Mice: Basic Handling and Technique Workshop
University of North Carolina, Chapel Hill

“First do no harm” – Greek Hippocratic Oath,
Great Watch Words of Medicine

Objectives:
1. Teach methods of safe, humane handling and restraint
2. Teach injection techniques: subcutaneous, intraperitoneal, intravenous and intramuscular
3. Teach blood collection techniques
4. Teach rodent identification methods
5. Teach anesthesia administration and monitoring
6. Teach proper euthanasia methods

Basic Information about working with Mice:
Proper Personal Protection Equipment (PPE) is a requirement for working with animals. In DLAM facilities, minimum requirements include disposable coveralls, shoe covers, head bonnet, mask, and gloves. Please review requirements before entering any animal area! Requirements may change from room to room so each door is posted with instructions. The use of a face mask reduces your risk of allergy to animals. We strongly recommend that you wear masks whenever you work with animals.

Training Information:
For additional training please contact the Training and Compliance Coordinators for the Institutional Animal Care and Use Committee (IACUC) at 966-5569. We offer training in both one on one and classroom settings.

For a look at IACUC Guidelines and dates of future classes, visit our website at http://research.unc.edu/Offices/iacuc/index.htm as well as http://research.unc.edu/Offices/NLAC/index.htm

If Bitten: **DO NOT PUNISH THE MOUSE FOR ITS NATURAL RESPONSE**
1. Calmly return the animal to its cage
2. Wash the wound with antibacterial soap and water
3. Bandage the wound
4. Notify your supervisor and contact the University Employee Occupational Health Clinic (UEOHC) 966-9119

Mouse Psychology:
1. Mice are usually mild in temperament and easy to handle. They are not usually aggressive, but can bite if frightened. There are some strains that are aggressive and can inflict painful bites. Mice groom themselves almost constantly to maintain a smooth, glossy haircoat.
2. Mice are nocturnal animals. Activities such as eating, drinking or mating are typically done at night.
3. Dominant mice exhibit a behavior called barbering. Barbering is the dominant mice biting or chewing on the fur of a more subordinate mouse. Barbering should not be confused with fur loss due to illness. Typically, barbering occurs around the face or back.

4. Male mice can be more aggressive and fight more often than females. Aggressive mice should be housed individually to avoid severe injury to cage mates. Generally male littermates may be housed together, but once separated, it is advisable to only house males with females.

5. Mice are creatures of habit. Everyday events do not tend to stress or excite the mice. However, handling and restraint can be stressful and result in the mouse being difficult to work with. Conditioning the mice to such handling (so they do not associate handling and restraint aversively) can make the animals much easier to work with.

Handling and Restraint:

When picking up adult mice, grasp them gently but firmly at the base or center of their tail. Do not pick them up by the tip of the tail. Place the animal on a surface such as the wire cage top or lid. It is best that the surface not be slick or smooth as mice will behave much more calmly if they have firm footing. While still holding the tail near the base, with your other hand firmly grasp the loose skin on the back starting near the ears using your thumb and first two fingers. The tail can then be held by the last two fingers as shown. Your grip should be firm enough to keep the mouse from struggling, but gentle enough for it to breathe comfortably.

For quick handling such as cage to cage transfers, it is acceptable to use forceps. Gently grasp the loose skin on the back and quickly transfer them to the new cage. This technique is useful for fractious or aggressive animals. Be sure to clean gloves or forceps with a sterilant such as MB-10 or Spor-Klenz between cages.

Gloves, either light leather, cloth or mesh greatly diminish sensitivity, increasing chance of injury to mice. They also make it more difficult to perform delicate procedures, and mice can often bite through them.
Examples of Commercial Restrainers:

**Snuggle:** [Lomir.com](http://Lomir.com) Various styles and sizes available: See Page 24 for vendors

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**Sex Determination**

Gender in mice is determined by comparing anogenital distance, or the distance between the urogenital opening and the anus. Male mice typically have a larger anogenital distance when compared with the females. Be aware there are variances in anogenital distance among strains. See diagrams below.

