IACUC POLICIES, PROCEDURES, and GUIDELINES

EUTHANASIA

Purpose:

This document establishes policies and provides guidelines for the performance of euthanasia on laboratory animal species maintained at the University of Kentucky and used in biomedical research. Many research and other factors may impact the method of euthanasia selected by the investigator in the animal use protocol. The intent of this document is to provide guidance to investigators regarding recommended methods when research requirements do not mandate specific methods.

Responsibilities:

All personnel conducting euthanasia should be trained and familiar with the procedure(s) used and the procedures used should minimize animal pain and distress to the extent possible in performance of the research. The Attending Veterinarian of the University of Kentucky has overall responsibility for providing guidance to investigators and animal care personnel regarding recommended and approved methods of euthanasia. The Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky must review and approve all methods of euthanasia proposed as a component of an animal use protocol. The research facility, through the veterinary care program and IACUC, is responsible for ensuring that all personnel performing euthanasia procedures are adequately trained in the procedures.

Investigators and animal technicians typically find the process of euthanizing animals to be an unpleasant and distasteful task. Laboratory workers who report that the process is a major source of personal stress should not be required to administer euthanasia. The euthanasia procedure should only be performed by individuals who can approach the task with a sense of responsibility and who can accept the nature of the task with a minimum of stress.

General Guidelines:

In the simplest terms, the word euthanasia means "good death." To the extent possible, animals being euthanized should not experience pain, fear, or other significant stress prior to their death. In some instances this may require that the animals be rendered unconscious through some other painless method prior to euthanasia.

a. Only personnel who have demonstrated proficiency through both training and experience will be allowed to perform euthanasia procedures. Such proficiency will include the following:

1) Familiarity with the normal behavior of the species being euthanized

2) How handling and restraint affects this behavior

3) An understanding of how the selected euthanasia techniques induce unconsciousness and death, i.e., (1) hypoxia, direct or indirect (e.g., CO_2); (2) direct depression of neurons vital for life function (e.g., barbiturate overdose); (3) physical disruption of brain activity and destruction of neurons vital for life (e.g., cervical dislocation)

Training in specific requirements and methods of euthanasia may be obtained through the AALAS Learning Library, through Division of Laboratory Animal Resources (DLAR) training seminars, or through direct instruction from DLAR supervisors and veterinarians.

b. Animals should not be euthanized in the presence of other animals, particularly animals of the same species (conspecifics). Euthanasia should not be performed in the animal housing room unless such action has been specifically approved by the IACUC as a component of the animal care and use protocol.

c. In all cases, death must be ensured. This determination may be made by auscultation for cessation of both heartbeat and respiration by a qualified individual in larger animals or by utilizing an unequivocal secondary means of ensuring death (decapitation, opening thoracic cavity, etc.) following euthanasia with an inhalant agent (anesthetic overdose or CO_2)

d. All euthanasia procedures will follow the guidelines in the "*AVMA Guidelines on Euthanasia*"[2] (June 2007) or more recent versions.

e. Prior to euthanizing an animal as a component of an animal use protocol, personnel must ensure that the method of euthanasia to be employed is the same as that described in the approved animal use protocol.

f. Personnel of the Division of Laboratory Animal Resources (DLAR) may assume the responsibility for euthanizing animals assigned to an approved protocol during or at the conclusion of the study pending the receipt of a written request for this service from the principal investigator. In these cases, only euthanasia methods recommended below will be used and the methods must have been approved in the animal use protocol.

Recommended Methods of Euthanasia:

There are a number of acceptable euthanasia agents and methods with the final specific agent and method of choice dependent upon the experimental design, the species involved, the familiarity of the individual with the specific agent or method, and the availability of the agent or method. The following brief list of recommended agents represents the agents recommended for use in the listed species where no specific experimental requirement exists.

