RAT
BIOMETHODOLOGY WORKSHOP

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DIVISION OF ANIMAL RESOURCES (DAR)

OBJECTIVES:

- Instruct participants in methods of safe, humane handling and restraint
- Instruct participants in substance administration to include {intramuscular (IM), intraperitoneal (IP), subcutaneous (SC), and intravenous (IV)} as well as the technique of gavage.
- Instruct participants in techniques associated with the collection of blood samples
- Instruct participants in the areas of sedation, anesthesia, and analgesia
- Instruct participants in methods of euthanasia
BASIC INFORMATION ABOUT WORKING WITH RATS

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 rat 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 Rat psychology:
   1. Rats are basically docile, curious animals.
   2. Rats respond positively to quiet, gentle handling. They are normally not aggressive (except for some strains/stocks), but if frightened or distressed can inflict painful bites.
   3. Like any animal, rats are creatures of habit. Everyday events do not tend to stress or excite the rats. However, out of the ordinary events such as being picked up, handled, and restrained are stressful and can result in the rats being fractious. Conditioning the rats to handling and restraint will prevent the rats from associating being handled with “negative” things (like being stuck with a needle) and often makes the animals much easier with which to work.
   4. 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 laboratory rat (*Rattus norvegicus*) is a mammal of the order Rodentia. The laboratory rat was the first animal in which the primary reason for domestication was for use in scientific endeavors.

Rats have several unique biological characteristics. The acute hearing of rats makes them sensitive to ultrasounds and high pitched sounds. Rats have poor vision; they are unable to detect color and are blind to long-wave (red) light. The rat’s tail is the principal organ for heat exchange.

Behavior

Rats are nocturnal and thus are active primarily during the night at which time they feed. During the daytime, rats tend to rest, sleep and digest their food. Handling animals during the night can be more difficult due to this increase in activity. The diurnal rhythm can be changed by a 12-hour shift in the light cycle. It takes approximately two weeks for rats to adjust to this shift.

Rats tend to get along well with other rats. However, please realize that introducing two rats of the same sex to each other after weaning age can result in fighting and, potentially, serious injuries. Similarly, rats of the same sex that have been housed together may fight if separated and later re-introduced. Male and female rats tend to accept each other. If one is to introduce post-weaning rats that are strangers, one should take the following precautions: introduce the rats together into a clean cage so that neither rat has established the cage as their home territory. Supervise the rats closely over the next hour or so to see how they do. Be prepared with another cage to separate the rats if needed. The Division of Animal Resources can assist by providing enrichment items for the cage that may serve as a distraction.

Biological Data

<p>| | | |</p>
<table>
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<tr>
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<td>Tidal Volume</td>
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**Basic Husbandry**

Most rats 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 rats 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/stock, sex, number of animals/cage, principal investigator, and IACUC protocol number. Cage cards should not be removed from the cage to avoid misidentification of the animals. Temporary identification of individual rats can be accomplished by pen marks on the tail, hair clipping or dyeing the fur. Pen marks may only last a few days whereas hair clipping may last up to 14 days. Ear punch identification and ear tags can also be readily utilized. Finally, microchips and tattoos have also been used for identification. Should you wish to individually identify your rats, please contact DAR for assistance.

**Handling**

When handling rats it is advisable to wear clean gloves to prevent the development of allergies due to direct contact with animal allergens. Rats typically become accustomed to repeated handling. In a naive animal, the temperament of the animal can be determined by placing the hand into the cage to allow exploration by the animal prior to touching. Initial gentle stroking of the animal followed by gradual grasping the animal will prevent startling the animal and initiating an aggressive response.

Rats are normally lifted by grasping the whole body with the palm over the back, with the fingers and the thumb behind the forearms. This extends the rat's forelimbs so that they may be controlled. Holding with one hand is usually adequate for control, but the tail, rear legs or lower part of body may be held by the other hand for close control, treatment, or examination. The use of both hands is often necessary for rats weighing over 350 grams. Young rats may be handled like mice (see mouse handout). One should exercise caution when lifting rats by the tail as they may strip the skin from the tail (de-gloving injury). This is particularly likely for heavy rats (>450 grams). When picking up a rat by the tail, be sure to grasp the base of the tail with the
thumb and forefinger. For transporting short distances it is helpful to support the rat with your arm or hand while holding the tail.

