

Guidelines for Euthanasia of Rodents

Rodents must be euthanized by trained personnel using appropriate techniques, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements. Death should be induced as painlessly and quickly as possible. 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. The euthanasia method must be appropriate to the species, approved in the animal study proposal and conform to the most recent AVMA Guidelines on Euthanasia.

CO₂ inhalation is the most common method of euthanasia used for adult mice, rats, guinea pigs and hamsters. A few important aspects of this procedure are:

1. "Dry Ice" is no longer an acceptable source of CO₂. CO₂ gas from compressed gas cylinders must be used¹. If scientifically justified, dry ice may be used to euthanize fetuses and neonates prior to immersion in chemical fixatives or liquid nitrogen.
2. The animal(s) should be placed into the euthanasia chamber. With an animal in the chamber, an optimal flow rate should displace at least 50% of the chamber volume per minute.
3. 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. Mice may not occupy more than 75% of the cage floor.
4. Each animal must be visually observed during the euthanasia procedure or directly following [for pre-programmed CRL euthanasia chamber] to ensure that animals receive adequate CO₂ concentrations and do not revive from the terminal procedure.
5. Alternatively, animals may be placed into a two-port euthanasia chamber. The animals will be placed into the chamber and the isoflurane port activated until the animals are anesthetized (approximately one minute or until evidence of stage III anesthesia is present/unconsciousness). The CO₂ port will then be activated to ensure euthanasia.
6. Mice should be maintained in the chamber for at least two minutes after apparent clinical death (lack of respiration, cyanotic appearance [bluish purple discoloration], pupils fixed and dilated). After animals are removed from the chamber each animal must be visually observed for an additional 5 minutes to ensure death prior to disposal. If clinical death cannot be assured, CO₂ narcosis must be followed up with another method of euthanasia (i.e., cervical dislocation or decapitation with surgical scissors).
7. Do not overcrowd dead animal bags prior to disposal or placement in the freezer.
8. Individual species should be euthanized separately.
9. Moribund and/or sick animals must be euthanized separately from healthy animals to alleviate any potential stress prior to euthanasia. In addition, these animals may need to be maintained in the chamber for longer time periods due to altered respiration.
10. Animals that are less than 15 days of age must be euthanized separately from adult animals as per Table 2.
11. Animals of varying adult ages, strains, etc., must be euthanized in a timely manner and cannot be maintained in the cage prior to euthanasia for an extended period of time.
12. Please see Table 2 (Adult Rats) for adult rat euthanasia recommendations.

EUTHANASIA OF MOUSE FETUSES AND NEONATES

The AVMA Guidelines on Euthanasia does not provide specific recommendations for the euthanasia of prenatal or neonatal animals. The following guidelines are suggested:

FETUSES:

Up to Day 14 Gestational Age:

Neural development at this stage is minimal and pain perception is considered unlikely. 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 fetuses at this stage of development.

Day 15 Gestational Age to Birth:

The literature on the development of pain pathways suggests the possibility of pain perception at this time. Whereas fetuses at this age are not sensitive to inhalant anesthetics, anesthesia must be induced by chilling on wet ice slurry. Chilling followed by decapitation with sharp surgical scissors or rapid freezing (immersion in liquid nitrogen) are acceptable physical methods of euthanasia.

TABLE 1 - EMBRYOS

AGE	RECOMMENDED METHOD OF EUTHANASIA for EMBRYOS	NOTES
Embryo < Day 15	Removal of fetus from uterus	Not applicable.
Embryo Day 15-Birth	Decapitation with sharp surgical scissors	Use is at the discretion of the individual performing euthanasia. Individual must demonstrate proficiency at this technique
	Anesthesia by hypothermia & decapitation	Fetus is placed in specimen cup or petri dish and submerged into an ice slurry for 20 minutes. <i>This method is used to decrease or terminate movement prior to decapitation, reduce residual nervous activity pre- and post-decapitation and reduce bleeding post-decapitation.</i>

NEONATES:

Acceptable methods for euthanasia of neonatal mice and rats are described below. In all cases, personnel performing the euthanasia must be fully trained in the appropriate methods. Please consult veterinary staff with additional questions or concerns.