Species	**Recommended Method of Euthanasia**
Cat	Pentobarbital[1] or pentobarbital containing euthanasia solution[2] (100 mg/kg IV)
Dog	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Goat	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Sheep	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Swine	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Rabbit	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) w/wo prior tranquilization or anesthesia
Primate	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)
Hamster	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO_2
Mouse	Pentobarbital or pentobarbital containing euthanasia solution (150 mg/kg IP), CO_2
Rat	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO_2
Guinea Pig	Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO_2
Amphibians	Tricaine methane sulfonate (MS-222) 1-3% solution buffered with sodium bicarbonate or sodium phosphate to a pH of 7.0-7.5
Avians	CO_2

[1] Pentobarbital is a CII controlled drug
[2] Pentobarbital containing euthanasia solutions are generally CIII controlled drugs

Euthanasia of Rodents using Carbon Dioxide:

The euthanasia method must be appropriate to the species, approved in the animal use protocol and conform to the most recent AVMA Guidelines on Euthanasia.[2] CO_2 inhalation is the most common method of euthanasia used for mice, rats, guinea pigs, and hamsters.

A few important aspects of this procedure are:

1. 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.

2. 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.

3. There is an ongoing debate as to the relative distress to the subject animals and the rapidity of unconsciousness when using either pre-filled carbon dioxide chambers for euthanasia or gradually increasing the carbon dioxide concentration in chambers not pre-filled with carbon dioxide. In the 2007 report, the AVMA Guidelines on Euthanasia[2] recommended the use of prefilled chambers only for species where this practice "has not been shown to cause distress." At present, the published literature indicates a lack of increased distress in rats when prefilled chambers are used,[4,5] making both methods acceptable. In mice the use of prefilled chambers is associated with increased distress in the animals prior to unconsciousness which is much less evident when the carbon dioxide concentration in the chamber is gradually increased.[6,7,8] Prefilled chambers are not recommended for the euthanasia of mice.

    i. Non-pre-filled chamber with gradual increase in carbon dioxide:

        Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide at the rate of 20% of the chamber volume per minute so as to minimize distress.

Cage Type	Cage Size (W x L x H)	Flow Rate
Mouse	7.5" x 11.75" x 5"	1.5 l/min
Rat	10.5" x 19" x 8"	5 l/min

After the animals become unconscious, the flow rate can be increased to minimize the time to death. The gradual introduction of carbon dioxide reduces the acute reactions seen in some species exposed to pre-filled chambers[6,8] but it does also significantly increases the exposure time required for unconsciousness. A two stage CO_2 regulator with a flow meter is required to control the rate of CO_2 introduction into the chamber.

ii. Pre-filled carbon dioxide chamber:

The chamber is pre-charged by flowing carbon dioxide into the chamber so as to effect a final concentration of greater than 70%. [For a 10-liter volume chamber, such as a standard rat cage, a flow rate of 3 liter(s) per minute for 3-4 minutes is sufficient.] The carbon dioxide flow is suspended and the top carefully opened to minimize turbulence and loss of carbon dioxide from the chamber. The animals are then placed into the chamber, the top replaced, and the carbon dioxide flow restarted. Two stage CO_2 pressure regulators or flow-meters may be used to introduce the CO_2 into the chamber.

4. Animals should be left in the container until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO_2 concentrations and the use of a secondary means to ensure death. The secondary methods may include decapitation, cervical dislocation, thoracotomy, etc.

5. Neonatal animals (up to 10 days of age) are resistant to the effects of CO_2, therefore, alternative methods are recommended[7]. Carbon dioxide may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Keeping neonates warm during CO_2 exposure may decrease the time to death[5].

6. If an animal is not dead following CO_2 exposure, another approved method of euthanasia (e.g. decapitation) must be added while the animal is under CO_2 narcosis to assure death. Please refer to Appendices 1 and 2 of the Report of the AVMA Panel on Euthanasia[2] for additional recommended methods.