![Diagrams](a) Young Rodent  (b) Adult Rodent

**Injections:**
- Use a fresh, sterile needle for each injection
- To avoid excessive leaking, keep the needle in the needle tract for a few seconds
- Always inject with the bevel of the needle facing up
- Do not reuse needles between animals
<table>
<thead>
<tr>
<th>Injection Type</th>
<th>Ideal Needle Size (Gauge)</th>
<th>Recommended Volume</th>
<th>Aspiration Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal (IP)</td>
<td>≤ 23G</td>
<td>2 – 3mL; depending on the animal size</td>
<td>No</td>
</tr>
<tr>
<td>Subcutaneous (SQ)</td>
<td>≤ 23G</td>
<td>2 – 3mL; ensure the skin at the injection site is not taunt</td>
<td>Yes; inject if no blood in the needle hub</td>
</tr>
<tr>
<td>Intramuscular (IM)</td>
<td>27G</td>
<td>50 - 100µL per site</td>
<td>Yes; inject slowly if no blood in the needle hub</td>
</tr>
<tr>
<td>Intravenous (IV)</td>
<td>≤ 27G</td>
<td>200µL</td>
<td>No; inject slowly</td>
</tr>
</tbody>
</table>

1. **Intraperitoneal injections**: Holding the mouse in dorsal recumbency, insert the needle in a position below the bend of the knees; left or right of the midline. Avoid the midline to prevent penetrating the bladder. Angle the needle approximately 45° to the body.

2. **Subcutaneous injections**: May be performed in any area of loose skin along the back or flank. Tenting the skin between the shoulder blades or over the rump creates an appropriate pocket for injection. Inserting the needle under the skin along the flank results in the outline of the needle clearly visible when correctly situated.
3. **Intramuscular injections:** Only to be used in instances where the other injections are not appropriate, since this is potentially painful. The muscle mass running along the back of the leg is used; with the needle angled parallel to the femur (avoiding the sciatic nerve).

4. **Intravenous injections:** Dilate the blood vessels by warming the mouse. Once warmed, place the mouse in a restrainer. An additional method to dilate the veins is rubbing alcohol on the tail. Locate one of the lateral veins. Insert the needle as low as possible towards the tip of the tail, since the vein is very superficial at the tip. As you move up toward the base of the tail the vein is located more deeply. The vein will clear from the injection site to the base of the tail if properly situated, whereas ballooning around the injection site will occur if the needle is not properly seated you will note ballooning at the injection site.
Various Helpful Tips for Injections:

1. When collecting blood from laboratory animals, the largest recommended amount is 1.5% of the animal’s total body weight. Collection should not occur again for two weeks. This will allow blood constituents to return to normal. If blood is needed weekly, 0.5% body weight is a safe amount.
2. When giving substances intravenously, inject slowly to avoid shock.
3. Injecting slowly when giving substances intramuscularly will cause the least amount of pain.
4. The volume of blood in an adult mouse is about 78-80 ml/kg. This is approximately 10% of its body weight. Only about half of this can be recovered in a terminal blood withdrawal procedure.
5. Check rodents’ teeth frequently. This will insure early detection of malocclusion. If ma-o-closed, teeth may become overgrown and interfere with eating.

Oral Gavage:

We recommend using stainless steel, ball tipped gavage needles. It is important to pre-measure the needle before gavaging. The tube should measure the distance from the tip of the nose to the last rib, so that the needle will pass down the esophagus into the stomach. If the tube is too short, the injected fluid may be aspirated by the mouse causing possible pneumonia and death. If the needle is too long, it may perforate the stomach.

The mouse should be firmly restrained. Insert the gavage needle into the mouth at one side. Slide the needle down the back of the throat while tilting the mouse’s head back, so that the neck is in a straight line. The needle should pass easily down the esophagus; with little to no resistance. If the mouse struggles or resistance is met, stop, back up and start over. Improper gavage technique can cause tearing of the esophagus or asphyxiation.

Observe the mouse carefully after the gavage is completed. No fluid should be coming from the mouth or nose and the mouse should not show signs of distress. Oral dosing should not exceed more than 10ml/kg.

The following table indicates appropriate needle sizes:

<table>
<thead>
<tr>
<th>Mouse Wt (g)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 14g</td>
<td>24</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td>15-20g</td>
<td>22</td>
<td>1 or 1.5</td>
<td>1.25</td>
</tr>
<tr>
<td>20-25g</td>
<td>20</td>
<td>1, 1.5 or 3</td>
<td>2.25</td>
</tr>
<tr>
<td>25-30g</td>
<td>18</td>
<td>1, 1.5 or 2</td>
<td>2.25</td>
</tr>
<tr>
<td>30-35g</td>
<td>18</td>
<td>2 or 3</td>
<td>2.25</td>
</tr>
</tbody>
</table>
Acknowledgements:

The University of North Carolina would like to thank:
The AALAS Learning Library
University of Minnesota, Research Animal Resources
University of Texas Medical Branch at Galveston

Rodent Identification

There are several IACUC approved methods used to identify rodents. Each method has both advantages and disadvantages. In long-term studies, it is important to choose a method that is permanent and easily read.

