Welcome to the course Working with Mice at the University of Washington.

This web course is designed to provide important information about using laboratory mice in biomedical research settings and to help ensure their humane care and use.

Please note that this course is the 1st of a 2-part series. After passing the online exam with a 100% please register for the hands-on portion of the series by submitting an Animal Use Training Registration Form. Credit for attending the Mouse Laboratory will only be given after completion of the hands-on training session. The hands-on portion will provide you with the opportunity to practice handling mice and perform techniques as well as become certified in procedures you may need when working in your lab.

Page 2. Course Goals

The goals of this course are to:

Provide important information about using mice in biomedical research settings.

Highlight the unique biological features of these animals.

Provide background information on the common signs of pain, illness and distress.

Provide instruction on procedures that may be used when working with mice in a research setting.

Function as a resource of information and contacts for working with mice at the University of Washington.
Upon completion of this course, and after passing the online exam with a 100%, personnel will be allowed to register for the hands-on portion of the mouse lab.

**Page 4. Exam**

To take the exam covering this course at any time, click on the Exam link on the previous webpage.

You must score **100%** to pass the **final 20 question exam**. If you do not pass, you may take the exam again as many times as you wish.
Before working with animals at the UW there are a few things you need to do. Complete the “University of Washington: Animal Use Laws and Regulations Training” which is an online course. This course needs to be updated every 5 years and completed prior to being added to an IACUC protocol.

You must also complete the Animal Use Medical Screening Form to enroll in the Occupational Health Program.

You must be listed and approved as personnel on each IACUC protocol you will be working under.

You should also read and understand the protocol you will be working under. It is important that you know what techniques you are allowed to perform and which you are not allowed to perform.
Lesson 3. Occupational Health Issues
Page 1. Occupational Health Responsibilities

It is the responsibility of principal investigators to ensure that their laboratory staff are informed of and participate in their institution's occupational health and safety program.

Page 2. Occupational Hazards

Working with mice is associated with several types of hazards, which are discussed here and on the next page:

Injuries
Personnel handling a mouse can be bitten if the animal is poorly restrained. Though mice are often inclined to bite when frighteneed, fortunately their incisors do not always penetrate disposable gloves to break the skin. Bites can be caused by poor handling and restraint technique, which can also cause injury to the mice. If you are nervous working with mice or do not know how to properly handle and restrain them, ask for help. Each institution must provide training as needed so that personnel know how to handle and restrain mice effectively and humanely, preventing injuries to both people and mice.

Allergies
People can develop an allergy to mice over time after having contact with them, even if they have no history of allergies. Mouse urine is particularly allergenic, and pelt proteins can also be allergenic. For this reason, you should wear the provided Personal Protective Equipment (PPE), disposable gloves, a dedicated lab coat or scrubs and a hair bonnet to prevent exposure to urine and pelt proteins. People who develop allergy symptoms should seek medical counseling, and may have to wear special protective equipment or even discontinue working with this species if symptoms are severe after exposure. Protect yourself! To see some references on allergies to mouse urine, click here.
Zoonoses (diseases transmitted by animals to humans)

In general, transmission of zoonotic disease from specific pathogen free (SPF) laboratory animals is uncommon. This is because of ongoing vendor efforts to improve the health status of animals, as well as routine periodic infection surveillance programs by facility staff. However, experimentally infected animals are a potential source of zoonotic transmission to humans, and contact with wild mice in field research may also expose humans to zoonotic agents. Animal infection surveillance programs, routine sanitation, training, and PPE all have important roles in preventing zoonoses.

Mice can be a reservoir of the following infectious agents that are transmissible to people. Here are some zoonotic agents carried by mice:

Viruses

- **Hantavirus**

  Hantavirus is a bunyavirus carried by wild mouse species. The virus is transmitted to man by excretions and aerosols from the lungs, saliva, and urine of infected animals.

  Humans are at risk for Hantavirus infection (Korean Hemorrhagic Fever) primarily from wild caught rodents (e.g., the deer mouse, *Peromyscus*). Strains vary in symptoms based on geographical origin (US, Asia, Scandinavian, and Europe). Hantavirus occurring in the southwestern U.S. causes a severe pulmonary syndrome. Strains originating in Asia produce a hemorrhagic fever and nephropathy. Strains originating in northern Europe produce renal symptoms of less severity.

