MICE BIOMETHODOLOGY WORKSHOP

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DIVISION OF ANIMAL RESOURCES (DAR)

OBJECTIVES

A. Instruct participants in methods of safe, humane handling and restraint

B. Instruct participants in substance administration to include {intramuscular (IM), intraperitoneal (IP), subcutaneous (SC), and intravenous (IV)} as well as the technique of gavage.

C. Instruct participants in techniques associated with the collection of blood samples

D. Instruct participants in the areas of sedation, anesthesia, and analgesia

E. Instruct participants in methods of euthanasia
BASIC INFORMATION ABOUT WORKING WITH MICE

A Wear a minimum of a clean laboratory coat and gloves. The use of surgical masks or respirators may assist in reducing allergen exposure.

B Keep records of each procedure performed on each mouse or group of mice on the Laboratory Animal Care Record located in the animal room or in your laboratory notebook (the latter must be accessible by the DAR staff (veterinarian or veterinary assistant) as well as the oversight individuals (e.g. IACUC, etc.) upon request. The conduct of surgical procedures must be documented on the surgical record located in the animal room.

C If Bitten:
- Don’t punish the mouse for its natural response! Calmly return the animal to its cage.
- Wash the wound thoroughly with soap and water.
- Cover the wound with a bandage. Please note that first aid kits are located in the animal facilities (PSC: located on the counter top in the breakroom; NSC: located in the restroom in the cabinet on the left side as you enter).
- Notify your immediate supervisor and/or the DAR office of the bite so that procedures appropriate to the injury can be followed consistent with university policy.

D Mouse psychology:
- Mice respond positively to quiet, gentle handling. They are normally not aggressive (except for some strains), but if frightened or distressed can inflict painful bites.
- Like any animal, mice are creatures of habit. Everyday events do not tend to stress or excite the mice. However, out of the ordinary events such as being picked up, handled, and restrained are stressful and can result in the mice being fractious. Conditioning the mice to handling and restraint will prevent the mice from associating being handled with “negative” things (like being stuck with a needle) and often makes the animals much easier with which to work.
- Work quietly among the animals, and avoid performing euthanasia as well as procedures requiring anesthesia in the animal housing room. Furthermore, when conducting these procedures in a procedural room, only have the cage of animals on which you are actively working in the procedural room at a given time (e.g. the other cages should be kept in the hallway or an adjacent room as opposed to their being in the same room where the invasive procedure is being conducted. This will minimize the excitement and physiological alterations experienced by the mice from smells (pheromones) and noises, will minimize the introduction of confounding variables which can adversely affect your research data, and will allow you to perform your tasks on a more tractable, less stressed animal.
GENERAL INFORMATION

General Biology

The genus and species of the laboratory mouse is *mus musculus* (order *Rodentia*). The laboratory mouse has been domesticated by man for many generations. Other notable biological characteristics are their very acute hearing, well developed sense of smell, poor vision, small size and short generation interval. Mice are by far the most common laboratory animal used for research.

Behavior

The laboratory mouse can be easily handled with appropriate training. Animals that grow up together or those grouped at weaning usually coexist peacefully. However, some strains of mice (i.e. BALB/CJ, SJL/J, HRS/J) may begin to fight even if grouped at weaning. Breeding males that have been removed from breeding cages and then caged together will usually fight. Wounds on the tail or along the back are a common sign of aggression between cage mates.

Biological Characteristics and Data

Mice, like most species, have a circadian rhythm. Investigators should be aware that this may affect biological data, and it is best to standardize the time of day that samples/measurements are taken to avoid this effect. The standard light/dark cycle in DAR animal rooms is 12/12. This light cycle can be modified upon request by the investigator.

The adult mouse weighs approximately 25-30 grams. The small size and relatively large surface area/body weight ratio makes mice susceptible to changes in environmental conditions. The core body temperature is easily affected by small changes in ambient temperature which may modify the physiologic responses of the animal. The acute hearing of mice makes them highly sensitive to ultrasounds and high pitched noises inducing a stress response that has been empirically related to cannibalism of pups by their dams. The well developed sense of smell is used to detect pheromones used in social interactions. Mice have poor vision and are unable to detect color. Red light is often used to observe animals during the dark cycle.

