PSU
Animal Resource Program
Mouse Biomethodology

Handling and Restraint
- Safety concerns: Bite wounds, needle puncture and exposure to animal allergens.
- Appropriate attire for handling mice in the laboratory includes lab coat and disposable gloves.
- Normal mouse behavior is best observed before the cage or animal is disturbed by movement or handling.
- Characteristics used to distinguish between the sexes:
  - Ano-genital distance greater in the male vs. female
  - Genital papilla is larger in the male
  - Nipples present in the female (only) by 9 days of age
- The typical weight range for an adult mouse is 20-40 gm.
- Mouse Handling
  - Exam gloves must be worn when handling mice.
  - Removal from the cage:
    - Mice may be caught and lifted out of the cage by grasping near the base of the tail.
    - A plastic cup or other container may be used to gently scoop up the mouse.
    - Mice may also be removed from the cage by cupping them inside your hands.
  - Mice can be restrained by the scruff of the neck with the tail held against the palm of the hand with your fingers.
  - Various restraint devices are available for specialized procedures.
  - See the following videos for demonstrations of correct technique:
    - http://www.research.psu.edu/arp/training/videos/handling-and-restraint-of-mice

Blood Collection
- Volume
  - Total blood volume of the average adult mouse is 2-2.75 ml.
  - Up to 1% of the body weight may be collected every 2 weeks. (0.25 ml from a 25 gm mouse)
  - Removal of blood in excess of the above guideline may lead to physiologic changes in the mouse in response to blood loss.

- Sites for blood collection in the mouse
Repeated blood collection:

- Saphenous vein-hind leg
- Mandibular vessels -lower jaw
- Retro-orbital sinus-requires anesthesia
- Central tail artery-requires anesthesia

Terminal procedures:

- Cardiac puncture-requires deep anesthesia or euthanasia
- Axillary bleed-requires deep anesthesia or euthanasia
- Decapitation-requires “scientific justification by the investigator” to perform without anesthesia

Video demonstrations of blood collection techniques are available on the ARP website: [http://www.research.psu.edu/arp/training/videos/blood-collection-in-mice](http://www.research.psu.edu/arp/training/videos/blood-collection-in-mice)

Administration of Substances by Injection

- Intraperitoneal (maximum volume = 2-3 ml).

- Subcutaneous (maximum volume = 2-3 ml).

- Intravenous (maximum volume = 0.2 ml)

- Intramuscular (maximum volume = 0.05 ml)
  - Intramuscular injections are not recommended in mice due to the small size of the musculature, possibility of secondary complications and small injection volumes achievable.


Administration of Substances by Oral Gavage

- Recommended oral gavage volumes range from 5-10 ml/kg.
- A video demonstration of oral gavage is available on the ARP website: [http://www.research.psu.edu/arp/training/videos/oral-gavage-in-mice](http://www.research.psu.edu/arp/training/videos/oral-gavage-in-mice)
Euthanasia

The following methods are acceptable for use on mice and rats. Deviations from these procedures or the use of other methods of euthanasia require scientific justification and IACUC approval before they may be used.

CO₂

- Acceptable under specific conditions outlined below.
- The animal is placed into a closed chamber in which carbon dioxide gas is introduced.
- Compressed CO₂ gas in cylinders is the only acceptable source of carbon dioxide.
- Separate males from females and do not overcrowd the cage or chamber.
- Do not combine unfamiliar male mice in a cage prior to euthanasia.
- Maintain gas flow for at least one minute after respiration ceases.
- Make sure the animal is dead (i.e., no spontaneous respiration, pale or blue mucous membranes) before removing it from the CO₂ chamber.
- An adjunct method of euthanasia must be used to assure death after exposure to CO₂. These include:
  - Cervical dislocation
  - Decapitation (neonates)
  - Stab incision between the ribs (each side of animal) to puncture the chest cavity and insure that the animal cannot breathe.

Neonatal Rodents

- Neonatal rodents up to 10 days of age are resistant to CO₂ and volatile (gas) anesthetics and take a long time to euthanize using these methods alone.
- Although neonates are resistant to volatile anesthetics and CO₂, prolonged exposure will induce anesthesia. Do not rely on exposure to CO₂ or volatile anesthetics such as halothane and isoflurane as the sole method of euthanasia for neonates. **Decapitation or incisions into the chest cavity must be used to ensure death following exposure to CO₂ or anesthetic gases.**

Recommended method for neonatal euthanasia:

Place neonates in a plastic bag from which all air has been removed. Fill the bag with CO₂, seal the bag and observe periodically until all signs of respiration and movement are gone (this may take from 5 to 10 minutes). The neonates must be decapitated after this period to ensure recovery does not occur.

Cervical Dislocation

- Acceptable with the following conditions:
  - Cervical dislocation may be performed on unconscious rodents.
Personnel using cervical dislocation must be adequately trained to use this method and investigators are responsible for ensuring all personnel are trained. ARP will provide training to those who request it.

- **Cervical dislocation on conscious rodents requires scientific justification and prior approval by the IACUC.**

**Injectable Anesthetics**

- Acceptable
- Anesthetics may be injected intraperitoneally to euthanize rodents.
- Agents available for use include sodium pentobarbital, other pentobarbital combinations and ketamine/xylazine combinations.
- Controlled drugs must be used under the supervision of personnel registered with the United States Drug Enforcement Administration (DEA).
  - Requires strict accounting of quantities used.
  - Requires locked storage.

**Inhalant Anesthetics**

- Acceptable under specific conditions for euthanasia of laboratory rodents.
- A number of inhalant anesthetics may be used for anesthesia. Contact an ARP veterinarian for information regarding the use of inhalant anesthetics for euthanasia.
- All inhalant anesthetics require some method of scavenging the waste anesthetic vapors (i.e., working in a biosafety cabinet).

**Exsanguination**

- Acceptable only under deep anesthesia.
- May only be used to euthanize unconscious animals.