- **Lymphocytic choriomeningitis virus (LCM)**

  The LCM virus is an RNA arenavirus. Human infection with LCM has been associated with laboratory animals and pets. Mice may be endemically infected (infected in the absence of clinical signs). In utero or early neonatal infection produces a subclinical infection in mice that is characterized by virus shedding (blood, urine). Tumor cell lines may be infected.

  Virus transmission occurs by direct contact as well as by inhalation. Pregnant women are at risk of transmission to the fetus.
Humans typically develop an influenza-like illness. Additionally, infection may cause a maculopapular rash, lymphadenopathy, meningoencephalitis, orchitis, arthritis, and epicarditis.

**Bacteria**

- *Leptospira* spp.

  Mice may be a reservoir for *Leptospira* spp. bacteria, which are shed in the urine. Transmission occurs by contact with urine and tissues, or inhalation or ingestion of aerosol droplets.

  Humans with leptospirosis may have influenza-like symptoms, orchitis, rash, skin and mucosal hemorrhage, hemolytic anemia, hepatorenal failure, jaundice, encephalitis, and pneumonia.

- *Salmonella* spp.

  Mice may carry *Salmonella* spp., which are ubiquitous in nature. These bacteria are transmitted via the fecal-oral route.

  Humans infected with *Salmonella* may have inapparent clinical signs (and be carriers) or may have a febrile enterocolitis, septicemia and focal infections in diverse tissues. Increased severity of the disease occurs due to reduced immunocompetence, e.g., in persons with AIDS, neoplasia, immunosuppression therapy, etc., and due to treatment with antibiotics.

**Fungi**


  Dermatophytic fungi grow in the skin and hair follicles and cause a condition of reddened skin and patchy hair loss known as ringworm. The symptoms are the same in animals and humans. Infection may be inapparent in individual animals.

  Dermatophytes are spread by direct contact. Fungal spores are long-lived and may become widely dispersed in the environment. Infections are treatable, but an extended period of therapy is often required to eliminate infection.

**Parasites**

- *Hymenolepis nana*

  *Hymenolepis nana*, otherwise known as the dwarf tapeworm, may be found in mice. It has both a direct and indirect (via flour beetles or fleas) cycle.

  *H. nana* is transmissible to man. Depending on the parasite burden, humans may have no
apparent clinical signs or may have nausea, anorexia, vomiting, diarrhea, and central nervous system signs (agitation).

For more information, refer to *Occupational Health and Safety in the Care and Use of Research Animals*, published by the National Research Council.
Lesson 4. Acclimation and Quarantine

Page 1. Acclimation

Upon arrival to your facility, your mice should have an acclimation period before they are used in research studies. This period of time allows animals to adapt to a new environment. Effects of transportation stress include alterations in various blood parameters, immune cell function and animal behavior. The period of time necessary for biological stabilization will depend on the parameters to be studied. Refer to your institution’s attending veterinarian for recommendations that are appropriate for your project. Typically, acclimation periods can range from days to over a week, depending on the studies involved.

Page 2. Quarantine

Routine quarantine procedures may prolong the holding of your animals in special facilities. An important goal of quarantining animals is to prevent transmission of diseases between new animals and animals already present at the facility in established colonies.

Many institutions quarantine all mice received from other institutions, no matter what certifications of health may accompany them. There are many reasons for this, but the following three are worth noting:

1. **Detecting viral, bacterial, and parasitic pathogens in mice can be challenging** because many infections are *asymptomatic* (cause no observable clinical signs), and thus infections can be
missed in animals prior to shipment. As an example, pinworm infections in mice are notoriously difficult to diagnose because eggs from the female nematodes are shed intermittently and sometimes in low number, leading to missed diagnoses.

2. The **cost** of controlling and eliminating infections once they escape into other colonies can be enormous.

3. And finally, huge amounts of **investigator time as well as priceless research data can be lost** due to infections.

Acclimation and quarantine periods can run concurrently, although they serve *different purposes*. Institutions may or may not allow experiments on animals while quarantined, depending on the circumstances.
Breeding mice as inbred strains and outbred stocks produce animals that are used for different purposes. The decision to use isogenic inbred strains or non-isogenic outbred stocks is determined by the experimental strategy.

**Inbred strains** are used for genetic engineering and finely controlled studies that capitalize on genetic isogenicity. Inbred strains with characteristics of human diseases or physiological conditions are generally preferred models for biomedical research.

