recommendations are vague, the analysis is not a good summary of the literature and it does not address some key issues raised by the literature or by differences in common practice.

Existing policies relevant to this issue

Government-funded institutions are required to follow the Public Health Service (PHS) Policy on the Humane Care and Use of Laboratory Animals. PHS policy requires that institutions follow Principle #4 of the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training. This principle states, “[p]roper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.” (emphasis added). These principles must also be followed by government agencies when developing policies about the use of laboratory animals, and it is the responsibility of the Institutional Official to ensure institutional adherence to the principles (PHS policy, 1993).

In addition, when research protocols pertain to species regulated by the U.S. Department of Agriculture (USDA) under the Animal Welfare Act, research institutions must also follow USDA regulations and policies. USDA Policy #11 regarding painful/distressful procedures echoes U.S. Government Principle #4, “[a] painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied.”

On the other hand, both the USDA and the PHS make use of the AVMA report as the source of appropriate practice regarding euthanasia. CO₂ does cause more than slight or momentary pain and distress in humans and, therefore, should be considered painful or distressful to animals. Consequently, Government Principle #4 and USDA Policy #11
are at odds with the AVMA report, specifically the routine use of CO₂ as a euthanasia agent.

**Physiological effects/actions of CO₂**

Carbon dioxide causes a range of neurochemical, respiratory, and vascular responses (Woodbury, 1960). The physiological effects caused by CO₂ are one reason that researchers, especially neuroscientists, argue that they cannot use CO₂ as a euthanasia agent for studies that involve physiological measurements.

Many studies have shown that CO₂ exposure causes a rise in pulse rate and respiratory rate in both unanesthetized humans (Dripps, 1947) and anesthetized rats (Thomas, 2000). Thomas et al. (2000) reported that it is generally accepted that CO₂ does not have a direct effect on breathing; rather, changes in hydrogen ion concentration mediate this effect. They hypothesized that there are receptors that are sensitive to changes in pH, which cause CO₂-evoked changes in breathing. It was concluded that inspiratory neurons “appear to have purinoceptors with pH sensitivity that can account for the actions of CO₂ in modifying ventilatory activity.” Carbon dioxide is also known to cause an increase in blood pressure (Dripps, 1947, Kety, 1947 & McArdle, 1959).

Thurauf et al. (1991) noted that anatomical studies of both humans and rodents have shown that non-myelinated nerve endings that sense chemicals are similar in humans and rats. Danneman et al. (1997) also stated that rats and humans are similar in the innervation of their nasal epithelia.

**Relevant human data**

There is evidence from human studies that inhalation of CO₂ at various concentrations can cause pain and/or distress. For example, Danneman et al. (1997) conducted a study in which twenty humans were asked to take a full breath of CO₂ at five different concentrations: 50%, 60%, 70%, 80%, 100% and also a breath of 100% O₂. The subjects were asked to score each concentration on a scale according to the level of discomfort, with a score of “1” representing “not at all unpleasant” and a score of “10” representing “extremely painful (intolerable).” The twenty subjects were given each concentration twice in random order, resulting in a total of 40 scores for each concentration. Results indicated that higher concentrations of CO₂ were perceived to be increasingly noxious (Figure 1).

**Figure 1.** The response of human subjects to various concentrations of CO₂. The subjects rated each gas/mixture twice, using a visual analog scale, for a total of 40 scores for each concentration (Danneman et al., 1997)
Danneman et al. further reported that the subjects used the following terms in reference to every concentration of CO₂ tested (including the lowest concentration of 50%): burning, tingling or prickling, and unpleasant taste or odor. These terms were used more frequently at higher concentrations. Many described 100% CO₂ as piercing, stabbing, painful or causing the eyes to burn or water, and 18 out of 20 subjects indicated that they were unable to take a full breath of this concentration.

Dripps et al. (1947) examined respiratory and circulatory responses in male humans to severe muscular exercise as well as “high” concentrations of CO₂ (7-10%) in oxygen. The study reported that CO₂ caused increases in pulse rate, respiratory rate and blood pressure. The recorded symptoms upon inhalation of CO₂ included: headache (55% of the subjects at 7.6% CO₂; 42% of the subjects at 10.4% CO₂), dizziness (33% of the subjects at 7.6%; 58% at 10.4%), and dyspnea (31% of the subjects at 7.6%; 32% at 10.4%). Other symptoms noted by the subjects were: irritation of the nose, palpitation, faintness, “generally uncomfortable,” muscle tremor and substernal pain. Due to presence of these symptoms, only two of the 31 subjects were able to tolerate 10.4% CO₂ for five minutes.

McArdle (1959) examined the effects of CO₂ on the heart rate of humans and reported that, contrary to a similar study conducted by MacDonald et al., no ventricular extrasystoles were observed. However, “evidence of delayed conduction and numerous auricular extrasystoles were observed.” Finally, the authors indicated that “[i]nhalation of 30 per cent carbon dioxide produces hyperventilation, severe acidosis and a marked rise in arterial pressure. The procedure is unpleasant, and in the conscious untrained subject
is associated with a considerable degree of mental stress. Each of these factors is capable of producing changes in the electrocardiogram and it is difficult to decide the relative importance of each.”

**Pain studies utilizing CO₂**

Carbon dioxide has been used as a stimulus in examining responses to pain in humans. Anton et al. (1992) examined noxious chemical stimulation induced by CO₂ in the nasal mucosa in humans. The first test aimed to determine pain threshold, which was tested in two different sessions. Subjects were exposed to pulses (durations of two seconds) of 5% CO₂ and the concentration increased by increments of 5% until a painful sensation was reported by the subjects. This stimulus was repeated two or three times and the direction of stimulus presentation then changed from ascending to descending concentration. A linear relationship was found between CO₂ concentration and pain sensation. Average individual tolerance thresholds ranged from 40-55% CO₂ in the first session and 32-55% in the second session.

In order to determine if duration of exposure to a painful stimulus affects pain sensation, Anton also exposed subjects to a fixed concentration of CO₂ (80% of the individual tolerance level determined in the first part of the study). In this part of the study, pain ratings increased with stimulus duration and then quickly reached a plateau at approximately 2 seconds, indicating that pulses of longer duration did not cause further increased pain intensity.

**Animal studies regarding CO₂ as a euthanasia agent**

Research regarding CO₂ use in different species has included several measurements such as blood pressure, heart rate, behavior, time to anesthesia, time to death, EEG activity, histology, blood pH and respiratory rate. Factors such as the concentration of CO₂, flow rate and the presence of oxygen have also been included in the assessment of CO₂. There are wide variations in the methods used and the resulting recommendations throughout the published literature.

