



## GUIDELINES FOR CO<sub>2</sub> EUTHANASIA OF POULTRY AND YOUNG SWINE

Institutional Animal Care and Use Committee (IACUC)

Office of the Vice Chancellor for Research

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The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition does not specifically address the best practices for CO<sub>2</sub> euthanasia of swine and poultry. Based upon scientific evidence and professional judgment, the following procedures for euthanasia of poultry and swine with CO<sub>2</sub> are appropriate. It is standard practice in all cases to assure that adequate quantities of CO<sub>2</sub> are available and that all equipment is functioning properly prior to beginning euthanasia procedures with any animals.

- A. Euthanasia of poultry using CO<sub>2</sub>: prefill the chamber prior to introducing the birds, and then continue to supplement CO<sub>2</sub> until death is confirmed. Although the actual percentage of CO<sub>2</sub> in the chamber will not reach theoretical values of 100%, immersion in high concentrations of CO<sub>2</sub> for poultry is preferred as it induces rapid loss of consciousness followed by death. This method does not require the precise metering of CO<sub>2</sub> into the chamber.
  
- B. Euthanasia of young swine using CO<sub>2</sub>: introduce the animals into the chamber and then rapidly flush the residual air from the chamber with CO<sub>2</sub> until death is confirmed. This method does not require the precise metering of CO<sub>2</sub> into the chamber.

### Background:

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (pg. 26) provides general recommendations for the use of CO<sub>2</sub>, however the discussion and literature cited refer to rodents:

*"...a displacement rate from 10% to 30% of the chamber volume/min is recommended."*

*"The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO<sub>2</sub>, is unacceptable."*

The AVMA Guidelines are not directly applicable to swine at 10 weeks of age or to poultry. Aversive responses to CO<sub>2</sub>, nociception and progression of anesthesia differ among rats, poultry and young pigs.

### Rats:

CO<sub>2</sub> is aversive to rats as evidenced by their avoidance of a reward chamber with CO<sub>2</sub> concentrations as low as 13% (Niel et al, 2007). Nociceptors in the nasal mucosa (Peppel and Anton, 1993) and cornea (Hirata et al., 1999) of rats are stimulated by CO<sub>2</sub> indicating that CO<sub>2</sub> is likely to cause pain in rats. Niel's (2007) experiments indicated that rats tolerated higher concentrations of CO<sub>2</sub> when it was introduced at an optimal flow rate of ~15% of the volume of the chamber, even though the rats still exhibited an aversion to the gas. The AVMA has taken the position that a prolonged exposure to CO<sub>2</sub> is a gentler method of euthanasia. Their recommendations, based on studies with rats, is extrapolated in the 2013 AVMA Guidelines for the Euthanasia of Animals to apply to all other species, while data gathered in other species indicates this may not be most appropriate.

### Poultry:

While rats do not lose consciousness prior to aversive responses to CO<sub>2</sub>, chickens become unconscious very quickly in high concentrations of CO<sub>2</sub> and somatosensory neuronal potentials are not evoked until several seconds after the birds are unconscious. Suppression of activity in electroencephalograms occurred at ~21 seconds and somatosensory evoked

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potentials were lost in ~30 seconds when hens were exposed to 45% CO<sub>2</sub> in air. Convulsions occurred 15 seconds after the loss of somatosensory potentials, indicating that birds convulsed while they were unconscious (Mohan Raj et al., 1990).

Additionally, the time to eye closure, which follows loss of somatosensory potentials in birds, occurred more rapidly in 35% CO<sub>2</sub> administered in an 8 second accession than when 45% CO<sub>2</sub> was administered in an accession of 18 seconds. Mohan Raj and Gregory (1990) concluded that the rate of accession is more important in the time to unconsciousness than the final concentration and that a faster rate of exposure would minimize any unpleasantness associated with CO<sub>2</sub> inhalation.

Webster and Fletcher (2004) reported that while White Leghorn chickens could detect atmospheres enriched with CO<sub>2</sub> at 30, 45, and 60% in air, there were no differences in behaviors associated with the various atmospheres. They concluded that most hens, given modest motivation, were not sufficiently deterred from entering any of the CO<sub>2</sub>-enriched atmospheres. Similarly, Mohan Raj and Gregory (1991) reported that hens' aversions to atmospheres containing 30 to 60% CO<sub>2</sub> were outweighed by aversions to a dominant hen, indicating the CO<sub>2</sub> was less aversive than experiences a chicken might normally encounter.

