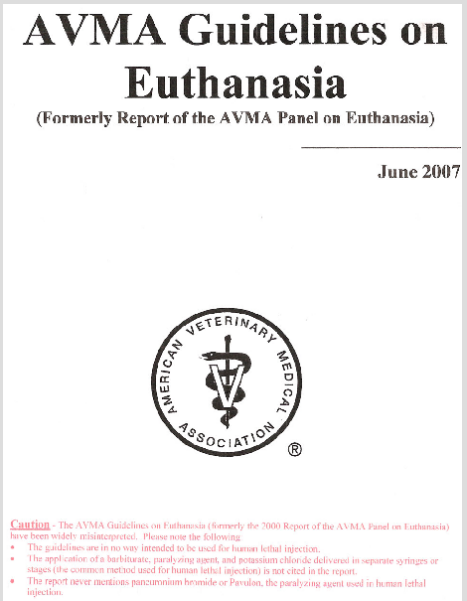
Carbon Dioxide (CO_2) Euthanasia

Euthanasia is defined as the act of killing an animal in a manner that will cause the least possible amount of pain, distress or anxiety.

The 2007 **AVMA Guidelines on Euthanasia** provides approved methods of euthanasia and states that carbon dioxide (CO_2) is acceptable for euthanasia of small laboratory animals including, mice, rats, guinea pigs, and chickens. This report can be accessed from the UCAR website (http://www.urmc.rochester.edu/ucar/manual/euthan.htm).

All euthanasia methods must be described in your approved UCAR Protocol.


AVMA Guidelines on Euthanasia

(Formerly Report of the AVMA Panel on Euthanasia)

June 2007

AMERICAN VETERINARY MEDICAL ASSOCIATION

Caution - The AVMA Guidelines on Euthanasia (formerly the 2000 Report of the AVMA Panel on Euthanasia) have been widely misinterpreted. Please note the following:

- The guidelines are in no way intended to be used for human lethal injection.
- The application of a barbiturate, paralyzing agent, and potassium chloride delivered in separate syringes or stages (the common method used for human lethal injection) is not cited in the report.
- The report never mentions pancuronium bromide or Pavulon, the paralyzing agent used in human lethal injection.

The purpose of this training session is to provide instructions for the operation of the Vivarium euthanasia equipment in order to perform humane euthanasia.

Euthanasia Methods

- Inhalant agents such as halothane, isoflurane, methoxyflurane and carbon dioxide (CO_2) are used to euthanize animals.

- Noninhalant agents such as barbiturate and anesthetic overdose can also be used.

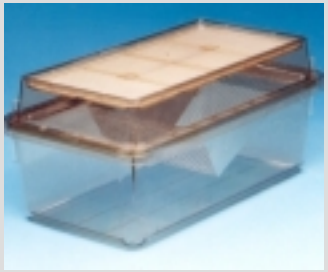
- Physical methods such as cervical dislocation and decapitation can be used as long as they are scientifically justified in an approved UCAR protocol.

- Recommended methods of euthanasia for all animal species are available on the UCAR website. You may contact a University Veterinarian at 5-2651 for additional information.

Appropriate Cage Density

- **Do not overcrowd**.
  - Place no more than 10 mice in small cages.
  - Place no more than 20 mice in large cages.
  - Place no more than 5 rats in a large cage.
  - Do not combine animals with a tendency to fight in the same cage (e.g. **unfamiliar hamsters** or aggressive mouse strains).
  - Do not combine species.

7½" wide x 11½" deep x 5" high



10½" wide x 19" deep x 6" high

10½" wide x 19" deep x 8" high

Fear, anxiety and apprehension must be minimized at all times.