Upon discussing CO₂ euthanasia with various people in laboratory animal medicine and care (e.g. veterinarians, vivarium directors, technicians), it has become evident that there are conflicting practices and recommendations within the animal research community. For example, there appear to be wide differences of opinion as to whether or not a euthanasia chamber should be prefilled. At the 2001 Public Responsibility In Medicine & Research meeting, some of those present at a workshop on CO₂ use indicated that they are required to prefill the chamber at their institution. Others indicated that they would never use a prefilled chamber because it appears to cause distress. Similar discrepancies were also noted in regards to concentration, flow rate, and presence of oxygen. [See also the section on Guidelines later in this report. The various euthanasia guidelines from around the world differ significantly from one another on reportedly important details.]
Concentrations of CO₂ for euthanasia
Danneman et al.’s study (1997) exposed rodents to various concentrations of CO₂ ranging from 50-100% via either pre-filled or gradual induction chambers. Oxygen was combined with CO₂ in all trials; therefore each concentration was actually a ratio of CO₂ to O₂. Time to recumbency, time to anesthesia and time to recovery (and time to death in the euthanasia study) were measured, as well as behavioral and histological effects. It took an average of sixteen minutes for rodents to reach a state of anesthesia at 50% CO₂ and an average of two minutes at a concentration of 100% CO₂ (regardless of method of chamber filling). A state of anesthesia was determined by “onset of slow, shallow breathing in 20 animals and by loss of response to toe pinch with hemostats in 16 animals.” Time to death took an average of 22 minutes at 60% CO₂ and approximately 5.5 minutes at 100% CO₂. The frequency and severity of the adverse reactions observed were inversely related to CO₂ concentration (see also section on Anesthesia).

Danneman also performed histological examinations. They found that every rat had edema and hemorrhage of the lungs, regardless of the concentration used. However, the severity of lesions was inversely correlated with CO₂ concentration. A study conducted by Fawell et al. (1972), which focused on the respiratory effects of CO₂ and pentobarbitone on lung tissue, also indicated that edema of perivascular connective tissue was observed in the lungs of all rats subjected to CO₂ euthanasia. The increase in incidence of extravasation and alveolar diameter was considered to be trauma-related.

Ambrose et al. (2000) found that a concentration of 60% CO₂ causes many adverse effects (as also observed by Danneman, et al.) in mice. The mice were exposed to a flow rate of 30% chamber volume per minute CO₂ with and without the presence of oxygen (presence of oxygen consisted of 20% chamber volume per minute oxygen in addition to the CO₂) and 60% CO₂ combined with oxygen at a flow rate of 20% chamber volume per minute (effects of oxygen discussed in a separate section below). The gas was introduced after the mice were put into the chamber. During the 60% CO₂ condition, distress behaviors (see below) were significantly increased in comparison to 30%. According to the authors, simply introducing carbon dioxide into the euthanasia chamber caused distress behaviors such as: increased locomotion, rearing, defecation and urination. The authors concluded, “behavioral results indicated that [60% CO₂] … caused an undue amount of stress.”

Coenen et al. (1995) conducted a study with rats that examined several factors: concentration of CO₂, pre-filling versus gradual induction, and the presence of oxygen. The authors reported that there were four phases observed in each case of CO₂ use: normal behavior (phase I); continuous abnormal activity, excitation and agitation at a higher rate than normal (phase II); sagging of hindlegs and loss of body control (phase III); disappearance of muscle tone and head sinking (phase IV). Two independent observers scored signs of behavioral agitation and asphyxia on a 3-point scale. Phase II (“strong behavioral agitation and excitation”) was seen more frequently when 100% CO₂ was used. The same adverse signs were also observed at lower concentrations (without O₂), but less frequently than 100% CO₂. It was indicated that animals suffered from
asphyxia in some conditions and “the animals gasped with their mouths opened wide and their heads turned up and backward….Animals in the CO2-100 groups showed most evidence of this behavior and undoubtedly suffered from serious asphyxia.”

There is evidence that low concentrations of CO2 can also lead to pain and distress. For example, according to the guidelines provided by the Canadian Council on Animal Care, “carbon dioxide also stimulates the respiratory center in the brain and in low concentrations of up to 10% of inspired gas is considered a potent respiratory stimulant causing a tenfold increase in the ventilation rate and a feeling of profound respiratory distress.” The CCAC cites one reference to this statement (CCAC, 1993).

Experienced veterinary clinicians have indicated that anesthesia sets in so quickly that there is not sufficient time for the animals to experience significant pain and distress. However, a review of the literature finds that times to anesthesia when using the AVMA recommended method vary. One problem in researching this issue is that many authors use different terminology or assess different behaviors, such as: anesthesia, collapse, recumbency, unconsciousness, and immobility. The following are examples of observations regarding the AVMA-recommended 70% prefilled condition: Carding (1968) recorded collapse of dogs at 0.4 minutes (24 seconds); Danneman (1997) recorded that anesthesia (onset of slow, shallow breathing and loss of response to toe pinch with hemostats) occurred at 4.01 minutes in rats; Glen (1973) recorded unconsciousness at 35 seconds in kittens; Mischler (1994) recorded anesthesia in rats at 10 seconds; Simonsen (1981) recorded immobility in cats at 90.5 seconds; and Smith (1972) reported loss of recumbency at .444 minutes (26.6 seconds) in rats.

While the AVMA recommends using 70% CO2 in a prefilled chamber, they do not reference any papers for this particular recommendation.