Basic Biological Data
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Adult body weight: male</td>
<td>20-40gm</td>
</tr>
<tr>
<td>Adult body weight: female</td>
<td>20-40gm</td>
</tr>
<tr>
<td>Body surface area (cm²)</td>
<td>10.5(wt. in grams)(^{2/3})</td>
</tr>
<tr>
<td>Life Span</td>
<td>1.5-3 years</td>
</tr>
<tr>
<td>Food consumption</td>
<td>15 gm/100gm/day</td>
</tr>
<tr>
<td>Water consumption</td>
<td>15 ml/100gm/day</td>
</tr>
<tr>
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<td>50 days</td>
</tr>
<tr>
<td>Breeding onset: female</td>
<td>50-60 days</td>
</tr>
<tr>
<td>Gestation Period</td>
<td>19-21 days</td>
</tr>
<tr>
<td>Body Temperature</td>
<td>36.5-38.0 °C</td>
</tr>
<tr>
<td>Heart rate</td>
<td>325-780 beats per minute</td>
</tr>
<tr>
<td>Respiratory Rate</td>
<td>84-230 per minute</td>
</tr>
</tbody>
</table>

### Basic Husbandry

Most mice are housed in shoe box cages composed of a plastic (polycarbonate) material with a lid and placed on a ventilated cage rack. Bedding is placed directly into the shoe box cage allowing the absorption of urine. The bedding also allows the animal to burrow and/or den.

The animal care staff change the cages on a fixed schedule (typically weekly or biweekly depending upon the number of mice in the cage), thereby providing the animal a clean cage with new bedding, food, and water. Water bottles and feed hoppers are checked daily by caretakers to insure the provision of food and water and to monitor for health or other problems.

Pelleted natural ingredient diets are used to feed all rodents and are composed primarily of cereal grains supplemented with additional protein, vitamins and minerals. The water provided to the mice is municipal tap water. For mice housed under sterile conditions, the water is autoclaved.

A health surveillance program is in place utilizing sentinel animals to detect the presence of rodent pathogens. Rodent pathogens often do not produce clinical signs in affected animals but their presence serves as an unwanted research variable.

### Identification
Cage cards are utilized to identify the strain of mouse, sex, number of animals per cage, principal investigator, research protocol number, etc. Cage cards should not be removed from the cage to avoid misidentification of the animals. Temporary identification of individual mice can be accomplished by pen marks on the tail, hair clipping or dyeing the fur. Pen marks will only last a few days whereas hair clipping may last up to 14 days. Ear punch identification and ear tags can be utilized but may be obliterated by fighting between individuals. Finally, microchips and tattoos have also been used for identification.

**Handling (General Information)**

When handling mice it is required to wear gloves. Mice are usually caught and lifted by the tail. The tail should be grasped between its midpoint and the mouse's body. The tail may be grasped with the thumb and forefinger or by the used of smooth-tipped forceps. With this simple method of holding, they may be transferred to another cage, a balance, identified, or casually examined. Pregnant mice or very obese mice may be handled by this method, but they should be supported by use of the second hand placed under their feet. However, such restraint is not sufficient for treatment and close examination. For more effective control, the mouse may be held by the tail and placed on a table or other surface and the loose skin over neck and shoulders grasped with thumb and fingers (see “handling and restraint” section). It is necessary to perform this maneuver expeditiously, or the mouse may turn and bite. Once the mouse is grasped correctly, the head is adequately controlled. Restraint is improved if the tail or the tail and rear legs are held by the third and little fingers of the same hand or with the other hand (see “handling and restraint” section). Mice should not be dropped into the cage as this may result in spinal fracture. Rather, they should be lowered into the cage and released upon contact with the bedding.

