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HAMSTERS

BIOMETODOLOGY WORKSHOP

DR. MIKE HART, DR. AMELIA WILKES, MRS. COURTNYE BILLINGSLEY, MS. JOI SCOTT

DEPARTMENT OF ANIMAL RESOURCES (DAR) Phone: 404-413-3560

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), 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 HAMSTERS

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 hamster or group of hamsters on the Laboratory Animal Care Record located in the animal room.

C If bitten:
   • Don’t punish the hamster for its natural response! Calmly return the animal to its cage.
   • Wash the wound thoroughly with an antiseptic soap and water.
   • Cover the wound with a bandage.
   • Notify your immediate supervisor of the bite so that procedures appropriate to the injury can be followed.

D Hamster psychology:
   • Hamsters respond positively to quiet, gentle handling. Before picking up the hamster, gently nudge the hamster with your finger so that the hamster is aware of your presence and not startled when you go to lift it from the cage. A startled hamster is more likely to bite.
   • If frightened or distressed, hamsters can inflict painful bites.
   • Like any animal, hamsters are creatures of habit. Everyday events do not tend to stress or excite the hamsters. However, out of the ordinary events such as being picked up, handled, and restrained are stressful and can result in the hamsters being fractious. Conditioning the hamsters to handling and restraint will prevent the hamsters 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 try to avoid performing procedures in the animal housing room. This will minimize the excitement experienced by the hamsters from smells and noises, 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 hamsters in use at Georgia State University are as follows: *Mesocricetus auratus* (Syrian hamster) and *Phodopus sungorus* (Siberian hamster). All are rodents (order Rodentia). The laboratory hamster 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, short generation interval, and presence of cheek pouches.
Behavior

The laboratory hamster can be easily handled with appropriate training. Hamsters tend to be compatible with one another, regardless of sex, if they have been weaned and raised together. Otherwise, hamsters are aggressive towards unfamiliar animals of their own and opposite gender when the new animal is introduced, with the exception being the female in heat (a female in heat will usually be receptive to an unfamiliar male).

Hamsters are escape-prone so the cage lid must fit securely or they will push their way out. Hamsters will hoard food in their cheek pouches and at specific sites within their cages.

Adult females are larger and more aggressive than males.

Biological Characteristics and Data

Hamsters, 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.

Hamsters have “flank organs” which are sebaceous glands located on the hip region in both males and females. They are responsive to androgens (the main one being testosterone) and thus the glands are more pronounced in males (coarse overlying hair, dark pigmentation).

Cheek pouches exist to hoard and carry food. If startled, a female will sometimes hide her babies in the cheek pouches.

The small size and relatively large surface area/body weight ratio makes hamsters 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 well developed sense of smell is used to detect pheromones used in social interactions. Hamsters have poor vision and are unable to detect color. Red light is often used to observe animals during the dark cycle.

Basic Biological Data

<table>
<thead>
<tr>
<th>Adult body weight: male</th>
<th>85-130 gm (Syrian), ~50 gm (Siberian)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult body weight: female</td>
<td>95-150 gm (Syrian), ~50 gm (Siberian)</td>
</tr>
<tr>
<td>Life Span</td>
<td>1.5-2.5 years</td>
</tr>
<tr>
<td>Food consumption</td>
<td>10 gm/100gm/day</td>
</tr>
<tr>
<td>Water consumption</td>
<td>7-10 ml/100gm/day</td>
</tr>
<tr>
<td>Breeding onset: male</td>
<td>~ 9 weeks</td>
</tr>
<tr>
<td>Breeding onset: female</td>
<td>~ 8 weeks</td>
</tr>
<tr>
<td><strong>Gestation Period</strong></td>
<td>15-18 days (Syrian), 18-19 days (Siberian)</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td><strong>Body Temperature</strong></td>
<td>37.0-38.5°C</td>
</tr>
<tr>
<td><strong>Heart rate</strong></td>
<td>~280-400 beats per minute</td>
</tr>
<tr>
<td><strong>Respiratory Rate</strong></td>
<td>~35-130 per minute</td>
</tr>
</tbody>
</table>

**Basic Husbandry**

Most hamsters are housed in shoe box cages composed of a plastic (polycarbonate) material with a stainless steel wire bar lid used to hold the water bottle and feed. Bedding is placed directly into the shoe box cage allowing the absorption of urine. Nestlets are routinely provided to allow the animal to make a nest. With the approval of the investigator, shelters and chew blocks may be afforded as well.