**Long Term Methods:**

- **Ear Notching** – This method is frequently used in both mice and rats. There are several tools that may be purchased to achieve this. Most resemble a hole puncher and are very cheap. There are previously created maps that serve as a numbering system, or the researcher may create a map. (See diagram below for universal numbering system that may be used for identification via ear notching)

  A) **Advantages** – Ear notching can be done quickly while causing very little pain or distress. The instruments are not costly and can be obtained easily.

  B) **Disadvantages** – This method can not be applied until the ears are fully developed. This may be too late for those that use young rodents. This may not work with fractious strains. Rips or tears caused by fighting may leave the pattern indiscernible. Tools used to notch ears dull easily so must be replaced frequently.

- **Ear Tagging** – Ear tags can be purchased with numbers and/or letters. Correct placement of the tag makes them fairly easy to read. (See images below of two commonly available ear taggers, with proper tag placement).

  A) **Advantages** – Ear tags are inexpensive and are fairly easy to apply. This method does not require the use of anesthesia. Tagging can be done quickly and does not seem to cause pain and only minor distress.
B) **Disadvantages** – Tags can fall out if not applied properly. They can also be lost if ears are ripped or torn in strains that fight. Different sized tags are available for different species. Tags are relatively heavy for weanlings and may cause young mice to tilt their head even when the proper sized tag is applied. Some strains are prone to scratching the tagged area which can lead to infection, hematomas, and granulomas. In a very limited number of cases, a member of the DLAM veterinary team has seen ear tags stimulate tumor growth.

- **Microchipping** – Microchips, electronic transponders, are safe and reliable.

  A) **Advantages** – Microchips may be applied without the use of anesthesia. Applying microchips seems to cause little or no pain. Even though the chip may migrate to a different area, they are not lost so prove to be a reliable method. Animals can be identified without handling and removing them from the cage. Some microchips are designed to provide other information such as core body temperature and heart rate.

  B) **Disadvantages** – The equipment used to read the chips is fairly expensive. Microchips cost five to ten dollars each. Despite the manufacturer’s recommendation, chips can be reused. In order to reuse the chips they must be sterilized by ethylene oxide. The hospital will do this as a service for fee. If not implanted properly there is a slight risk of infection. In a very limited number of cases, a member of the DLAM veterinary team has seen microchips stimulate tumor growth.

- **Micro tattooing** – This method seems to be growing in popularity. It is both permanent and fairly easy to apply.

  A) **Advantages** – This method works in all strains, even the more fractious. The cost is very reasonable after the initial expense. Tattoos can be applied to rodents of any age. The markings are easily read, especially when applied to the tail of light colored rodents. When placed in the proper area, it is not necessary to handle the animal to read the tattoo. Tattooing causes only minor pain and distress and does not require the use of anesthesia.

  B) **Disadvantages** – The identifying marks may be a little difficult to read in young pigmented mice. This improves as the mice age. The initial cost is rather expensive. There is a small chance of inducing infection if the tattoo is not applied correctly.

- **Toe Clipping** – “Toe-clipping, as a method of identification of small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates.”¹ The IACUC allows a maximum of four toes and no more that two per foot. Do not cut the hallux (“dew-claw” or “little toe”) as this may decrease the rodent’s grasping ability.

  A) **Advantages** – Toe clipping can be done at a very early age, post natal day one. The ideal time is between post natal day five and seven when the toe is large enough to work with yet the bones are not calcified. The tissue can be used for genotyping. Toe clipping may not be performed after post natal day ten. No anesthesia is needed. This seems to cause little or no pain when performed early enough. The young react to being removed from their mother, but do not react to the clipping of the toe.

  B) **Disadvantages** – The young show signs of distress when removed from their mother and siblings. This may cause a small amount of pain. The general public views this as a
form of brutal mutilation. There is a small possibility of infection. A reduction in the number of toes may reduce the ability to grasp objects.

**Short Term Methods:**

- **Hair Clipping** – Trim patterns into the fur. Keep a record or picture to identify rodents.
  
  **A) Advantages** – Causes no pain.  
  **B) Disadvantages** – This is very temporary. The hair will grow back within ten days and the clipping must be repeated.

- **Permanent Markers and Fur Dyes** – It is easy to apply marks or dyes to different body parts.
  
  **A) Advantages** – This method is non-invasive. It causes only minor stress due to restraint.  
  **B) Disadvantages** – This method can be time consuming, since it must be repeated soon after the previous marking. If working with nursing pups, the mothers will groom the neonates excessively and the markings may disappear overnight. This could result in a loss of identity.

