Introduction

Welcome to the Working with Mice in Research at UAB (AU_M) Course Material. The goal of this course material is to introduce you to working with mice in research at UAB. However, some procedures may be different according to your approved protocol. If you have questions or need further instruction, please contact the ARP Veterinary Staff.

Objectives

At the conclusion, participants should be able to:

1. Care for and monitor mice used in research.
2. Comprehend the physiological and environmental issues considered when working with mice.
3. Identify who to contact for guidance if there are questions.

Care and Husbandry

Housing

Caging

Rodents are social animals that burrow and nest, and these behaviors are supported by the use of contact bedding on solid flooring as is provided by shoebox caging. Contact bedding absorbs liquid wastes while providing comfort, warmth, the opportunity to burrow, and protection of newborn pups from chilling.

Minimum Space Recommendations

The Guide for the Care and Use of Laboratory Animals describes the minimum space recommendations for laboratory mice. For more information, see the IACUC Housing Density SOP for mice.

Once a cage is out of compliance with the UAB Density SOP, it’s tagged as overcrowded, and the PI is charged. PI’s are given three days to correct any non-compliance. ARP Staff will remediate the overcrowding, and then PI is charged for the service if any non-compliance is not fixed in the allotted time.
Acclimation and Quarantine

**Acclimation**

Acclimation is the process of becoming adjusted to a new environment or situation. Acclimation is necessary due to factors like stress from transportation. This stress could alter various blood parameters, immune cell function, and animal behavior.

Upon arrival at UAB, mice **must** have an acclimation period of at least 48 hours before use in research studies. It may be desirable to extend the acclimation period beyond 48 hours. If possible, one week of acclimation is best.

**Quarantine**

The **quarantine period** is eight to ten weeks. The goal of quarantining animals is to prevent transmission of diseases between new animals and animals already present at the facility in established colonies.

A Quarantine Period is vital for avoiding the:

- Loss of investigator time and valuable research data due to the introduction of disease.
- Cost of controlling and eliminating infections once they escape into established colonies.

Acclimation and quarantine periods can run at the same time even though they serve different purposes. Mice from new or unknown sources require a quarantine period. UAB does not allow animals from unapproved sources. Animals from approved sources do not require quarantine. For more information about acclimation and quarantining your mice, please contact the ARP Veterinarians.
Health Surveillance

The Sentinel System

Monitoring for the appearance or reoccurrence of a causative pathogen or parasite defines a sentinel. Health surveillance of animals at UAB monitors for the presence or recurrence of causative pathogens or parasites. The rodent health surveillance program uses a dirty bedding sentinel program. Colonies maintained by Principal Investigators (PIs) must participate in the Sentinel Program. For instructions and information, contact the ARP Veterinarians.

This system protects all UAB rodent colonies by:

- Identifying potential outbreaks as quickly as possible.
- Limiting the spread of potential pathogens.
- Mitigating the disruption of research.
- Facilitating the export of animals from UAB colonies to other institutions.

The Sentinel Cage

Colony animals do not have to be tested directly to provide colony health information. A sentinel cage containing a specific pathogen-free, the immune-competent rodent is used to represent colony animals. One sentinel cage represents up to 150 colony cages.

How This Works

- A portion of dirty bedding is added to the sentinel cage from each colony cage change.
- Every six months:
  - Survival samples are collected from sentinels and tested for viral pathogens only.
  - Sentinels are euthanized and replaced. At that time the gross exam is performed and tests for internal and external parasites, and viral pathogens.
- If ARP identifies a problem within a colony, they will contact the PI to arrange suitable management solutions.
Animal Identification

Temporary

Temporary identification examples are marking the tail, back, or head with a permanent marker. However, these are temporary due to hair growth and loss, or grooming.

Permanent

The most common permanent method is the ear punch. Other means of permanent identification include microchips, tattooing, or ear tagging. The use of anesthesia may facilitate some of these methods. Check with the ARP Veterinarians for more information.

