Euthanasia (from the Greek, meaning "good death") is a critical component of humane animal care. In general, the recommendations of the AVMA Guidelines on Euthanasia (2013) serve as the standard for acceptable methods on euthanasia.

The following guidelines provide important criteria for the successful implementation of inhalant anesthesia.

1) Animals must be euthanized only by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements.

2) Death must be induced as painlessly and quickly as possible.

3) Upon completion of the procedure, death must be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting an animal's fixed and dilated pupils (described more fully below).

4) Euthanasia must not be performed in the animal room.

5) The euthanasia method must be appropriate to the species, approved in the animal study proposal, and conform to the most recent Report of the AVMA Panel on Euthanasia\(^1\). The use of inhalant agents for euthanasia must observe the conditions and precautions spelled out in the pertinent sections of that report and to NIH guidelines in their latest revisions\(^2-4\).

As a means of euthanasia, administration of inhalant overdose results in deep depression of all life signs prior to death. It is possible that animals could revive from this state, which can be mistaken for death during a cursory examination. To prevent such an occurrence, the TTUHSC Animal Care and Use Committee has instituted the following policy:

**Administration of an inhalant overdose must be followed by one of the following procedures:**

- Cervical dislocation
- Decapitation
- Exsanguination
- Bilateral thoracotomy

**I. Adult Animals:**

Acceptable agents for vertebrate animals include inhalant anesthetics (enflurane, isofluroane, sevoflurane, or desflurane). These inhalants may be supplemented with nitrous oxide. For adult rodents (>10 days old, see section III, below), \(CO_2\) may be used as an inhalant anesthetic.

**II. Rodent Fetuses:**
- **0-14 days gestation (mouse, rat and hamster) or 0-34 days gestation (guinea pigs):** Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the dam will ensure rapid, painless death of fetuses.
- **15 days gestation to birth (mouse, rat and hamster) or 35 days gestation to birth (guinea pigs):** Literature suggests the possibility of pain perception during this time. At this stage, fetuses are resistant to inhalant euthanasia, so other forms of euthanasia must be used (i.e., decapitation) to assure rapid, painless death of these older fetuses.

### III. Rodent Neonates:

- **0-10 days old mice, rats and hamsters:** At this stage, inhalant anesthetics will accomplish acceptable euthanasia following guidelines for adult inhalant euthanasia (excluding CO₂), although prolonged exposure is required, when followed by an acceptable physical method (i.e. decapitation). CO₂ is not considered an acceptable method of euthanasia at this age. Other acceptable methods include barbiturate overdose, decapitation while anesthetized (i.e. inhalant anesthetics, hypothermia, barbiturates, etc), and other anesthetic overdoses (i.e. injectable Ketamine/xylazine, barbiturates, avertin, etc) followed by an acceptable physical method to assure death. Note: the use of hypothermia must protect the skin from freezing (i.e. paper towel or saran wrap overlying wet ice).
- **10 days old and older mice, rats and hamsters:** Follow guidelines for adults.
- **Guinea pig neonates:** Guinea pigs are born precocious; therefore, CO₂ euthanasia can be administered from birth. Follow guidelines for adults, CO₂ may also be used.

### IV. Guidelines for Euthanasia of Rodents Using Carbon Dioxide:

CO₂ inhalation is the most common method of euthanasia used for mice, rats, guinea pigs and hamsters and must be used as follows:

- The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber. All animals in the chamber must be able to make normal postural adjustments.
- Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide at a low flow (little or no hissing noise) which is equivalent to a rate of 10-20% of the chamber volume per minute so as to optimize reduction in distress. For a 10-liter volume chamber, use a flow rate of approximately 1-2 liter(s) per minute. After the animals become unconscious, the flow rate can be increased to minimize the time to death. Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful.
- Animals should be left in the container until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and exposure times or by other means.
- The use of dry ice for CO₂ euthanasia is not permitted.

Exceptions to these guidelines will be considered by the IACUC on a case-by-case basis.
References

1) AVMA Guidelines on Euthanasia, 2013

2) National Institutes of Health. Office of Animal Care and Use: Guidelines for the Euthanasia of Rodents Using Carbon Dioxide, 2001: revised 05/08/13
[http://oacu.od.nih.gov/ARAC/index.htm]

3) NIH Notice NOT-OD-02-062 (7-27-2002)

4) National Institutes of Health. Office of Animal Care and Use: Guidelines for the Euthanasia of Rodent Fetuses and Neonates, 1997; revised 04/10/13
[http://oacu.od.nih.gov/ARAC/index.htm]