



THE HUMANE SOCIETY
OF THE UNITED STATES

Comments Submitted by The Humane Society of the United States Regarding the NIH's Office of Laboratory Animal Welfare's Proposed Implementation of the Updated AVMA Guidelines for the Euthanasia of Animals: 2013 Edition; NIH Guide Notice NOT-OD-13-048

1. **Part I - Introduction and General Comments**
(No suggested comments)
2. **Part II - Methods of Euthanasia: M1. Inhaled Agents**

M1.3 Inhaled Anesthetics

The AVMA states that "Inhaled anesthetics can be administered as the sole euthanasia agent or as part of a 2-step process, where animals are first rendered unconscious through inhaled anesthetic agent exposure and then subsequently killed by a secondary method. "

For euthanasia by overdose of an inhalation anesthetic, time to death can take an extended amount of time; therefore, we urge OLAW to recommend that only a 2-step process be used so that once the animal is unconscious as a result of the anesthesia, a second procedure should *always* be performed to ensure death of the animal. This recommendation is also the current guideline recommended by the Canadian Council on Animal Care (<http://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>).

M1.6 Carbon Dioxide

We are pleased to see that the AVMA guidelines recognize that carbon dioxide euthanasia can cause animal distress and, further, that the document describes the mechanisms by which CO₂ does cause distress. We also support the AVMA's statement that the use of carbon dioxide immersion as a sole euthanasia method (where conscious animals are placed directly into a container pre-filled with 100% CO₂) is unacceptable, and urge OLAW to adopt this part of the guidelines, at minimum.

The AVMA further states that "Carbon dioxide is conditionally acceptable for euthanasia in those species where aversion or distress can be minimized." The guidelines then continue by describing the gradual fill method as "less likely to cause pain," but do not conclude that this method is likely to cause distress and thus should be avoided. The guidelines do indicate that there are conflicting results when one examines the gradual-fill method in various species, but the strong evidence that exposure to a gradual-fill method is distressful to humans should not be dismissed. A meeting of experts at the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals concluded that both pre-fill and rising concentrations of

CO2 cause welfare problems. The full report is available at:
<http://www.nc3rs.org.uk/downloaddoc.asp?id=416&page=292&skin=0>

Further, a review by Conlee, et al (2005) demonstrates the wide range of results regarding use of carbon dioxide euthanasia and concludes that “CO₂ is painful and/or distressful in humans at concentrations ranging from 7% to 100%.”

Current US policies regarding the use of animals for research purposes indicate that if something is determined to be painful or distressful to humans, it must be assumed that it may causes pain and distress in other animals, unless the contrary is established. It is important to emphasize that the contrary has not been established in this case, and furthermore, that there is sufficient evidence that gradual exposure to CO₂ causes distress in a number of species.

Gradual displacement methods *should not* be used given their potential to cause unrelieved distress. **Therefore, we urge OLAW to indicate that carbon dioxide euthanasia should *only* be done as a two-step process where animals are rendered unconscious prior to exposure to carbon dioxide, as is already indicated by the AVMA as the best option “when gradual displacement methods cannot be used.” We further urge OLAW to adopt guidelines stating that the use of carbon dioxide as a sole euthanasia agent is unacceptable.**

3. Part II - Methods of Euthanasia: M2. Noninhaled Agents

M2.2 Routes of Administration

We strongly disagree with the statement that paralytic immobilizing agents can be used when IV injection of sodium pentobarbital will follow ‘immediately,’ particularly as it pertains to companion animals. More appropriate alternatives to paralytics are readily available. We urge OLAW not to adopt this language.

Additionally, OLAW should recommend that the use of immersion methods should be followed by another method of euthanasia to cause brain death, and that immersion methods may be ineffective on fish that breath-hold or breathe air.

4. Part II - Methods of Euthanasia: M3. Physical Methods

5. Part III - Methods of Euthanasia by Species and Environment: S2. Laboratory Animals

S2.2.1.1 Noninhaled Agents

Since the AVMA guidelines state that, “Pain may be associated with [Barbiturate and barbituric acid derivative] injections given via the IP route (Svendsen, 2007; Ambrose et al. 2000),” **we urge OLAW to recommend that the solution be buffered, diluted, and**

combined with a fast acting local anesthetic like lidocaine. This is also the recommendation of the Canadian Council on Animal Care (<http://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>).

S2.2.2.1 Inhaled Agents

The AVMA states that “Carbon dioxide, with or without premedication with inhaled anesthetics, is acceptable with conditions for euthanasia of small rodents.” The strong evidence that exposure to a gradual-fill method is distressful to humans should not be dismissed. A meeting of experts at the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals concluded that both pre-fill and rising concentrations of CO₂ cause welfare problems.

(<http://www.nc3rs.org.uk/downloadaddoc.asp?id=416&page=292&skin=0>)

Further, a review by Conlee, et al (2005) demonstrates the wide range of results regarding use of carbon dioxide euthanasia and concludes that “CO₂ is painful and/or distressful in humans at concentrations ranging from 7% to 100%.”

Current US policies regarding the use of animals for research purposes indicate that if something is determined to be painful or distressful to humans, it must be assumed that it may cause pain and distress in other animals, unless the contrary is established. It is important to emphasize that the contrary has not been established in this case, and furthermore, that there is sufficient evidence that gradual exposure to CO₂ causes distress in a number of species. Gradual displacement methods *should not* be used given their potential to cause unrelieved distress. **Therefore, we urge OLAW to indicate that carbon dioxide euthanasia should *only* be done as a two-step process where animals are rendered unconscious prior to exposure to carbon dioxide. We further urge OLAW to adopt guidelines stating that the use of carbon dioxide as a sole euthanasia agent is unacceptable.**

S2.2.2.3 Physical Methods

We urge OLAW to recommend that animals be anesthetized prior to cervical dislocation, to reduce potential pain and distress in case of unplanned personnel error.

