Recommended Methods of Euthanasia for Laboratory Animals

Euthanasia derives from the Greek eu or good, and thanatos or death. Euthanasia is the act of painlessly terminating life. The criteria that have been used for determining that death is painless are rapid loss of consciousness followed by cardiac and respiratory arrest. There are additional criteria that should be considered when euthanizing research animals including the emotional effect the technique has on the person(s) performing the method, and the resultant effect on the tissues or samples that may be collected post mortem. There are a variety of acceptable methods that can be used for each species. The advantages and disadvantages of each technique should be considered in selecting the most appropriate method for a particular project. These guidelines review techniques which are recommended by the University's Institutional Animal Care and Use Committee (IACUC) and the American Veterinary Medical association's (AVMA) Panel on Euthanasia. Additional methodologies are available. Consult the "2000 Report of the AVMA Panel on Euthanasia" which is available in the ACS administrative offices in CB-159.

ACS veterinary staff are available to demonstrate and/or discuss these techniques.

Suggested Techniques

1. Carbon dioxide (C02)

C02 followed by cervical dislocation or opening of the chest cavity are the preferred techniques for euthanizing rodents and other small laboratory animals. C02 can be used with a tank and airtight chamber. The only acceptable source is compressed CO2 in cylinders. Ninety to 100% CO2 should be utilized for euthanasia. It is imperative that the animals be placed in the chamber for sufficient time, as C02 initially anesthetizes the animal causing it to be unconscious and unresponsive. You should verify by observation that the animal is not breathing and palpate for lack of a heartbeat. Neonates require prolonged exposure to the gas because their hemoglobin has a higher affinity for oxygen and may require 15 minutes or more of exposure to ensure death. C02 is not recommended for euthanasia of amphibians and reptiles.

All ACS managed facilities contain a C02 chamber for rodent euthanasia except in the metabolic building at VMC and Progress Center. Please review instructions on or near the chamber for proper use or request assistance from ACS personnel.

Animals should remain within the chamber for approximately 5-10 minutes. Death should be verified by lack of breathing and heartbeat. This can be verified by observing for the absence of respiration and palpation of the heartbeat by placing your thumb and index finger on opposing sides of the animal’s chest. As stated above, neonates require considerably longer periods of gas exposure and should lose their pink color before death is recognized. For additional assurance, cervical dislocation or opening of the chest cavity provides a physical verification of euthanasia.

2. Barbituric Acid Derivatives Sodium

Pentobarbital is the most common barbiturate agent for euthanasia. Barbiturates depress the CNS producing sequential unconsciousness, deep anesthesia, apnea, and cardiac arrest. Dosage for euthanasia (120 mg/kg) is usually at least twice that required for anesthesia. Concentrated formulations are available and are recommended for euthanasia. The following chart provides recommended routes for administration by species:
### Species Route of Administration

- **mouse**: IP
- **rat**: IP
- **guinea pig**: IP
- **hamster**: IP
- **rabbit**: IV (lateral ear vein)
- **cat**: IV (cephalic or saphenous veins)
- **dog**: IV (cephalic or saphenous veins)
- **swine**: IV (ear vein)
- **primate**: IV (after immobilization with ketamine IM)

Sodium pentobarbital is a Schedule II drug which is regulated by the Federal Drug Enforcement Agencies (DEA). Federal regulations require you to maintain records which include the date, purpose and amount of use. Investigators who require routine use of controlled substances are encouraged to obtain their own DEA permit. Euthanasia agents should only be administered before their expiration date. All expired agents should be disposed of as instructed by the DEA.

#### 3. Inhalational Anesthetics

Overdose with isoflurane, halothane, or other types of inhalational anesthetics in an appropriate bell jar is an acceptable method of euthanasia for rodents and other small animals. Inhalational anesthetics should be used in a fume hood or with appropriate scavenging as they may be toxic to personnel. As with CO2, death should be confirmed by verifying the absence of respiration and a heartbeat. The use of ether is prohibited in ACS facilities (see special considerations below).

#### 4. Cervical Dislocation

Cervical Dislocation is a conditionally acceptable technique for euthanizing mice and rats weighing less than 200 g when scientifically or clinically justified. This technique must be approved by the IACUC during protocol review. Personnel must demonstrate proficiency with this technique.

#### 5. Decapitation by Guillotine

Decapitation by guillotine is a conditionally acceptable technique for euthanasia of rodents when scientifically or clinically justified. This technique must be approved by the IACUC during protocol review. Personnel must demonstrate proficiency. Decapitation is a potential hazard to personnel. Additionally, many animal species react adversely to the smell of blood. Animals should not be decapitated in the presence of other animals and the investigator should wash his/her gloved hands and the guillotine between animals. The blade on the guillotine should be sharpened regularly.

#### 6. Potassium chloride

Overdosage with KCl is permissible only in anesthetized animals. Rapidly rising serum potassium levels result in cardiac arrest.
7. Exsanguination

Rabbits and larger laboratory animals may be exsanguinated to collect large volumes of blood or blood products. Animals must be anesthetized to reduce the distress associated with hypovolemia.

Special Considerations

Death verification regardless of the technique utilized; it is essential that death be confirmed. Many of the agents utilized induce deep anesthesia prior to death. Therefore the animal must be carefully evaluated for absence of respiration and heartbeat. Additional signs that can be used to assess death in larger animals include dilated and fixed pupils and lack of the corneal reflex. ACS policy must be followed for carcass disposal. Animals must be placed in leak-proof bags and placed in an appropriate morgue. Animals exposed to hazardous agents may require special identification and storage. Please contact the ACS @ 2-2977 for additional information.

Ether Use

Ether is not permitted in ACS facilities because it is extremely flammable and explosive. It should be stored in an explosion proof refrigerator, chemical fume hood, or flammable liquids cabinet if used in investigator’s laboratories. Animals should not be routinely sacrificed with ether because of safety concerns regarding carcass disposal.

Poikilotherm euthanasia

Differences in amphibian and reptile metabolism, respiration, and tolerance to cerebral hypoxia may preclude some euthanasia techniques that would be acceptable in homeotherms. Pentobarbital or MS-222 (tricaine methanesulfonate; pH balanced - 7.0) overdose is recommended. Amphibians should be anesthetized if physical euthanasia methods such as pithing or decapitation are utilized. The animals may be chilled in a 4oC refrigerator until torpid to aid in handling if anesthesia is precluded because of post mortem effects. Pithing of the brain should follow decapitation, as brain death may not be immediate.