Prefilled chamber vs. gradual induction/times to anesthesia and death

The mode by which animals are exposed to CO2 has been examined fairly extensively. In most of these studies, gradual induction (GI) means that the animals are put into a chamber that is free of added CO2 and then CO2 alone (100%), or CO2 in conjunction with various concentrations of oxygen, is gradually introduced to the chamber at a fixed rate. Prefilled (PF) means that the chamber is pre-filled with CO2 and the animal is added to the chamber once a predetermined concentration and rate of flow of CO2 has been reached. [Note: in such studies, if the lid is open for too long, the concentration will change dramatically and will affect results]

Smith and Harrap (1997) compared GI versus PF methods in rats. The PF study used a concentration of 75% CO2, 3% O2 and air. During the GI study, it took seven minutes for the chamber to reach 80% CO2 (plus 3% O2). Blood pressure, pulse rate and behavior were measured. PF caused an immediate fall in blood pressure while GI caused increased blood pressure for the first 4 minutes and then a rapid decline. Additionally, the PF subjects reached loss of consciousness within 30 seconds but the GI group took 99 seconds. The time to death for PF was 5.4 minutes and for GI was 9 minutes. The authors
reported, “carbon dioxide produced few overt signs of distress in either treatment group.” However, head-raising (in 50% of the GI subjects), urination (in 100% of the PF subjects and 20% of the GI subjects), defecation (in 25% of PF subjects and 60% if the GI subjects), and gasping or labored breathing (in 50% of the PF subjects) were reported. Head-raising, defecation, urination, difficult respiration and gasping were behaviors predetermined by the authors to be indicators of stress, pain, distress, anxiety and fear. Despite the above findings, the authors concluded, “CO2 gas in either a precharged chamber or given gradually was satisfactory as an agent for euthanasia, but that the rapid loss of consciousness and rapid death associated with the ‘plunge’ method [i.e., PF] was preferred.”

Danneman et al (1997) reported that pre-charging caused no significant effect on times to anesthesia, recovery or death in rats in comparison to gradual induction. However, time to recumbency (“ceased to make any effort to raise its head off the floor”) was shorter for most concentrations in the precharged chambers. In regards to the use of prefilled versus gradual induction, Danneman made three suggestions “for those who must use CO2.” One method recommended was to “place the animal(s) in a non-precharged chamber and gradually introduce 70% CO2 into the chamber…” The two other methods also suggested use of a non-precharged chamber, but one indicated that 100% could be delivered after the animals become unconscious and the third suggested 100% at a low flow rate after placing the animals in the chamber.

Hewett et al. (1993) examined the differences between PF and GI methods in rats at a final concentration of 100%. Times to ataxia, immobility, loss of righting ability and loss of pedal reflex were determined by reviewing videotapes of the animals. Blood gas quantitation was also measured. The authors reported that, “the animals’ responses documented in this study are consistent with anesthetic properties of CO2. These responses did not indicate that the animals experienced distress by either method. While hypoxemia occurs in rats placed in prefilled chambers, the reactions observed were consistent with cortical depression.” Time to anesthesia was assessed as two consecutive negative pedal reflexes (no response when the foot was pinched with forceps). The shortest amount of time to loss of pedal reflex was 28 seconds, which occurred in the subjects exposed to the prefilled chamber. Time to loss of pedal reflex in the GI study was approximately 140 seconds or six times longer compared to the PF method.

Finally, at a symposium organized by Universities Federation for Animal Welfare (UFAW), Britt (1986) presented information on the humaneness of CO2 use, which was based on a study during which he specifically compared pre-filling and gradual induction methods using rats and mice. He noted that even though time to collapse was shorter with rapid induction, there were more signs of distress with that method. The abnormal behaviors included: shaking (frequent), moving in reverse, tail thrashing (uncommon) and increase in frequency of urination and defecation. Behavioral responses varied between species and individuals. The author concluded that “[n]either of the two methods of CO2 application can be considered stress-free.” However, he favored gradual induction, “[w]hile it may be desirable and convenient to complete the whole process
quickly, any increased speed in securing unconsciousness which is at the expense of gentleness, fails to serve the purposes of humanitarianism.”

Presence of Oxygen

The use of oxygen supplementation in order to minimize pain and distress during CO2 use has also been a point of debate. Existing studies contradict each other, depending on the effects examined.

As previously mentioned, Coenen et al. reported that there were four phases in each case of CO2 use: normal behavior (phase I); continuous abnormal activity, excitation and agitation (phase II); sagging of hindlegs and loss of body control (phase III); disappearance of muscle tone and head sinking (phase IV). Phase II was completely absent in the presence of oxygen. While 100% CO2 produced the shortest time to death, the authors concluded that a longer time to death with a minimization of adverse effects when oxygen is present is the preferable method.

In a study of rapid vs. gradual induction conducted by Hewett et al., there was one condition in which rats were exposed to the gradual induction of a combination of 75% CO2, 20% oxygen and 5% nitrogen. The author concluded, based on behavior and blood gas quantitation results, that “[t]here appeared to be no advantage to combining oxygen with the carbon dioxide.” However, it should be considered that a concentration of 20% O2 might be too small to prevent hypoxia, which may be the reason that O2 at this concentration was not found to be advantageous by the author.

Ambrose et al. (2000) also examined whether the presence of O2 reduces the adverse effects of CO2 and their results conflict with those of Coenen. The concentrations used in this particular comparison were: a combination of a flow rate of 816 ml/min (30% of euthanasia chamber volume per minute) CO2 and 544 ml/min (20% of euthanasia chamber volume per minute) oxygen or only 816 ml/min (30% of euthanasia chamber volume per minute) CO2 with no oxygen. There were no behavioral differences observed between the two conditions. Presence of oxygen was found to increase alveolar consolidation. The authors concluded that “[a]lso haemorrhage is likely to be stressful to the mice by inducing a feeling of ‘drowning’, any alveolar consolidation above basal level indicates potential poor welfare.” Furthermore, “the use of exogenous oxygen at 20% of box volume per minute cannot be recommended as a refinement to carbon dioxide euthanasia.”

Finally, Woodbury et al. (1958), when studying the effects of CO2 on brain excitability and electrolytes in rats, found that when higher than normal oxygen concentrations were combined with 35-40% CO2, incidence of seizures in the chamber decreased, but incidence of seizures upon withdrawal increased. During mild hypoxia conditions (15% O2 with 35% CO2) the incidence of seizures in the chamber and upon withdrawal slightly increased, but severe hypoxia (5-10% O2 with 30% CO2) decreased incidence of seizures and protected against withdrawal seizures.
Summary

Thus far, we have seen that the published literature on CO₂ as a euthanasia agent reports conflicting findings. It is also evident from the literature that CO₂ is painful and/or distressful in humans at concentrations ranging from 7-100%. According to PHS policy and USDA Policy #11, if pain and distress is experienced by humans, that same technique should be assumed to cause pain and distress in animals, unless it has been proven otherwise.

In summary, the combination of conflicting evidence in the animal literature and the evidence of pain and distress in the human literature clearly signals that the use of CO₂ by itself may be problematic.

Studies that compare the use of CO₂ alone to other euthanasia methods

A fair amount of the published data on CO₂ was derived from studies that compared CO₂ euthanasia to other methods. Once again, concentration, induction method and presence of oxygen are repeated factors/concerns.