**Outbred mice** are used when outbred vigor is desirable, e.g., as foster females for a transgenic colony, or when genetic heterogeneity and phenotypic variability are not a concern.
Though mice share many anatomical and physiological features with humans, mice have many unique biological characteristics. Knowledge of species-specific characteristics is helpful to effectively manage these animals and to plan experimental procedures for their use.

Researchers should be aware of the following practical features of mouse anatomy and biology:

Anatomy

- **Muscular Skeletal System**

  Mice are quadrupeds, this means they walk on all four feet. Quadrupeds are more likely to scratch at incision sites. Also, many times an incision site will rub along dirty bedding in a cage, such as an abdominal incision. This contact with dirty bedding can lead to
infection of the site.

- **Ocular System**

  Rats and mice may develop red staining around the eyes and nostrils when they are distressed, e.g., by disease, trauma, etc. This staining is due to the accumulation of porphyrins produced by the Harderian gland, a lacrimal gland (see photo right). Though a normal constituent of tears in rodents, lacrimal porphyrin is produced in limited amounts and healthy rodents keep themselves clean of debris through frequent grooming. Porphyrin staining in distressed animals occurs because stress stimulates porphyrin production in tears and distressed animals groom themselves less often.

- **Teeth**

  Mice have incisors that are open rooted, meaning that these teeth grow continuously throughout adult life. A diet of soft foods, i.e. in liquid or powder form, or a developmental jaw malformation will cause tooth overgrowth (see photo right). In particular, transgenic or knock-out mice may have unintended genetic anomalies that cause jaw malalignment and result in tooth overgrowth. Staff must be alert to detect any signs of this condition and to provide appropriate treatment. Mice with this condition often present with dehydration, weight loss and failure to thrive.

**Gastrointestinal System**

- **Inability to vomit**

  Mice do not vomit because they lack the neurophysiological mechanisms for doing so. Therefore, withholding food and water before surgery is not usually necessary in mice.

- **Gall Bladder**

  Unlike rats, mice *do* have a gall bladder.

- **Coprophagy**

  In mice, herbaceous foodstuffs are broken down by microbial action in the cecum, which is a large organ in the mouse. To assimilate the microbial byproducts of digestion, the mouse regularly eats its own feces, a habit known as coprophagy. *If a study does require fasting for scientific reasons, be aware that mice will consume their own feces and thus*
there may be fecal material in the GI tract in the absence of food. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the mouse.

Metabolism

• **Albinism**

Many mice used in research are albinos, whether an inbred strain such as the Balb/C or an outbred stock such as the Swiss Webster. Albinism in mice is an inherited disorder of melanin metabolism caused by the lack of the enzyme tyrosinase, which has an impact both on melanocytes and neurons. Neuronal morphological abnormalities and functional impairments involve the following sites: medial vestibular nucleus, cochlear nuclei and retina. Studies comparing albino and pigmented animals have shown differences even in pharmacotoxic kinetics in these tissue areas. The lack of pigment in the eyes of albinos can result in retinal damage in brightly lit caging rooms. Consequently, animal care staff are obligated to monitor light levels.

• **High rate of metabolism** – impact on drug clearance

The mouse’s high rate of metabolism produces a rapid clearance of drugs from the body. Drugs administered at dose rates used in larger species (with lower metabolic rates) will likely reach lower blood concentrations and exert less pharmacological effect in the mouse. This includes analgesics given postoperatively to control pain. As a result, mice should receive drug doses that have been scaled to the mouse’s metabolism. Through a discipline known as allometry, mathematical formulas have been developed to adjust dose rates between species of disparate size.

Investigators are advised to obtain mouse dose rates from **Veterinary Services**.

• **High surface area** – impact on hypothermia

Mice have a large body surface area (relative to body volume) plus many hairless body parts (tail, ears, feet). As a result, mice are vulnerable to profound hypothermia under conditions of sedation and anesthesia. Sedation and anesthesia induce hypothermia due to drug effects on the hypothalamus and a rapid drop in core body temperature. If surgery is being performed, additional heat is lost by convection from an open incision during surgery, and placement of the mouse on a heated surface will be necessary during the surgery to maintain a healthy body temperature.

Mice should have a source of warmth throughout a procedure that lowers their body
temperature (e.g., anesthesia, surgery) and afterwards until they recover the ability to thermoregulate themselves.

