

GUIDELINES FOR EUTHANASIA

Background. Euthanasia is defined as the rapid and painless humane killing of an animal. The Office for Protection from Research Risks (OPRR) *Institutional Animal Care and Use Committee Guidebook* (NIH Publication No. 92-3415) and the *Guide for the Care and Use of Laboratory Animals* (NRC, revised 2010) state that euthanasia methods should conform to the recommendations of the AVMA Guidelines on Euthanasia, 2007.

Guidelines – General

Methods of euthanasia must conform to the *AVMA Guidelines on Euthanasia* (June, 2007). This document can be accessed on line at http://www.avma.org/issues/animal_welfare/euthanasia.pdf. Acceptable methods are those that depress the central nervous system to ensure insensitivity to pain before induction of death. They include carbon dioxide inhalation (rodents), anesthetic overdose, or anesthesia followed by other (chemical or physical) methods. If the principal investigator feels it is necessary to use alternate methods to obtain scientifically valid data, the methods must be described and justified in detail in writing.

Guidelines for Use of Carbon Dioxide (CO₂) for Euthanasia

Carbon dioxide is the preferred method of euthanasia for rodents. Exposure to high concentrations of CO₂ has an initial rapid depressant and anesthetic effect followed by death through asphyxiation. Unconsciousness is induced most rapidly by exposing animals to a CO₂ concentration of 70% or more.

1. Method

- a. Hard sided chambers should be filled with CO₂ gas for at least 45-60 seconds before placing animals inside the chamber and securing the lid. However, if a plastic bag is used, the animal may be placed in the bag before expelling the resident air and re-filling the bag with CO₂. With hard sided chambers, additional gassing for approximately one minute is usually sufficient. When using a plastic bag, the animal should be observed frequently; euthanasia may take as long as 5 minutes.
- b. Chambers (except plastic bags) should be filled with CO₂ from the bottom up. This is accomplished by having the gas inlet port at the bottom of the chamber or by tubing entering the top of the chamber and extending at least 3/4 of the way to the bottom. These measures are necessary because CO₂ is ~50% heavier than air. Using these methods, lethal levels of CO₂ are maintained easily with a minimum of additional gas. Incomplete filling of a chamber may allow tall or climbing animals to avoid exposure to high concentrations of the gas, leading to prolonged distress to the animals.

- c. If the chamber is opened a number of times during use (e.g., for sequentially euthanizing several animals), it is necessary to either maintain a small inflow of gas to replace that which escapes or to recharge the chamber periodically.
 - d. Gas flows can be high when pre-filling an empty chamber but should be lowered when animals are in the chamber (to avoid excessive noises or drafts within the chamber).
 - e. Several animals may be euthanized simultaneously, although the animals should be of the same species and there should be adequate space in the chamber to avoid crowding or piling.
 - f. The chamber should be kept clean to minimize odors that might cause distress to animals subsequently euthanized.
 - g. CO₂ should not be used in neonates, because their inherent resistance to hypoxia will prolong substantially the time of death.
 - h. CO₂ gas should be provided from a compressed gas cylinder with an appropriate regulator. Use of dry ice is discouraged due to imprecise/suboptimal gas concentrations and risk of thermal distress/injury.
2. To ensure that animals do not recover from CO₂ narcosis, it is important to confirm that the animals are dead when removed from the chamber. This can be accomplished by:
 - a. keeping the animal in the chamber for at least 5 minutes after the last detectable movement, or
 - b. physical methods, such as thoracotomy or cervical dislocation (animals <200 g).
3. Examples of chambers:
 - a. A large plastic or glass dessicator jar with a tubulature in the top and a two-holed rubber stopper inserted in the tubulature may be used. The hose from the CO₂ regulator is connected to a 6-8 inch piece of rigid tubing that passes through one hole of the stopper and allows CO₂ to flow into the bottom of the chamber. A 3 inch piece of tubing is passed through the other hole and is connected to a short length of hose that has an adjustable screw clamp to regulate the escape of gas from the chamber. Before administering gas, any ground glass surfaces should be sealed with silicon grease.
 - b. Commercially available or custom made tops allow glass aquaria or plastic rodent cages to be used. Inlet/outlet holes in the top are fitted as described above for a dessicator. Alternatively, a single hole in the top may be fitted with tubing with

an external diameter smaller than the diameter of the hole, thus allowing gas to escape around the tubing. The tubing should extend down to the bottom of the chamber.

- c. Any inexpensive plastic container (e.g., garbage can) of suitable size with a lid that can be secured, but is not airtight, can be used. A hole is punched in the lid through which tubing is passed to the bottom of the container for administration of CO₂. Use of a durable transparent plastic for the lid will ensure that the animals can be observed during euthanasia. Plastic bags may be used as liners to facilitate carcass disposal and to keep the chamber clean.
- d. A heavy duty non-opaque plastic bag may be used. Animals are placed in the bag, a majority of the air is squeezed out, and the bag is carefully filled with 100% CO₂ and tied shut. The animals should be observed frequently during euthanasia.

Guidelines for Use of Anesthetic Overdose for Euthanasia

Anesthetic overdose may be used for virtually any species, but is most commonly used for non-rodents.

1. Intravenous injection of barbiturates (e.g., B-Euthanasia D, pentobarbital) at at least twice the anesthetic dose may be used. Intraperitoneal injections are not advised, because there is a large amount of variability in dose and effect. If the animal is excitable or difficult to restrain for administration of the euthanatizing agent, a sedative or tranquilizer may be used first.
2. Inhalation anesthetics (e.g., isoflurane, halothane) may be used for euthanasia, provided care is taken to vent the fumes safely in a fume hood or scavenging device. As with carbon dioxide, death of the animal must be verified following administration of the anesthetic gas.

Guidelines for Use of Combined Methods for Euthanasia

1. Deep anesthesia followed by thoracotomy, exsanguination, cervical dislocation (animals <200g), decapitation, or other physical methods may be utilized.
2. Deep anesthesia followed by intravenous administration of a saturated potassium chloride (KC) solution is allowed. KC should not be used for euthanasia without first anesthetizing the animal.

Guidelines for Use of Other Methods of Euthanasia

1. Cervical dislocation without anesthesia or CO₂ narcosis requires justification in writing (attach to these guidelines) and must be approved by the IACUC.

- a. Cervical dislocation should only be used for mice and rats with body weights <200g.
 - b. Individuals performing cervical dislocation should have had previous training. The veterinary staff can provide training if necessary. Inexperienced individuals should practice cervical dislocation on carcasses or anesthetized animals until facile with the technique.
 - c. The rodent should be restrained on a firm, flat surface, and the tail grasped with one hand. A stick-type pen, rod-shaped piece of metal or wood, or the thumb and first finger (of the other hand) should be placed against the back of the neck at the base of the skull. Dislocation is produced by quickly pushing forward and down with the hand or object restraining the head while pulling backward with the hand holding the tail.
2. Decapitation without anesthesia or CO₂ narcosis requires justification in writing and must be approved by the IACUC.