Enrichment

Enrichment promotes an animal’s natural behaviors by providing a stimulating and interactive environment. Environmental enrichment leads to improved health in your mouse colony.

The Handbook of Laboratory Animal Management and Welfare identifies three points to consider when assessing enrichment:

1. It is relevant to the species and will positively impact welfare and normal species-specific behaviors.
2. It will not significantly impact the science.
3. It will not interfere with the animal housing management.
**Examples**

The provision of nesting material, such as nestlets, is a standard form of enrichment provided for rodents at UAB. Enviropaks are bags of shredded paper used for nest building and supplied in clean rodent cages.

Disposable or sanitizable huts can also be used in the cage to provide a place to hide and feel secure, improve breeding success, reduce aggression between animals, and promote natural activities.

Some mice even enjoy having objects designed for chewing. Please do not add enrichment items without first checking with the Animal Behaviorist. For more information on environmental enrichment options, please contact the ARP Animal Behaviorist or see the Enrichment and Social Housing of Animals SOP.

**Biological Features**

Though mice share many anatomical and physiological features with humans, mice have many unique biological characteristics. Researchers should be aware of the practical elements of mouse anatomy and biology.

**Anatomy**

**Ocular System**

Mice can develop red staining (called porphyrin staining) around the eyes and nostrils due to the accumulation of porphyrins. Lacrimal porphyrin is a regular component of tears in rodents and usually removed through frequent grooming. When distressed, an animal will groom less often, and the porphyrin will visibly accumulate around the eyes.
Teeth

Mice have incisors that are open-rooted, meaning that these teeth grow continuously throughout adult life. In particular, genetically modified mice may have unintended anomalies that cause jaw misalignment and result in tooth overgrowth.

A diet of soft foods (liquids or powders) or developmental jaw malformations can also cause tooth overgrowth.

Staff must be alert to detect any signs of this condition and to seek appropriate treatment.

Gastrointestinal

Inability to Vomit

Mice do not vomit; therefore, withholding food and water before surgery is not usually necessary in mice.

Gall Bladder

Mice do have gallbladders.

Coprophagy

In mice, plant material is broken down by microbial action in the cecum. To utilize the microbial byproducts of digestion, the mouse regularly eats its feces, a habit known as coprophagy. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the mouse.

If a study does require fasting for scientific reasons, be aware that mice will consume their feces and thus there may be fecal material in the GI tract (even when withholding food).
Metabolism

High Rate

The mouse’s high rate of metabolism may increase their ability to utilize and break down drugs. The mouse’s high rate of metabolism may enhance their ability to utilize and break down drugs. This high rate of metabolism means that drug dosages used in larger species (with lower metabolic rates) will likely be inappropriate in mice (this includes analgesics given postoperatively to control pain).

Investigators are advised to obtain mouse dosages from laboratory animal references or the ARP Veterinary Staff.

Albinism

Albinism is an inherited disorder, which has an impact not only on pigmentation (skin, fur, eye color, etc.) but also on other body systems. Neurologically albinos have abnormalities and functional impairments affecting hearing, equilibrium, and vision. The lack of pigment in the eyes of albinos can result in retinal damage in brightly lit rooms. Studies comparing albino and pigmented animals have shown differences even in drug metabolism.

Body Temperature Regulation

- **Hypothermia:** Mice have a high body surface area (relative to body volume), and many hairless body parts (tail, ears, and feet) which make them vulnerable to profound hypothermia when under sedation and anesthesia. Mice need a source of warmth throughout the procedure until they recover.

- **Hyperthermia:** Mice have no sweat glands and cannot pant, so they are susceptible to overheating if the room temperature is not appropriately regulated or they are unable to escape a heat source.
Behavior

Mice are social animals and typically live in groups. When group housed, mice will sleep together in a pile. Mice are nocturnal, meaning they are more active at night. Most mouse births occur at night.