For more information on mice please refer to the Veterinary Services Fact Sheet.
Lesson 7. Gender Determination

Page 1. Gender Determination

When working with mice in a research setting it is very important to be able to determine the gender of each animal to avoid unwanted breeding or to encourage breeding.

The best way to determine gender is to compare the anogenital distance of males versus females (distance between the anus and the genital papilla is ~ 2 times longer in males). It is best to perform gender determination when animals are weaned (approximately 3 weeks of age). Accuracy may be difficult if younger than 3 weeks.

Mice are sexually mature at 5-7 weeks of age. Improper gender determination may result in unwanted pregnancies or could result in a lack of pregnancies.
There are several different methods that may be used to identify your animals. Some are temporary while others are considered to be permanent.

If you only need to temporarily mark your mice you can make a mark on the tail or hair coat with a non-toxic “Sharpie” pen (this will be removed by the mouse during normal grooming, usually within 24-48 hours).

Methods of permanent identification are ear tags, ear punch, micro-chips and tattooing. **NOTE:** Amputation of toes is generally not acceptable. This techniques requires a strong scientific justification and approval from the IACUC.
Rodents being used in non-terminal procedures must be transported in secure cages with filter tops. Please flip up the water bottles during transport to avoid flooding the cages. Transport boxes may be used for terminal procedures.

All cages (including empty cages) MUST be completely covered with a drape during transport through public areas (i.e., outside the animal facility). The drape reduces allergen exposure to the public and helps to reduce the spread of pathogens. The drape also aids in keeping the use of animals discreet.
Lesson 10. Humane Standards

Page 1. Humane Standards

The core intent of all of the federal laws, regulations, policies and guidelines applicable to animal research is to ensure the humane treatment of the animals involved in a study. Accordingly, your IACUC will have requirements for the proper care of your animals prior to, during and after a research procedure.

Page 2. Humane Standards - Housing

Part of meeting humane standards is providing adequate housing for the animals you will be working with. Animals must not be overcrowded. Below are the cage density limits for mice.

- 5 adult mice
- 1 adult male, 2 adult females, and 2 litters up to 21 days of age
- 1 adult female, 1 litter up to 28 days of age and 1 litter under 21 days of age

Adequate housing includes keeping cages clean and dry. Occasionally a cage may flood from a leaky bottle or a stuck open lixit. When you discover a cage that is flooded it is very important that the animals be put in a new dry cage. Failure to do so can result in the mice going into shock and possibly dying.

Page 3. Humane Standards - Procedures

What is a procedure? A procedure is any activity performed on the animal, such as controlled behavioral observation (e.g., use of a maze), venipuncture, or surgery. Requirements for peri-
procedural care include:

- Properly preparing the animal to undergo the procedure humanely;
- Supporting the animal's physiological functions during the procedure; and
- Providing appropriate supportive care to aid the animal in recovering from the procedure.

**Page 4. Humane Standards - Training**

![Image](https://example.com/image1)

The investigator has the responsibility to see that staff working with the animals are properly trained not only to perform the procedure humanely but also to provide the necessary supportive care to the animals.

**Page 5. Humane Standards - Planning**

![Image](https://example.com/image2)

When performing any procedure, you should think through the steps that are necessary to protect the animal's welfare. For example, for blood collection, you should limit the volume taken to a safe minimum and you should realize that safe volumes will differ for acute or chronic collections. With any venipuncture, you should be prepared to care for the animal in the event of trauma to the vein or excess hemorrhage.

The saphenous vein shown in the photo is useful only for small volume collections.

Refer to the [UW IACUC policies](https://example.com/policies) for specific guidelines.
Lesson 11. Handling and Restraint

Page 1. Handling and Restraint

Learning proper handling and restraint technique is crucial in preventing injury to the animal and the handler. For this reason it is recommended that you complete the second portion of this course. Contact the Training Coordinator to register for the hands-on portion of the course.

When restraining mice it is important that you restrain them for as short a period as possible. That means that for whatever procedure you are doing you should have your equipment set up and ready to use prior to picking up the mouse. A mouse can be supported by the base of its tail for 2-3 seconds. This will give you enough time to move it from one cage to the top of a wire rack cage.
Lesson 12. Detecting Pain and Distress

Page 1. Detecting Pain and Distress - Challenges

If your proposed study involves a painful procedure, the protocol form will ask for a method of assessing if the mice are experiencing pain or distress.