Blackshaw et al. (1988) assessed the behavioral response of rats, mice and chickens to CO₂, ether and chloroform. Dry ice was used for the carbon dioxide condition and was measured as 97% CO₂ before the animals were placed in the chamber. Times to ataxia, collapse and death were recorded. The authors argued that the shortest time to death is preferable when choosing a euthanasia agent. Time to collapse and death were shortest with CO₂ for all three species in comparison to the other two agents. In addition, adverse behavioral signs were not observed with the use of CO₂, except for wing flapping in chickens. Therefore, the authors concluded that CO₂ was the agent of choice for rats and mice, but not chickens. One potential problem with this study is that the behaviors observed - movement (all four limbs change position), partial body movement (hindlimbs stationary with movements of head and body), stationary, touch wall, and climb wall - would not necessarily have included other stress behaviors that were observed in other studies examining the use of CO₂. It should also be noted that Artwohl et al. (2001) have demonstrated that the use of dry ice as a source of CO₂ “can create environmental conditions that conform with the recommendations of the AVMA Panel, but these conditions can change relatively rapidly with prolonged use, repeated openings to remove animals, and the amount of dry ice that is used.” The 2000 AVMA Report considers the use of dry ice as an unacceptable source of CO₂.

Simonsen, et al. (1981) compared use of carbon monoxide (CO) and carbon dioxide for euthanasia of cats. Twenty-one cats were killed by 4-5% CO and four cats by 70% CO₂/30% oxygen by means of gradual induction. Behavior was videotaped and phases were timed. Phase I was the time from first contact with the gas until it was “evident that the cat was affected by it, i.e. was yawning, staggering or trembling.” Phase II was from the end of Phase I until the cat was no longer on its feet. Finally, Phase III was from the end of Phase II until the cat was completely immobile.
Restlessness and fear were recorded during Phase I for both CO and CO₂, but was more prevalent for CO₂ (100% exhibited restlessness and 75% exhibited fear). The average duration of Phase I for the cats exposed to CO₂ was 30 seconds. Fear was defined “as a defensive posture (Leyhausen, 1979) or attempts at escape.” Sounds and convulsions were recorded in all phases for each method also, but were more prevalent during Phases II and III. The authors concluded, “[t]he behaviour reactions during phase I indicate that CO₂ + O₂ causes more discomfort than exhaust fumes.”

Carding (1968) compared carbon monoxide (CO) and CO₂ for mass euthanasia of dogs. Twenty-seven dogs were euthanized with CO, eleven with CO₂ and two by a combination of CO and CO₂. Various initial concentrations of CO₂ were used, from 0-80%. It was determined, “[w]ith CO₂ the most satisfactory result was achieved at 70% with air when death occurred after 5 min.” At this concentration, collapse occurred at approximately 40 seconds. The most severe struggling and hyperpnea occurred when CO₂ was admitted after the dogs entered the chamber, “this test was disagreeable to watch and should not be repeated under any circumstances.” Other behaviors reported in all dogs, except for the one exposed to 70% CO₂, included hyperpnea, dyspnea and a “paddling” movement of the limbs. The author did stress the importance of proper technique and apparatus as well as further investigation into these methods of euthanasia.

Finally, Hackbarth et al. (2000) examined whether sedation or anesthetization prior to the use of CO₂ would decrease the stress-induced effects of CO₂ euthanasia. Acepromazine placed in chopped meat was used for sedation and pentobarbital via intraperitoneal injection was used for anesthetization. Rats were subjected to one of four conditions: meat with acepromazine, meat without acepromazine, injection with pentobarbital or injection with saline. Behavioral observations were recorded and adrenocorticotropic hormone (ACTH), glucose, and corticosterone measurements were taken at various times (30, 75 and 120 seconds) after CO₂ induction began. Each animal to be injected was handled daily for three weeks prior to the experiment in order to minimize the stress response to handling. All subjects were exposed to CO₂ via gradual induction at a rate of 6 liters/minute. No groups showed any behavioral signs of pain or distress; glucose and corticosterone levels did not differ between groups and finally, ACTH was higher in the subjects given injections in comparison to those given oral treatments. The increase in ACTH was assumed to be a result of handling for the injection. The authors concluded that, “…euthanasia with CO₂ [without prior sedation or anesthetization] is in concordance with animal welfare as it is rapid and does not cause distress in the animal and therefore can be recommended as ‘humane’.”

**Literature on Stunning for Slaughter**

Raj and Gregory (1995 & 1996) compared the use of argon and carbon dioxide for stunning at different concentrations in pigs. In the first study, aversion to various concentrations was assessed: 90% argon in air, 30% CO₂ in air and 90% CO₂ in air. Aversion was assessed from the pigs’ reluctance to enter the gaseous atmospheres for a reward of apples. It was concluded that “inhalation of high concentration of carbon dioxide is aversive to the majority of pigs and, given a free choice, they refused to obtain
the reward presented in the carbon dioxide atmosphere even after 24h fasting. By contrast, pigs did not perceive any aversion to the presence of argon and the majority of them did not perceive any aversion to the presence of 30 per cent carbon dioxide in air” (Raj and Gregory, 1995). A second study (1996) examined the severity of respiratory distress at various concentrations of argon with or without oxygen and/or CO$_2$ and carbon dioxide (20, 30, 40, 50, 60, 70, 80 or 90 per cent) in air. It was found that argon with 2% oxygen induced minimal respiratory distress, the combination of 30% CO$_2$ in argon with either 2 or 5% residual oxygen induced moderate distress and “exposure to all the concentrations of carbon dioxide in air induced severe respiratory distress in the pigs” (Raj and Gregory, 1996).

Hoenderken (1983) compared CO$_2$ and electrical stunning methods in pigs in order to determine if CO$_2$ is an acceptable method of stunning in regards to animal welfare. Behavior and EEG were recorded while CO$_2$ was used. Specific concentrations and induction methods were not clearly indicated in the publication. It was found that it takes approximately 12 seconds following exposure to the gas for the pigs to show signs of excitation and this excitation lasted 26 seconds on average (isoelectric EEG was recorded at 56 seconds). In ten of the 16 experiments, the EEG remained isoelectric after treatment, therefore indicating that the animals were dead. The author concluded that there is a long period of excitement during CO$_2$ exposure. It was further reported that, “[c]arbon dioxide stunning of pigs has not been allowed in the Netherlands since 1980, in the knowledge that immobilization by means of CO$_2$-gas leads to unconsciousness under very disturbing and stressful conditions for the animal.” As an aside, it should be noted that the 2000 AVMA Report does indicate that CO$_2$ is an acceptable form of euthanasia for swine.