Barbering

Mice often spend a great deal of time grooming, and they groom each other. Sometimes a dominant mouse grooms its cage mates so much that it will ultimately remove areas of their fur, resulting in a bald spot, usually in the facial region. This behavior is called barbering.

Fighting

**Fighting is the number two clinical issue for mice.** Dominance hierarchy and territoriality are linked to fighting. It is thought to occur primarily because of environmental factors, but genetics may contribute. Pheromones and hormones contribute significantly to aggressive behavior.

The most critical step to prevent fighting is never to mix sexually mature male mice (mainly after they have been housed alone or in a breeding cage). Common sites for fight wounds include the tail, the tail base, along with the back, and around the front legs.

Male mice weaned together can be housed together; however, some males will fight as they mature. Males from strains such as FVB, SJL, and BALB/c are predisposed to fighting. Female mice do not fight and can often be kept together indefinitely. If your mice are fighting, please contact the ARP Veterinarian Staff for recommendations and refer to the [ARP Fighting Mouse Guidelines](#).
Handling

Mice will avoid being handled and can bite. Proper handling will minimize the risk of injury to personnel and animals.

- Grasp the base of the tail to initially catch mice. Do not grab mice by the tip of the tail as it can lead to injuries in which the skin of the tail tip comes off.
- Tongs can also be used to pick up adults at the base of the tail.
- Newborns should be picked up by scooping them up with the hand.

Scruffing or gentle handling can restrain mice. The key is to handle the mouse in such a way as to not harm the mouse and avoid bites or scratches. For some procedures, the mouse’s head can be covered with a disposable drape or clean towel to minimize distress.

Sometimes procedures may require the use of a commercially available restraint device. There are several different models available. Contact the ARP Veterinary Staff for more information.

Detecting Pain and Distress

Assessing pain and distress in mice is difficult at times because mice commonly conceal outward signs of moderate pain and suffering. Behavioral changes that reveal a mouse's pain and suffering may be subtle and elude detection unless observations are thorough and made by a trained observer. Severe pain and suffering cause overt clinical signs in mice. Therefore, laboratory staff working with mice should be taught to recognize the following abnormalities: activity level, behavior, appearance, body condition, and vital signs. For more information, see this comprehensive checklist.
The research indicates that mice can express pain through facial expressions just like humans. The Mouse Grimace Scale (MGS) looks at five “pain faces”: orbital tightening, nose bulge, cheek bulge, ear position, and whisker change. For more information, see this study.

Example

The mouse shown here has scruffy fur, a hunched posture, and porphyrin staining around the eye. The ears, feet, and tail appear pale, suggesting blood vessel constriction or anemia.

Procedures for Injections and Blood Collection

Volume Recommendations

Exceeding 0.1 ml per 10 grams of body weight, or expressed, about 10% of total blood volume produces a hypovolemic shock. Removal of up to 10% of blood volume from multiple collections over a two week period has the same effect. The regeneration time for blood cells is approximately two weeks. When removing large quantities of blood, it may be necessary to administer warmed physiological fluid to replace the volume of blood collected. For more information, see volume recommendations.

Methods Commonly Used for Blood Collection or Fluid Administration

For hands-on training, please see the ARP Veterinarian Staff. The recommended gauge size of a needle in blood collection and fluid administration is 25 to 29. Larger needles may be necessary for injecting large volumes or thick materials. When accessing the tail, lateral saphenous, and cheek veins:

- Anesthesia is not required.
- Sedation may be used to increase vein visibility by peripheral vein dilation and may help immobilize the animal.
### Method of Blood Collection