If you would like to inquire about equipment used in methods discussed above, please email the OACU Training/Compliance Team. You may send questions to the general IACUC email account iacuc@med.unc.edu

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Ear Tagging:
Ear Notching:

REM   MEM    ER   R   REM   MEM
EMBER    BER    B   BER    BER
THAT    THAT
RIGHT   RIGHT
IS      IS
THE     THE
RODENT'S
RIGHT
RODENT'S
LEFT   LEFT
ACCEPTABLE METHODS OF RODENT BLOOD WITHDRAWAL

Each laboratory must designate a Laboratory Animal Coordinator (LAC) who may train research personnel in their laboratory in various animal-handling techniques, including blood collection. The LAC must be certified by the Office of Animal Care and Use (OACU) or the Division of Laboratory Animal Medicine (DLAM) and demonstrate proficiency before training others within their lab. Alternatively, laboratory personnel may register for a hands-on techniques class here or one on one session with the OACU Training and Compliance team (966-5569).

- **Chronic Blood Withdrawal:** For sequential blood sampling (over a period of time), the maximum survival blood withdrawal for most mammals is 1.5% of lean body weight every 14 days.

- **Acute or Single Blood Withdrawal:** The maximum survival amount of an acute blood withdrawal is 1% of the lean body weight. [e.g.: For a 20 gram adult mouse, no more than 4 X 50 ul micro capillary tubes (200 ul), may be withdrawn].

To facilitate blood collection, warm the rodent first. When using the tail veins or artery, you may dip the tail in warm water (45°C). The entire animal can be warmed with a carefully placed heat lamp for 5-10 minutes or by placing the housing cage on a circulating water pad. Alternatively, alcohol may be used initially as a vasodilator, but it should not be used on broken skin.

1) **Submandibular:**
A relatively simple way to obtain blood from a mouse is to puncture the area behind the hinges of the jawbones. The superficial temporal vein is a large vessel positioned behind the eye, which can be traced backward to the temporal vein, the maxillary vein, and finally, the jugular vein.

Scrub the mouse and pierce the skin in the relevant area. A mouse bleeding lancet is strongly recommended for use. However, an 18 gauge needle may also be used. Information on the lancets and a video of this procedure may be seen by going to the following URL:
The submandibular bleed is one of the easiest methods of collecting blood from a mouse. Your LAC may train in this technique or you can contact the OACU at 966-5569 to arrange for training.

2) **Saphenous Vein:**
This method of obtaining blood is often used when a series of small samples is required. Place the mouse in a conical tube and shave the caudal surface of the thigh. The saphenous vein can be seen in this area. It is advantageous to apply a lubricant to prevent wicking. Place a tourniquet above the knee and enter the vein with a 25 gauge needle. Micro-hematocrit and microvette tubes work well to collect the blood. This method of blood withdrawal does not require anesthesia, however, the method of restraint is cumbersome. For detailed instructions and pictures of this procedure please visit http://www.uib.no/dyreavd/Vivarium-blood-sampling.pdf

3) **Tail Artery / Vein (NICK):**
Tail veins and artery can be used for serial bleedings. Use the central tail artery or lateral tail veins. Anesthesia is not required for tail nick. Start midway up the tail and nick the artery or vein (usually with a needle or lancet). You may collect blood with micro capillary tubes, a
micropipette or various microtainer collection tubes. Move cranially 0.5 cm at a time applying pressure after the bleed.

4) Tail Clip Bleed and/or Tail Biopsy for Genotyping:
Perform on (un)anesthetized or anesthetized animals depending on amount of tissue needed (see below):

- Anesthesia is optional for the removal of up to 4 mm from the tail tip. It is strongly recommended that no more than 2 mm be removed at a time. Anesthesia may be used as a means of animal restraint and its use must be described in the approved animal care application.

- Harvesting greater than 4 mm requires written permission from the IACUC. Depending on the age of the animal, removal of greater than 4 mm from the tail tip may involve cutting into the vertebral column. Therefore, anesthesia is always required when removing this much tail, irrespective of the age of the animal. The use of anesthesia must be described in the approved animal care application. Requests to perform tail biopsies or successive tail cuts totaling greater than 4 mm without anesthesia must be scientifically justified and must receive IACUC approval prior to implementation.

The IACUC has approved the tail cut method for both rats and mice to obtain blood and/or tissue. This method must be described in the animal use application and approved by the IACUC prior to use. See policy below.

