Purpose of Policy: This policy is intended to ensure that the euthanasia of any animal is done in as humane a manner as possible and in accordance with the guidelines set forth in American Veterinary Medical Association’s AVMA Guidelines for the Euthanasia of Animals.

Policy Information: The AVMA Guide cited above presently considers carbon dioxide (CO₂) to be a conditionally acceptable means of euthanasia for many animal species. It has many advantages in that it is readily available in compressed gas cylinders; it is inexpensive; it is not flammable or explosive; and it produces a rapid depressant, analgesic and anesthetic effect when used in appropriate concentrations. Moreover, it poses minimal risk to personnel when used properly.

However, Part II, section M1.6 of the AVMA guidelines states that “Inhalation of CO₂ causes respiratory acidosis” and that “Carbon dioxide has the potential to cause distress in animals via three different mechanisms: (1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of so-called air hunger and a feeling of breathlessness, and (3) direct stimulation of ion channels within the amygdala associated with the fear response.” The time it takes for an animal to become unconscious depends in part on the rate of oxygen displacement. Rodent models suggest that gradual displacement of oxygen by CO₂ is less likely to produce pain prior to unconsciousness than immersion in a tank prefilled with 100% CO₂. Furthermore, neonates and other immature animals may be exceptionally tolerant to CO₂.

Policy: The use of CO₂ as a depressant, analgesic, anesthetic or for euthanasia is not permitted without scientific justification and prior approval of the Institutional Animal Care and Use Committee (IACUC). Should the use of CO₂ be approved, the following requirements will be in effect:

1. Immersion of conscious animals in 100% CO₂ is not permitted. Instead, a gradual displacement method must be used with a CO₂ flow rate that will displace 10-30% of the chamber volume per minute.

2. If more than one animal is to be euthanized together, they must be of the same species.

3. Chambers must not be overcrowded.
4. Immature animals must be exposed to a high concentration of gas for an extended period of time to ensure death.

5. Purified CO$_2$ must be supplied in compressed gas cylinders or tanks that are precisely regulated. CO$_2$ generated from dry ice or fire extinguishers is not permitted as gas flow cannot be regulated and may be of unreliable or undesirable composition.

6. The top or walls of the gas chamber must be transparent in order to observe the animals during euthanasia.

7. **Death must be verified after euthanasia and prior to disposal.** Thoracotomy after apparent death is one way to ensure the procedure is irreversible.