

<b>TITLE:</b>	<b>Use of Carbon Dioxide (CO<sub>2</sub>) for Euthanasia</b>
<b>Policy Number:</b>	2014-005
<b>Responsible Department:</b>	Institutional Animal Care and Use Committee
<b>Policy Contact:</b>	<a href="mailto:IACUCOffice@westernu.edu">IACUCOffice@westernu.edu</a>
<b>Approval Date:</b>	6/11/14
<b>Reviewed Date:</b>	4/12/17; 1/21/2020
<b>Revised Date:</b>	4/12/17 (added secondary methods for verification); 1/21/2020 (expanded requirement for use; added PHS policy and consequences of noncompliance)

**Purpose of Policy:** 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 (CO<sub>2</sub>) 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 CO<sub>2</sub> 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 CO<sub>2</sub> is less likely to produce pain prior to unconsciousness than immersion in a tank prefilled with 100% CO<sub>2</sub>. Furthermore, neonates and other immature animals may be exceptionally tolerant to CO<sub>2</sub>.

Disposal of an animal that has not been properly euthanized is a serious animal welfare concern and a significant breach of the conditions of WesternU's approved Public Health Service (PHS) Animal Welfare Assurance and could result in reportable non-compliance.

**Policy:** The use of CO<sub>2</sub> 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). Any rodent used for research may be conditionally euthanized by CO<sub>2</sub> in accordance with the guidance below. This includes mice of the genus *Mus*, rats of the genus *Rattus*, hamsters, gerbils, and other laboratory rodents.

Should the use of CO<sub>2</sub> be approved, the following requirements will be in effect:

1. Immersion of conscious animals in 100% CO<sub>2</sub> is not permitted. Instead, a gradual displacement method must be used with a CO<sub>2</sub> flow rate that will displace 10-30% of the chamber volume per minute.
2. Purified CO<sub>2</sub> must be supplied in compressed gas cylinders or tanks that are precisely regulated. CO<sub>2</sub> generated from dry ice or fire extinguishers is not permitted as gas flow cannot be regulated and may be of unreliable or undesirable composition.
3. The euthanasia chamber must not be pre-filled with CO<sub>2</sub> prior to placing rodents in the chamber. Sudden exposure of conscious animals to CO<sub>2</sub> concentrations of 70% or greater has been shown to be distressful and potentially painful. CO<sub>2</sub> is heavier than room air, therefore always empty (by turning it over) and clean the chamber with a regular disinfectant between uses to remove residual CO<sub>2</sub> (to avoid a pre-fill situation).
4. Animals should be euthanized in their home cages whenever possible and, if more than one animal is to be euthanized together, they must be of the same species. Animals in the euthanasia chamber should be readily visible and personnel must remain present for the entire euthanasia procedure. Animals must not be overcrowded in the chamber and must not be mixed with unfamiliar or incompatible animals, i.e., animals from different cages must not be mixed in the chamber during euthanasia. All animals in the chamber must be able to make normal postural adjustments.
5. Fetal and neonatal animals must be exposed to a high concentration of gas for an extended period of time to ensure death as they are resistant to CO<sub>2</sub> induced hypoxia and may take up to 60 minutes to reach unconsciousness. Therefore, it is recommended that neonatal rodents be placed into the CO<sub>2</sub> chamber for 10-15 minutes until fully anesthetized as evidenced by no observable movement and no response to a toe pinch. A secondary method, such as decapitation with a sharp blade or scissors, can then be used to ensure death.
6. **Animals should be left in the euthanasia chamber for at least one minute after spontaneous movements have ceased, with CO<sub>2</sub> continuing to flow at the same rate.** Animals must be continuously monitored for adverse reactions for the duration of the euthanasia procedure. It is important to contact Animal Care staff promptly if the procedure appears to produce excessive agitation or other complications.
7. **Death must be verified after euthanasia and prior to disposal.** Thoracotomy after apparent death, cervical dislocation or decapitation are acceptable secondary methods to ensure that the procedure is irreversible.

**Noncompliance:** As stated in the PHS Policy, “Unintended recovery of animals after apparent death from CO<sub>2</sub>...constitute[s] serious noncompliance with the PHS Policy and serious deviation from the provisions of the Guide...the IACUC, through the Institutional Official, must promptly provide OLAW with a full explanation of the circumstances and actions taken.” Failure to confirm death of a euthanized rodent is therefore a significant noncompliance at WesternU and will be reported to the appropriate regulatory and accrediting agencies, and will require corrective actions (i.e., re-training) and possible additional steps (i.e., prohibition of certain personnel from performing CO<sub>2</sub> euthanasia) depending on the circumstances of the noncompliance.