<table>
<thead>
<tr>
<th>Method of Blood Collection</th>
<th>Images</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tail Vein</strong></td>
<td>![Tail Vein Image]</td>
</tr>
<tr>
<td>Primarily the tail vein is used for intravenous injection, but small amounts of blood can be obtained using tail nick technique.</td>
<td></td>
</tr>
<tr>
<td><strong>Lateral Saphenous Vein</strong></td>
<td>![Lateral Saphenous Vein Image]</td>
</tr>
<tr>
<td>Blood collection from the lateral saphenous vein requires puncturing the vein through the skin.</td>
<td></td>
</tr>
<tr>
<td><strong>Cheek Vein</strong></td>
<td>![Cheek Vein Image]</td>
</tr>
<tr>
<td>Blood collection from the cheek vein requires puncturing the vein through the skin.</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiac Puncture</strong></td>
<td>![Cardiac Puncture Image]</td>
</tr>
<tr>
<td>A Cardiac Puncture is a terminal procedure because of potential damage to the heart. The IACUC must approve it. The animal is required to be under anesthesia.</td>
<td></td>
</tr>
</tbody>
</table>
Retro-Orbital Puncture

The Retro-Orbital Puncture is required to be performed by skilled personnel because of the risk of injury to the eye, and surrounding structures is high. *This method is considered to be painful and requires anesthesia.* The topical ophthalmic anesthetic may provide pain relief after the procedure.

### Procedures for Injections

**Intraperitoneal (IP)**

The Intraperitoneal (IP) injection is appropriate for the administration of up to 2-3 ml of fluids or liquids and given to the lower left or right area of the abdomen.

**Subcutaneous (SQ or SC)**

The volume recommendation for this route in adults is 2-3 ml total or a maximum of 0.5 ml per site.

**Oral (PO)**

This technique, also called gavage, requires a specialized feeding needle and may require anesthesia.
Analgesics and Anesthetics

You should check with the ARP Veterinary Staff for assistance in determining a dose rate appropriate for use in mice for analgesics and anesthetics.

**Analgesics**

An analgesic is a medical term used for pain medicine or those drugs that help to control pain.

The analgesic regimen should be chosen to match the expected duration of pain or discomfort caused by the procedure. If you have any questions, contact the ARP Veterinary Staff.

**Perform injection bevel side up. The bevel is the sloping side of the needle.**

Under no circumstances, should pain medications not be provided following a painful procedure unless justified in your approved IACUC Protocol!
An anesthetic regimen should be chosen to match the duration of drug effects with the length of the procedure. Explosive agents, such as Ether, **cannot** be used.

- For long or short procedures, gaseous anesthesia using a non-explosive agent such as Isoflurane is often the most practical method to sustain uniformly adequate levels of anesthesia.

- You should not use short-acting agents (and regimens) for lengthy procedures. Repeat drug administrations, necessary to prolong anesthesia, will produce uneven blood concentrations and periodically inadequate anesthesia.

**Neonates**

Hypothermia may be used as an anesthetic for neonates (newborn) seven days of age or younger. Alternatively, inhalation anesthesia with an agent such as Isoflurane administered using a non-rebreathing system may be an acceptable alternative to hypothermia in neonatal rodents. For instruction in this technique, please consult the [ARP Veterinary Staff](#).

**Surgery Techniques and Requirements**

If you are planning to perform surgical procedures on rodents, you must complete [Rodent Surgery](#). We also strongly recommend you complete [Post-Procedure Care of Mice and Rats in Research](#), as well.
Euthanasia

UAB requires two methods of euthanasia listed on your approved IACUC Protocol. If you have not been trained to euthanize rodents, please contact one of the ARP Veterinary Staff before attempting the procedure.

**Primary Methods**

Following are examples of acceptable primary methods of euthanasia with IACUC approval:

1. Inhalants (e.g., CO₂ and Isoflurane)
2. Injectables (e.g., Fatal Plus, Ketamine/Xylazine)
3. Physical, (e.g., rapid decapitation, microwave euthanasia)

**Secondary Methods**

To ensure death, you must follow primary euthanasia (e.g., inhalants and injectables) with a secondary method such as:

*Cervical Dislocation*

Cervical dislocation is used in unconscious rodents and involves the physical separation of the skull and vertebrae by application of force and results in severing the cervical spinal cord.