Mice less than two weeks of age can be handled by grasping the loose skin over the neck and shoulder with thumb and forefinger or smooth tipped forceps. Handling neonatal mice should be avoided especially during the first few days after birth to avoid triggering cannibalism or litter abandonment by the dam. If it is necessary to handle the litter, remove the dam to a separate cage and handle the neonates using plastic gloves to avoid contamination with human scent. Multiparous females are less likely to cannibalize if they have historically been successful mothers.

Numerous types of restraint devices are commercially available to restrain mice. Quality devices prevent the animal from turning around yet allow easy access to strategic parts of the mouse. Devices should also be easy to clean and provide adequate ventilation.

**Gender Differentiation**
Male and female mice can be differentiated by observing the distance from the anus and genital papilla which is greater in males. This difference is also present in neonatal mice. In addition, one can usually determine gender by looking for the presence of testicles. However, one must realize that rodents have the ability to retract their testicles into the abdominal cavity (thus the apparent absence of testicles does not necessarily mean the mouse is female).

**HANDLING AND RESTRAINT**

A  **Mouse Restraint Technique I - For removal from caging**

Materials: Disposable gloves

Procedures:

1. Grasp mouse near base of tail (grasping near the tip of the tail may induce a “de-gloving” injury in which the skin on the tip of the tail is removed).
2. Lift animal out of cage and place in new caging or on firm surface.
3. DO NOT suspend mouse by the tail for a prolonged time period.
B  Mouse Restraint Technique II - For technical manipulation

Materials: Disposable gloves

Procedures:

2. Lift animal out of cage and place on firm surface.
3. Grasp nape of neck with opposite hand.
4. Place tail between fingers to secure and control animal.
5. Mouse is now ready for technique manipulations.

C  Mouse Restraint Technique III - For technical manipulation using mechanical restraint

Materials: Disposal gloves, Plexiglas restraint box

Procedures:

1. Restrain mouse by grasping near base of tail.
2. Grasp nape of neck with opposite hand.
3. Place mouse’s head into opening of the restraint box.
4. Release hold on neck while maintaining grasp on tail.
5. Place securing block on appropriate slot for necessary restraint.
INJECTION TECHNIQUES AND BLOOD WITHDRAWAL

Always use sterile syringes and needles for all procedures. To insure aseptic techniques and sharp needles, the one time use of disposable supplies is strongly recommended. When administering injections, select the smallest gauge needle possible to minimize tissue trauma and injection discomfort. Before injecting the solution, always check for correct placement of the needle by slightly pulling back the plunger of the syringe to create a vacuum. This is known as aspiration. The signs to look for will vary with the injection site. If blood or other fluids are aspirated, placement may be incorrect.

Due to the small muscle mass of many rodents, an intramuscular injection may cause discomfort and local tissue irritation, especially if too large a volume of a solution or a solution with an acidic or alkaline pH is administered. An understanding of anatomy and careful technique are necessary to avoid the ischiatic nerve. Injection into or close to the nerve may lead to unnecessary discomfort, temporary lameness, or permanent paralysis of the leg. As a result of nerve damage, an animal may chew off the affected extremity.

If too much blood is withdrawn too rapidly, or too frequently without replacement, one may induce hypovolemic shock and/or anemia. As a general guide, up to 10% of the circulating blood volume can be taken on a single occasion from normal healthy mice with minimal adverse effect (mice blood volume = 70 ml/kg body weight). This volume may be repeated after 2-3 weeks. For repeat bleeds at shorter intervals, a maximum of 1% of an animal's circulating blood volume can be removed every 24 hours. However, care should be taken in these calculations, as the percentage of circulating blood will be about 15% lower in obese and older animals.

INJECTIONS

BASIC PROCEDURE

1. Clean the drug bottle septum with alcohol before withdrawing the dose.
2. Slowly withdraw the dose and tap the air bubbles out of the syringe. Air bubbles injected into vessels can potentially cause air emboli and associated problems.
3. Always check specified route of administration on drug bottle.

