EUTHANASIA GUIDELINES

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Background

According to the Guide for the Care and Use of Laboratory Animals (Guide) and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy), euthanasia of animals must use appropriate techniques that are consistent with the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. UC Berkeley's Office of Laboratory Animal Care (OLAC) and Animal Care and Use Committee (ACUC) strictly adhere to these guidelines. A copy of the most current AVMA Guidelines is available on the ACUC website.

The purpose of this document is to provide additional clarification and information for methods of euthanasia commonly used in animals at UC Berkeley. Recommended euthanasia techniques for small mammals (e.g., rodents), birds, and ectotherms as well as prenatal and neonatal animals are outlined below.

Training

Only trained individuals may perform euthanasia. Individuals who receive ACUC approval for euthanasia must have their technical competence certified by authorized OLAC staff in advance of performing the procedure independently.

For supplementary information, assistance, or certification/training in any euthanasia method, please contact OLAC at 642-9232.

Minimizing Pain and Distress

Pain and distress prior to and during euthanasia should be avoided as much as possible. Regardless of the technique used, the animal should be carefully handled and/or gently restrained in an appropriate manner prior to euthanasia.

General Considerations

Following euthanasia and prior to carcass disposal, an additional physical means of ensuring euthanasia must be performed. These physical methods vary by species but may include cervical dislocation for small rodents, bilateral thoracotomy, decapitation, exsanguination, double pithing for amphibians and reptiles, freezing for small ectotherms, or another AVMA-approved method. These must occur after the animal has been rendered non-responsive to noxious stimuli by the primary euthanasia agent.

Rodents and Other Mammals

Chemical Methods

A. Pentobarbital-based Euthanasia Solution

1. Chemical euthanasia with an overdose of intravenous pentobarbital-based euthanasia solution is the preferred euthanasia method for mammals with practical vascular access (e.g., rabbits).
2. Intraperitoneal pentobarbital is acceptable for smaller mammals when intravenous access is not practical.

B. Isoflurane Inhalation

1. The preferred method of delivering isoflurane is via precision vaporizer. These should be calibrated per the manufacturer's recommendation (no less than every 3 years). At the time of calibration, the associated flow meters, valves, and scavenging systems should also be tested and refurbished if necessary.
2. . When using an open-drop system (application of liquid isoflurane to an absorbent material which is then placed into the bottom of the chamber), the animal must be separated from the absorbent material by a physical barrier (e.g., screen or platform). If using an open drop system, isoflurane should be adequately scavenged to prevent personnel exposure.

C. Carbon Dioxide (CO_2) Inhalation

1. Compressed CO_2 gas in cylinders is the only allowable source of carbon dioxide because the inflow to the chamber can be regulated precisely. CO_2 delivery must be monitored to ensure CO_2 does not displace air by more than 10-30% of the chamber volume per minute. CO_2 generated by other methods (e.g., dry ice) is unacceptable.

2. Procedure:

    a) Euthanasia of animals in the home cage is preferred. Animals must never be euthanized in an overcrowded cage or with unfamiliar individuals.

    b) Remove lid and wire/wire bar and place euthanasia lid over cage.

    c) CO_2 will be delivered from a pressurized tank into a cage. The flow rate will be set to displace 10-30% of the chamber or cage volume/minute.

    d) Animals will be monitored for cessation of respiration and will remain in chamber for at least an additional 60 seconds after respiration has ceased.

    e) Never leave a euthanasia chamber with flowing gas unattended.

Physical Methods

A. Unless an exception is approved by the ACUC, physical methods of euthanasia must be performed under anesthesia. Scientific justification for performing a physical method of euthanasia without anesthesia and documentation of experience and/or training of the person(s) who will be performing the procedure must be provided in the AUP.

B. Cervical Dislocation

1. When properly performed by trained personnel, manual cervical dislocation is a humane technique for euthanasia of mice, other small rodents, and rats weighing less than 200 grams.

2. The AVMA Guidelines on Euthanasia require anesthesia for animals undergoing cervical dislocation unless the individual performing the task is highly proficient.

C. Decapitation

1. Decapitation when performed properly is virtually instantaneous and is considered humane.

2. Guillotines that are designed for decapitation in adult rodents are commercially available. These guillotines are recommended for all animals and must be used for any animals greater than 50 grams. Please refer to ACUC Guidelines on Guillotine Maintenance for additional information.

3. Alternatively, regularly sharpened surgical scissors may be used for smaller animals, such as neonates and mice, if described in an AUP and approved by the ACUC. Scissor blades must be rust-free, sharp, and decapitate with minimal force.

