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### **1. PURPOSE**

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This Standard Operating Procedure (SOP) describes acceptable procedures for rodent euthanasia. It meets the AVMA 2021 guidelines for euthanasia and ensures that animals are euthanized in the most humane way possible, minimizing pain and distress while ensuring compatibility with research objectives.

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### **2. RESPONSIBILITY**

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Principal investigator (PI) and their research staff.

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### **3. GENERAL CONSIDERATIONS**

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- 3.1. All animal euthanasia must be performed by appropriately trained personnel.
  - 3.2. Euthanasia procedures should not be performed in the same room where rodents are housed.
  - 3.3. All euthanasia procedures must be continuously monitored by the person(s) performing the procedure until confirmation of euthanasia is complete.
  - 3.4. Animals must not be left unattended until they die.
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### **4. MATERIALS**

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- 4.1. CO<sub>2</sub> euthanasia station.
  - 4.2. General anesthetic or commercial euthanasia solution.
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### **5. CHEMICAL METHODS FOR ADULT RODENT**

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#### **5.1. CO<sub>2</sub> asphyxiation (First method of choice):**

- 5.1.1. To minimize pain and distress, it is preferable to anesthetize rodents with isoflurane before exposure to CO<sub>2</sub>.
- 5.1.2. To minimize stress, animals should be euthanized in their home cage. The maximum cage density must be respected, and do not pool animals from different cages.
- 5.1.3. Neonatal animals (up to 10 days of age) are resistant to hypoxia-induced by high anesthetic gas concentrations and exposure to CO<sub>2</sub>; therefore, alternative methods are recommended.
- 5.1.4. The placement of animals into chambers pre-filled with carbon dioxide is unacceptable.
- 5.1.5. Procedure:

- 5.1.5.1. Place the animal cage in the euthanasia station.
- 5.1.5.2. Open the CO<sub>2</sub> tank and set the flowmeter to a 2% flow rate (ensuring a gradual-fill rate of less than 40% and greater than 30% of the chamber volume per minute).
- 5.1.5.3. Maintain the CO<sub>2</sub> flow until the animal loses consciousness.
- 5.1.5.4. Once unconsciousness increases the CO<sub>2</sub> flow to 10%, maintain the CO<sub>2</sub> flow until the animal has stopped breathing.

**Note that the time required for euthanasia can be several minutes.**

- 5.1.5.5. Close the CO<sub>2</sub> flow meter and the valve on the CO<sub>2</sub> tank.
- 5.1.5.6. Leave the animals in contact with CO<sub>2</sub> for an additional 2 minutes.
- 5.1.5.7. Confirm death as described in section 9 before disposing of the animals.

## **5.2. Barbiturate, Tamax (T61), or injectable anesthetic overdose:**

- 5.2.1. Pentobarbital: inject at a dose of 120mg/kg intravenously or intraperitoneally.
- 5.2.2 T61: 0.5-2ml per animal, intravenously or intrapulmonary.
- 5.2.2. Other injectable anesthetics: inject three times the anesthetic dose intravenously or intraperitoneally.
- 5.2.3. Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.
- 5.2.4 Use general injectable or gas anesthesia before injecting the euthanasia solution.
- 5.2.5. Confirm death as described in section 9 before disposing of the animals.

## **5.3. Overdose of inhalant anesthetic:**

- 5.3.1. Anesthetic chambers should not be overloaded and must be kept clean to minimize odors that might distress animals subsequently euthanized.
- 5.3.2. The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate anesthetic. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Avoid direct contact between the animal and the liquid anesthetic. Procedures should be conducted in a chemical fume hood or hard ducted Type II B2 Biological Safety Cabinet to prevent inhalation of the anesthetic by personnel.
- 5.3.3. The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter.
- 5.3.4. Sufficient air or oxygen must be provided during induction to prevent hypoxemia. In the case of small rodents placed in a large container, there will be sufficient oxygen in the chamber to prevent hypoxemia.
- 5.3.5. Neonatal animals (up to 10 days of age) resist hypoxia induced by high anesthetic gas concentrations; therefore, alternative or adjunct methods are recommended.
- 5.3.6. Confirm death as described in section 9 before disposing of the animals.

- 6.1. Anesthesia is necessary before physical methods of euthanasia unless holding anesthesia was approved in the animal protocol by BGU's Animal Care and Use Committee.
- 6.2. Only trained and certified personnel can perform these procedures.
- 6.3. Physical methods of euthanasia are also an appropriate means to ensure death after euthanasia with anesthetics or CO<sub>2</sub> used as euthanasia agents.