A Intramuscular (IM) Injection
Materials: Disposable gloves, Syringe (1 ml), Hypodermic needle (23-30 g), Injection article, Isopropyl alcohol, Gauze

Procedures:

★ Maximum injection volume = 0.05ml.
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain mouse.
3. Prep area with alcohol swab.
4. Insert needle into hind leg muscles (either in front of the thigh bone or behind it with the needle directed towards the back of the leg).
5. Aspirate syringe to insure proper placement. Any sign of blood in the syringe indicates improper placement - reposition.
6. Administer article in a steady, fluid motion. DO NOT administer rapidly because of tissue trauma.

B Subcutaneous (SC) Injection
Materials: Disposable gloves, Syringe (1-3 ml), Hypodermic needle (22-30 g), Injection article, Isopropyl alcohol, Gauze

Procedures:

★Maximum injection volume = 2-3ml.
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain mouse.
3. Prep area with alcohol swab.
4. Insert needle at base of skin fold between thumb and opposing finger.
5. Aspirate syringe to insure proper placement. Any sign of blood indicates improper placement; also, a lack of negative pressure in the syringe indicates the needle has punctured out through the opposite side of the skin - reposition.
6. Administer article in a steady, fluid motion.

C Intraperitoneal (IP) Injection

Material: Disposable gloves, Syringe (1-3 ml), Hypodermic needle (23-30 g), Injection
article, Isopropyl alcohol, Gauze

Procedures:

★ Maximum injection volume = 2ml
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain mouse for technical manipulation. Tilt the body at a 45-degree angle with the head down. This will position the intestines cranial to the injection site.
3. Prep area with alcohol swab.
4. Insert needle into the mouse’s right lower quadrant of the abdomen at a 30-degree angle.
5. Aspirate syringe to insure proper placement. Any sign of blood or other fluid indicates improper placement. To prevent inducing peritonitis, remove syringe, discard, and use new syringe, needle, and article in the event that fluids other than blood are aspirated.
6. Administer article in a steady, fluid motion.

D Intradermal (ID) Injection

Materials: Anesthetic, Disposable gloves, Syringe (1 ml), Hypodermic needle (25-30 g), Gauze, Clippers, #40 blade, Isopropyl alcohol
Procedures:

★ Maximum injection volume = 0.1ml
1. Intradermal injection MUST be done UNDER ANESTHESIA!
2. Clip hair on back and prep with alcohol swab.
3. Insert needle between layers of skin on the back at a 30-degree angle.
4. Aspirate syringe to insure proper placement. Any sign of blood or other fluid indicates improper placement- reposition.
5. Administer article slowly to avoid tissue trauma. Successful injection results in a small circular skin welt.

E. Intravenous (IV) Injection Utilizing Lateral Tail Veins

Materials: Disposable gloves, Plexiglas restraint box, Syringe (1 ml), Hypodermic needle
Note: The lateral tail veins of the tail are the most frequently used site for intravenous injection. The secret of successful injection of the tail vein is to dilate the veins. This has been accomplished in various ways such as the following: placing the tail in warm water (47 degrees Celsius for about 1 minute (do not exceed 47° C as this can result in thermal injury to the tail); placing the animal in an incubator (37° C) for 10 – 15 minutes; or wrapping the tail in an electric heating pad that is warm (not hot) to the touch. In addition one can place a tourniquet around the base of the tail to facilitate visualization of the vein (a rubber band and mosquito hemostat are suitable for this purpose).

The veins can be seen when the tip of the tail is lifted and rotated slightly in either direction. The tip of the needle can be followed visually as it penetrates the vein. Trial injection verifies proper needle placement. Also, accurate placement can be confirmed when the vessel is visually flushed when the compound is administered. The formation of a bleb at the site indicates improper placement of the needle. A second attempt can be performed by removing the needle and trying a site on the same vessel in a more proximal (closer to the animal’s body) location on the tail. Practice is essential.