Rodent Neonates and Fetuses

A. Neonates (up to 10 days of age)

Euthanasia of rodent neonates is as for adults described in the above methods. Neonates of altricial rodents (e.g. mice, rats, hamsters) are relatively resistant to the effects of CO_2; therefore, primary CO_2 euthanasia of this age group is not recommended. Anesthesia via isoflurane or CO_2 followed by decapitation is the preferred method for euthanasia of neonates up to 10 days of age. Any exceptions must be scientifically justified in an AUP and approved by the ACUC.

B. Fetuses

1. For mouse, rat, and hamster fetuses up to 15 days of gestation, and guinea pig fetuses up to 35 days of gestation – euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to blood loss and non-viability, thus no additional method of euthanasia is required.
2. For mouse, rat, and hamster fetuses >15 days of gestation, and guinea pig fetuses >35 days of gestation - after general anesthesia or euthanasia of the mother, decapitation of the fetus must be performed.

Non-Mammalian Vertebrates, including Birds, Reptiles, and Amphibians

Chemical Methods

A. An overdose of pentobarbital-based euthanasia solution can be used to euthanize ectotherms and birds via intracoelomic/intraperitoneal injection. The actual time to death may be prolonged for ectotherms.

B. pH-neutralized pharmaceutical grade tricaine methanesulfonate (MS-222) immersion may be used to euthanize fish, aquatic and semi-aquatic amphibians. Reptiles may be euthanized by intra-coelomic (IC) injection of 250-500 mg/kg pH-neutralized 1% MS-222, followed by 0.5-1 ml 50% unbuffered MS-222 (Conroy et al, 2009).

C. Benzocaine immersion may also be used to euthanize fish, aquatic and semi-aquatic amphibians.

D. Small birds may be euthanized with carbon dioxide inhalation, but all recommendations described above for rodents must be followed.

Physical Methods

A. Freezing – Rapid freezing following prolonged immersion in MS-222 and verification of cessation of respiration is acceptable. Freezing is NOT allowed as a sole method of euthanasia. Lowering the ambient temperature may facilitate handling; however, there is no evidence that it

raises the pain threshold in ectotherms. Formation of ice crystals on the skin and in the tissues of an animal may cause pain and distress.

B. Rapid Chilling – It is acceptable for zebrafish (*Danio rerio*) to be euthanized by rapid chilling (2° to 4°C, until loss of orientation and operculum movements and subsequent holding times in ice-chilled water specific to size and age. Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4-7 days post-fertilization (dpf) for at least 20 minutes following loss of operculum movement.

C. Decapitation – The central nervous system of reptiles is extremely tolerant to anoxia. Therefore, methods of euthanasia that induce unconsciousness by interruption of blood supply to the head (e.g., decapitation, cervical dislocation, and exsanguinations) are inappropriate for reptiles when used alone. These methods can only be used on small reptiles that are already unconscious by a chemical agent or concussion, or when followed immediately with double-pithing, or freezing in liquid nitrogen or dry ice.

D. Double-pithing – Double-pithing can only be carried out on unconscious animals and performed by trained personnel.

<u>Birds and Reptiles: Hatchlings, Embryos, and Eggs</u>

A. Hatchlings

1. Avian and reptile embryos that hatch, either intentionally or unintentionally, are live vertebrate animals and are regulated by PHS Policy. For this reason, any project in which bird or reptile eggs may hatch must include a contingency plan for humane euthanasia of hatchlings in the AUP.

2. Acceptable methods of euthanasia of avian or reptile hatchlings include overdose of CO_2 or anesthetic agents. A secondary physical method is required to ensure death, for example decapitation, cervical dislocation (birds) or decapitation/pithing (reptiles).

B. Embryos & Eggs

1. For embryos/eggs >50% gestation, use methods appropriate for hatched birds or reptiles (see above).

2. For embryos/eggs <50% gestation, prior to neural tube formation (Close et al, 1997; AAZV guidelines), destroy the viability of the egg via one of the following methods:

a) Shaking
b) Puncturing
c) Freezing (<4°C for 4 hours)
d) Coating eggs with oil

Amphibians and Fish: Larvae and Embryos

A. Larvae –Acceptable methods of euthanasia for larvae of these species are the same as for adult amphibians and fish as described above (i.e., chemical agent followed by secondary physical method).

B. Embryos – Amphibians and fish are embryos prior to the larval stage (i.e., up to hatched embryos being able to independently feed), and therefore have yet to develop pain systems (AHAW Panel Report). Embryos of these species are disposed of depending on their experimental treatment and transgenic status, but care should be taken to recognize the variation among these species of the stage at which independent feeding begins. Use of rapid chilling and buffered MS-222 alone have been shown to be unreliable euthanasia methods for zebrafish embryos <3 dpf. To ensure embryonic lethality, immersion in sodium hypochlorite is recommended after immersion in MS-222 has been completed.

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