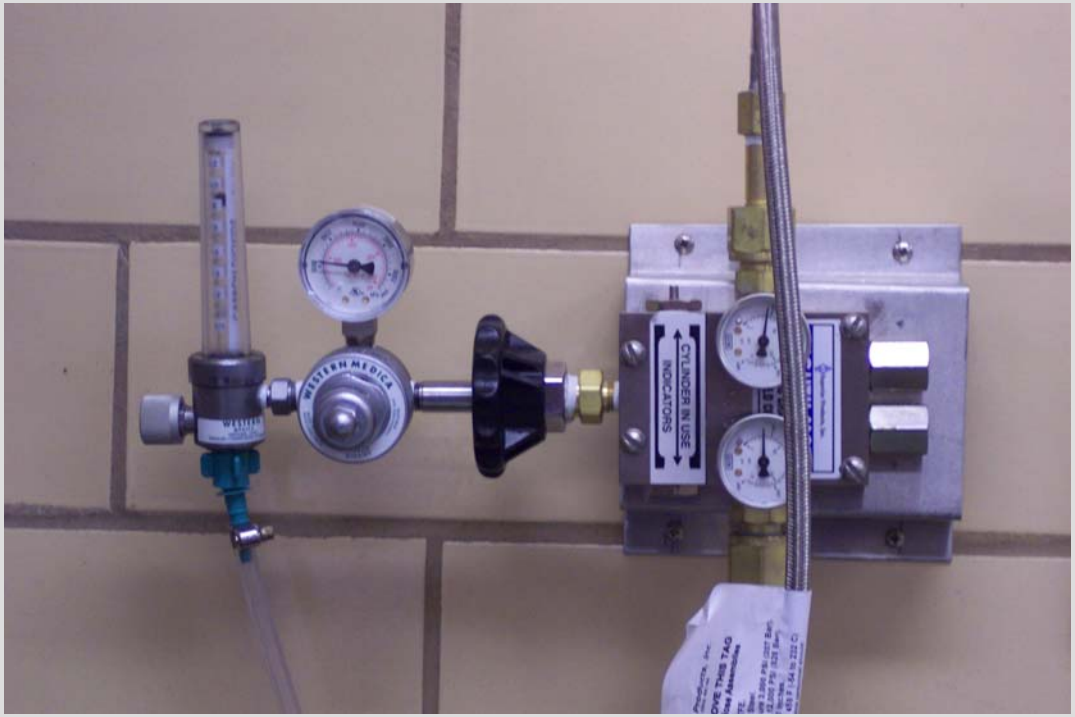
- Never place animals awaiting euthanasia near or next to those actually being euthanized.

The 2007 AVMA Euthanasia Guidelines state that the only acceptable source of CO_2 is from pressurized cylinders because the gas flow can be regulated.

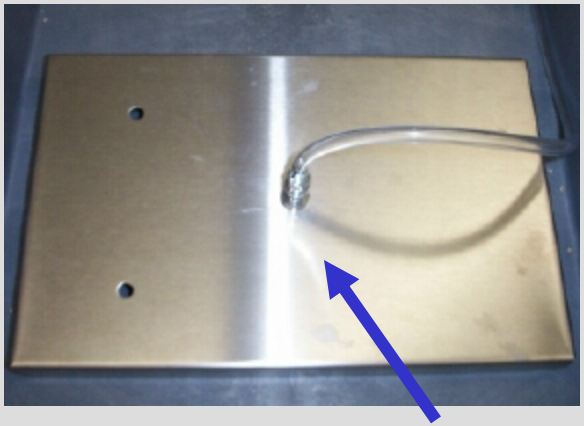
Unacceptable sources of CO_2 are:

- Dry ice
- Natural gas
- Fire extinguisher
- Chemical means (e.g. antacids)

OPERATION OF CO_2 EUTHANASIA EQUIPMENT



The euthanasia equipment provided by the Vivarium is obtained from the Euthanex company. The components are:

