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\(^1\). 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 AVMA Guidelines for the Euthanasia of Animals: 2013 Edition\(^2\) (or more recent editions) establishes the acceptable methods of euthanasia\(^1\)-\(^3\). 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\(^1\).

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 for the Euthanasia of Animals: 2013 Edition 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.
g. Cadavers must be appropriately disposed after euthanasia. In the case of rodents or other small laboratory animals, the cadavers must be placed in leak resistant plastic bags, clearly marked with first and last name of PI and placed in the appropriate refrigerator for disposal.
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended Method of Euthanasia</th>
</tr>
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<tbody>
<tr>
<td>Cat</td>
<td>Pentobarbital(^1) or pentobarbital containing euthanasia solution(^2) (100 mg/kg IV)</td>
</tr>
<tr>
<td>Dog</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)</td>
</tr>
<tr>
<td>Goat</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)</td>
</tr>
<tr>
<td>Swine</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV) w/wo prior tranquilization or anesthesia</td>
</tr>
<tr>
<td>Primate</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IV)</td>
</tr>
<tr>
<td>Hamster</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO(_2)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (150 mg/kg IP), CO(_2)</td>
</tr>
<tr>
<td>Rat</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO(_2)</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Pentobarbital or pentobarbital containing euthanasia solution (100 mg/kg IP), CO(_2)</td>
</tr>
<tr>
<td>Amphibians</td>
<td>Tricaine methane sulfonate (MS-222) 1-3% solution buffered with sodium bicarbonate or sodium phosphate to a pH of 7.0-7.5</td>
</tr>
<tr>
<td>Avians</td>
<td>CO(_2)</td>
</tr>
</tbody>
</table>

\(^1\) 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 for the Euthanasia of Animals: 2013 Edition. CO₂ 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 approved source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled.

3. Historically there has been an ongoing debate as to the relative distress to different species 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 their 2013 report, the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition reviewed the current literature and concluded that “the practice of immersion, where conscious animals are placed directly into a container pre-filled with 100% CO₂, is unacceptable” and mandated the gradual displacement method of CO₂ euthanasia as the only acceptable method.

It is preferable that animals are euthanized in their home cage to minimize stress. If this is not possible, the euthanasia chamber must be emptied and cleaned between uses. CO₂ must be supplied from either gas cylinders or building CO₂ gas distribution systems equipped with an appropriate pressure reducing regulator and flow meter combination (or equivalent) to permit precise regulation of gas flow to the chamber. The CO₂ flow should be set to displace 10-30% of the chamber volume per minute:

<table>
<thead>
<tr>
<th>Cage Type</th>
<th>Cage Size (W x L x H)</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>7.5” x 11.75” x 5”</td>
<td>1.5 l/min</td>
</tr>
<tr>
<td>Rat</td>
<td>10.5” x 19” x 8”</td>
<td>5 l/min</td>
</tr>
</tbody>
</table>
After the animals become unconscious, the flow rate can be increased to minimize the time to death.

4. 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 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 CO₂, therefore, alternative methods are recommended. 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 CO₂ exposure may decrease the time to death.

6. If an animal is not dead following CO₂ exposure, another approved method of euthanasia (e.g. decapitation) must be added while the animal is under CO₂ narcosis to assure death. Please refer to Appendices 1 and 2 of the Report of the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition 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 CO₂ delivered through a precision flowmeter. Following [unconsciousness/death] the animals will be subject to [cervical dislocation/decapitation/thoracotomy] as a secondary means to ensure death.”

Euthanasia of Rodents using Physical Methods:

The euthanasia of rodents using physical methods (decapitation, cervical dislocation, etc.) must be approved in the animal use protocol and conform to the most recent AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

A few important aspects of this procedure are:

1. These methods may be aesthetically displeasing, however in certain conditions, they may be the most appropriate choice.

2. Personnel using physical methods of euthanasia must be well trained on the method performed to ensure euthanasia is conducted appropriately.

3. Guillotines for adult rodent decapitation are commercially available. For
neonatal rodents sharp scissors/blades can be used for decapitation. Care must be exercised to prevent injuries when using sharp instruments.

4. The equipment used to perform decapitation must be maintained in good working order. Guillotines must be serviced on a yearly basis to ensure sharpness and functionality of the instrument. Service records must be maintained.

5. The use of plastic cones to restrain animals is recommended as they appear to reduce stress, minimize the chance of injury to personnel, and improve positioning of the animal.

**Euthanasia of Rodent Feti and Neonates:**

The *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* provides additional recommendations for the euthanasia of prenatal or neonatal animals. Scientific data indicate that mammalian embryos and fetuses are in a state of unconsciousness throughout pregnancy and birth and cannot suffer while dying *in utero* after the death of the dam, whatever the cause.9

The 2013 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 CO2 is the usual method of euthanasia for neonates and the mechanism of eventual death when using CO2 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.

**Feti:** Scientific data indicate that mammalian embryos and fetuses are in a state of unconsciousness throughout pregnancy and birth and cannot suffer while dying *in utero* after the death of the dam, whatever the cause.9 Chemical inhibitors (eg, adenosine, allopregnanolone, pregnanolone, prostaglandin D2, placental peptide neuroinhibitor) and hypoxic inhibition of cerebrocortical activity render the fetuses unconscious prior to birth.

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 it be removed from the *in utero* environment should be considered.10,11 Reflexive behavior in response to painful stimuli has been observed in feti and correlates with adult behaviors10. However, fetal behavioral arousal and awareness may be suppressed by low arterial oxygen limiting higher cortical processing12.

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.\textsuperscript{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\textsuperscript{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.\textsuperscript{11,13,14} 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 as is rapid chilling.\textsuperscript{2} The veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. Feti at this age are resistant to hypoxia\textsuperscript{15} and require extended exposure to inhalant anesthetics, including CO\textsubscript{2}.\textsuperscript{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\textsuperscript{16}. Recommended methods are CO\textsubscript{2} exposure with or without cervical dislocation of the mother.\textsuperscript{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.

**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.\textsuperscript{17-19} Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO\textsubscript{2} is used as a euthanasia agent.\textsuperscript{2,7} Death must be verified after euthanasia, preferably by use of an adjunctive method (decapitation, cervical dislocation, etc.), prior to disposal.\textsuperscript{20}

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. Rapid chilling is acceptable for euthanasia of pups six days of age or less.\textsuperscript{2,6,21,22}

b. Guinea Pig Neonates: Follow guidelines for adults.

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