S3.3 Swine: S3.3.1.2.1 Inhaled Agents

Since carbon dioxide is an acidic, pungent gas, and is aversive to many animals, OLAW should consider it unacceptable as a sole euthanasia agent. The following references support this notion:

- Raj AM and Gregory NG. 1995. Welfare implications of gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Animal Welfare* 4:273-80.
- Lagerweij E. 1990. CO₂ inhalation in the pig. In: Lambooy E (ed.), *The Use of CO₂ for the stunning of slaughter pigs*. *Fleischwirtsch* 70(10):1174.

- Velarde A, Cruz J, Gispert M, Carrión D, Ruiz de la Torre JL, Diestre A, and Manteca X. 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. *Animal Welfare* 16(4):513-22.

6. Part III - Methods of Euthanasia by Species and Environment: S5. Avians

S5.2.2.1 Inhaled Agents

Given that birds have intrapulmonary chemoreceptors that detect CO₂, and that they show signs of respiratory distress when exposed to CO₂ and will often avoid an atmosphere containing high CO₂ concentrations, **carbon dioxide euthanasia should be discouraged by OLAW**. Please see supporting references:

- Ludders, J. W. (2001). Inhaled anesthesia for birds. In R. D. Gleed & J. W. Ludders (Eds.), *Recent advances in veterinary anesthesia and analgesia: Companion animals*. Ithaca, NY: International Veterinary Information Service.
- Raj, A. B. M. (1996). Aversive reactions of turkeys to argon, carbon dioxide and a mixture of carbon dioxide and argon. *The Veterinary Record*, 138, 592–593.
- Raj, A. B. M. (1998). Welfare during stunning and slaughter of poultry. *Poultry Science*, 77, 1815–1819.

7. Part III - Methods of Euthanasia by Species and Environment: S6. Finfish and Aquatic Invertebrates

(1) Immersion in solutions of buffered tricaine methanesulfonate, buffered benzocaine, quinaldine sulfate and 2-phenoxyethanol: Care must be taken using immersion as a single step procedure, since it may render fish immobile without actually killing them. For euthanasia purposes, **we urge OLAW to recommend two-stage procedures in order to be absolutely confident the fish has been killed**. OLAW should also include additional detail on how benzocaine can alter water chemistry and how changes in parameters such as pH may negatively impact the welfare of fish.

(1) Immersion in CO₂-saturated water:

OLAW should consider that CO₂ will reduce the pH of water, which in turn may cause distress in fish.

8. General Comments - All Other Parts

S7.3 Captive Amphibians and Reptiles

S7.3.4 Acceptable Methods: S7.3.4.1 Noninhaled Agents

Injectable agents: Intracoelomic, subcutaneous lymph spaces, and lymph sacs should be considered acceptable alternate routes of administration only if venous access is limited or impossible.

External or Topical Agents

When using buffered tricaine methanesulfonate (MS 222), it is important to monitor pH of water to ensure correct buffering and to avoid large changes in pH which may be irritating and stressful.

S7.3.5.1 Inhaled Agents

Inhaled anesthetics-

The guidelines state that regardless of the species or taxonomic group, death must be verified prior to terminating the use of the inhalant, or a second, guaranteed lethal procedure (e.g., decapitation) should be performed to ensure death. **We urge OLAW to recommend that decapitation should always be followed immediately by destruction of the brain.** Some reptiles are thought to retain consciousness for minutes or more following decapitation alone.

S7.3.5.2 Physical Methods

Penetrating captive bolt or firearm—

The guidelines state that “crocodilians and other large reptiles can be euthanized by a penetrating captive bolt or gunshot (free bullet) delivered to the brain.” **We urge OLAW to state that this method should always be followed by destruction of the brain** since animals can recover consciousness following the application of captive bolt methodology.

Rapid freezing—

We urge that OLAW recommend that this method not be considered conditionally acceptable without some form of pre-stunning or anesthesia.

S7.3.6 Adjunctive Methods

Pithing—

The AVMA guidelines include a specific description of this method for frogs. The method should be specified by OLAW for all species or, alternatively, a statement should be made by OLAW that the pithing site will vary with species and reference should be made to the anatomy of the species and relevant expertise.

S7.3.7 Unacceptable Methods

Hypothermia—

The guidelines state that hypothermia is an inappropriate method of restraint or euthanasia for amphibians and reptiles unless animals are sufficiently small (< 4 kg; Close, 1997) to permit immediate and irreversible death if placed in liquid nitrogen. The maximum weight recommended here is actually a sizeable animal and the surface area to volume ratio is too small to ensure immediate and irreversible death. **We urge OLAW to remove the reference to allowing euthanasia by hypothermia for animals less than 4kg.**

S7.3.8 Special Cases and Exceptions

The guidelines state that “injectable agents such as lidocaine hydrochloride, potassium salts, or magnesium salts may be useful as an adjunctive method to prevent recovery.” **We urge OLAW to recommend that these methods should only be used as a means of ensuring death in an otherwise anesthetized or unresponsive animal.**

The guidelines state that perfusion with fixative of a deeply anesthetized animal can be used to euthanize amphibians and reptiles when scientifically justified. It is not acceptable to compromise the welfare of the animal concerned because the scientific procedure or experiment warrants it. **OLAW should recommend that tissue fixation methodology should only be performed on animals in which death has been confirmed.**