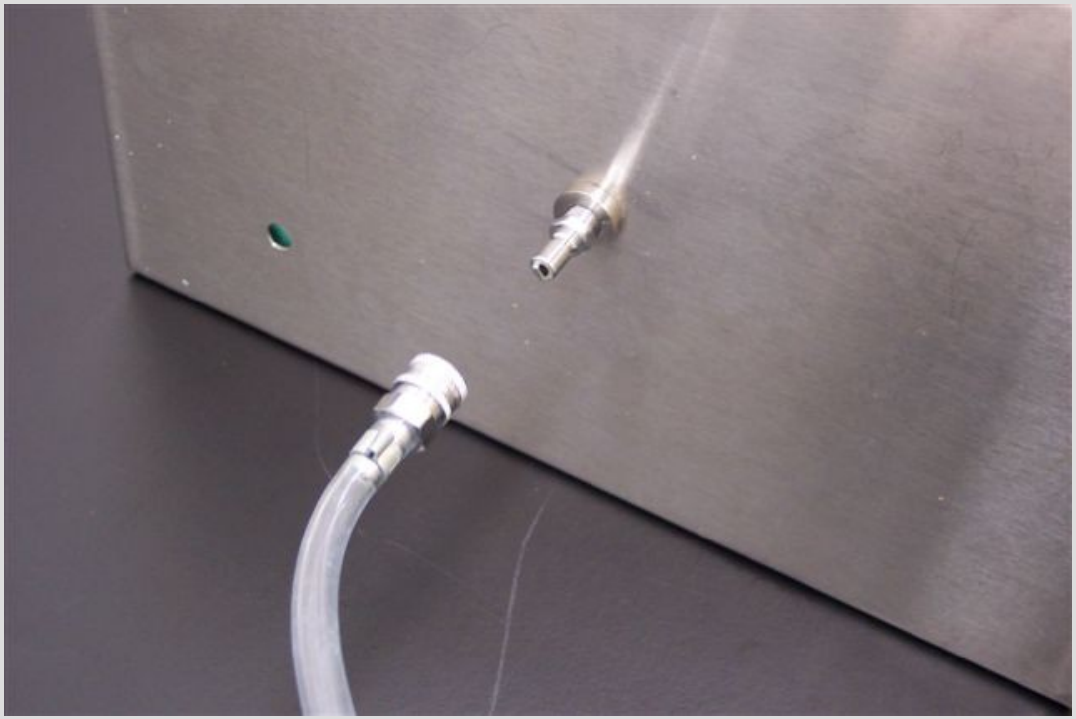


Euthanex Lid with
Quick Disconnect Fitting

Gate
Valve

CO_2 Regulator
Valve

CO_2 Tank



The Quick Disconnect Fitting is located at the distal (far) end of the hose from the CO_2 tank.

The Quick Disconnect Fitting is attached to or removed from the nipple connector on the center of the Euthanex lid by sliding the knurled outer collar of the Quick Disconnect Fitting above the inner valve connector, and with the collar still in the raised position, sliding the inner valve connector either on or off of the lid nipple connector.

When the knurled collar is released with the valve fully seated, the connection is made.

It is <u>NOT</u> necessary to remove the filter top and water bottle from the cage of animals to be euthanized. Simply place the Euthanex lid directly on the filter top of the cage.