TABLE 2 – NEONATES AND ADULTS

AGE	RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS <i>(In order of recommended use)</i>	NOTES
Day 1-6	Decapitation with sharp surgical scissors	Use is at the discretion of the individual performing euthanasia. Individual must demonstrate proficiency at this technique
	Anesthesia by hypothermia & decapitation	Rodent is placed in specimen cup (not petri dish) and submerged into an ice slurry for 20 minutes. <i>This method is used to decrease or terminate movement prior to decapitation, reduce residual nervous activity pre- and post-decapitation and reduce bleeding post-decapitation.</i>
	CO ₂ followed by decapitation with surgical scissors	Verify state of unconsciousness (cessation of movement) immediately followed by decapitation
	CO ₂ alone <i>(not recommended as sole method)</i>	Not a recommended method as it requires that animal remain in the CO ₂ chamber >20 min. after cessation of all movement is observed; after removal from CO ₂ chamber an additional >15 min. of observation is required to ensure death has occurred. A recommended method for use prior to decapitation with sharp surgical scissors.
Day 7-14	CO ₂ followed by decapitation with sharp surgical scissors	Verify state of unconsciousness (cessation of movement) immediately followed by decapitation
	CO ₂ alone	Requires that animal remain in the CO ₂ chamber >10 min. after cessation of all movement is observed; after removal from CO ₂ chamber an additional >15 min. of observation is required to ensure death has occurred.
Day15-Adult	CO ₂ alone	Requires that animal remain in the CO ₂ chamber >2 min. after cessation of all movement is observed. After animals are removed from the chamber each animal must be visually observed for an additional 5 minutes to ensure death prior to disposal or placement in the freezer.
	Cervical dislocation (see note)	Requires scientific justification; requires that individual demonstrate proficiency at this technique.

AGE	RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS <i>(In order of recommended use)</i>	NOTES
Adult Rats	CO ₂ alone	Requires that animal remain in the CO ₂ chamber >2 min. after cessation of all movement is observed. After animals are removed from the chamber each animal must be visually observed for an additional 5 minutes to ensure death prior to disposal. Rats may require longer exposure time than mice.

LIQUID NITROGEN AND CHEMICAL FIXATIVES

NOTE: Fixatives should be chilled prior to and during use with rodent embryos and neonates

Fetuses Day 18-Birth and Neonates Day 1-6

The preferred euthanasia method for neonates is decapitation prior to immersion (in liquid nitrogen or chemical fixative) or perfusion (in chemical fixatives). If decapitation is not possible, the following methods are recommended: Immersion in liquid nitrogen (for >2 min.) may be used only in anesthetized/euthanized mice. Similarly, anesthesia must precede immersion or perfusion with chemical fixatives. Anesthesia/euthanasia may be induced by CO₂, wet ice, dry ice* or injectable anesthetics; the attending veterinarian must be consulted for appropriate agents and dosages.

* *Scientific justification required*

Neonates Day 7-14

Older neonates (Day 7-14) must be anesthetized/euthanized prior to immersion (in liquid nitrogen or chemical fixative) or perfusion (in chemical fixatives). Consult with LAM prior to initiating an animal study proposal for appropriate method.

TABLE 3. – FIXATION OR HARVEST

AGE	RECOMMENDED METHOD OF EUTHANASIA for FIXATION or HARVEST <i>(In order of recommended use)</i>	NOTES
Embryo Day 15 - Birth	Decapitation with surgical scissors prior to immersion or perfusion	Use is at the discretion of the individual performing euthanasia. Individual must be proficient at demonstrating this technique

	Anesthesia by hypothermia prior to fixation	Fetus is placed in specimen cup or petri dish and submerged in an ice slurry for 20 minutes prior to immersion (in liquid nitrogen or chemical fixative) or perfusion (in chemical fixatives). After submersion in fixative an additional >15 min. of observation is required to ensure that recovery does not occur.
	For Organ Harvest: anesthesia by hypothermia	Fetus is placed in specimen cup or petri dish and submerged in an ice slurry for 20 minutes. Remove to chilled PBS and open the thoracic and abdominal cavities.
Neonate Day 1-6	Decapitation with surgical scissors prior to immersion or perfusion	Use is at the discretion of the individual performing euthanasia. Individual must be proficient at demonstrating this technique
	Anesthesia by hypothermia prior to fixation – dry ice*	Animal is placed in specimen cup (not petri dish) and placed in dry ice (for >3 minutes after cessation of all movement is observed) prior to immersion (in liquid nitrogen or chemical fixative).
	Anesthesia by hypothermia prior to fixation – wet ice	Animal is placed in specimen cup (not petri dish) and submerged in an ice slurry for 30 minutes prior to immersion (in liquid nitrogen or chemical fixative) or perfusion (in chemical fixatives). After submersion in fixative an additional >15 min. of observation is required to ensure that recovery does not occur.
Neonate >Day 7	Consult with LAM	Consult with LAM

* *Scientific justification required*

References

1. American Veterinary Medical Association [AVMA] Guidelines on Euthanasia [June 2007]. http://www.avma.org/issues/animal_welfare/euthanasia.pdf
2. Phifer CB, Terry LM. 1986. Use of hypothermia for general anesthesia in preweaning rodent. *Physiol & Behav* 38:887-890.