Assessing pain and distress in mice is difficult at times because mice, like many other species, commonly conceal outward signs of moderate pain and distress. Accordingly, behavioral changes that reveal a mouse’s pain and distress may be subtle and elude detection unless observations are thorough and made by a trained observer.

Page 2. Detecting Pain and Distress - Signs

Severe pain and distress causes overt clinical signs in mice. Laboratory staff working with mice should be trained to recognize these abnormalities in:

- Activity level: hypoactivity (abnormally low), hyperactivity (abnormally high), restlessness.
- Behavior: vocalization, self-trauma, aggressiveness, isolation from cage mates, ataxia and decreased exploratory behavior.
- Appearance: unkempt or greasy fur, porphyrin (reddish brown) staining around eyes, nostrils, paws and/or hair, hunched posture, cyanosis (blue or purple coloring of skin), pale mucous membranes, soiled anogenital area.
- Vital Signs: e.g. respiratory distress (rapid shallow breathing or slow deep breathing).
- Body Condition: weight loss, emaciation, dehydration.
- Intake: reduced intake of food and water.

A chronic state of pain or distress may be more subtle and difficult to detect. A good knowledge of the animal’s normal appearance and behavior is especially important to recognize chronic pain
or distress.

The mouse shown above right has scruffy fur, a hunched posture, and porphyrin staining around the orbit. The ears, feet, and tail have a blanched coloration, suggesting vasoconstriction (blood vessel constriction), hypoperfusion (abnormally low levels of blood in tissue) or anemia (reduced red blood cell count). This mouse is showing signs of severe pain and/or distress. **NOTE: Your animals pain and distress may not be this obvious. It is important that researchers be able to detect the subtle signs of pain and distress.**

If the animal is showing signs of pain, distress or illness, contact Vet Services immediately.
Lesson 13. Procedures for Injections and Blood Collection

Page 1. Injections and Blood Collection - Volumes

Volume recommendations (ml) for acute intravenous fluid administration and blood collection in adult mice (average 20 g):

<table>
<thead>
<tr>
<th>IV Fluid Volume (ml) max. acute admin.</th>
<th>Total Blood Volume (ml)</th>
<th>Safe Bleeding Volume (ml)</th>
<th>Total Bleed-out Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1.0 - 2.4</td>
<td>0.1 - 0.2</td>
<td>0.6 - 1.4</td>
</tr>
</tbody>
</table>

*a* Removing greater quantities of blood (exceeding 0.1 ml per 10 grams of body weight, or alternately expressed, about 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, it may be necessary to administer warmed physiological fluid to replace the volume of blood collected, please consult with Vet Services.

*b* Animals should be exsanguinated only under anesthesia.


When collecting blood the following rule is helpful to assess how much blood you can take, how often you can take that volume and what is the exsanguination amount for a specific animal.

1-3-6% Rule for Venipuncture:

1% of body weight = maximum volume per collection every 2 weeks

3% of body weight = amount expected at exsanguination

6% of body weight = approximate total blood volume

Example: 25 gram mouse

1% = 0.25 ml every 2 weeks

3% = 0.75 ml at exsanguination

6% = 1.50 ml approx. blood volume

Page 2. Blood Collection - Sites

Below are peripheral vessels that are commonly accessed for blood collection. Recommended needle sizes are 25 to 30 gauge.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Comment</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>Instructions</th>
</tr>
</thead>
</table>
| Tail vein                     | 1. Accessing the tail vein and the lateral saphenous vein:  
   o Does not require anesthesia.  
   o May be aided by sedation because vein visibility is enhanced by peripheral vasodilation (drug effect).  
   o May be aided by sedation to reduce animal struggling due to restraint.  
   2. Blood collection from the lateral saphenous vein does not involve cannulation of the vein lumen. Instead, the vein is punctured percutaneously and blood is passively collected as it pools on the skin. |
| Submandibular vein            | 1. Accessing the submandibular vein:  
   o Does not require anesthesia.  
   2. Blood collection from the submandibular vein does not involve cannulation of the vein lumen. Instead, the vein is punctured percutaneously and blood is passively collected. |
| Tail tip amputation           | 1. These two methods generally require anesthesia, but UW IACUC allows tail tip amputations (for genotyping) without anesthesia prior to weaning. Click here to read the UW IACUC policy on tail tip amputation.  
   2. Due to potential damage to the heart, cardiac puncture is allowed only as a terminal procedure and only when the animal is in a surgical plane of anesthesia. |
| Retro-orbital puncture        | 1. Retro-orbital bleeding must be performed by skilled personnel and the risk of injury to the eye and surrounding structures is high. You must be certified by a designee of the Attending Veterinarian of the UW to perform this procedure.  
   2. This method is considered to be painful and may cause blindness. Generally requires anesthesia.  
   3. Topical ophthalmic anesthetic may provide pain relief after the procedure.  
   4. If only a small amount of blood is needed consider saphenous bleeding. |
Page 3. Injections - Sites

