

Guidelines for the Euthanasia of Rodent Feti and Neonates

The Report of the AVMA Panel on Euthanasia provides limited recommendations for the euthanasia of prenatal or neonatal animals. The 2007 report states: "When ovario-hysterectomies are performed, euthanasia of feti should be accomplished as soon as possible after removal from the dam." It also states "Neonatal animals appear to be resistant to hypoxia, and because all inhalant agents ultimately cause hypoxia, neonatal animals take longer to die than adults."¹ The following guidelines are suggested to assist Animal Care and Use Committees at the NIH in reviewing proposals which involve the use of rodent feti or neonates². In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

Feti (unborn animals that have not breathed):

- a. **Mouse, Rat and Hamster Feti up to 15 days' Guinea Pig Feti up to 34 days' gestation:** Neural development at this stage is minimal and pain perception is considered unlikely.^{3,4} 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.⁵
- 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 signals may be biochemically or neurophysiologically processed by the maturing neural circuitry.^{3,4,6} However, recent evidence implies that feti are neither sentient nor conscious prior to birth and thus incapable of actually perceiving pain.^{7,8,9} When feti are required for study, euthanasia of individual feti may be induced by decapitation with surgical scissors or cervical dislocation which are acceptable physical methods of euthanasia. Alternatively, if the mother is euthanized as described in "c" below, the uterus with the pups or the pups with the amniotic sac intact can be removed from the dam. This results in immediate cessation of uterine blood flow and oxygen delivery to the feti and rapid progression to an isoelectric EEG.⁸ If, at any point, the feti are allowed to breathe, then they must be decapitated or cervically dislocated. Rapid freezing, without prior anesthesia, as a sole means of euthanasia is not considered to be humane¹. Animals should be deeply anesthetized prior to freezing. When chemical fixation of the whole fetus is required, feti should be deeply anesthetized prior to immersion in, or perfusion with, fixative solutions. Anesthesia may be effectively induced by hypothermia of the fetus, which can be achieved by submerging the fetus (with the amniotic sac intact) in cold (4-8°C/35-
immobile.
- 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 arousal⁸. Recommended methods for euthanasia of the mother are CO₂ exposure followed by a secondary method of euthanasia, which may include cervical dislocation, decapitation or bilateral pneumothorax. Death of the mother must be verified after euthanasia and prior to disposal. The institute veterinarian should be consulted for considerations of other euthanasia agents.

Neonates (newborn animals that are breathing): 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 life¹⁰⁻¹⁴. Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO₂ is used as a euthanasia agent^{1,5,15}. A secondary physical method of euthanasia is recommended to ensure death (e.g. cervical dislocation, decapitation, bilateral pneumothorax). Death must be verified after euthanasia and prior to disposal¹⁴.

- 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., CO₂, halothane or isoflurane (used with appropriate safety considerations) although prolonged exposure may be necessary. A secondary physical method of euthanasia is recommended to ensure death (e.g. cervical dislocation, decapitation, bilateral pneumothorax). 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 institute 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 (however 3-4 days of age is more typical).^{16,17,18}
- b. **Guinea Pig Neonates:** Follow guidelines for adults¹.
- c. **Mouse, Rat and Hamster Neonates over 10 days of age:** 1.

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