Procedures:

★ Maximum injection volume = ~1% of the animal’s body weight in mls (i.e., 0.3 mls for a 30 g mouse)
1. Restrain mouse in plexiglass restrainer.
2. Warm the tail as mentioned above to facilitate dilation.
3. Needle placement should be no closer to the body than half the length of the tail.
4. With tail under tension, insert needle into skin approximately parallel with the vein.
5. Insure proper placement by inserting needle at least 3 mm into lumen of vein.
6. Administer article in a slow fluid motion to avoid rupture of vessel.
7. Upon completion, insure good hemostasis before returning to cage.
GAVAGE

Gavaging the Mouse

Materials: Disposable gloves, gavage tubes, syringes (1-3 ml), injection article

Procedures:

★ Maximum administration volume = 10 ml/kg (this equals 0.30 ml for typical adult mice)
1. Measure the distance from the tip of nose to the last rib. This is the length the needle should be inserted.
2. Fill syringe with appropriate amount of article to be dosed.
3. Restrain mouse (Refer to Restraint Technique II).
4. Place tip of needle in the rear of the mouse’s mouth to induce swallowing.
5. Slide tip down back of mouth, moving tip forward in one fluid motion.
6. Take your time, any resistance felt indicates improper placement. Needle should slide down into esophagus easily. A violent reaction (coughing, gasping) usually follows accidental introduction of the tube into the larynx or trachea.
7. Using the gavage tube to gently extend the neck facilitates introduction into the stomach.
8. Once the needle is properly placed, administer the article.

Recommended Standard Gavage Tube Sizes for Mice

<table>
<thead>
<tr>
<th>Wt. range (grams)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball Diameter (mm)</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>To 14</td>
<td>24</td>
<td>1</td>
<td>1 ¼</td>
<td>Straight</td>
</tr>
<tr>
<td>15-20</td>
<td>22</td>
<td>1, 1 ½</td>
<td>1 ¼</td>
<td>Straight</td>
</tr>
<tr>
<td>20-25</td>
<td>20</td>
<td>1, 1 ½, 2</td>
<td>2 ¼</td>
<td>Straight, Curved</td>
</tr>
<tr>
<td>25-30</td>
<td>18</td>
<td>1, 1 ½, 3</td>
<td>2 ¼</td>
<td>Straight, Curved</td>
</tr>
<tr>
<td>30-35</td>
<td>18</td>
<td>2, 3</td>
<td>2 ¼</td>
<td>Straight, Curved</td>
</tr>
</tbody>
</table>
**BLOOD COLLECTION**

A  **Blood Withdrawal Utilizing the Lateral Saphenous Vein**

Materials: +/- Anesthetic, Disposable gloves, Hypodermic needle (22 gauge), Gauze, Electric clippers, #40 blade, Hematocrit tube or Microvette, petroleum jelly (e.g. vaseline) can be applied to the puncture site to prevent blood clotting during sample collection.

Procedures:

1. Restrain or anesthetize mouse.
2. Clip hair from lateral aspect of lower leg. **When clipping the leg, be sure to use small clippers like you will use in the lab. Large clippers can easily induce trauma by cutting the leg. One can also learn to properly use a straight razor for this purpose.**
3. Apply a small amount of petroleum jelly to the clipped region and lightly constrict the saphenous vein above knee joint.
4. Puncture the vein with a needle. Collect the blood via a hematocrit tube or Microvette.
5. Upon completion, insure good hemostasis by applying gentle pressure to the collection site.

B  **Blood Withdrawal Utilizing Orbital Sinus**

*Note: This technique has been largely replaced with less invasive blood collection techniques such as from the lateral saphenous vein.

Materials: Anesthetic (systemic and local), Disposable gloves, Hematocrit tubes, Collection vessel, Gauze

Procedures:

1. Anesthetize mouse. After the mouse is anesthetized, place a drop of the Proparacaine Hydrochloride (local anesthetic) in the eye from which the sample is to be collected. The Proparacaine Hydrochloride takes effect in about 30 seconds and lasts for about 15 minutes.
2. Place hematocrit tube at the medial canthus of the eye and insert behind the eye.
3. Rotate tube on back of orbit until blood flows. Please note that this is a finesse procedure and does not require force.
4. Instill sterile eye ointment when finished.
5. Upon completion, insure good hemostasis by holding eyelids closed.

