Guidelines for the Euthanasia of Rodent Feti and Neonates

1. 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 neonates[2]. In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

2. **Feti:** At approximately 60 percent of the gestation period, the neural tube has developed into a functional brain and the likelihood that a fetus may perceive pain should be considered[3,4]. Reflexive behavior in response to painful stimuli has been observed in feti and correlates with adult behaviors[5]. However, fetal behavioral arousal and awareness may be suppressed by low arterial oxygen limiting higher cortical processing[6].

   a. **Mouse, Rat and Hamster Feti up to 15 days' and Guinea Pig Feti up to 34 days' gestation:** Neural development at this stage is minimal and pain perception is considered unlikely[7,8]. 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 [9].

   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 may be perceived[4,7,8]. When feti are required for study, euthanasia of individual feti may be induced by the skillful injection of chemical anesthetics. Decapitations with surgical scissors or cervical dislocation are acceptable physical methods of euthanasia. Rapid freezing, without prior anesthesia, as a sole means of euthanasia is not considered to be humane[1]. 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 induced by hypothermia of the fetus[10,11], or by injection of the fetus with a chemical anesthetic[12]. If hypothermia is used, the feti should not come in direct contact with the cold agent. The institute veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. Feti at this age are resistant to hypoxia[13] and require extended exposure to inhalant anesthetics, including CO_2 [9].

   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 arousal[6]. Recommended methods for euthanasia of the mother are CO_2 exposure with or without cervical dislocation. 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.

3. **Neonates:** 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[14-17]. Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO_2 is used as a euthanasia agent[1,9, 19]. 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[18].

   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, 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).[10,11]

   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].

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