Below are the routes of injection that are commonly used in mice. Included are volume recommendations for the safe administration of fluids acutely in adults (average 20 g). Recommended needle sizes are 25 to 30 gauge. Larger needles may be necessary for injecting large volumes, cells or viscous materials.

Note – **Injection of non-pharmaceutical and pharmaceutical compounds requires inclusion in an IACUC approved protocol.**

Subcutaneous (SQ or SC)
1-2 ml total; maximum of 0.25 ml per site.

Intraperitoneal (IP)
0.5-1.0 ml

Retro-orbital (IV)
0.1-0.2 ml

Intra venous (IV) tail vein
0.1-0.3 ml

Oral Gavage (PO)
0.25 ml

Intradermal (ID)
0.05 ml/site

Intramuscular (IM)
0.05 ml/site Note – **Intramuscular (IM) injection is not generally recommended in mice because these animals lack sufficient muscle mass for an injection. An IM injection in mice would be likely to cause muscle injury, and should be done with the mouse under anesthesia.**
Lesson 14. Analgesics, Sedatives, and Anesthetics

Page 1. Analgesics, Sedatives, and Anesthetics
Because mice have a high rate of metabolism, drugs are rapidly eliminated from their bodies. Dose rates appropriate for larger species produce ineffective drug concentrations when used in mice. Consult with Vet Services for dose recommendations.

Page 3. Analgesics, Sedatives, and Anesthetics
- Agents
Click on the drug types for information on each of the common agents that may be used in mice:

Analgesics:
Available in two drug types – the opioids and the nonsteroidal anti-inflammatory drugs (NSAIDs). The rapid clearance of many of these drugs in mice results in the need for an increased frequency of administration.

Sedatives:
Sedatives may obtund consciousness but in normal doses do not do so sufficiently to ablate the perception of pain or other sensations. When combined with general anesthetics, they may be used to induce a "balanced" anesthesia where muscle relaxation, unconsciousness, and analgesia are enhanced.

Anesthetics:
Because mice metabolize drugs so rapidly, many anesthetic agents have brief durations of effect. An anesthetic regimen should be chosen to match the duration of drug effects with the length of the procedure. In particular, short acting agents (and regimens) should not be used for long procedures because repeat drug administrations, necessary to prolong anesthesia, will produce uneven blood concentrations and therefore periodically inadequate anesthesia. For long procedures, gaseous anesthesia using a non-explosive agent such as isoflurane in a calibrated vaporizer is often the most practical method to sustain uniformly adequate levels of anesthesia. Potentially explosive agents, such as ether, are not recommended.

Page 4. Analgesics, Sedatives, and Anesthetics
- Hypothermia
Hypothermia is not allowed for anesthesia in adult rodents.

The practice of using hypothermia as an anesthetic for neonates is generally discouraged in pups older than 6 days of age.

It is not clear whether the depression of neural function by hypothermia is sufficient to prevent the sensation of pain related to a surgical procedure. Also, the recovery from hypothermia may be a painful experience in animals, as it is known to be in humans.

Inhalation anesthesia with an agent such as isoflurane is an acceptable alternative to hypothermia in neonatal rodents.
Lesson 15. Surgery

Page 1. Surgery

Aseptic technique should be used when performing surgery on mice. The standards described here are consistent with the *Guide for the Care and Use of Laboratory Animals*. If you will be performing surgery on mice it is recommended that you take the AUTS Surgery course, [Click Here](#) for the registration form.

Page 2. Surgery - Location

Surgery on mice should be performed in a location that allows for a physical separation of the operative field from other functions of the procedure (such as animal preparation and anesthetic recovery) and other laboratory activities.

- The isolation of the operative field avoids contaminating sterile areas with animal fur, bedding, non-sterile supplies, etc.
- The location used for the operative field should be cleaned and sanitized before use.
- Materials and supplies used in support of the procedure should be positioned and managed to avoid contaminating sterile areas.