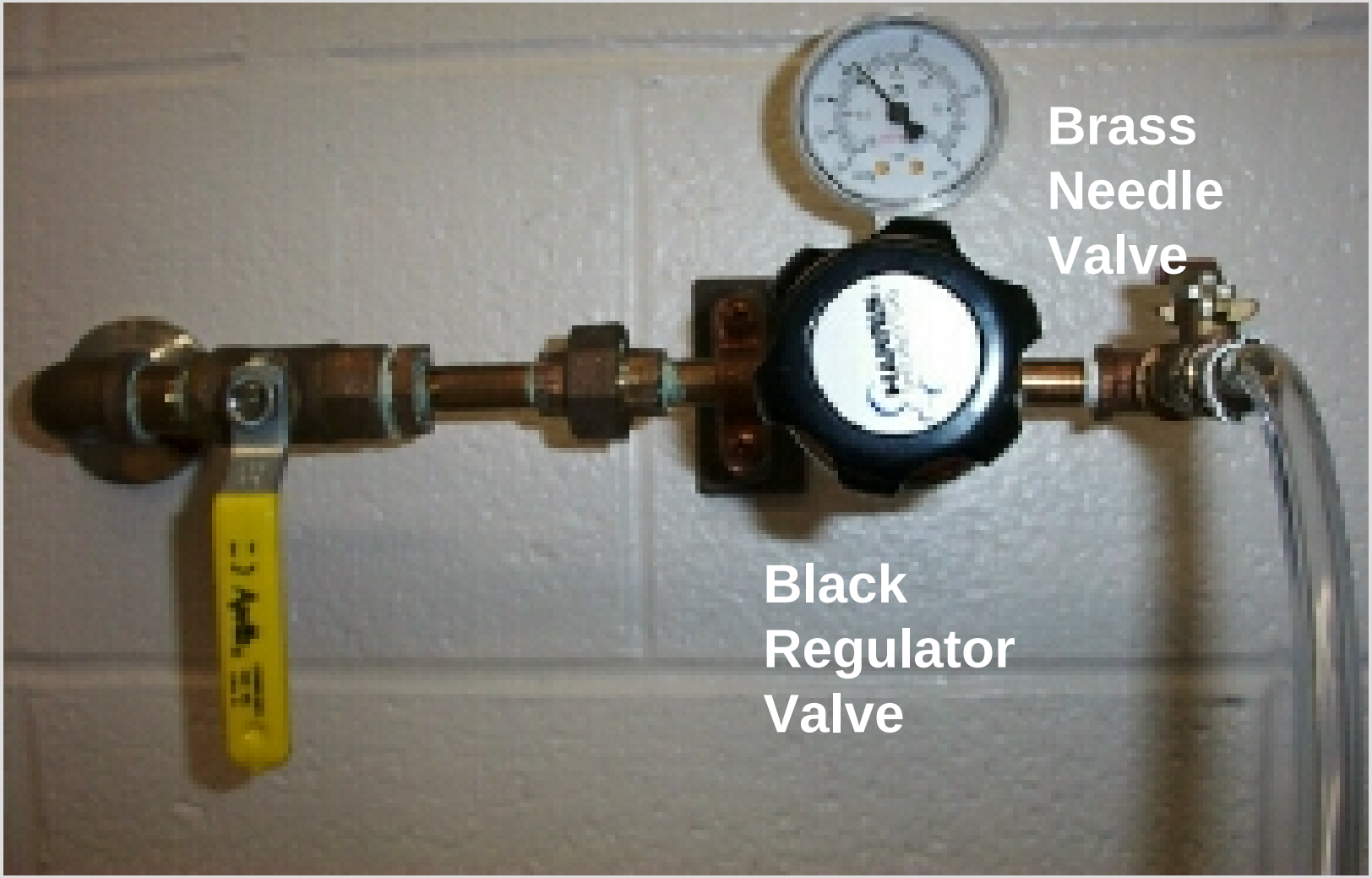
KMRB Procedure Room Stations

Open

Closed

In the KMRB, the CO_2 tanks themselves are not accessible to the user. However, valves that are connected to the tanks are mounted on the wall and can be turned on and off by opening and closing the valves as shown above.

Open the Gate Valve (colored handle) on the CO_2 supply line. The valve is open when the handle is parallel to the CO_2 supply line. It is closed when perpendicular to the CO_2 supply line.



The Brass Needle Valve to which the hose is connected and the Black Regulator Valve should be open at all times. Do not adjust these valves.

CO_2 Regulator Valve

The regulator valve you will use to actually control the flow rate of CO_2 is the small knurled knob left of the vertical flow meter. This valve allows you to control the flow of CO_2 in liters per minute.

CO_2 in high concentrations is a noxious stimulant and can cause a burning sensation when in contact with mucous membranes. CO_2 must be administered slowly in order to anesthetize the animal prior to achieving a high enough concentration of CO_2 to cause distress. Once the animal is anesthetized, the flow of CO_2 may be increased.

Flow Rates

- A flow rate such that 33% of the volume of the cage is replaced by CO_2 after one minute has been determined not to cause stress in rats and mice.

