General Information

Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. PHS policy requires Institutional Animal Care and Use Committees (IACUCs) to determine that methods of euthanasia utilized in research proposals are consistent with the recommendations of the 2000 Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia.

The criteria used as the basis for the Panel’s recommendations include:

1. Minimum pain, distress, anxiety or apprehension
2. Minimum delay until unconsciousness
3. Reliability and irreversibility
4. Safety of personnel; emotional effect on personnel
5. Species and age limitations

Principal Investigators are responsible for ensuring all personnel performing animal euthanasia have been properly trained to consistently apply the technique(s) in a humane and effective manner.

Euthanasia for Rodents of >10 Days Old

THE PERSON PERFORMING THE EUTHANASIA MUST BE FULLY TRAINED IN THE APPROPRIATE PROCEDURES.

Euthanasia must be performed in a compassionate manner to avoid animal distress. Depending on the species involved, some animals being euthanized may vocalize, release pheromones or behave in a manner, which may be distressing to other animals. For those reasons, animals should not be euthanized while crowded or in the presence of animals not being euthanized.

Intact animals: CO₂ inhalation using compressed CO₂ gas in cylinders, inhalant anesthetic overdose using (halothane, enflurane, isoflurane, sevoflurane, and desflurane) or barbiturates at 150mg/kg of body weight.

Additional Recommended Practices to Ensure Death in Rodents:

Death must be verified after euthanasia and prior to disposal. Confirmation of death is verified by absence of respiration, heartbeat and toe/tail pinch reflexes. However, the assessment of heart beat, respiratory pattern can be very difficult in rodents due to their small size. Consequently, these criteria may be difficult to apply to these species and there is a risk of animals recovering. The following additional steps are recommended after euthanasia using inhalant or non-inhalant pharmaceutical agents to ensure that animals are euthanized:
1. Gas flow should be maintained for at least 1 minute after apparent clinical death.
2. Euthanized animals must be monitored closely for 10 minutes before bagging and transferring to the freezer.
3. To ensure the irreversibility of the procedure after apparent death, it is recommended that animals may further undergo a physical method of euthanasia (i.e., exsanguinations, thoracotomy, cervical dislocation or decapitation).

**Anesthetized, laparotomized (non survival surgery) animals:**

While animals are fully anesthetized, they should be euthanized using one of the following methods of physical euthanasia:
Exsanguinations - i.e., great vessels severed, cardiac perfusion, removal of vital organs
Incision of the chest cavity or diaphragm to produce a pneumothorax (collapsed lung) and cessation of respiration. Decapitation or cervical dislocation

**Method of euthanasia**

The AVMA Panel categorizes each method of euthanasia as acceptable (methods which consistently produce a humane death when used as the sole means of euthanasia), conditionally acceptable (methods which by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature) and unacceptable (methods deemed inhumane under any conditions or that the panel found posed a substantial risk to the human applying the technique). Inhalant, noninhalant pharmaceutical agents or physical methods can be used for euthanasia.

IACUC approval of such deviations must be project-specific and include critical review of assertions of scientific necessity. If conditionally acceptable techniques are planned, they must be scientifically justified and approved by the IACUC prior to implementing.

**Inhalant Agents**

**Carbon dioxide** (Acceptable)
Carbon dioxide has a rapid depressant, analgesic and anesthetic effect. Carbon dioxide is nonflammable, nonexplosive, and poses minimal hazard to personnel when used with properly designed equipment. Because CO₂ is heavier than air, incomplete filling of a chamber may permit animals to climb or raise their heads above the higher concentrations and avoid exposure.

High concentrations of CO₂ may be distressful to some species. Accordingly, pre-filling the chamber is recommended only under circumstances in which such use has not been shown to cause distress.
Chambers must not be overcrowded (maximum of 20 mice and 5 rats per chamber). In this regard, it is important to also consider that mixing unfamiliar or incompatible animals in the same container may be distressful. Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (i.e., antacids) is unacceptable.

Death must be verified after euthanasia and prior to disposal. Unintended recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times or by other means. To ensure the irreversibility of the procedure after apparent death from CO₂, animals may further undergo a physical method of euthanasia (i.e., exsanguinations, thoracotomy, cervical dislocation or decapitation).

**Inhalant Anesthetics (Acceptable)**

Inhalant anesthetics are particularly valuable for euthanasia of smaller animals or for animals in which venipuncture may be difficult. Since the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors and prevented from contacting the anesthetic agent in its liquid form. In order of preference, halothane, enflurane, isoflurane, sevoflurane, and desflurane are generally acceptable for euthanasia of small animals (< 7 kg). Halogenated anesthetic agents should only be used if they are appropriately scavenged to avoid personnel exposure, i.e., fume hoods or exhausted biosafety cabinet class II, type B.

**Inhalant Anesthetics (Conditionally Acceptable)**

*Ether* is irritating to the mucous membranes and poses serious risks associated with its flammability and explosiveness. Explosions have occurred when animals, euthanatized with ether, were placed in an ordinary (not explosion proof) refrigerator or freezer and when bagged animals were placed in an incinerator. Ether should only be used after IACUC approval in carefully controlled situations in compliance with all applicable safety policies and regulations.