C Intracardiac (IC) Puncture

Materials: Anesthetic, Disposable gloves, Syringe (1-3 ml), Hypodermic needle (21-25g), Isopropyl alcohol, Gauze

Procedures:

1. Anesthetize mouse.
2. Prep area with alcohol swab.
3. Insert needle at base of sternum on a 20-30 degree angle just lateral of the midline on the mouse’s left side. Use your thumb and index finger to feel the heart. This will assist in directing your needle.
4. Aspirate syringe slowly. A good puncture should allow the collection of approximately 1 ml of blood from an adult mouse.
5. This procedure must be followed by euthanasia as it is only permissible as a terminal procedure.

D Axillary Cutdown

Materials: Anesthetic, Disposable gloves, Syringe (1-3ml), Isopropyl alcohol, Gauze, Scissors

Procedures:

1. Anesthetize mouse.
2. With the mouse in dorsal recumbency (lying on its back), prep axillary (armpit) area with alcohol swab.
3. Cut axillary region with scissors or a scalpel blade to expose the subclavian artery and vein which are deep in the armpit.
4. Cut the subclavian artery and vein with the scissors or a scalpel blade.
5. Collect the blood sample with the syringe (no needle) as the blood pools in the axillary region.
6. This procedure must be followed by euthanasia as it is only permissible as a terminal procedure.
Submandibular Puncture

Materials: Individually Wrapped & Sterile Goldenrod Lancets, Disposable Gloves, Isopropyl alcohol, Collection Tube, Gauze

Proper lancet point length corresponds to the age of the mouse and the volume of blood needed (http://www.medipoint.com/html/animal_lancets.html)

4mm - 2 to 6 weeks
5mm - 2 to 6 months
5.5mm - over 6 months

Procedures:

1. Hold the animal securely.
2. Locate the back of the jaw bone, the submandibular area.
3. Prep area with alcohol swab.
4. Puncture the vein with the Goldenrod lancet.
5. Collect the sample.
6. Press a clean compress to the site for a few seconds.
F Inferior Vena Cava

Materials: Anesthetic, Disposable gloves, Syringe (1-3ml), Isopropyl alcohol, Scissors

Procedures:

1. Anesthetize mouse
2. With the mouse in dorsal recumbency (lying on its back), prep the abdomen with an alcohol swab. The area may be shaved, but it is not necessary.
3. Make an incision in the abdominal skin with a scalpel blade or scissors, exposing the abdominal wall.
4. Make an incision in the abdominal wall with the scalpel blade or scissors, exposing the abdominal cavity.
5. Gently push the contents of the abdominal cavity to one side, exposing the inferior vena cava lying just ventral to the spinal cord.
6. Clean away the fascia from the vein gently with a pair of cotton swabs to allow for better visibility.
7. Insert a slightly bent needle into the vein and aspirate the syringe to collect the sample.
8. This procedure must be followed by euthanasia as it is only permissible as a terminal procedure.

PERFUSION

Perfusing the mouse

Materials: Anesthetic, Disposable gloves, Syringe (1-3ml), Isopropyl alcohol, Scissors

Procedures:

1. Anesthetize mouse
2. With the mouse in dorsal recumbency (lying on its back), prep the abdomen with an alcohol swab. The area may be shaved, but it is not necessary.
3. Make an incision in the abdominal skin with a scalpel blade or scissors, exposing the abdominal wall.
4. Make an incision in the abdominal wall with the scalpel blade or scissors, exposing the abdominal cavity.
5. Cut the abdominal wall along the border of the rib cage on either side of the mouse.
6. Using scissors, cut along both sides of the sternum to expose the contents of the thoracic cavity.
7. Insert a blunted needle into the left ventricle and, subsequently (preferably) into the ascending aorta. Cut a small hole in the right atrium. The needle can be secured in place if desired by using hemostats to clamp across the heart and onto the needle.
8. Inject saline slowly into the heart, flushing out the blood until the animal expires.
9. This procedure must be followed by euthanasia as it is only permissible as a
terminal procedure.

