



Florida State University Animal Care and Use Committee

Euthanasia Guidelines

Euthanasia is the use of a humane method to induce rapid unconsciousness and to kill animals without pain or distress. The word euthanasia is derived from the Greek "eu" meaning good and "thanatos" meaning death, thereby giving us a word which translates as "good death". The object of euthanasia is to quickly render the animal unconscious, therefore insensitive to pain, without or with only momentary fear or anxiety, progressing quickly to cardiac or respiratory arrest and then to brain death. Humane euthanasia is to be carried out only by trained personnel using techniques which have been approved by the institution and in accordance with applicable laws and regulations.

There are three basic mechanisms of death:

1. Hypoxia. Leading to cellular anoxia and death.
2. Direct depression of neurons vital for life function. Depression of the cortical neural system causes loss of consciousness followed by death.
3. Physical disruption of brain activity. The midbrain centers controlling respiration and cardiac activity fail and death follows.

Euthanasia methods fall into 3 basic categories:

1. Inhaled Agents - carbon dioxide, isoflurane, argon, etc. *"Inhaled agents must be supplied in purified form without contaminants or adulterants."*
2. Physical Methods - cervical dislocation, decapitation, gunshot, electrocution, etc. *"Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately."* Some physical methods are not to be used as a sole method of euthanasia but need to be combined with another method or agent. Physical methods should be used only if inhalation or chemical agents are excluded for the purpose of research.
3. Noninhalation Agents – barbiturates, MS-222, etc. *"Chemical agents that are introduced into the body by means other than through direct delivery to the respiratory tract. The primary routes of their administration are parenteral injection, topical application, and immersion."*

Selection of the method for euthanasia should be based on the species involved; the animal's behavior, presence of any injury or disease; the ability to restrain the animal; any requirement for tissue collection before or after death; compatibility with subsequent evaluation, examination, or use of tissue; the relative rapidity of the technique; irreversibility of the method; reliability of the method; drug or equipment availability, human abuse potential and the skill/proficiency of the personnel involved. The method chosen should also take into account the sensitivity and emotional impact on the technician, as certain methods are esthetically stressful for person performing the job.

Personnel must demonstrate proficiency in the technique before being allowed to perform euthanasia without supervision.

As the method for euthanasia may impact the research objectives for the animals being euthanized (e.g. metabolic or histologic artifacts), researchers should determine if/how a chosen method might affect their results. An excellent table compiling currently known effects can be found in the [Report of the ACLAM Task Force on Rodent Euthanasia](#).

Equipment used for euthanasia must be maintained in good working order at all times. Equipment used for decapitation must be kept in good condition (closes readily) with sharp blades and clean. See also the FSU ACUC Policy for Use and Maintenance of Guillotines and other Equipment Used for Decapitation.

Ideally animals which are to be euthanized should be transferred to a room without other animals, particularly those of the same species. This avoids inducing stress in other animals. Also, where possible, animals should remain in their normal housing environment (cage or tank) and social groups to reduce stress.

Altricial rodent fetuses and neonates often present a challenge for euthanasia and may require different techniques than those for adults of the same species.

- Fetuses \leq E 14 - In utero fetuses rely upon euthanasia of the dam or simple removal of the fetuses from the uterus (loss of blood supply). *“...embryos and fetuses cannot consciously experience feelings such as breathlessness or pain. Therefore, they also “cannot suffer while dying in utero after the death of the dam, whatever the cause.”* . Based upon current knowledge, fetuses E14 and younger need no special secondary method following euthanasia of the dam.
- Fetuses \geq E15 - However, altricial neonates are resistant to hypoxia and begin to develop pain perception pathways in later stages of gestation, so when considering a method using hypoxia for these fetuses, E15 and older fetuses should either not have the uterus opened for a prolonged time (approximately 1 hour) or should undergo a second method of euthanasia such as decapitation to ensure death immediately following opening of the amniotic sac
- Neonates \leq P10 - Neonatal rodents also typically take prolonged periods of time to death from exposure to carbon dioxide or other inhalation agents. Adequate exposure time to the inhalant should be provided (up to 50 minutes depending upon age). Due to their resistance to hypoxia, the use of a two-step method (CO₂ exposure for sedation followed by decapitation) is the recommended technique. Preferred methods of euthanasia for neonatal rodents (mice, hamsters, rats) < 10 days of age include cervical dislocation, decapitation and injectable chemical agents.
- Precocial rodent neonates and altricial rodents older than 10 days should undergo methods approved for adult rodents.

IMPORTANT NOTE: The 2013 AVMA Guidelines state: *“Death must be confirmed before disposal of any animal remains.”* Researchers must make sure the animal is dead before disposal, tissue collection or leaving the animal’s body unattended. Failure

to ensure death of animals after euthanasia procedures violates PHS policy and the AWA and is a reportable instance of non-compliance.

Signs of death include:

- Absence of respiration
- Absence of pulse or heartbeat
- Loss of color in mucous membranes
- Lack of corneal reflex
- Lack of response to hard toe pinch
- Glazing of eyes
- Rigor mortis

Only rigor mortis is a confirmative sign of death; a combination of the above signs can, however, be used as a reliable means to confirm death. Researchers should take the precaution of using a secondary procedure to ensure that an animal is dead if the primary method is not one that guarantees irreversible death (such as decapitation or bilateral thoracotomy). Secondary procedures includes cervical dislocation, exsanguination (severing of heart or major arteries), destruction of the brain (via direct concussion, disruption of the brain stem, lack of blood supply) or bilateral opening of the chest (bilateral thoracotomy).

Principal Investigators are responsible for ensuring that all personnel performing animal euthanasia have been properly trained to consistently use the technique(s) in a humane and effective manner. The 2013 AVMA Guidelines on Euthanasia *state "Personnel who perform euthanasia must demonstrate proficiency in the use of the technique in a closely supervised environment. Each facility or institution where euthanasia is performed (whether a clinic, laboratory, or other setting) is responsible for training its personnel adequately to ensure the facility or institution operates in compliance with federal, state, and local laws. Furthermore, experience in the humane restraint of the species of animal to be euthanized is important and should be expected, to ensure that animal pain and distress are minimized. Training and experience should include familiarity with the normal behavior of the species being euthanized, an appreciation of how handling and restraint affect that behavior, and an understanding of the mechanism by which the selected technique induces loss of consciousness and death."*

Methods of euthanasia used will be consistent with the recommendations of the AVMA Guidelines on Euthanasia, unless a deviation is justified for scientific reasons. Any departures from these guidelines will require the investigator to provide strong scientific and clinical justifications for the exemption and must be approved the ACUC prior to use.

See Also:

FSU ACUC Guidelines on Euthanasia Using Carbon Dioxide

Standard Operating Procedure Euthanasia Of Rodents And Birds Using Carbon Dioxide

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