

# 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. 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.

CO<sub>2</sub> 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 CO<sub>2</sub>. CO<sub>2</sub> gas from compressed gas cylinders must be used<sup>1</sup>.
2. The animal(s) should be placed into the euthanasia chamber. With an animal in the chamber, an optimal flow rate should displace at 20% 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 to ensure that animals receive adequate CO<sub>2</sub> 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 CO<sub>2</sub> port will then be activated to ensure euthanasia.
6. If CO<sub>2</sub> is used as the only method of euthanasia, the mice or rats must remain in the chamber for thirty minutes (including fill and holding time).
7. A secondary method of euthanasia, such as cervical dislocation or thoracotomy, must be used if mice and rats are placed in the euthanasia chamber for less than thirty minutes (i.e. fill period only). Cervical dislocation cannot be used as a secondary method for rats weighing greater than 200 grams.
8. Mice must demonstrate signs of clinical death (lack of respiration, cyanotic appearance [bluish purple discoloration], pupils fixed and dilated, cardiac arrest) after they are removed from the chamber. If clinical death cannot be assured, CO<sub>2</sub> narcosis must be followed up with another method of euthanasia (i.e., cervical dislocation or decapitation with surgical scissors).
9. All bags containing dead animals must be sealed prior to disposal and labeled with the date and building number.
10. Individual species should be euthanized separately.
11. 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.
12. Animals that are less than 15 days of age must be euthanized separately from adult animals as per Table 2.
13. 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.
14. Please see Table 2 (Adult Rats) for adult rat euthanasia recommendations.

## EUTHANASIA OF MOUSE FETUSES AND NEONATES

### 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 without direct contact with the ice. 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 without direct contact with the ice 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**

<b>AGE</b>	<b>RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS</b> <i>(In order of recommended use)</i>	<b>NOTES</b>
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 without direct contact with the ice 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 <sub>2</sub> followed by decapitation with surgical scissors	Verify state of unconsciousness (cessation of movement) immediately followed by decapitation
	Isoflurane	Animals are placed in a specially designed jar with an internal gauze holder that will prevent the animals from coming in contact with the anesthetic. The jar is placed in a fume hood or other scavenging device, and precharged with isoflurane soaked gauze (5% vaporizer). Animals are then placed in the jar and observed for at least 10 minutes after the cessation of movement. After removal from the jar the animals are observed for an additional minute to ensure that death has occurred.
Day 7-14	CO <sub>2</sub> followed by decapitation with sharp surgical scissors	Verify state of unconsciousness (cessation of movement) immediately followed by decapitation

AGE	<b>RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS</b> <i>(In order of recommended use)</i>	NOTES
	CO <sub>2</sub> alone	Requires that animal remain in the CO <sub>2</sub> chamber for 30 minutes if a secondary method is not used. Requires that animal remain in the CO <sub>2</sub> chamber >10 min. after cessation of all movement is observed; after removal from CO <sub>2</sub> chamber an additional 30 minutes of observation is required prior to placing the bag in the freezer or refrigerator to ensure death has occurred.
	Isoflurane	Animals are placed in a specially designed jar with an internal gauze holder that will prevent the animals from coming in contact with the anesthetic. The jar is placed in a fume hood or other scavenging device, and precharged with isoflurane soaked gauze (5% vaporizer). Animals are then placed in the jar and observed for at least 10 minutes after the cessation of movement. After removal from the jar the animals are observed for an additional minute to ensure that death has occurred.
Day15-Adult	CO <sub>2</sub> alone	Requires that animal remain in the CO <sub>2</sub> chamber for 30 minutes if a secondary method is not used. Animals must remain in the chamber for >2 min. after cessation of all movement is observed. After animals are removed from the chamber each animal must be visually observed for at least 1 minute to ensure death prior to disposal or placement in the freezer.
	CO <sub>2</sub> followed by cervical dislocation	Requires that animal remain in the CO <sub>2</sub> chamber >10 min. after cessation of all movement is observed; after removal from CO <sub>2</sub> chamber cervical dislocation is required.

AGE	<b>RECOMMENDED METHOD OF EUTHANASIA for NEONATES and ADULTS</b> <i>(In order of recommended use)</i>	NOTES
Day 15 - Adult	Cervical dislocation alone (see note)	Requires scientific justification; requires that individual demonstrate proficiency at this technique.
	Isoflurane followed by CO <sub>2</sub>	Requires that animal remain in the CO <sub>2</sub> chamber for 30 minutes if a secondary method is not used. Requires that animal remain in the CO <sub>2</sub> 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 30 minutes of observation prior to placing the bag in the freezer or refrigerator to ensure death has occurred.
Adult Rats	CO <sub>2</sub> alone	Requires that animal remain in the CO <sub>2</sub> chamber for 30 minutes and at least 2 additional minutes after cessation of all movement is observed. After animals are removed from the chamber each animal must be visually observed for an additional minute to ensure death prior to disposal. Rats may require longer exposure time than mice.
	Isoflurane followed by CO <sub>2</sub>	Requires that animal remain in the CO <sub>2</sub> chamber for 30 minutes. Requires that animal remain in the CO <sub>2</sub> chamber >2 min. after cessation of all movement is observed. After animals are removed from the chamber each animal must be visually observed for at least 1 minute to ensure death prior to disposal or placement in the freezer.

## 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 may be induced by CO<sub>2</sub>, wet ice or injectable anesthetics; the attending veterinarian must be consulted for appropriate agents and dosages.

### 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 without direct contact with the ice 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 without direct contact with the ice for 20 minutes. Remove to chilled PBS and open the thoracic and abdominal cavities.

AGE	RECOMMENDED METHOD OF EUTHANASIA for FIXATION or HARVEST <i>(In order of recommended use)</i>	NOTES
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 – wet ice	Animal is placed in a closed specimen cup (not petri dish) and submerged in an ice slurry without direct contact with the ice 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 [2013]. <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
2. Phifer CB, Terry LM. 1986. Use of hypothermia for general anesthesia in preweaning rodent. *Physiol & Behav* 38:887-890.
3. Pritchett K, Corrow D, Stockwell J, Smith A. 2005. Euthanasia of Neonatal Mice with Carbon Dioxide. *Comp Med.* 55:275-281.
4. Klaunberg B, O'Malley J, Clark T, Davis J. 2004. Euthanasia of Mouse Fetuses and Neonates. *JAALAS* 43:29-34.
5. American Veterinary Medical Association [AVMA] Public Statements: Report of the ACLAM Task Force on Rodent Euthanasia [August 2005]. [http://www.aclam.org/Content/files/files/Public/Active/report\\_rodent\\_euth.pdf](http://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf)
6. National Institutes of Health Animal Research Advisory Committee [NIH-ARAC] Guidelines for the Euthanasia of Rodent Feti and Neonates [February 1997].