Page 3. Surgery - Aseptic Technique

Survival surgical procedures in mice should be conducted using aseptic technique including using sterile instruments and wearing a surgical mask. Non-aseptic methods are not acceptable. Rodents have been shown to develop subclinical infections, a consequence which has led to an outdated belief that rodents tolerate nonaseptic technique without developing postoperative infections. The *Guide* recommends methods for adapting aseptic technique to the scale of rodent surgery. In this way, efficiencies and economies can be realized without sacrificing asepsis.
Lesson 16. Supportive Care and Monitoring

Page 1. Supportive Care and Monitoring - Goals

Supportive care aims to:

- Maintain the animal's physiological status as nearly normal as possible.
- Minimize animal pain and distress.

Supportive care includes monitoring of both physiological parameters and analgesia during anesthetic and surgical procedures. Monitoring of vital signs and pain perception should be conducted throughout the procedure and the recovery period.

Page 2. Supportive Care and Monitoring - Concerns

Keep in mind that:

- General anesthesia causes disturbances of thermoregulation and other physiological functions. Maintaining body temperature, e.g., via insulating materials and supplemental heating sources, is an important objective of supportive care.
- During surgery, the animal may experience pain if anesthesia is inadequate at any time during the procedure.
- Postoperatively, the animal may experience pain, discomfort, and distress unless treated with analgesics and appropriate supportive care measures.

Due to the interaction of metabolic factors and drug effects that can cause animal mortality, mice should receive good supportive care and monitoring during anesthesia, whether or not the procedure involves surgery.

Page 3. Supportive Care and Monitoring: Procedures - During the Procedure
During anesthesia and surgery, the following procedures are recommended.

Supportive Care:

- Provide a source of warmth to mice from the onset of anesthesia to the end of anesthetic recovery. Care needs to be taken to avoid heating sources that may cause thermal injuries to the mice. Examples of acceptable heat sources are rubber gloves filled with warm water and isothermic heating pads. Whatever method is used place a towel between the animal and the heat source in order to avoid thermal burns. Heat sources such as electrical heating pads can cause thermal burns on the skin of the animals and their use is usually discouraged. If you are using an electrical heating pad great care must be taken to ensure the mice do not develop burns. If you are using a heat lamp take care to ensure the lamp is not so close as to burn the skin of the mouse (the ears are especially susceptible to burns from heat lamps).
- Inject sterile physiological fluid, such as 0.9% normal saline (warmed to body temperature) to compensate for blood loss during a procedure and depressed fluid intake post-procedure.

Monitoring during Anesthesia:

- Anesthetic depth – toe pinch.
- Respiration – gross changes in rate, character of breathing. Increased respiratory rate may mean the animal is too light, abdominal effort may indicate the animal is too deep.
- Color of mucous membrane and skin – blue (poor oxygenation), pale (poor blood perfusion).

After anesthesia and surgery, the following procedures are recommended for:

Monitoring Post Anesthesia:

- Mice must be monitored until fully recovered from anesthesia as indicated by the ability to ambulate and maintain core body temperature.

Monitoring Post Procedure:

- Assess appearance, activity, and behavior as indications of pain and discomfort (see screen Detecting Pain and Distress).
- Assess food and water intake.
- Provide floor-level access of food and water (via water gel) post procedure if stretching
overhead for these items (in the cage wiretop) may be painful.

• Assess wound repair/healing.
• Wound clip/suture removal in 10-14 days. (See the IACUC policy)
• Routine use of antibiotics is not indicated after uncomplicated, aseptic surgery.
Lesson 17. Euthanasia

Page 1. Euthanasia
The term euthanasia is derived from Greek and means "good death." Animals should be euthanized when killed for any purpose, including research. To euthanize a mouse, you must be trained in the concepts of euthanasia, the method to be used, and the proper handling of mice.

Euthanasia methods are classified as acceptable or conditionally acceptable, as set by the American Veterinary Medical Association. The inclusion of conditionally acceptable methods in your protocol requires scientific justification in addition to IACUC approval.

Page 2. Euthanasia - Methods
Acceptable Methods:

- Barbiturates
- Inhalant anesthetics
- Carbon dioxide (compressed tanks only)

Conditionally Acceptable Methods:

- Cervical dislocation*
- Decapitation*

*NOTE: Individuals performing decapitation of awake adult mice and/or cervical dislocation of awake or anesthetized mice at the UW must be certified by a designee of the Attending Veterinarian.