In summary of this stunning information, it appears that when given a choice, animals choose to avoid CO$_2$ and appear to find CO$_2$ aversive. Furthermore, results of some of these studies do indicate that CO$_2$ is associated with adverse reactions, some of which result in distress.

**Pain and acute stress studies utilizing CO$_2$**

Carbon dioxide has been used as a stimulus in studies of pain in animals, as it has been in humans. Thurauf et al. (1991) exposed rats to various concentrations of CO$_2$ (from 0-90%) and measured evoked potentials in the EEG recordings in an attempt to determine the origin of “negative mucosal potential” (NMP—negative potential recorded from the respiratory mucosa following painful stimulation with CO$_2$) from the nasal mucosa in the animal model. It was reported that local anesthetics eliminated NMPs and EEG cortical responses, therefore signifying that the pain response ceased. When no anesthetic was administered, the result was increased NMPs, indicating an increased pain response.

Barbaccia et al. (1996) examined the effects of acute stress on brain steroid concentrations in studies where CO$_2$ was used to elicit a stress response in rats. A concentration of 35% CO$_2$ and 65% O$_2$ for one minute caused a sufficient stress response
for the study. It was concluded that exposure to CO₂ is correlated with an increase in brain neuroactive steroid concentrations

**Use of CO₂ as an anesthetic**

CO₂ is used not only for euthanasia and stunning, but also as an anesthetic. As with CO₂ for euthanasia, there are conflicting results in the literature reporting on its welfare aspects when used as an anesthetic.

Danneman et al. (1997) examined CO₂ as both a euthanasia agent and as an anesthetic. A total of 43 adverse reactions (ADRs) occurred among the 116 rats used in the study (some subjects had no adverse reactions while others had 2 or 3). Seizures, convulsive chewing, unexpected death, and need for resuscitation with oxygen were observed. One rat was euthanized for humane reasons because of “severe serosanguinous nasal discharge.” It was noted that “the time to anesthesia was so long and the incidence and severity of adverse reactions so great with 50% CO₂ in the early anesthesia trials that this concentration was tested no further.” After the 50% concentration was discontinued, the 60% CO₂ concentration resulted in the highest number of adverse reactions (16 out of 28 animals) while 100% CO₂ resulted in the lowest number of adverse reactions (1 out of 28 animals) The authors indicate that the frequency and severity of ADRs were inversely related to CO₂ concentration.

Kohler (1999) examined the use of CO₂ as an anesthetic in mice, rats and guinea pigs. The subjects were exposed to either a combination of 80% CO₂ and 20% O₂ or 80% CO₂ and ambient air (this decision was based on results from a previous study by Meier, 1994) and exposure times were predetermined. It was concluded, “a high inspiratory CO₂ concentration quickly induces unconsciousness in small laboratory animals, followed by a stage of surgical tolerance, and fast recovery.” The authors point out that CO₂ induces unconsciousness more quickly than other inhalational anesthetics, such as isoflurane.

Mischler et al. (1994) studied the effects of CO₂ anesthesia in the laboratory rat. In this study, the rats were exposed to either 70% CO₂/30% O₂ or 100% CO₂ in a prefilled chamber for 30 seconds. According to the authors, all of the animals “were clinically anesthetized within 10 s[seconds] after initial exposure to either 70 or 100% CO₂.” Animals regained motor control within one and a half minutes and complete recovery from anesthesia occurred within 5 minutes. These animals were then subjected to thermal and mechanical nociceptive tests following recovery from CO₂ and it was concluded that CO₂ use results in prolonged mild antinociception.

The cause of this antinociceptive response was closely examined. It was hypothesized that a release of opioids plays a role in this response; therefore some rats were exposed to an opiate antagonist (naltrexone). Naltrexone did not prevent the antinociceptive response indicating that opioids are not involved. These results, in conjunction with results from other studies, indicate that CO₂ inhalation produces characteristics of a generalized stress response. Therefore, the authors concluded that “complete recovery
from CO₂ anesthesia is associated with a prolonged, mild antinociceptive response in the laboratory rat that may represent a novel form of stress-induced analgesia.”

Urbanski and Kelly (1991) examine the use of CO₂ to sedate rats prior to decapitation. According to this study, the animals lost consciousness within one minute with a concentration of 50% CO₂/50% O₂ in a prefilled chamber. No adverse reactions to this concentration were reported. It is important to note that the concentration recommended by Urbanski and Kelly reportedly caused a high number of adverse reactions in the study by Danneman, et al. (1997).

Eisele et al. (1967) examined the narcotic effects of CO₂ in dogs and determined the minimum anesthetic concentration needed to prevent movement in response to a painful stimulus in each group. They reported a high incidence of seizures in the CO₂-alone group. There were four groups of dogs in this study, one of which was treated solely with CO₂ in combination with O₂. Other groups were first anesthetized with halothane and then exposed to CO₂. The CO₂-alone group of dogs was given 50% CO₂/50% O₂ until consciousness was lost. Cyclopropane was then added to the mixture to facilitate intubation and catheterization. Cyclopropane was discontinued and “anesthesia was maintained with 30-40 per cent inspired CO₂ for 30-60 minutes while cyclopropane was eliminated. At this level of CO₂, there was complete CO₂ narcosis.”

“The results showed that CO₂ exerts a narcotic influence in dogs at a concentration above 95 mm of mercury and at a level of 245 mm mercury CO₂ produces anesthesia.” It was reported that seizure activity occurred in “approximately one-fourth of the animals during the period of highest CO₂ concentration. These seizures were unsustained and untreated. They [seizures] reportedly did not affect the dog’s ability to make a purposeful movement in response to painful stimuli at CO₂ levels below CO₂ narcosis.” This high incidence of seizures did not occur in the groups who were anesthetized with halothane first. (According to the USDA, the occurrence of seizures should be considered as Column E, i.e., associated with pain/distress (http://www.aphis.usda.gov/ac/iacucaugust.pdf).

Klemm (1964) examined whether 15 minutes of sustained CO₂ anesthesia can be used in cats without causing brain damage. There were 85 trials with 13 cats. Both solid and compressed gas sources (flow rate of 5-6 liters per minute) were used. In general, the cats were not excited during induction but some did cry out and scratch at the door. Other observations included marked salivation, rapid respiratory rate and gasping. The adults collapsed within 138 seconds and anesthesia set in at approximately 203 seconds. Collapse occurred in kittens at 67 seconds and anesthesia at 90 seconds.

Klemm found that immediate removal from the chamber was essential in order to avoid respiratory or cardiac arrest. Overdose occurred in 10 of the 40 kitten trials and in 8 of the 45 adult trials; two of which resulted in death; the others were resuscitated. Recovery time (time after removal of gas until the cat could walk without staggering) was 134 and 168 seconds for cats and kittens respectively. Recovery was “often characterized by defecation, urination, some crying out and some brief clonic muscle seizures of the face,
jaw and limbs.” Finally, no brain damage as a result of frequent exposure to CO₂ occurred.

In the most recent study on this topic, Leach et al. (personal communication, paper submitted, - 2001) reported on the aversiveness of various gas anesthetics (each at 3 concentrations: low, medium and high) including halothane (1.8-7.4%), isoflurane (1.7-7.2%), enflurane (2.7-8.1%), argon (93.7-99.2%), CO₂ (25.5-50.8%), CO₂ with argon and 5% O₂ (15.5-59.2 CO₂) and, CO₂ with argon and 7% O₂ (19.1-54.2% CO₂). The animals were placed in the gaseous atmosphere chamber but were given the choice to stay in the chamber or escape, through a door and a tube, to a chamber that contained air only. Times recorded included times that the animals remained in the chamber (dwelling time) and time to escape (escape time).

According to the results, escape and dwelling times were significantly lower than for air for the following conditions: high halothane, medium and high enflurane and isoflurane, all CO₂ concentrations and all CO₂/argon concentrations. Both rats and mice found CO₂ (at all concentrations) more aversive than halothane or isoflurane at all corresponding concentration levels. For example, the dwelling time for the lowest concentration of CO₂ (25%) was two seconds whereas they stayed in the lowest concentration of isoflurane for 40 seconds, in halothane for over 50 seconds and argon for 15-20 seconds. Therefore, it appears clear that the animals find CO₂ to be more aversive than the other agents.

The authors also found that CO₂ causes hemorrhage and filling of alveolar spaces of the lungs with blood while the animals are still conscious, making them unable to absorb oxygen. From this, the authors concluded that the animals are likely rendered unconscious by hypoxia instead of narcosis and suggest that the use of CO₂ is no longer appropriate.

**Guidelines for euthanasia**

Various organizations, such as the American Veterinary Medical Association (AVMA), Canadian Council on Animal Care (CCAC), the European Commission, the Australia and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) and the Universities Federation for Animal Welfare (UFAW) provide guidelines on euthanasia. These guidelines provide conflicting recommendations in regards to CO₂ (Table 1).

The AVMA report (2000) recommends, “an optimal flow rate should displace at least 20% of the chamber volume per minute.” (Note: the AVMA indicates that compressed CO₂ gas is the only recommended source of carbon dioxide.) The report further recommends that, “loss of consciousness may be induced more rapidly by exposing animals to a CO₂ concentration of 70% or more by prefilling the chamber for species in which this has not been shown to cause distress.” The CCAC (1993) and UFAW (1986) cite a study by Britt (1986) that found that neither slow nor swift CO₂ induction is stress-free, but that slow induction is preferable to using a precharged chamber, which can produce obvious signs of distress in the animals. The 1993 ANZCCART guidelines
indicate that a 1988 UFAW report recommends a 70% CO₂/30% oxygen ratio concentration for euthanasia of rats and mice (induction method was not indicated).

A European Commission report (1996) cited studies that found that “in most animals it is recommended to place the animals immediately into >70% CO₂ where the animals lose consciousness very quickly due to narcotic effect of the high intake of CO₂ on the brain without causing hypoxia.” The report further indicates, “one hundred per cent CO₂ may cause severe dyspnoea and distress in conscious animals.” It is then stated that “the chamber must be prefilled with up to 70% CO₂ before placing the animals in it.” Nonetheless, the Commission report adds, “others feel that it may be better to fill the chamber once the animals have been placed in it.”

ANZCCART (1993) considers the use of CO₂ as a “recommended technique” (in rats and mice, guinea pigs, and birds) but various recommendations are made as to the best method. While the document notes the short time to collapse when 100% CO₂ is used for rats and mice, it also states, “[o]ther workers have indicated that exposure to 100% carbon dioxide may cause discomfort due to hypoxia…. The document also notes, “the optimal flow rate appears to be one which displaces approximately 20% of the chamber volume per minute.” Finally, a 70:30 ratio of CO₂ to oxygen is ultimately recommended as “more appropriate” (induction method is not indicated).

Both ANZCARRT and the CCAC permit the use of dry ice as a source of CO₂ for the chamber whereas the AVMA (following Artwohl et al., 2001) permit only the use of compressed gas cylinders.

Table 1. Recommendations regarding the use of CO₂ for euthanasia of rodents

<table>
<thead>
<tr>
<th>Organization (year)</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANZCCART (1993)</td>
<td>Dry ice can be used; flow rate of 20% chamber vol/min; 70% CO₂ with 30% oxygen “would seem to be more appropriate.”</td>
</tr>
<tr>
<td>AVMA (2000)</td>
<td>Cylinders only; flow rate at least 20% chamber vol/min; more rapid loss of consciousness=prefilled with 70%</td>
</tr>
<tr>
<td>CCAC (1993)</td>
<td>Dry ice can be used; sufficient concentration should be used to produce narcosis=maintain oxygen level close to normal air levels and increase % of CO₂ in the air.</td>
</tr>
<tr>
<td>European Commission (1996)</td>
<td>“..place the animals immediately into &gt;70% CO₂;..”; 100% CO₂ may cause distress in conscious animals</td>
</tr>
</tbody>
</table>

Discussion of CO₂ use

The evidence that CO₂ causes pain and distress in both humans and animals can be summarized as follows:
1. The fact that CO₂ has been used in pain studies in animals as a painful stimulus (Hummel, 1994; Thurauf, 1991) and in studies to elicit an acute stress response (Barbaccia, 1996) supports the claim that CO₂ causes pain and distress in rodents.

2. We would argue that the conflicting findings within the literature requires erring on the side of caution (as happened in the decapitation discussion). While some of the studies reviewed above conclude, based on empirical data, that CO₂ causes distress in animals, other studies report few or no signs of distress in response to CO₂. There is no obvious explanation of the differences in the results from these various studies, although there may well be strain, species, age, as well as methodological differences.