When removing the lid from this type of cage it is important to remove the water bottle to prevent spillage. If the cage is to be transported the bottle should be turned sipper tube up to prevent spillage during transport. However, you should remember to turn the bottle back over to allow access to water after transport.

The animal care staff change the cages on a fixed schedule (frequency depends upon the type of housing, number of animals per cage, and demands of the experimental protocol), 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 hamsters is municipal tap water. For hamsters housed under sterile conditions, the water is autoclaved.

Two levels of barrier housing of hamsters are available. One level involves the housing of hamsters in a cage as described above. However, in addition, the cage can be fitted with a filter top (microisolater top). This filter top allows pathogen exclusion and containment. DAR also maintains ventilated cage racks which provide HEPA filtered supply and exhaust air to each individual cage. This type of housing also provides for pathogen exclusion and containment. Depending upon the needs of the investigator, a HEPA filtered change-out hood can be placed in the animal room to allow for pathogen containment and exclusion when the filter top is removed for cage change-outs or animal manipulations. Also, arrangements can be made to have the entire cage and its contents autoclaved to allow for a sterile environment in which the hamsters can live. Please contact DAR to discuss the details of these housing options.

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 hamster, sex, number, principal investigator, and research protocol. Cage cards should not be removed from the cage to avoid misidentification of the animals. Temporary identification of individual hamsters 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. Should you wish to individually identify your hamsters, please contact DAR for assistance.

**Handling (General Information)**

When handling hamsters it is advisable to wear gloves to prevent the development of allergies due to direct contact with animal allergens. Hamsters are usually lifted from the cage by either scooping with cupped hands or using one hand to gently press down on the hamster’s back while it resides on the floor of the animal cage and then scruffing the loose skin (see “handling and restraint” section). Before handling the hamster, gently nudge the hamster with your finger so that the hamster is aware of your presence and not startled when you go to lift it from the cage. A startled hamster is more likely to bite. Hamsters 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.

Hamsters 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 hamsters 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.

**Gender Differentiation**

Male and female hamsters 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 hamsters. In addition, one can usually determine gender by looking for the presence of testicles. The testicles impart a convex appearance to the male hamster’s hind quarters with whereas the hind quarters of females do not have this convex protrusion. 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 hamster is female).

**HANDLING AND RESTRAINT**

**Hander Restraint Technique I - For removal from caging**

Procedure:
1. Gently nudge the hamster with your finger so that the hamster is aware of your presence and not startled when you go to lift it from the cage. A startled hamster is more likely to bite.

2. Use two hands to scoop the hamster from the cage with cupped hands. As opposed to using one’s hands, one can instead use a plastic scoop for this purpose.

Hamster Restraint Technique II - For technical manipulation

Procedure:

1. Use one hand to gently press down on the hamster’s back while it resides on the floor of the animal cage. Use the thumb and fingers of this hand to grasp the loose skin along the hamster’s neck and back.

2. The animal can now be removed from the cage for technical manipulations.

Hamster Restraint Technique III - For technical manipulation

Procedure:

1. Use one hand to gently press down on the hamster’s back while it resides on the floor of the animal cage. Specifically grip the hamster over the back with the palm of the hand over the hamster’s head and the fingers towards the hamster’s tail end.

2. The animal can now be removed from the cage for technical manipulations.
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, one should 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 when injecting into the hind leg. Injection into or close to this 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 hamsters with minimal adverse effect (hamsters 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 = 0.10ml.
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain hamster.
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 = 3-5 ml.
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain hamster.
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 = 3-5ml
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain hamster 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 hamster’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 = 0.1 ml
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.

GAVAGE

Gavaging the Hamster

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

Procedures:

★ Maximum administration volume = 5 ml/kg (this equals 0.15 ml for typical adult hamsters)
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 hamster (Refer to Restraint Technique II).
4. Place tip of needle in the rear of the hamster’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 Hamsters

<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>
</tbody>
</table>
**BLOOD COLLECTION**

A  Blood Withdrawal Utilizing the Lateral Saphenous Vein

Materials: +/- Anesthetic, Disposable gloves, Hypodermic needle (20-23 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 hamster.
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. Alternatively, a razor can be carefully used to shave the leg.**
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  Intracardiac (IC) Puncture

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

Procedures:

1. Anesthetize hamster.
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 hamster’s left side. Use your thumb and index finger to feel the heart. This will assist in directing your needle.
4. Aspirate syringe slowly.
5. **This procedure must be followed by euthanasia as it is only permissible as a**
terminal procedure.