1. Place animal in approved animal restrainer. (Experienced handlers may be able to perform technique in habituated rats with light or no restraint).
2. Remove any bedding material or feces from the tail. The tail tip must be disinfected with an approved disinfectant (i.e. Betadine)
3. Place the animal on a clean work surface.
4. Using a fresh scalpel blade, cut 1-2 mm of the distal tail at an angle perpendicular to the work surface.
5. Apply gentle pressure proximal to the collection site to occlude venous return and ease collection. Collect the blood in a suitable collection device.
6. Apply gentle digital pressure to the wound for 30-45 seconds with a clean gauze pad to stop any hemorrhaging. For persistent bleeding, apply a silver nitrate stick, styptic powder or a cautery pen to the wound to stop bleeding.
7. Return the animal to its cage only after bleeding has stopped.
8. Serial blood samples can be obtained over a short time frame by gently removing the scab without performing an additional cut.
9. Only the fleshy portion of the tail tip should be cut. Cutting into the vertebrae is NOT permitted. As only a small portion of the tail does not contain vertebrae, the use of the tail cut procedure should be limited.
10. This procedure should be performed only by individuals trained and certified in the technique and comfortable with rodent handling.

5) Retro Orbital Bleeding:
Retro-orbital or orbital sinus/plexus bleeding (permitted in rats, mice, gerbils, guinea pigs, hamsters) must be proposed to and approved by the IACUC before implementation. The IACUC
will permit orbital sinus bleeding when it is scientifically justified, performed with appropriate technique and anesthesia. Veterinary staff experience indicates that this method may lead to orbital damage, blindness and potentially death if not performed correctly. The IACUC encourages the primary use of the submandibular, tail artery or veins; specifically the nick or cut techniques. These methods are less likely to harm the animal and may be used repeatedly for bleeding. LACs may not train in this technique so training and certification must be obtained from OACU Training and Compliance team or DLAM veterinary services.

Alternating eyes for each bleeding is mandatory, and a week must separate each bleeding. A maximum of two (2) bleedings per eye is permitted. Maximum volume withdrawn within a two week period is 1.5% body weight. Orbital sinus bleeding requires training and must be performed on anesthetized animals only with IACUC approval.

“Following blood collection, the eyelids should be held closed for a few seconds to allow the punctured blood vessel to clot. It is also common practice to place a small amount of ophthalmic ointment into the eye following this procedure.” excerpt from Laboratory Animal Technician Training Manual

6) Cardiac Puncture: Always a terminal procedure conducted under anesthesia!
Cardiac puncture as a method of blood withdrawal permitted in all species provided the following conditions are met:
1. Animal is under a surgical plane of anesthesia when procedure is conducted.
2. Animal is NOT allowed to recover from anesthesia following the puncture.
3. If the animal is euthanized prior cardiac puncture, training and certification in the technique is not required.
A needle is inserted into the heart and blood is extracted until a sufficient volume is collected or the animal is exsanguinated. This procedure must be followed by a physical euthanasia method.

PDF format available on the IACUC website:
Click HERE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
MOUSE EUTHANASIA POLICY

Performing euthanasia correctly is an ethical imperative. Proper euthanasia is quick, minimizes pain/distress and reliably causes death. Practical issues such as degree of technical difficulty, time required to perform the procedure, readily available equipment/resources to perform the procedure, as well as aesthetics and human emotion must be considered. Standardized guidelines for humane euthanasia are detailed in the June 2013 AVMA Guidelines on Euthanasia and available HERE.

In addition to Division of Laboratory Animal Medicine (DLAM) personnel, only trained research personnel, listed in the IACUC approved Animal Care Application (ACAP), may euthanize animals. All animals slated for euthanasia must be housed according to UNC-Chapel Hill cage density standards, and should have access to food and water if they are being housed for more than 3 hours prior to euthanasia. Unweaned animals that are slated for euthanasia should stay with the lactating female until final preparation(s) for euthanasia are complete. Cages marked for euthanasia should not be overcrowded or stacked on top of each other, as this blocks air flow into the cage. Euthanasia must follow the method(s) described in the approved ACAP. Euthanasia must be confirmed by the physical method described in the approved ACAP. A confirmation of death by a physical method is required for all animals, irrespective of age.

The DLAM staff can, for a fee, perform euthanasia of research animals. When requesting this DLAM service, research personnel must do the following:

1) Complete and submit a “Request for Euthanasia in Animals” form [available on the DLAM website]. Ensure all euthanasia instructions are very clear (e.g. ‘euthanize dam and neonates’ or ‘euthanize only the pre-weanling animals, not the dam’);
2) Leave the animal(s) requiring euthanasia in the cage. All unweaned animals should stay with the lactating female until the time of euthanasia.
3) Place a euthanasia card on the cage so that DLAM can readily identify the animal(s) slated for euthanasia.

The investigator is responsible for ensuring proper documentation on a euthanasia request form. DLAM is not responsible for errors on the form or miscommunications that may occur during the euthanasia process. Do not make verbal arrangements with DLAM staff.