3. High concentrations of CO₂ have been found to cause more pain and distress in comparison to low concentrations, but pain and distress have also been reported at low concentrations (as low at 7.6% in humans). Leach et al. (2001) found that even 15% CO₂ was highly aversive to rats and mice. Reported findings that there is increased noxiousness with increased concentrations of CO₂ (Danneman, 1997) are of concern because some literature and most available euthanasia guidelines recommend the use of higher concentrations of CO₂ due to its shorter time to recumbency, anesthesia and euthanasia (Enggard, 1991; Glen 1973; Mischler, 1994).

4. There is debate within the laboratory animal medicine community as to which methods of CO₂ use are painful and/or distressful. For example, some individuals have commented that prefilling the chamber causes distress; therefore those individuals refuse to prefill the chamber. Some institutions require that the chamber be prefilled. These same conflicts are reflected in the various guidelines on CO₂ euthanasia (e.g. the AVMA, the CCAC, the European Commission, ANZCCART, and UFAW). The inconsistency between guidelines and within actual practice might reflect the conflicts found in the literature but also raise questions about the appropriateness of CO₂ use.

The U.S. Government PHS Principles and the USDA policy both indicate that, if a procedure causes pain and distress in humans, it must be considered to cause pain and distress in animals (PHS Policy also indicates “in the absence of evidence to the contrary”). The evidence that CO₂, at least at concentrations of approximately 50% and above, elicits a painful response in humans (Anton, 1992; Danneman, 1997; Dripps, 1947; Hummel, 1994; McArdle, 1959) is very strong. Furthermore, the evidence that CO₂ does not cause pain and distress does not rise to the level of appropriate “evidence to the contrary.” Therefore, absent unequivocal evidence to the contrary, both policies require that CO₂ use should be considered painful and distressful in animals.

**Time to unconsciousness**

Time to anesthesia has been recorded in a number of the CO₂ studies and is of course a critical issue. The finding that brain activity occurs for an average of 13 seconds following decapitation in rodents was the main reason why the AVMA did not recommend decapitation as a method of euthanasia (and still questions it). However, using the AVMA-recommended concentration and method of CO₂ euthanasia, the time
until unconsciousness ranged from 10 seconds to 4 minutes in published studies. Therefore, the potential for 13 seconds or more of stress or distress in CO₂ euthanasia is high.

In conclusion, according to the literature, carbon dioxide does not, used by itself, appear to meet the standard of what a euthanasia agent should do. Alternatives resulting in better welfare while also taking into account ease of technique should be considered.

**Alternatives to use of CO₂**

Discontinuing use of CO₂ alone as a euthanasia agent is unlikely to happen soon because it is so widely used. However, the literature raises questions that need to be addressed and there are also reasonable alternatives to CO₂ by itself.

**Pre-anesthetic**

David Morton (personal communication, 2001) recommends using halothane as a pre-anesthetic, followed by CO₂ once all the animals have lost consciousness. This is because halothane was found to be the least aversive by Leach et al. (2001). However, isoflurane is also not particularly aversive and could be used instead. These combinations of anesthetic gases with CO₂ should be acceptable in the euthanasia of large numbers of animals not involved in research protocols or for the euthanasia of small numbers of animals where the effect of the anesthetic gas is not a problem. Where the research protocol calls for tissues unaffected by anesthetics, then decapitation may be the best method for small numbers of rats or mice. However, this method is still questioned because of the debate over the meaning of the EEG trace following decapitation and also because of the high chance for error due to inadequate operator training or guillotine maintenance.

Eisele et al. (1967) found that induction with halothane prior to CO₂ for use as an anesthetic resulted in a lower incidence of seizures. Gas agents are particularly advantageous because they require minimal handling of the animals and larger numbers of animals can be euthanized simultaneously. As Hackbarth et al. (2000) report, an injectable pre-anesthetic can cause a handling-induced stress response. The inhalant anesthetic should have a fast uptake to minimize the excitation phase.

One concern about the use of volatile anesthetics is exposure of lab personnel to the gas. There are portable scavenging units that would allow for safe use of both a pre-anesthetic and carbon dioxide. The European Commission recommendations for euthanasia indicate that halothane, enflurane and isoflurane “are all acceptable agents with appropriate gas scavenging apparatus.”

**Decapitation**

Decapitation is a possible alternative to the use of CO₂ for the euthanasia of (relatively) small numbers of animals. However, since 1986, it has been somewhat controversial because of a recommendation that it not be used without justification (see paragraph below regarding
former AVMA recommendations). It is instructive to review the history of the issue to contrast the level of concern raised regarding the decapitation issue on the basis of a single published report and the lack of similar concerns regarding CO₂ even though there have been multiple publications questioning its use.

In 1972 and 1978, the AVMA euthanasia panel deemed decapitation an appropriate euthanasia method for small rodents. The 1978 recommendation was then challenged (Warren, 1979) because of a single study reporting an EEG trace in the decapitated heads of eight rats for up to 29.5 seconds after decapitation (Mikeska & Klemm, 1975). The average duration of the EEG trace was 13.6 seconds. In 1986, the AVMA panel, relying on this study, recommended that any animal to be decapitated should be lightly anesthetized or sedated. Because the AVMA report on euthanasia has become a de facto regulatory standard for laboratories, the downgrading of decapitation as an acceptable euthanasia method for small rodents was challenged by several authors (Allred & Berntson, 1986; Derr, 1991; Holson, 1992; Vanderwolf et al., 1988).

Allred & Berntson (1986) presented a counterargument to Mikeska & Klemm (1975), citing references that demonstrated an activated EEG pattern after hypoxia or damage to the lower brain stem as evidence that the Mikeska and Klemm data did not necessarily provide evidence of consciousness and distress. Vanderwolf et al. (1988) cited additional references that reported the same sort of LVFA (low voltage fast activity) EEG trace during surgical anesthesia with volatile anesthetics. They then performed a series of experiments looking at the source of the LVFA (coming from either the basal forebrain or from the midbrain). The forebrain traces were atropine-sensitive whereas those from the midbrain were atropine-insensitive. The atropine-sensitive EEG trace occurs under surgical anesthesia whereas the atropine-resistant EEG trace occurs in response to noxious stimulation. In their experiments, the EEG traces of the decapitated heads were atropine-sensitive. The atropine-resistant traces (that might indicate consciousness of noxious stimuli) were not present. Derr (1991) reported that it would take 2.7 seconds for a decapitated rat head to become anoxic (indicating a presumed loss of consciousness) and for anoxia to occur.