Page 3. Euthanasia - Barbiturates

Barbiturates
Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia in most species; however, intraperitoneal injection is commonly used in rodents due to the difficulty and stress associated with vascular access. Intracardiac injection may also be used, but intracardiac injection is only acceptable if the animal is unconscious or anesthetized.

Pentobarbital Combinations
Several euthanasia products are formulated to include a barbituric acid derivative (usually sodium pentobarbital) with other added agents. The pharmacologic properties and recommended use of products that combine sodium pentobarbital with lidocaine or phenytoin are interchangeable with those of pure barbituric acid derivatives.

Pentobarbital combined with a neuromuscular blocking agent is not acceptable for euthanasia.

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Page 4. Euthanasia - Inhalant Anesthetics
With inhalant anesthetics, the animal is placed in a closed receptacle and the anesthetic is introduced from a vaporizer. Vapors are inhaled until respiration ceases and death ensues. Animals should not be exposed to inhalant anesthetics in their liquid state. In addition, sufficient
oxygen must be provided during the induction period to prevent hypoxemia.

In order of preference, halothane, enflurane, isoflurane, sevoflurane, and desflurane are acceptable for euthanasia of rodents. Nitrous oxide may be used in combination with other inhalants to speed up the onset of anesthesia, but is not effective to induce anesthesia when used alone and cannot be used alone for euthanasia.

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Page 5. Euthanasia - Carbon Dioxide

Compressed CO₂ gas in cylinders is the only acceptable source of carbon dioxide because the inflow to the chamber can be regulated precisely.

Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable for use in euthanasia of rodents according to the AVMA Guidelines on euthanasia.

Species should be separated and chambers should not be overcrowded. All animals need to have adequate floor space.

With an animal in the chamber, turn on the CO₂ at a low flow rate (20% of the chamber volume per minute). Gas flow should be maintained for at least 1 minute after apparent clinical death (at least 5 minutes total for mice, 10 minutes total for rats). It is important to verify that an animal is dead before removing it from the chamber.

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Page 6. Euthanasia - Carbon Dioxide Secondary Methods

To ensure death, the University of Washington IACUC requires a secondary method of euthanasia when euthanizing with CO₂, and this method must be approved in your IACUC protocol.

A secondary method is required because mice can stop breathing for a minute or more, then regain respiratory function and survive. This is particularly true of younger mice, which are resistant to carbon dioxide asphyxiation.

The following methods are acceptable secondary euthanasia methods:
• Filling carcass disposal bag with carbon dioxide and sealing prior to disposal
• Cervical dislocation by UW certified individuals
• Exsanguination
• Thoracotomy
• Decapitation
• Anesthetic overdose

Click here for the UW IACUC policy on carbon dioxide euthanasia.

Page 7. Euthanasia - Cervical Dislocation

Manual cervical dislocation is a humane technique for euthanasia of mice when performed by individuals with a demonstrated high degree of technical proficiency.

This technique can be used only when scientifically justified by the user and approved by the Institutional Animal Care and Use Committee.

At the UW, cervical dislocation can be performed only by those individuals certified to perform this technique by a designated trainer. A list of certified individuals is maintained in the UW Animal Use Training Office.

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Page 8. Euthanasia - Decapitation

Decapitation is a humane technique for euthanasia of mice when performed by individuals with a demonstrated high degree of technical proficiency.

This technique can be used only when scientifically justified by the user and approved by the Institutional Animal Care and Use Committee. The equipment used to perform decapitation should be regularly maintained to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

At the UW, decapitation of unanesthetized rodents greater than 14 days of age can be performed only by those individuals certified to perform this technique by a designated trainer. A list of certified individuals is maintained in the UW Animal Use Training Office.

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Page 9. Euthanasia - Emotional Distress

Euthanasia can cause some degree of emotional distress to the people performing or witnessing the euthanasia. This is a normal reaction to euthanasia. If you need extra support in this area, the following resources are available to you:

• Current UW students can contact the UW Counseling Center
• Current UW employees can contact the Employee Assistance Program.
Lesson 18. References

Federal Laws, Regulations, Policies:

1. Animal Welfare Act, as Amended (7 USC, 2131-2156)

Guidelines:


Texts: