# Guidelines for the Euthanasia of Rodent Fetuses and Neonates

The following guidelines are suggested to assist Animal Care and Use Committees at the NIH in reviewing proposals which involve the use of rodent fetuses or neonates. In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

The AVMA Guidelines for the Euthanasia of Animals, 2013 Edition states that **"**Scientific data indicate that mammalian embryos and fetuses are in a state of unconsciousness throughout pregnancy and birth." It also states that "The precocious young of guinea pigs remain insentient and unconscious until 75% to 80% of the way through pregnancy and remain unconscious until after birth due to chemical inhibitors" and "embryos and fetuses cannot consciously experience feelings such as breathlessness or pain. Therefore, they also cannot suffer while dying in utero after the death of the dam, whatever the cause." [1]

1. **Fetuses**

   a. **Mouse, Rat, Hamster, and Guinea Pig Fetuses to birth:** Recent evidence implies that fetuses are neither sentient nor conscious prior to birth and thus incapable of actually perceiving pain.[1,4,5,6] When fetuses (mouse, rat & hamster > E15, or Guinea pigs > E35) are required for study, euthanasia of individual fetuses 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 "b" below, the uterus with the pups or the pups with the amniotic sac intact can be removed from the dam. However, it will take 1 hour or longer before the fetuses are dead.[1] If, at any point, the fetuses are allowed to breathe, then they must decapitated or cervically dislocated. When chemical fixation of the whole fetus is required, fetuses should be euthanized 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$^{o}$C/35-39$^{o}$F) physiological saline until the fetus becomes completely immobile.

   b. When fetuses 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.[5] Recommended methods for euthanasia of the mother are $CO_2$ 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.

2. **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 life.[7-11] Resistance to hypoxia at this age results in a prolonged time to unconsciousness when $CO_2$ is used as a euthanasia agent.[1,3,12] 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 disposal.[11]

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., $CO_2$, or isoflurane from a vaporizer (used with appropriate safety considerations) although prolonged exposure, up to 50 minutes[1], may be necessary. A secondary physical method of euthanasia is recommended to ensure death (e.g. cervical dislocation, decapitation, bilateral pneumothorax). "Fetuses that are believed to be unconscious and altricial neonates < 5 days of age ... may be quickly killed by rapidly freezing in liquid N2."[1] For neonates 5 days or greater, immersion in liquid nitrogen may be used only if preceded by anesthesia. 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).[13,14,15]

b. **Guinea Pig Neonates:** Follow guidelines for adults.[1]

c. **Mouse, Rat and Hamster Neonates over 10 days of age:** Follow guidelines for adults.[1]

**References**


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