**ANESTHESIA AND ANALGESIA (See Table 1 for Methods)**

METHODS OF ANESTHETIC DELIVERY/EQUIPMENT (OVERVIEW)

There are basically two methods of anesthetic delivery to rodents, parenteral and inhalation.

A. Parenteral Anesthesia involves the injectable routes of administration (typically intraperitoneal in rodents).

B. Inhalation Anesthesia involves the delivery of volatile anesthetic agents to the patient via the respiratory tract.

**METHODS OF DELIVERY OF INHALANT AGENTS TO RODENTS**

The best method for the delivery of volatile agents to rodents involves the use of a precision vaporizer and an anesthesia chamber alone or in combination with a face mask appropriately sized for rodents. **DAR has the equipment to safely and effectively administer inhalant anesthetics (isoflurane) to rodents using a precision vaporizer. Please contact DAR for details regarding use of this equipment.** The rodent is placed within the chamber for induction, then removed from the chamber with anesthesia maintained by delivery through a face mask. Both chamber and mask delivery incorporate the use of a precision vaporizer for precise control of the concentration of anesthetic gas delivered to the patient. Because oxygen flow is required to volatilize the liquid anesthetic placed within the vaporizer, oxygen is also delivered to the patient and helps to maintain the blood oxygen saturation. Because fairly high fresh gas flows are required for either chamber or mask delivery, adequate scavenging of waste anesthetic gases is necessary to avoid exposure to personnel. In general, isoflurane anesthesia is superior to injectable anesthesia. Animals are more quickly induced and recovered, and close to 100% of the gas is eliminated through the lungs without being metabolized, (<<1% of isoflurane is metabolized). This allows for greater control of the anesthetic depth and tends to minimize experimental variables.

**ANESTHETIC MONITORING OF RODENTS**

Parameters that can be used to assess the depth of anesthesia in rodents include:

- recumbency and loss of purposeful movements
- muscle relaxation
- lack of vocalization
- loss of response to aversive stimulation (e.g. pinching the toes)

In most instances, cardiovascular and respiratory assessments are limited to observations of chest wall movement to determine respiratory rate and palpation of the apical pulse through the chest wall.
Because the ratio of body surface area to body mass is greater in rodents than in larger species, thermal support can be critical to the successful recovery of rodents from anesthesia. Particularly with rats and mice, body heat may be dissipated from the tail, soles of the feet and ears with a resultant profound decline in the core and surface body temperature. This hypothermia may, in turn, lead to a decline in both anesthetic metabolism and any urinary excretion of the anesthetic agent.

**SUPPORTIVE CARE OF ANESTHETIZED RODENTS**

Methods to minimize heat loss to the environment during anesthesia of rodents include increasing the ambient temperature of the operating room; placement of a thermal blanket (e.g. recirculating warm water blanket) or drape between the animal and the stainless steel operating table; use of heat lamps (carefully placed!); minimization of organ exposure from body cavities during surgery; recovery of the animal on a warming blanket or within a temperature-supported cage; administration of warmed subcutaneous or intraperitoneal fluids intra and/or postoperatively; housing on bedding during recovery to provide thermal insulation; and recovery with cage mates to permit animals to huddle together and thus provide thermoregulation. Do not place an anesthetized mouse in a cage with an awake mouse as the awake mouse will tend to mutilate the anesthetized mouse.

Rodents have high energy requirements due to their small size and high metabolic rate, yet they have minimal fat reservoirs which can be mobilized to supply needed energy. Nutritional support is critical upon recovery to avoid hypoglycemia. Nutritional support can be provided by simply providing a high-quality pelleted rodent diet as soon as the animal has recovered sufficiently to ambulate and eat (remember - rodents do not vomit so pre-anesthetic fasting is not typically performed).

Fluid deficits can be corrected by subcutaneous or intraperitoneal injection of warmed saline, Lactated Ringers solution or replacement fluids (e.g., Normosol®).

Because rodents are frequently anesthetized with injectable agents that inhibit blinking (e.g., ketamine), ocular lubrication is important to protect against corneal ulceration.