Rats may bite and certain strains/stocks are more aggressive than others (e.g., F344 rats tend to be more aggressive than Sprague-Dawley), so care and experience are essential to safe handling. Various commercial restraint devices are available for use with rats (see below).

**Restraint Devices**

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

**Sexing**

Male and female rats can be differentiated by observing the distance between the anus and genital papilla, which is greater in males. This difference is also present in neonatal rats. 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 rat is female).
HANDLING AND RESTRAINT

A. Rat Restraint Technique I - For removal from caging and transport

Materials: Disposable gloves

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away.
2. With firm but gentle pressure, grasp rat around thorax with thumb and fingers under each of the front legs. Alternatively, one or two fingers can be in front of the foreleg.
3. Lift rat out of cage and place in new caging or on firm surface.
4. For aggressive rats, pick them up by grasping the base of the tail.
5. DO NOT suspend rat by the tail or the upper body for a prolonged time period because of the stress on the animal. Support body weight quickly.

B. Rat Restraint Technique II - For technical manipulation

Materials: Disposable gloves

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away.
2. With firm but gentle pressure, grasp rat around thorax with thumb and fingers under each of the front legs. Alternatively, one or two fingers can be in front of the foreleg.
3. For a greater degree of restraint, slide the thumb across the ventral thorax and underneath both forelegs. This allows greater control of the head.
4. Animal is now ready for technical manipulations.
5. The base of the tail may be held or the rear quarters supported by the other hand for additional control.

C. Rat Restraint Technique III - For technical manipulation using mechanical restraint
Materials: Disposable gloves, Plexiglas restraint box

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away.
2. With firm but gentle pressure, grasp rat around thorax with thumb and fingers under each of the front legs. Alternatively, one or two fingers can be in front of the foreleg.
3. Place rat's head into opening of the restraint box.
4. Release hold on body, while maintaining grasp on tail.
5. Place securing block in appropriate slot for necessary restraint.
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 in the hind leg. 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 rats with minimal adverse effect (rat 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 intravenously or intraarterially 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 (22-30 g), Injection article, Isopropyl alcohol, Gauze

Procedures:
   ♦ Maximum injection volume = 0.2 – 0.3 mls in an adult rat
1. Fill syringe with appropriate amount of article to be administered.
2. Restrain rat.
3. Prep area with alcohol swab.
4. Insert needle into hind leg muscles (preferably quadriceps muscle group in front of the thigh bone).
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 = 5 – 10 mls in an adult rat

1. Fill syringe with appropriate amount of article to be administered.
2. Restrain rat.
3. Prep area with alcohol swab.
4. Insert needle at base of skin fold between thumb and forefinger.
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. Remove syringe and reposition.
6. Administer article in a steady, fluid motion.

C. **Intraperitoneal (IP) Injection**

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

Procedures:

- Maximum injection volume = no more than 10 mls in an adult rat

1. Fill syringe with appropriate amount of article to be administered.
2. Restrain rat by tilting 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 rat’s lower right quadrant of abdomen at a 30-degree angle.
5. Aspirate syringe to insure proper placement. Any sign of blood or other fluid indicates improper placement; if these are seen then to prevent inducing peritonitis, remove syringe, discard, and use new syringe, needle, and article.
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:

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 with a maximum volume of 100 ul per injection site 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 (25-30 g), Injection article, Isopropyl alcohol, Gauze, method to dilate the tail vein (see below)

Procedures:

- Maximum injection volume = ~1% of the animal’s body weight in mls (i.e., 3 mls for a 300 gm rat)

1. Restrain rat.
2. 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 for about 1 minute (47 degrees Celsius works nicely); 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).
3. Prep tail with alcohol swab.
4. Needle placement should be no closer to the body than half the length of the tail.
5. With tail under tension, insert needle into skin approximately parallel with the vein.
6. Insure proper placement by inserting needle at least 3 mm into lumen of vein.
7. Administer article in a slow fluid motion to avoid rupture of vessel.
8. Upon completion, insure good hemostasis before returning to cage.
GAVAGE