Euthanasia of sick or injured animals
Sick or injured animals that cannot be successfully treated or relieved of pain and distress should be euthanized promptly. Research personnel are responsible for euthanizing sick, injured or moribund animals as soon as these conditions are noted. These animals should not be held for later euthanasia by DLAM personnel. To investigate unexpected illnesses, research personnel may contact Veterinary Services to arrange for euthanasia and necropsy of the animals.

DLAM veterinarians have the authority to euthanize moribund animals, as well as animals experiencing more than momentary or slight pain and/or distress. If the DLAM veterinarian is unable to contact research personnel regarding the care or treatment of a moribund animal, DLAM veterinarians or designated representatives are authorized
to euthanize the animal. Ensure appropriate emergency contact numbers for all research personnel are posted in the animal facility.

**MOUSE EUTHANASIA**

**Section 1: Terms and Definitions**

**Secondary physical method to ensure death** — in order to confirm that animals are dead, one of the following secondary physical methods must be performed on animals that have been anesthetized with approved agents: 1) cervical dislocation; 2) decapitation; 3) thoracotomy [open the chest cavity using sharp scissors or scalpel]; or 4) collection of vital organs.

**Note:** In addition to DLAM personnel, only research personnel who have been properly trained and are listed on the approved ACAP, can perform these physical methods.

**Unanesthetized Physical Euthanasia** — Individuals who perform physical euthanasia on unanesthetized animals must first be trained and certified by IACUC approved designees. Laboratory Animal Coordinators (LAC) **may not** certify personnel in unanesthetized physical euthanasia. Physical euthanasia on unanesthetized animals, irrespective of age, can only be done if the procedure is described in the approved ACAP.

- **Cervical Dislocation** — cervical dislocation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, its use is scientifically justified, and it is described in an approved ACAP. Manual cervical dislocation is a humane method of euthanasia when limited to rodents weighing less than 200 grams. Personnel using cervical dislocation must be adequately trained, demonstrate their technical proficiency, and must consistently apply this method humanely and effectively.

- **Decapitation** — decapitation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, its use is scientifically justified, and it is described in an approved ACAP. When performed properly this technique is nearly instantaneous and is considered humane. Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available. Sharp scissors can be used to decapitate neonatal rodents. Check guillotine and scissor blades frequently to ensure sharpness. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to minimize stress from handling, minimize the chance of injury to personnel, and improves positioning of the animal in the guillotine. *(2013 AVMA Guidelines on Euthanasia)*

**Note:** The Physics Department's Instrument Shop, located in Phillips Hall 115A, will sharpen blades for a small fee [(919) 962-1183].

**Gaseous Carbon Dioxide** ($\text{CO}_2$): must be supplied using a compressed gas tank. The use of dry ice as a source of $\text{CO}_2$ for euthanasia is not permitted. (Refer to section 2A below.)

**Inhalant Anesthesia**: anesthetic agent(s) delivered as a volatile gas to the respiratory tract to induce anesthesia. Personnel should minimize their exposure to these agents.
as some are considered chemical hazards. These agents should only be used in a chemical fume hood, ducted biosafety cabinet or in a system with an active gas scavenging device. (Refer to section 2B below.)

Injectable Anesthesia: chemical agent(s) administered by injection with a needle and syringe to induce anesthesia. Common routes of injection include, but are not limited to, intraperitoneal (IP), intramuscular (IM) or intravenous (IV). Injectable anesthetics are easy to administer, require minimal equipment, and avoid safety concerns associated with inhalants. (Refer to section 2C: Section 2: Procedures

A. Gaseous Carbon Dioxide (CO2): The 2013 AVMA Guidelines on Euthanasia recommends that the gradual displacement rate of CO2 into the euthanasia chamber should be 10-30% to minimize pain and distress. All calculations described below are for a DLAM shoe box style rat cage at 30% displacement.

Note: DLAM procedure rooms have dedicated CO2 euthanasia chambers equipped with acceptable flow meters. Investigators who wish to perform CO2 euthanasia outside of DLAM facilities must adhere to all of the following principals and must purchase the same equipment utilized by DLAM. Appropriate flow meters must be purchased from VWR and can be found through the UNC purchasing system, E-Pro, or at the following website: https://us.vwr.com (part number: 89012-426). To purchase appropriately sized euthanasia chambers, contact DLAM at (919-843-7992).

1. Place the Euthanex stainless steel lid over the plastic cage. The lid should be connected to a CO2 tank via a plastic hose.
   a. Make sure the two holes on the top of the lid are not blocked, as these holes allow air to be pushed out by the heavier CO2.
   b. Make sure the plastic cage does not have an automatic watering opening.

2. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia chamber. Do not place different animal species in the chamber at the same time. Do not overcrowd the chamber. Each animal should have enough floor space available to lie down.

3. Turn on the valve located on top of the CO2 tank. Next, set the flow meter by adjusting the regulator valve on the left side of the flow meter (see photo on the next page):
   a. Standard DLAM Shoebox style RAT cage: 8 liters per minute (lpm)
   b. Standard DLAM Shoebox style MOUSE cage: 1.8 liters per minute (lpm)
   c. Other CO2 Chambers: Use the following formula to calculate the appropriate flow rate:

      \[
      \text{height} \times \text{width} \times \text{length} = \text{liters} \times .20 = \text{flow rate/minute}
      \]

4. Continue to allow CO2 to flow into the chamber for one minute after breathing stops (approximately 6 minutes for mice and 8 minutes for rats). Young animals, certain strains of mice, and sick animals may require more time to become deeply anesthetized.
5. Once animals are fully anesthetized, **immediately** perform a physical method of euthanasia (i.e. cervical dislocation, thoracotomy, major organ harvest, or decapitation) to confirm death.

6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.

7. Place dead animals into a non-PVC containing bag. DLAM provides these bags in a variety of sizes. Label the bag with the ACAP ID#. Seal the bag securely. Place the bag with dead animal(s) into the DLAM carcass freezer available in each animal facility. Please see the Policy on Rodent Carcass Disposal for more information.

8. Disinfect the euthanasia chamber bottom after each use.

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**B. Inhalant Anesthetics (e.g. Isoflurane)**

Induction chambers for inhalational anesthetics must allow animals appropriate floor space without being too large. Chambers that are too large require increased volumes of the anesthetic agent and may result in slow induction time. Where applicable, induction chambers must prevent animals from coming into direct contact with an anesthetic soaked material. The lid should fit snugly and the chamber must be used in a fume hood, a ducted biosafety cabinet, or with a properly functioning active scavenging system.

1. Pre-charge the anesthetic chamber by opening the vaporizer or placing two to three pieces of absorbent material on the bottom of the chamber. Add approximately 3-5 mls of isoflurane liquid to the absorbent material (amount of isoflurane is determined...
by the size of the chamber). Close the lid and wait 5 minutes for the liquid to form a volatile gas within the chamber.

2. Remove the lid of the chamber, quickly place the animals in the chamber, ensure the absorbent material is not in direct contact with the animal, and immediately close the lid.

3. The animals should become anesthetized in 2-5 minutes. Neonates require a longer period of time to anesthetize and should remain in the chamber for at least five (5) minutes.

4. When animals are completely recumbent and obviously deeply anesthetized, remove them from the chamber.

5. **Immediately** perform a physical method of euthanasia. Isoflurane is highly volatile and animals will quickly regain consciousness once removed from the chamber. Therefore, it is imperative that physical euthanasia be performed immediately.

6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.

C. **Injectable Anesthetics**

Injectable anesthetics can be effectively used to anesthetize animals prior to performing physical euthanasia. The agent should be an anesthetic recommended for the species, and the dosage used should be equal to or greater than the standard published reference dose for anesthesia (e.g., a common dose of pentobarbital for euthanasia is 100 mg/kg, which is approximately twice the anesthetic dose for rats and mice). Once the injectable anesthetic is administered, allow sufficient time for the animal to lose consciousness.

Injectable anesthetics intended for use in adult rodents may not have the desired effect in neonates. In a pilot study conducted at UNC-Chapel Hill, few anesthetics were found to be reliably effective in neonates. The drugs that provided the most effective anesthesia are available only to veterinarians and as a result were considered impractical for use by the scientific community. Contact a DLAM veterinarian for more information about appropriate doses of injectable anesthetics.

### Section 3: Euthanasia of Rodent Fetuses

Fetuses **up to 14 days** in gestation:
Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus at this stage of development.

Fetuses **15 days** in gestation to birth:
The literature on the development of pain pathways suggests the possibility of pain perception at this point in gestation. Whereas fetuses at this age are not sensitive to inhalant anesthetics, anesthesia may be induced by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g., pentobarbital. Decapitation with sharp scissors and cervical dislocation are acceptable physical methods of euthanasia when used by a trained person. The specific technique(s) employed must be described in the approved ACAP. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Consult with one of the institutional veterinarians to learn more about fetal sensitivity to specific anesthetic agents. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.

PDF format available on the IACUC website:
Click [HERE](http://www.avma.org/issues/animal_welfare/euthanasia.pdf).

### EUTHANASIA REFERENCES


evoked dopamine release from striatal synaptosomes in the rat. *Anesthesiology* 80(2), 352-363.


### Supply and Vendor Information

Isoflurane can be purchased from the UNC CH Hospital pharmacy: Call 919-966-1366 for more information or go to Ground Floor, room NG10B. A grant number and department number is required for purchase.

