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**STANDARD OPERATING PROCEDURE**

## EUTHANASIA GUIDELINES FOR RODENTS

Approval(s):

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Chair, IACUC

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Date

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Executive Director, IACUC

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Exec Director, DLAR

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Clinical Director, DLAR AV

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### 1) SCOPE

Rodent euthanasia must be performed by trained personnel using appropriate techniques, equipment, and agents. Death must be induced as quickly and painlessly as possible. Upon completion of the procedure, death **must** be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest via physical methods. Euthanasia should not be performed in the animal room or in view of conspecifics. The euthanasia method must be appropriate to the species, approved in the protocol by the IACUC and be performed by properly trained individuals.

CO<sub>2</sub> inhalation is the most common method of euthanasia used for mice, rats, guinea pigs and hamsters. This SOP describes important aspects of this procedure. This SOP also provides guidelines for the euthanasia of rodent fetuses or neonates.

### 2) PROCEDURES FOR EUTHANASIA OF ADULT MICE, RATS and other rodents.

#### **CO<sub>2</sub> Euthanasia:**

- A. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber. All animals in the chamber must be able to make normal postural adjustments.
- B. Compressed CO<sub>2</sub> gas in secured cylinders is the only recommended source of CO<sub>2</sub> as it allows the inflow of gas to the induction chamber in a controlled manner. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% CO<sub>2</sub> at a displacement rate of 50% to 55% of the chamber volume per minute. This rate of CO<sub>2</sub> introduction will minimize the distress for the animals. Sudden exposure of conscious animals to CO<sub>2</sub> concentrations of 70% or greater have been shown to be distressful. After the animals become unconscious, the flow rate can be increased to minimize the time to death. Depending on the euthanasia equipment setup, either the flow meter setting or the flow gauge component of the pressure regulator must be adjusted to control the euthanasia chamber displacement rate between 50 - 55%. Refer to the following link to ensure proper [CO<sub>2</sub> euthanasia equipment recalibration](#).

- C. Animals must be left in the container until clinical death has been ensured by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting fixed and dilated pupils. Then a physical method to assure euthanasia needs to be performed.
- D. Expected time to unconsciousness is usually within 2 minutes (6). Observe each rodent for lack of breathing and faded eye color. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO<sub>2</sub>. If unconsciousness has not yet occurred within two minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO<sub>2</sub> supply, and/or leaks. Appropriate CO<sub>2</sub> concentrations and exposure times will prevent unintended recovery. Again, death must be verified after euthanasia and prior to disposal.
- E. Neonatal animals (up to 14 days of age) are resistant to the effects of CO<sub>2</sub>, therefore additional alternative methods are mandatory to assure euthanasia is complete (see below).
- F. To ensure death in an animal following CO<sub>2</sub> exposure, another method of euthanasia (e.g. decapitation, thoracotomy, perfusion etc.) must be added while the animal is under CO<sub>2</sub> narcosis.

**Other methods of euthanasia:**

- A. Other commonly used methods of euthanasia include inhalant anesthetics (e.g. isoflurane), non-inhalant pharmaceutical agents (e.g. sodium pentobarbital); the institutional veterinarian should be consulted for appropriate agents and dosages. Physical methods (i.e. cervical dislocation, decapitation, and thoracotomy) must be scientifically justified to obtain IACUC approval, but are acceptable when used as an additional or secondary method of euthanasia.

3) PROCEDURES FOR EUTHANASIA OF FETUSES AND NEONATES

**Fetuses:**

- A. Fetuses up to 14 days in gestation: Neural development at this stage is minimal and pain perception is considered unlikely. 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 fetuses at this stage of development.
- B. Fetuses 15 days in gestation to birth: the literature on the development of pain pathways suggests the possibility of pain perception at this time. Whereas fetuses at this age are resistant to inhalant anesthetics including CO<sub>2</sub>, euthanasia 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. Animals should be anesthetized prior to freezing. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus, by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g., pentobarbital. The institutional veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.

**Neonates:**

- A. Birth up to 14 days of age: Acceptable methods for the euthanasia of neonatal mice and rats include: overdose 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).

Neonatal animals up to 14 days of age are resistant to the effects of CO<sub>2</sub>, therefore alternative methods are recommended. CO<sub>2</sub> may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (i.e. decapitation by sharp blades).

**Use of any inhalant euthanasia agent requires a secondary physical method to assure irreversibility of the euthanasia and is to be described in the approved IACUC protocol.**

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 institutional 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.

- B. Older than 14 days: Follow guidelines for adult rodents (see above).

In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

**Assurance of Death:**

Death of fetuses and neonates should be assured subsequent to the euthanasia procedure used. This can be done via assessment of physiological indicators (absence of spontaneous movement, respirations and heartbeat/pulse). As these parameters can sometimes be difficult to accurately evaluate in animals of this size, investigators must use physical methods post euthanasia such as cervical dislocation, decapitation or perform thoracotomies on animals after the primary euthanasia method is employed.

4) APPROVAL

The University of Pittsburgh's Institutional Animal Care and Use Committee has reviewed and approved this SOP as attested by the signature of the Committee Chairperson.