- The approximate appropriate flow rate for each size of rodent cage used at the U or R is as follows:

  - **Small Mouse Cage: 2.0 liters per minute.**
  - **Large Mouse Cage: 5.5 liters per minute.**
  - **Rat Cage: 6.5 liters per minute.**

- Once the animal appears to be anesthetized you may increase the flow rate.

Allow CO_2 to continue to flow for at least five minutes after the animal appears to be dead. Turn the **CO_2 Regulator Valve** off by rotating the knob all the way to the left.

In the MRB, be sure that the colored handle of the **Gate Valve** is in the off position, which is perpendicular to the CO_2 supply line. This valve is present only at MRB euthanasia stations.

Signs of Death

For animals that are euthanized by exposure to carbon dioxide and/or anesthetic overdose, the following criteria must be met:

- Adult animals should be left in a CO_2 environment for at least 5 minutes after you believe that they are dead.
- Breathing must stop; heartbeat must cease.
- Other signs of death are: the corneas become glassy in appearance, the pupils fully dilate and anal/urinary sphincters relax which may result in the animals passing urine and/or feces.

Confirm Death

One of the following secondary physical methods must be performed to ensure that the animals are dead:

- Decapitation
- Perfusion of a histological fixative via the major blood vessels
- Opening the thorax
- Complete severing of the spine just below the base of the skull using a dorsal approach
- Cervical dislocation for animals **under 200g.**

Do not leave animals unattended during euthanasia.

Demonstration of opening the thoracic cavity after euthanasia to assure that the animal is dead.

Euthanasia of Neonates

Neonatal rodents (up to ten days old) and newly hatched birds (less than 1 week old) are resistant to hypoxia and are able to tolerate relatively high CO_2 concentrations.

- Approximate flow rates for euthanasia of neonate rodents and newly hatched birds in vivarium cages are as follows:
  - **Small Mouse Cage: 4.0 liters per minute**
  - **Large Mouse Cage: 10.6 liters per minute**
  - **Rat Cage: 13 liters per minute**
- Use CO_2 exposure times of at least 10 minutes.

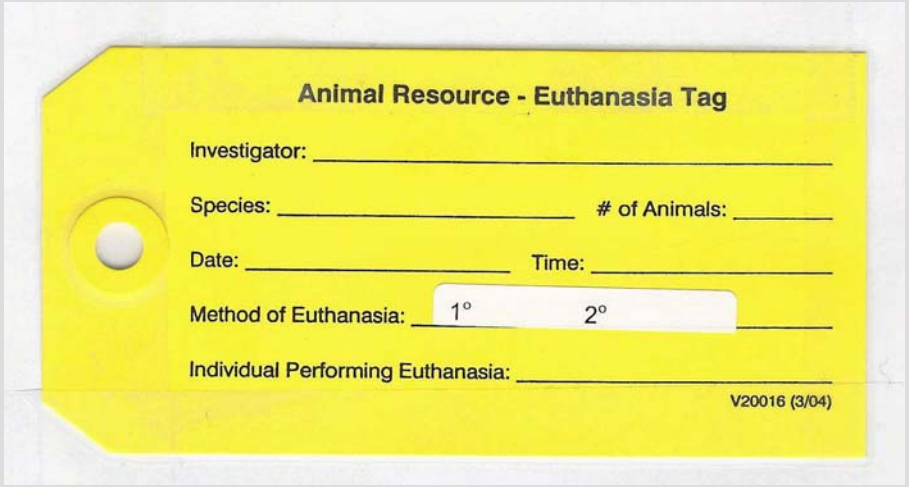
As with adults, euthanasia of neonates must be confirmed by a secondary physical method:

- Cervical dislocation
- Decapitation
- Removal of major organ
- Severing a major vessel
- Opening the chest
- Freezing.

Euthanasia of Embryos and Fetuses Planned for Tissue Harvest

- Once the mother has been euthanized by an AVMA method, embryos and fetuses may be euthanized by the following approved methods:

  Cervical dislocation, decapitation, removal of major organ/opening a major vessel, opening the chest, hypothermia followed by freezing or one of the above methods.