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Phone Number</th>
<th>Website</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Braintree Scientific</td>
<td>781-843-2202</td>
<td><a href="#">www.braintreesci.com</a></td>
<td>Instruments, lab equipment, isothermal pads, tattoo paste</td>
</tr>
<tr>
<td>Fisher Scientific</td>
<td>800-766-7000</td>
<td><a href="#">www.fishersci.com</a></td>
<td>Lab equipment, chemicals, instruments, pharmaceuticals</td>
</tr>
<tr>
<td>Henry Schein</td>
<td>800-872-4346</td>
<td><a href="#">www.henryschein.com</a></td>
<td>Veterinary supplies, instruments, pharmaceuticals Need Vet License or Researcher DEA license</td>
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<tr>
<td>JA Webster</td>
<td>800-225-7911</td>
<td><a href="#">www.jawebster.com</a></td>
<td>Veterinary supplies, instruments, pharmaceuticals Need Vet License or Researcher DEA license</td>
</tr>
<tr>
<td>Kent Scientific</td>
<td>888-572-8887</td>
<td><a href="#">www.kentscientific.com</a></td>
<td>Surgical equipment, telemetry equipment</td>
</tr>
<tr>
<td>Med-Vet International</td>
<td>800-544-7521</td>
<td><a href="#">www.shopmedvet.com</a></td>
<td>Veterinary supplies and instruments (discounted) Need Vet License</td>
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<tr>
<td>National Band and Tag</td>
<td>859-261-2035</td>
<td><a href="#">www.nationalband.com</a></td>
<td>ID tags, ear tags</td>
</tr>
<tr>
<td>Roboz</td>
<td>800-424-2984</td>
<td><a href="#">www.roboz.com</a></td>
<td>Specialize in instruments</td>
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<tr>
<td>TW Medical</td>
<td>888-787-4487</td>
<td><a href="#">www.twmedical.com</a></td>
<td>Veterinary supply (Bill Forrester)</td>
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<tr>
<td>UNC-CH Materials Management and Distribution</td>
<td>966-5671</td>
<td>Scientific Storeroom, General Storeroom, Chemical Storeroom</td>
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<tr>
<td>Veterinary Medical Supply</td>
<td>800-533-8674</td>
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<td>Veterinary Supplies out of Zebulon, NC</td>
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UNC Chapel Hill     Revised 05/2014
Need Vet License

Southern Anesthesia  800-456-0757  http://www.sasvet.com/
This is a human source company that has a Veterinary division, will set up an account without a vet license.
# Mice: Handling and Basic Techniques

<table>
<thead>
<tr>
<th>Technique needed?</th>
<th>Technique</th>
<th>Comments</th>
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<tbody>
<tr>
<td></td>
<td>Handling and Restraint</td>
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<td></td>
<td>Intraperitoneal Injection</td>
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<td>Subcutaneous Injection</td>
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<td>Intramuscular Injection</td>
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<td>Intravenous Injection</td>
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<td></td>
<td>Oral Gavage</td>
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<tr>
<td>Retro-orbital Injection</td>
<td><em>Individual appointment during class</em></td>
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<tr>
<td>Retro-orbital Bleed</td>
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<td>Tail Nick Bleed</td>
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<td>Tail Clip Bleed / Genotyping</td>
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<tr>
<td>Submandibular Bleed</td>
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<td>Cardiac Puncture, <strong>terminal</strong></td>
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<tr>
<td>Ear Notch (punch)</td>
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<td>Ear Tag</td>
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<td>Anesthesia Injectables</td>
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<tr>
<td>CO₂ Flow meter w/ Phys. Euthanasia</td>
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<tr>
<td>Cervical Dislocation without Anesthesia</td>
<td><em>Individual appointment during class</em></td>
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<tr>
<td>Decapitation without Anesthesia</td>
<td><em>Individual appointment during class</em></td>
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<tr>
<td>Isoflurane <strong>Euthanasia / Anesthesia</strong> Drop Method</td>
<td>*Provided during a 1-on-1 training session. Please call the OACU to schedule an appointment 966-5569</td>
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<tr>
<td>Inhalational Anesthesia Training-Vaporizer Machine Training</td>
<td>Contact DLAM Vet Services at 966-2906</td>
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## Proficiency Rating

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<th>II</th>
<th>III</th>
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Please add email address if you are a Lab Coordinator: _______________________________

Lab Phone #: _______________________________

I certify that I have received the above training:

Signature: _______________________________

PID: _______________________________

Print Name: _______________________________

Pt: _______________________________

Instructor Signature: _______________________________

Date: _______________________________