Coenen et al, (2000) reported that exposures to CO<sub>2</sub>-enriched atmospheres were not observed to cause heart rate increases in chickens or pigs beyond what might have been caused by restraint and handling.

The Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) addresses use of CO<sub>2</sub> for euthanasia in poultry (page 121):

*"Atmospheres containing a significant amount of carbon dioxide, with or without the presence of oxygen, cause birds to head shake and breathe deeply, but scientific evidence indicates that these behaviors are not associated with distress."*

*"Furthermore...most chickens and turkeys will voluntarily enter carbon dioxide concentrations as high as 60-80%."*

*"Because poultry can be rendered unconscious with 30% carbon dioxide in air, or less if enough time is allowed, and concentrations of carbon dioxide above 50% quickly kill adult birds, it is not necessary to measure the carbon dioxide concentration closely when performing euthanasia. However it is important that the process be observed and carbon dioxide added if necessary to ensure that death is attained without undue delay."*

Although the AVMA Guidelines (pg. 25) acknowledge that poultry do not exhibit aversion to concentrations of CO<sub>2</sub> at 60% and will voluntarily enter a chamber of 60%-80% CO<sub>2</sub> to gain access to food, their general recommendations do not specifically exclude these species.

### Swine:

Similar to chickens, EEG tracings indicated that convulsions in pigs exposed to carbon dioxide stunning occurred after the animals lost consciousness (Forslid, 1987). Also like chickens, high concentrations of CO<sub>2</sub> caused less aversion in pigs than other motivators. When aversion was measured in market aged pigs by comparing repeated exposures to 90% CO<sub>2</sub> with the use of an electric prod, pigs did not hesitate to re-enter the test chamber when they had been previously exposed to 90% CO<sub>2</sub> but they did resist re-entry into the test chamber if they had received two electrical shocks with a

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prod. Jongman et al. (2000) concluded that pigs did not find an atmosphere of 90% CO<sub>2</sub> as aversive as shocks with an electric prod.

Exposure to 80-90% CO<sub>2</sub> in air produces unconsciousness in swine within 13-30 seconds without signs of pain and suffering as determined by behavior, physical signs, and electroencephalographic activity Forslid, 1987; Martoft et al, 2002). In addition, cortisol levels were not further increased compared with levels observed during transport while awake, indicating that no additional emotional strain was imposed by CO<sub>2</sub> inhalation (Forslid et al., 1988). Martoft et al (2003) also concluded that that rapid decreases in intracellular pH by exposure to high (90%) concentrations of CO<sub>2</sub> in swine leads anesthesia soon after the start of inhalation.

Although page 25 of the AVMA Guidelines acknowledges that 60-90% CO<sub>2</sub> causes unconsciousness in 14 to 30 seconds in pigs and that unconsciousness is attained prior to the onset of signs of excitation, the guidelines do not specifically exclude pigs from the recommendation for a slower administration of CO<sub>2</sub> for euthanasia. In contrast, the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010, pg. 153) addresses the use of CO<sub>2</sub> for young swine at less than 10 weeks of age in stating:

*“Carbon dioxide is a suitable method for euthanating pigs less than 10 wk of age providing that residual oxygen is removed quickly from the CO<sub>2</sub> chamber.”*

The recommendations in the Ag Guide arise from the National Pork Board in collaboration with the American Association of Swine Veterinarians.

### Summary

The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition offers appropriate recommendations for CO<sub>2</sub> euthanasia for rodents but these recommendations are not most appropriate for chickens and pigs. The Illinois IACUC has approved guidelines stated herein which allow chickens to be euthanized by prefilling the chamber and then rapidly supplementing the chamber with CO<sub>2</sub> until death is confirmed. The Illinois IACUC has also approved guidelines which allow pigs less than 10 weeks of age to be euthanized by rapidly flushing the chamber with CO<sub>2</sub> until death is confirmed.



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