Place all carcasses in the plastic bags provided, tie the bags securely and completely fill out the euthanasia tag.

Carcass Disposal

SMD

- Place carcasses in the morgue cooler (B-6915), unless they are intended to be necropsied in which case they should be placed in the necropsy cooler (B-6911).

MRB

- Animals euthanized in MRB and MRBX laboratories should be placed in the refrigerator in room B-9636.

ANNEX

- Animals are euthanized in 1-B141 and 3-B324. Carcasses must be returned to B-6915 or B-6911

CVRI

- Carcasses are place in the refrigerator in room E111.

Meliora

- Animal carcasses should be placed in the refrigerator in room 139.

EDC

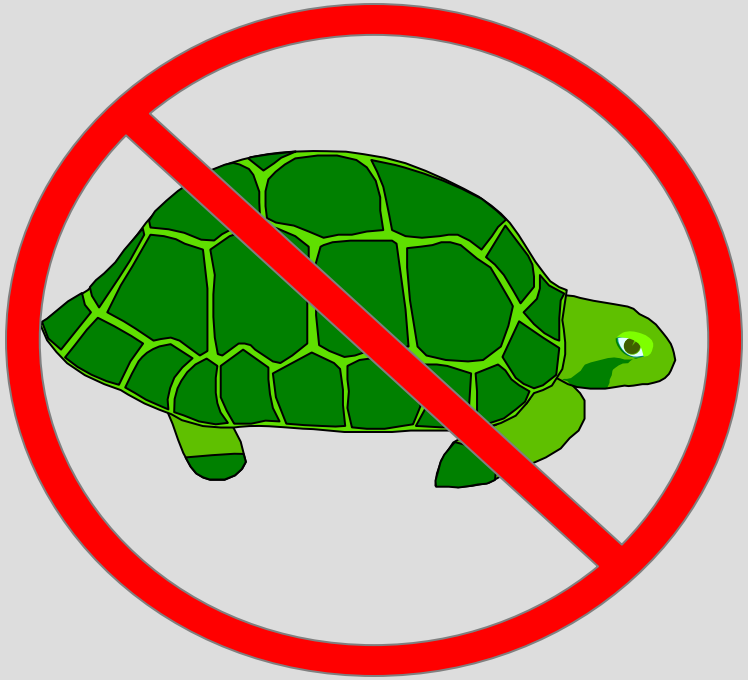
- Place carcasses in the refrigerator in 701.

Cage Return

- Empty cages generated in SMD laboratories should be returned to the dirty dumping station in the basement dirty freight elevator lobby.
- In the MRB procedure rooms, used cages should be neatly stacked in the area of the euthanasia station.
- Empty cages generated in MRB or MRBX laboratories should be returned to B-9636.
- Any questions about where soiled cages should be returned, contact the Animal Resource Office (5-2651).

Cage Return for other Animal Resource Locations

- **EDC:** return cages to 701.
- **Annex:** return cages to 1B-132.
- **Meliora:** return cages to 139.



CO_2 is **NOT** an appropriate method for euthanizing most reptiles and amphibians.

Remember

- Small laboratory animals (rats, mice, birds) that are euthanized by CO_2 or an approved anesthetic overdose, must undergo a second, physical method to ensure the animal is dead.

- Unintended recovery of animals after apparent death from CO_2 (e.g., in necropsy coolers) is an occurrence that must be documented. Research personnel are reminded that such incidents constitute serious noncompliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and a serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. All such incidents MUST be reported to PHS.

End of Euthanasia Module

- Euthanasia training will be complete upon successfully passing the quiz.
- UCAR will document your completion of this training requirement.
- If you have CO_2 equipment in your laboratory, please contact the Animal Resource Office for a copy of the vivarium CO_2 SOP. The SOP should be posted next to the equipment in your laboratory.

If you have any questions, please contact the UCAR Office at 5-1693.