**Noninhalant Pharmaceutical Agents**

**Barbiturates (Acceptable)**

A primary advantage of barbiturates is speed of action, which depends on the dose, concentration, route, and rate of injection. Barbiturates induce euthanasia smoothly, with minimal discomfort to the animal. Intravenous injection is the preferred route of administration, however intraperitoneal injections may be used in situations when intravenous injections would be distressful or impractical. Intracardiac injection must only be used if the animal is heavily sedated, unconscious, or anesthetized. Barbiturates may be administered intraperitoneally and induce rapid, smooth euthanasia with minimal animal discomfort. As with all controlled substances, barbiturate usage requires having
appropriate licensure and registration, ensuring secure storage and maintaining accurate
drug accountability.

**Potassium chloride in conjunction with general anesthesia (Acceptable)**

Although unacceptable when used in unanesthetized animals, the use of potassium
chloride administered intravenously or intracardially in animals under general anesthesia
is an acceptable method of euthanasia. It is important for personnel performing this
method of euthanasia to be trained and knowledgeable in anesthetic techniques, and
competent in assessing anesthetic depth. Administration of potassium chloride requires
animals to be in a surgical plane of anesthesia characterized by loss of consciousness,
loss of reflex muscle response, and loss of response to noxious stimuli.

**Physical Methods (Conditionally Acceptable)**

Physical methods of euthanasia when properly used by skilled personnel with well
maintained equipment, may result in less fear and anxiety and be more rapid, painless,
humane, and practical than other forms of animal euthanasia. Exsanguinations and
stunning are not recommended as sole means of euthanasia, but should be considered
adjuncts to other agents or methods. Personnel performing physical methods of
euthanasia must be well trained and monitored for each type of physical euthanasia
technique performed. Since most physical methods involve trauma, there is inherent risk
for animals and humans, therefore extreme care and caution should be used. Methods not
performed correctly can result in animal and personnel injuries. Inexperienced persons
should be trained by experienced persons and should practice on carcasses or
anesthetized animals to be euthanatized until they are proficient in performing the method
properly and humanely.

**Cervical Dislocation (Conditionally Acceptable)**

Cervical dislocation is a technique that is rapidly accomplished and can induce rapid loss
of consciousness without chemically contaminating tissue but requires technical
proficiency. Manual cervical dislocation is a humane technique for euthanasia of
rodents weighing ≤200 g when performed correctly. In lieu of demonstrated technical
competency, animals must be anesthetized prior to cervical dislocation.

**Decapitation (Conditionally Acceptable for adult rodents)**

Decapitation is a technique that is rapidly accomplished and appears to induce rapid loss
of consciousness without chemically contaminating tissues. Personnel performing this
 technique should recognize the inherent danger of the guillotine or other sharp
instruments and take adequate precautions to prevent personal injury. This method of
euthanasia should be used only when its use is required by the experimental design and
approved by the IACUC (except for animals younger than 14 days old). The equipment
used to perform decapitation should be maintained in good working order and serviced
on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain
animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine. Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so.

**Unacceptable Methods (all procedures are used alone)**

Air embolism, blow to the head, burning, chloral hydrate, chloroform, cyanide, decompression, drowning, exsanguinations, formalin, household products and solvents such as acetone, quaternary compounds (including CCl4), laxatives, clove oil, dimethylketone, quaternary ammonium products (i.e., Roccal D Plus), antacids, and other commercial and household products, hypothermia, neuromuscular blocking agents, rapid freezing, strychnine, stunning.

**Any exceptions to the above policy must have IACUC and Veterinary approval.**

**References:**


Euthanasia of Rodent Feti and Neonates Under 10 Days Old


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1. The Report of the AVMA Panel on Euthanasia provides limited recommendations for the euthanasia of prenatal or neonatal animals. The 2000 report states: “When ovarian 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. 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 (2, 3). Reflexive behavior in response to painful stimuli has been observed in feti and correlates with adult behaviors (4). However, fetal behavioral arousal and awareness may be suppressed by low arterial oxygen limiting higher cortical processing (5).

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 (6, 7). 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 (8).

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 (3, 6, 7). 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 anesthetized prior to freezing. When chemical fixation of the whole fetus is required, feti should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus (9, 10), or by injection of the fetus with a chemical anesthetic (11). The institute veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. Feti at this age are resistant to hypoxia (12) and require extended exposure to inhalant anesthetics, including CO2 (8).

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 (5). Recommended methods for euthanasia of the mother are CO2 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 (13-16). Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO2 is used as a euthanasia agent (1, 8). Death must be verified after euthanasia and prior to disposal (17).

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., halothane or isoflurane (used with appropriate safety considerations) although prolonged exposure may be necessary. 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 (9, 10).

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).

Any exceptions to the above policy must have IACUC approval.

References