**CLINICAL ASSESSMENT OF PAIN IN RODENTS**

Behavioral changes:

- ☐ Reluctance to move or groom properly
- ☐ Lack of appetite
- ☐ Abnormal vocalization
- ☐ Abnormal posturing
  - ☐ Aggressiveness

Physiologic signs:

- ☐ Pupillary dilation
EUTHANASIA (See Table 2 for Methods)

Proper euthanasia technique includes a follow-up exam to confirm the absence of a heartbeat, which is a reliable indicator of death. Monitoring respiration is not considered sufficient since with some euthanasia techniques heartbeat may be maintained after visible respiration has ceased.

The need to minimize fear and apprehension must be considered in determining the method of euthanasia. Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, whenever possible, animals should not be exposed to euthanasia of others.

The euthanasia methods listed in Table 2 are consistent with the American Veterinary Medical Association (AVMA) Panel on Euthanasia, 2014.

MICE EUTHANASIA METHODS

Table 2

<table>
<thead>
<tr>
<th>Method of Euthanasia</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide*</td>
<td>Method of choice</td>
</tr>
<tr>
<td>Barbiturate overdose (150mg/kg IV or IP)</td>
<td>Method of choice</td>
</tr>
<tr>
<td>Inhalant Anesthetic overdose</td>
<td>Method of choice</td>
</tr>
<tr>
<td>Exsanguination in anesthetized animal</td>
<td>Other acceptable method</td>
</tr>
<tr>
<td>Decapitation in anesthetized animal</td>
<td>Other acceptable method</td>
</tr>
<tr>
<td>Cervical dislocation in anesthetized animal</td>
<td>Other acceptable method</td>
</tr>
<tr>
<td>Decapitation in awake animal</td>
<td>Acceptable only with scientific justification in writing on the Animal Subjects Review Form and subsequent IACUC approval</td>
</tr>
<tr>
<td>Cervical dislocation in awake animal</td>
<td>Acceptable only with scientific justification in writing on the Animal Subjects Review Form and subsequent IACUC approval</td>
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</tbody>
</table>

*Carbon dioxide (CO₂), when used properly, is classified by the 2014 Report of the American Veterinary Medical Association Panel on Euthanasia as a safe method of euthanasia for many small laboratory animals. CO₂ has many advantages including: (1) rapid depressant, analgesic, and anesthetic effects; (2) easy availability in compressed gas cylinders; and (3)
inexpensive, nonflammable, nonexplosive, and poses minimal hazard to personnel when used with properly designed equipment.

Although CO2 is generally considered an acceptable form of euthanasia for small laboratory animals when properly administered, its acceptability is predicated on the following:

It is not desirable to prefill (precharge) the euthanasia chamber with CO2, since high concentrations (>70%) can cause nasal irritation, discomfort, and excitability. Rather, the animals should first be placed into the chamber, followed by the addition of CO2 at a low flow rate (e.g. a rate sufficient to displace approximately 30% of the chamber volume per minute) to complete the process. Rapid gas flows should be avoided since excessive noises ("winds") can develop and induce excitement and distress in the animals. Gas flow should be maintained for at least 1 minute after apparent clinical death. (e.g. at least one minute after the animal has quit breathing). It is important to confirm that an animal is dead after removing it from the chamber. It is important to confirm that an animal is dead after removing it from the chamber. Unintended recovery must be obviated by the use of a secondary method of euthanasia which will be specified in your IACUC protocol.

According to the 2014 Report of the AVMA Panel on Euthanasia, "Compressed CO2 gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. CO2 generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g. antacids) is unacceptable." Only one species at a time should be placed into a chamber, and the chamber must not be overcrowded. When placed into the chamber, all animals must have floor space. Euthanasia should always be done in cohorts (live animals should not be placed in the chamber with dead animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.

Neonates: Since the time period for euthanasia is substantially prolonged in neonatal animals due to their inherent resistance to hypoxia, CO2 narcosis is generally not recommended.

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