C Axillary Cutdown

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

Procedures:

1. Anesthetize hamster.
2. With the hamster 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. This is 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.

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 hamsters, 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 hamster in a cage with an awake hamster as the awake hamster will tend to mutilate the anesthetized hamster.

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
- Increased heart rate
- Increased rate of breathing
- Increased body temperature
<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Duration of Anesthesia</th>
<th>Sleep Time (min)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SEDATIVES/TRANQUILIZERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylpromazine (Acepromazine®)</td>
<td>5</td>
<td>IP</td>
<td></td>
<td></td>
<td>Light Sedation</td>
</tr>
<tr>
<td>Diazepam (Valium®)</td>
<td>5</td>
<td>IP</td>
<td></td>
<td></td>
<td>Light Sedation</td>
</tr>
<tr>
<td>Xylazine (Rompun®)</td>
<td>5</td>
<td>IP</td>
<td></td>
<td></td>
<td>Light Sedation, some analgesia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Duration of Anesthesia</th>
<th>Sleep Time (min)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INJECTABLE ANESTHETICS</strong></td>
<td></td>
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<tr>
<td>Pentobarbital (Nembutal®) (combine 1 ml Nembutal with 9 ml sterile saline and dose at 1.0 – 1.5 ml/100 grams body weight).</td>
<td>50 - 90</td>
<td>IP</td>
<td>30 - 60</td>
<td>120 - 180</td>
<td>Light Anesthesia</td>
</tr>
<tr>
<td>Ketamine and Xylazine* (Note: combine 10 ml of 100 mg/ml Ketamine and 0.5 ml of 100 mg/ml Xylazine and dose at 0.20 ml/100 grams body weight.).</td>
<td>K:200 X: 10</td>
<td>IP</td>
<td>30 - 60</td>
<td>90 - 150</td>
<td>Surgical Anesthesia</td>
</tr>
<tr>
<td>Ketamine and Medetomidine* (Note: combine 4.0 ml of 100 mg/ml Ketamine and 1.0 ml of 1.0 mg/ml Medetomidine and dose at 0.125 ml/100 g body weight).</td>
<td>K: 100 M: 0.25</td>
<td>IP</td>
<td>30 - 60</td>
<td>60- 120</td>
<td>Surgical Anesthesia</td>
</tr>
<tr>
<td>Drug</td>
<td>Induction</td>
<td>Maintenance</td>
<td></td>
<td></td>
<td></td>
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<td>------------------------------------</td>
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<tr>
<td><strong>INHALATIONAL ANESTHETICS</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fluothane (Halothane®)</td>
<td>1 - 3%</td>
<td>0.5 - 1.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflurane (Aerrane®, Isoflo®)</td>
<td>Up to 5%</td>
<td>1 - 3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANALGESICS</strong>*</td>
<td>mg/kg</td>
<td>Duration of Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carprofen</td>
<td>5</td>
<td>SQ q 24h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butorphanol (Torbutrol®, Stadol®)</td>
<td>1 - 5</td>
<td>SQ q 4h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buprenorphine (Buprenex®)</td>
<td>0.05 - 0.1</td>
<td>SQ q 12h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flunixin</td>
<td>2.5</td>
<td>SQ q 12h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5</td>
<td>SQ q 24h</td>
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</tbody>
</table>

*Consideration should be given to administering the analgesic preemptively to prevent the stimulation of pain receptors. This has been shown to reduce the severity and duration of pain following a surgical procedure in humans and other species. Rodents have been shown to return to a state of normal behavior and food consumption more quickly if given preemptive analgesia.

**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, 2000.
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 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>Decapitation in awake animal</td>
<td>Acceptable only with scientific justification in writing on the IACUC protocol form and subsequent IACUC approval</td>
</tr>
</tbody>
</table>

*Carbon dioxide (C02), when used properly, is classified by the 2000 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 20% 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. Unintended recovery must be obviated by the use of appropriate CO2 concentrations and exposure times or by other means.

According to the 2000 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 must be followed by a physical means of euthanasia after the animals lose consciousness to ensure irreversibility of the procedure (e.g. decapitation, cervical dislocation, or thoracotomy).