Gavaging the Rat

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

Procedures:
♦ Maximum injection volume = 10 ml/kg

1. Measure the distance from the tip of nose to the last rib. This is the length of needle that should be used.
2. Fill syringe with appropriate amount of article to be dosed. Wet the outside of the gavage tube with water to facilitate its passage.
3. Restrain rat. It is important that the neck be extended to facilitate passage of the gavage tube.
4. Place tip of tube into the mouth.
5. Slide tip down back of mouth, moving tip forward in one fluid motion. Using the tube to extend the neck facilitates passage into the stomach.
6. Take your time, any resistance felt indicates improper placement. Tube should slide down into esophagus easily. Do not force the tube.
7. Once the needle is properly placed, administer the article slowly. With proper placement of the tube the rat should not struggle during administration of the article.

Recommended Standard Gavage Tube Sizes for Rats

<table>
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<tr>
<th>Wt. range (grams)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball Diameter (mm)</th>
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<td>50-75</td>
<td>20</td>
<td>1, 1 ½</td>
<td>2 ¼</td>
<td>Straight</td>
</tr>
<tr>
<td>75-120</td>
<td>18</td>
<td>1, 1 ½</td>
<td>2 ¼</td>
<td>Straight, Curved</td>
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<tr>
<td>100-200</td>
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<td>2, 3</td>
<td>2 ¼</td>
<td>Curved</td>
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<tr>
<td>150-300</td>
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<td>3, 4</td>
<td>3</td>
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<tr>
<td></td>
<td>13</td>
<td>3</td>
<td>4</td>
<td>Straight</td>
</tr>
</tbody>
</table>
BLOOD COLLECTION

A. Blood Withdrawal Utilizing Orbital Sinus

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

Procedures:

1. Anesthetize rat. After the rat 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 then slide the tube to the top of 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.

B. Blood Withdrawal Utilizing the Lateral Saphenous Vein

Materials: Disposable gloves, Gauze, Electric clippers or razor blade, petroleum jelly, hypodermic needle (20-23 gauge), Microvette or capillary tubes

Procedures:

1. Restrain rat.
2. Clip hair from lateral aspect of lower leg (between the ankle and knee joints). Do not prep the skin with an alcohol swab or otherwise wet the leg as this will interfere with this particular blood collection procedure. 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.
3. Place a small dab of petroleum jelly over the anticipated puncture site to facilitate the flow of blood.
4. Constrict saphenous vein above knee joint by lightly pinching the skin.
3. Puncture the vein with a needle. Collect the blood via a capillary tube or Microvette.

2. Upon completion, insure good hemostasis by applying gentle pressure to the collection site before returning to cage.

C. Intracardiac (IC) Puncture (Terminal Collection)

Materials: Anesthetic, Disposable gloves, Syringe (3 - 5 ml), Hypodermic needle (21-25 g), Isopropyl alcohol, Gauze

Procedures:

1. Intracardiac puncture MUST be done UNDER ANESTHESIA.
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 rat’s left side
4. Aspirate syringe slowly.
5. Alternatively, the procedure may be performed with the rat lying on its right side. In this case the needle is inserted just behind the forelimb where the heartbeat is easily felt.
6. This procedure must be followed by euthanasia as it is only permissible as a terminal procedure.

E. Axillary  
(Terminal Collection)

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

Procedures:

1. Anesthetize rat.
2. With the rat lying on its back, prep axillary (armpit) area with alcohol swab.
3. Cut axillary region with scissors to expose the subclavian artery and vein.
4. Cut the subclavian artery and vein with the scissors.
5. Collect the blood sample with the syringe (no needle) as it fills 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. 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 rat in a cage with an awake rat as the awake rat will tend to mutilate the anesthetized rat.

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

**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 accepted by the IACUC for humane killing of animals. These methods are consistent with the American Veterinary Medical Association (AVMA) Panel on Euthanasia, 2014.

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</tr>
<tr>
<td>Barbiturate overdose (150mg/kg IP)</td>
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<td>Decapitation in anesthetized animal</td>
<td>Other acceptable method</td>
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<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>
</tr>
</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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