*Thoracotomy*

Thoracotomy is also used in unconscious rodents and involves cutting open the thoracic (chest) cavity to induce a pneumothorax and prohibits the expansion of the lungs and leads to irreversible oxygen deprivation and death.

*Exsanguination*

Exsanguination is also a secondary method and involves allowing an unconscious animal to “bleed out.” In essence, this acute loss of a large volume of blood will result in death.
Decapitation

Decapitation is also used in unconscious rodents and involves physical removal of the head from the body. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure such.

Other Techniques and Information

Restraint devices are available that minimize the chance of injury to personnel and improve positioning of the animal for this procedure.

Other techniques are available for rodent euthanasia and may be acceptable when scientifically justified by the user and approved by the IACUC.

For more information on euthanasia techniques, please consult with the ARP Veterinary Staff, and see the IACUC SOP on Animal Euthanasia.

Carcass Disposal

Before placing euthanized rodents in a bag, you must make sure the mice are dead! Mice can stop breathing for a minute or more then can regain respiratory function and survive. Younger mice are resistant to Carbon Dioxide asphyxiation and take longer to succumb than adult mice.

Locations with an Animal Morgue

Non-Hazardous Carcass

1. Place the carcasses in a black plastic bag.

2. Place the black bags in the red-lined Stericycle box in the morgue.

Hazardous Carcass

1. Place the black bags in the red-lined Stericycle box in the morgue.
Locations without an Animal Morgue

Non-hazardous carcasses:

1. Place the carcasses in a black plastic bag.
2. Place the bag in the designated refrigerator.

Hazardous carcasses:

1. Place the bag inside a red bag marked with the hazard.
2. Place it in the designated refrigerator.

Carcasses Contaminated with Radioactive Hazards

Carcasses contaminated with radioactive hazards should be disposed of as indicated by the PI’s Radioactive Materials License.

Occupational Health Issues

Personal Protective Equipment (PPE) is required when working with mice. Always check the AUSI on the door (if posted) to ensure that you have on the right PPE. PPE is available at the entrance of each facility.

- To enter an animal housing room, you must wear a clean, buttoned lab coat, a designated uniform or disposable gown.
- You should wear the appropriate gloves when handling mice. Never handle mice ungloved.
- When opening cages, wear a face mask, or conduct work in a changing station or Biosafety Cabinet.
- Respiratory equipment requires annual checks.
- Remove your PPE in the proper place. Never wear possibly contaminated PPE outside the facility.

Allergens

People can develop an allergy to mouse dander after having contact with them for some time. Persons who establish allergy symptoms should seek medical counseling and may have to discontinue working with this species.
Zoonotic Agents

The risk of transmission of zoonotic agents (diseases passed from animals to humans) from laboratory rodents to research personnel is very low. Lymphocytic Choriomeningitis Virus (LCMV) and Campylobacter species are common agents that can be found in rodents and passed to humans. Avoid these and other agents by using proper Personal Protective Equipment (PPE) and consistent hand washing. Injuries should be cleaned with soap and water immediately, and reported to your supervisor.

Conclusion

This section concludes Working with Mice in Research at UAB (AU_M) course material. You should take the assessment now. The passing score is 80% or higher. For a glossary, reference guides, and other information visit the IACUC website.

Other Required Training

- If you are responsible for breeding mice or rats at UAB, you must complete Rodent Breeding at UAB (AU_RBR).
- If you are responsible for monitoring rodents after any procedure, you must complete Post-Procedure Care of Mice and Rats in Research at UAB (AU_PP).
- If you are responsible for performing any procedures on a rodent, you must complete Rodent Surgery (AU_RS).
- If you are working with any animal at UAB, you must complete Using Animals for Teaching, Testing, and Research at UAB (AU_UA).