Finally, Holson (1992) reviewed of all the literature dealing with decapitation and also included some findings on cervical dislocation. Holson reports that, by 1986, there were eight papers reporting on the EEG of decapitated rodent heads. He states that the reports are very consistent. All agree that decapitation triggers an immediate, slow direct current EEG trace of 2-4 seconds duration followed by an LVFA trace that is usually gone in 10-13 seconds. Decapitation of anesthetized rats produces the same EEG pattern with the exception that the LVFA trace lasts longer. He concludes that this last finding “incontrovertibly establishes” that the LVFA is not associated with consciousness. He also cites studies of ischemia at the cortical surface of the decapitated rat brain. These indicate that hypoxia would occur in 3-6 seconds (close to the value calculated by Derr). Holson also noted that cervical dislocation (which was recommended as an approved euthanasia technique for laboratory rats and mice by the AVMA in 1986) resulted in the same type of EEG traces while still retaining blood flow; therefore hypoxia would not be expected to intervene to induce loss of consciousness.
Despite the range of papers that took issue with Klemm and Mikeska (1975), the 1993 AVMA euthanasia panel continued to raise questions about the meaning of the EEG activity after decapitation and recommended that decapitation should be permitted only when approved by the IACUC. However, they also raised questions about the use of cervical dislocation. Carbone (2000) looked for data on the effectiveness of cervical dislocation and was able to find only one report on five mice that had been x-rayed after cervical dislocation (Keller, 1982). One of the mice had the spinal fracture in the mid-thoracic region rather than the high neck region.

In sum, decapitation (and cervical dislocation) produces an EEG trace that can last for 10-13 seconds. The weight of the evidence would indicate that consciousness following decapitation is unlikely to persist for more than 3-6 seconds and maybe not even that. It seems clear that decapitation produces a much quicker loss of consciousness than recommended carbon dioxide protocols. Therefore, from an animal welfare perspective, decapitation properly performed may be preferable to CO₂. (NOTE: decapitation is not an easy procedure and technicians who use the guillotine must be properly trained and warned against overconfidence and carelessness for both their own and the animals’ sakes).

One final point needs to be made about the use of decapitation. The researchers most eager to retain decapitation as a euthanasia technique are neuroscientists who wish to study brain function and chemistry without interference from the artefacts introduced by the use of anesthetics and other chemicals. It is well known that anesthetics do alter a variety of brain metabolism parameters (see, e.g. Miller et al, 1988). However, it is also well known that stress is an important (and rapid - within ten seconds) modulator of metabolite levels (e.g. Faupel et al, 1972). Any neuroscientist who presents an argument to an IACUC that he or she needs to use decapitation in order to obtain in vivo metabolite levels uncontaminated by anesthetic effects, should be required to explain how the effects of handling and subsequent decapitation will prevent changes in metabolite levels. Unless the scientist uses the sort of technique developed by Veech et al (1973) and used later by Nishihara and Keenan (1985), they will simply be exchanging one problematic technique (preanesthesia) for another (handling and decapitation). There may, of course, be occasions where the stress of handling is unlikely to change the parameters the scientist wishes to measure but these should be readily justifiable to the IACUC. It is likely that decapitation is now used too often because IACUCs routinely accept the argument that anesthesia cannot be used because it will change the brain’s state.

**Recommendations and final discussion**

The evidence of potential pain and distress associated with the use of carbon dioxide as an anesthetic or as a sole agent for euthanasia indicates that its routine use should, at the very least, be questioned. We recognize that many clinical veterinarians and laboratory animal technicians are comfortable with CO₂ alone as a euthanasia agent. However, we would argue that the conflicting data in the literature and the arguments around prefilled chambers versus gradual induction, the presence or absence of oxygen supplementation, the lack of agreement on time to unconsciousness, and the fact that CO₂ produces
significant physiological changes, all demand a careful reassessment of the use of the gas by itself as a euthanasia agent.

In order to comply with the guidelines set out in the PHS policy and in USDA Policy #11 (if a technique causes pain or distress in humans its should be assumed to do so in the animals), it is recommended that CO₂ be coupled with an inhalant pre-anesthetic, such as isoflurane or halothane, if it is to be used for euthanasia. The inhalant anesthetic should be used to induce anesthesia, followed by use of CO₂ for euthanasia. However, if a protocol calls for the euthanasia of only a small number of animals where use of an anesthetic is contraindicated on scientific grounds, then decapitation under appropriate conditions may be acceptable.

Personnel play a big role in the issues discussed in this paper. Proper training of personnel in all techniques is essential for improved animal welfare. Centralized facilities in which the veterinary care staff performs such techniques are recommended; it is easier to ensure adequate training in such facilities and the expertise is typically higher among those who are performing euthanasia.

The AVMA also considers “emotional effect on observers or operators” when evaluating euthanasia techniques. Earlier, it was mentioned that the AVMA refers to 13.6 seconds of brain activity following decapitation as the reason that it is not a recommended method of euthanasia. However, it may be that people express concern about the possible 13.6 seconds of consciousness after decapitation because of the violence of the procedure whereas they would be far less concerned about thirty seconds of consciousness during gas euthanasia because it is typically less problematic for the person carrying it out. Distress caused by indirect action (for example, using a gas chamber) often receives less attention than distress caused by direct action (such as decapitation); therefore, there may be an inclination to recommend techniques that involve indirect action.

Clinical practice may be advanced in comparison to published reports regarding the use of CO₂, but that still does not explain the debates currently expressed in various settings, such as the Public Responsibility in Medicine and research conference. The following table, based on the review of available literature and not on anecdotal reports, shows our recommendations of various euthanasia methods according to various situations. It should be noted that there is very little information published on cervical dislocation and its outcomes. The ability to comply with PHS and USDA policies has been taken into consideration when comparing these techniques.

Table 2. A comparison of recommendations of various euthanasia methods, based on existing literature.
| Few-moderate # of rodents where no contamination is permitted | No | No | Maybe | When justified | No |
References


Kline BE, Peckham V and Heist, HE (1963) Some aids in the handling of large numbers of mice. Laboratory Animal Care, 13: 84-90.


MacDonald FM and Simonson, E (1953) Human electrocardiogram during and after inhalation of thirty per cent carbon dioxide. *Journal of Applied Physiology*, 6:304-10