#### 6.4. Cervical dislocation:

- 6.4.1. Cervical dislocation, as a primary or secondary method of euthanasia, is not allowed on rats weighing over 200g.
- 6.4.2. Procedure:
  - 6.4.2.1. Perform the procedure on a flat surface or surface where the animal can grip (e.g., the wire bar grid of the cage).
  - 6.4.2.2. Hold the base of the tail with one hand and allow the animal to stand in a normal position.
  - 6.4.2.3. With the other hand, the thumb and index finger are placed on either side of the neck at the base of the skull.
  - 6.4.2.4. To accomplish the cervical dislocation, quickly push down and forward with the hand, or the object pressed at the base of the skull while pulling backward with the hand holding the base of the tail.

Note: A 2-4 mm space should be palpable at the base of the skull, between the occipital condyles and the first cervical vertebra, or within the upper third of the neck.
- 6.4.3. A cervical dislocation should not be performed as a one-handed technique.
- 6.4.4. Confirm the animal's death by observing the following clinical signs: absence of breathing, pale eyes, and no reflexes; the animal may urinate.

#### 6.5. Decapitation :

- 6.5.1. Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly rapid manner are commercially available.
- 6.5.2. The use of plastic cones to restrain animals is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves the positioning of the animal in the guillotine.
- 6.5.3. Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g., scissors) can be used.
- 6.5.4. Consider using solid and sharp scissors, e.g., surgical scissors, for decapitating adult mice to reduce the risk of injury to personnel.
- 6.5.5. The equipment used to perform decapitation should be maintained in good working order and regularly serviced to ensure the blades' sharpness.

#### 6.6. Exsanguination:

- 6.6.1. Training and skill are required to obtain enough blood to ensure true exsanguination. Therefore, this method is not considered a valid physical method of euthanasia.
- 6.6.2. Animals may be exsanguinated to obtain blood products, **but only when they are profoundly anesthetized or immediately following euthanasia using isoflurane and CO<sub>2</sub> or CO<sub>2</sub>.**
- 6.6.3. Procedure:
  - 6.6.3.1. Deeply anesthetize the animal according to Rodent Anesthesia SOP.
  - 6.6.3.2. Collect blood from the heart.
  - 6.6.3.3. Confirm death as described in section 9 before disposing of the animals.

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## 7. METHODS FOR NEONATAL RODENTS

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- 7.1. Euthanize rodents under ten days old by one of the following procedures:
  - 7.1.1. CO<sub>2</sub> asphyxiation under isoflurane anesthesia followed by decapitation.
  - 7.1.2. CO<sub>2</sub> asphyxiation followed by decapitation.

- 7.1.3. Barbiturate overdose by intraperitoneal injection, under general anesthesia, or cooling in crushed ice.
- 7.1.4. Overdose of inhalant anesthetic followed by decapitation.
- 7.1.5. Decapitation (using sharp scissors) under general anesthesia or cooling in crushed ice.
- 7.2. The same procedures can euthanize rodents over 10 days old as adult rodents.

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## **8. METHODS FOR GESTATING RODENTS**

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- 8.1. The same procedures can euthanize gestating rodents with fetuses before two-thirds of the gestation period as adult rodents. Euthanasia of the mother or removal of the fetus should ensure rapid death of each fetus due to loss of blood supply and non-viability of the fetus at this stage of development.
- 8.2. During the final third of gestation, fetuses should be given the same ethical considerations as fully mature animals.
- 8.3. Gestating rodents with fetuses over 12 days, >E12:
  - 8.3.1. CO<sub>2</sub> asphyxiation under isoflurane anesthesia of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.
  - 8.3.2. CO<sub>2</sub> asphyxiation of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.
  - 8.3.3. Overdose of injectable anesthetics by intraperitoneal injection to the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.
- 8.4. When the gestational age of the fetuses is unknown, follow the procedures detailed in section 8.3.

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## **9. CONFIRMATION OF DEATH**

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- 9.1. All rodents must be confirmed dead before disposal of the carcass.
- 9.2. To confirm the death, monitor the animal for the following signs: no breathing, no palpable heartbeat, poor mucous membrane color, no response to a toe pinch, and color change in eyes—stiffing of the body after 5-10min.
- 9.3. Death can be confirmed using one of the following methods:
  - 9.3.1. Neonates and fetuses over 12 days, >E12:
    - 9.3.1.1. Decapitation
  - 9.3.2. Adult rodents:
    - 9.3.2.1. The physical method is described in section 6.

**SOP 401 RODENT EUTHANASIA**

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*Approved by the BGU Animal Policy and Welfare Oversight Committee*