Guidelines for Euthanasia of Rodent Fetuses and Neonates

The following guidelines and general references are suggested to assist NIH intramural Animal Care and Use Committees in reviewing proposals which involve the use of rodent fetuses or neonates.[1-22] 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." [2] There is also literature which indicates that there is the likelihood that pain may be perceived relative to the neural development in mouse, rat and hamster fetuses over 15 days, and in guinea pig fetuses over 35 days and that behavioral responses to sensory stimulation do occur. [18-22]

1. **Fetuses - Mouse, Rat, Hamster, and Guinea Pig Fetuses to Birth:** When fetuses (mouse, rat & hamster > E15, or guinea pigs > E35) are required for study, euthanasia of individual fetuses may be induced by acceptable physical methods of euthanasia, such as decapitation with surgical scissors or cervical dislocation. Although physical methods are considered the most effective and humane because of the speed, intraplacental injection of pentobarbital may be justified for procedures which require preservation of anatomical and histological mouse fetal structures or avoid hypoxia associated with physical methods. [5]

   When fetuses are not required for study, the method chosen for euthanasia of a pregnant dam should ensure rapid cerebral anoxia to the fetus with minimal disturbance to the uterine milieu minimizing fetal arousal.[7] Methods for euthanasia of the dam should follow recommendations of the ARAC Guidelines for Euthanasia of Rodents Using Carbon Dioxide and the AVMA Guideline for the Euthanasia of Animals.

   Alternatively, if the mother is euthanized as described above, the uterus with the pups or the pups with the amniotic sac intact can be removed from the dam, however, it may take 1 hour or longer before the fetuses are dead.[8] If, at any point, the fetuses are allowed to breathe, then they must be euthanized by methods which may include decapitation, cervical dislocation, hypothermia, anesthesia followed by exsanguination, or chemical anesthetic overdose. If believed to be unconscious, fetuses may be immersed in liquid nitrogen. When chemical fixation of the whole fetus is required, fetuses must be euthanized prior to immersion.

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.[10-14] Resistance to hypoxia at this

age results in a prolonged time to unconsciousness when CO_2 is used as a euthanasia agent.[2,4,15] If CO_2 is used, a secondary physical method of euthanasia is required to ensure death (e.g. cervical dislocation, decapitation, bilateral pneumothorax). Death must be verified after euthanasia and prior to disposal. [14]

3. **Fetuses and Altricial Neonates (note NOT Guinea Pigs)**: Hypothermia is an acceptable method of euthanasia for fetuses and altricial neonates up to 7 days of age as long as direct contact with ice/cold surfaces is avoided. Injectable anesthetic agent overdose can also be used.

4. **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, or isoflurane from a vaporizer (used with appropriate safety considerations) although prolonged exposure, up to 50 minutes may be necessary. [2,15-17] A secondary physical method of euthanasia is recommended to ensure death (e.g. cervical dislocation, decapitation, bilateral pneumothorax). Death must be verified prior to disposal. "Fetuses that are believed to be unconscious and altricial neonates <5 days of age may be quickly killed by rapidly freezing in liquid nitrogen"[2] For neonates 5 days or greater, immersion in liquid nitrogen may be used only if preceded by anesthesia.
5. **Guinea Pig Neonates:** Follow guidelines for adults.[2]
6. **Mouse, Rat and Hamster Neonates over 10 days of age:** Follow guidelines for adults.[2]

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