The following statement is recommended for inclusion in an Animal Use Protocol for any species where CO2 euthanasia is to be used:

> **"Animals will be euthanized by slow (20%/minute) displacement of chamber air with compressed CO_2. Following [unconsciousness/death] the animals will be subject to [cervical dislocation/decapitation/thoracotomy] as a secondary means to ensure death."**

The following statement may alternatively be used for rats and other species where exposure to a pre-filled chamber has not been shown to be distressful:

> **"Animals will be euthanized by placement in a chamber prefilled (~70%) with compressed CO_2 followed by CO_2 introduction until [unconsciousness/death]. Following [unconsciousness/death] the animals will be subject to [cervical dislocation/decapitation/thoracotomy] as a secondary means to ensure death."**

Euthanasia of Rodent Feti and Neonates:

The AVMA Guidelines on Euthanasia provides limited recommendations for the euthanasia of prenatal or neonatal animals. The 2007 guidelines state: "When ovarian hysterectomies are performed, euthanasia of feti should be accomplished as soon as possible after removal from the dam." It also states "Neonatal animals are relatively resistant to hypoxia."[2] Since CO_2 is the usual method of euthanasia for neonates and the mechanism of eventual death when using CO_2 is hypoxia, neonatal animals take much longer to die than adults. The following guidelines have been developed to assist investigators in developing proposals which involve the use of rodent feti or neonates. In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

1. **Feti:** At approximately 60 percent of the gestation period, the neural tube has developed into a functional brain and the likelihood that a fetus may perceive pain should be considered.[9,10] Reflexive behavior in response to painful stimuli has been observed in feti and correlates with adult behaviors[9]. However, fetal behavioral arousal and awareness may be suppressed by low arterial oxygen limiting higher cortical processing[12].

    a. **Mouse, Rat and Hamster Feti up to 15 days' and Guinea Pig Feti up to 34 days' gestation:** Neural development at this stage is minimal and pain perception is considered unlikely.[13,14] Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of feti at this stage of development[7].

b. Mouse, Rat and Hamster Feti 15 days' gestation to birth and Guinea Pig Feti 35 days' gestation to birth: The neural development at this stage supports the likelihood that pain may be perceived.[10,13,14] When feti are required for study, euthanasia of individual feti may be induced by the skillful injection of chemical anesthetics. Decapitations with surgical scissors or cervical dislocation are acceptable physical methods of euthanasia. Rapid freezing, without prior anesthesia, as a sole means of euthanasia is not considered to be humane[2]. Animals should be anesthetized prior to freezing. When chemical fixation of the whole fetus is required, feti should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus.[15,16] or by injection of the fetus with a chemical anesthetic[17]. The veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. Feti at this age are resistant to hypoxia[18] and require extended exposure to inhalant anesthetics, including CO_2.[7]

c. When feti are not required for study, the method chosen for euthanasia of a pregnant mother should ensure rapid cerebral anoxia to the fetus with minimal disturbance to the uterine milieu minimizing fetal arousal[11]. Recommended methods are CO_2 exposure with or without cervical dislocation of the mother.[7] Death of the mother must be verified after euthanasia, preferably by use of an adjunctive method (decapitation, cervical dislocation, etc.), prior to disposal. The veterinary staff should be consulted for considerations of other euthanasia agents.

2. Neonates: Maturation of nociceptors and the development of excitatory and inhibitory receptor systems occur during the period just prior to birth and into the second week of postnatal life.[19,20,21] Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO_2 is used as a euthanasia agent.[2,7] Death must be verified after euthanasia, preferably by use of an adjunctive method (decapitation, cervical dislocation, etc.), prior to disposal.[22]

a. Mouse, Rat and Hamster Neonates up to 10 days of age: Acceptable methods for euthanasia include: injection of chemical anesthetics (e.g., pentobarbital), decapitation, or cervical dislocation. Additionally, these animals are sensitive to inhalant anesthetics; e.g., halothane or isoflurane (used with appropriate safety considerations) although prolonged exposure may be necessary. Immersion in liquid nitrogen may be used only if preceded by anesthesia. Similarly, anesthesia should precede immersion or perfusion with chemical fixatives. Anesthesia may be induced by inhalant or injectable anesthetics; the veterinarian should be consulted for appropriate agents and dosages. Alternatively, when adequately justified, hypothermia may be used to induce anesthesia in pups six days of age or less.[15,16,25]

b. Guinea Pig Neonates: Follow guidelines for adults.

c. Mouse, Rat and Hamster Neonates over 10 days of age: Follow guidelines for adults.

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