Care and Use of Laboratory Animals: Working With Mice in Research Settings

Lessons:

1. Introduction .................................................................2
2. Occupational Health Issues ...........................................3
3. Humane Standards and Alternative Searches ..................6
4. Genetics and Biological Features ....................................8
5. Housing, Acclimation and Quarantine ..............................10
6. Detecting Pain and Distress ............................................12
7. Injections and Blood Collection .....................................13
8. Analgesics, Sedatives and Anesthetics ............................16
9. Surgery, Supportive Care and Monitoring .......................18
10. Euthanasia .................................................................20

References ........................................................................21
Welcome to Working with Mice in Research Settings.
This is the mouse module one in a series on the use of animals in research settings. The information in this course will also help in the preparation of an animal use protocol.

The goal of this course is to cover important information about using mice in biomedical research settings. If you are responsible for handling mice or if you must write an animal use protocol, this course will be useful by providing you with:

- Information on key regulatory issues.
- Guidance on searches for alternatives in the care and use of animals.
- Highlights of unique biological features of these animals.
- Overviews of acceptable basic methodologies.
- Requirements for supportive care procedures.

Hypertext links in this course provide you with supporting information, such as regulatory sources, drug doses, practical tips, etc.

This course will not provide you with detailed information on how to conduct the methods and procedures described. For this, you should use other courses offering in-depth information and hands-on instruction from your institution's animal facility staff.

Research Mandates
To ensure the humane treatment of laboratory animals, animal research is regulated by two federal agencies:

- The United States Department of Agriculture (USDA) / Animal Care; and
- The Public Health Service / Office of Laboratory Animal Welfare.

The USDA and PHS mandates on animal welfare differ greatly with respect to the laboratory strains of mice and rats. These species are not covered by the USDA but are included in PHS regulations and policy. However, the USDA may eventually regulate these species as well.

Because we receive funding from the PHS or are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC), our research must also comply with the National Research Council publication, the Guide for the Care and Use of Laboratory Animals. This document will simply be referred to as the Guide in this course.
Occupational Health Issues

Zoonoses
Zoonoses are diseases transmitted by animals to humans. In general, transmission of zoonotic disease from naturally infected laboratory animals is uncommon. This is because of ongoing vendor efforts to improve the health status of animals, as well 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 personal protective equipment 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, immunosupression therapy, etc., and due to treatment with antibiotics.

**Fungi**

**Microsporum spp., Trichophyton spp.**
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**

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

Working with mice is also associated with other hazards.

**Injuries**
Personnel handling a mouse can be bitten if the animal is poorly restrained. Though mice are often inclined to bite when frightened, 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. Training is provided through the animal facility 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. Mouse urine is particularly allergenic, and pelt proteins can also be allergenic. For this reason, you should consider always wearing disposable gloves and a protective gown or scrubs to prevent skin contamination, and a mask to prevent aerosol exposure to urine and pelt proteins. People who develop allergy symptoms
should seek medical counseling, and they may have to wear special protective equipment or even discontinue working with this species if symptoms are severe after exposure. Protect yourself!

**Occupational Health Plans**
The Public Health Service Policy requires institutions to have an occupational health and safety program for individuals working with laboratory animals. This requirement is also reiterated in the *Guide*. It is the responsibility of principal investigators to assure that their laboratory staff is informed of and participate in their institution's occupational health and safety program.

Elements of an occupational health and safety program, including institutional responsibilities, are described in the National Research Council publication *Occupational Health and Safety in the Care and Use of Research Animals*. 
Lesson 3: Humane Standards and Alternative Searches

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, the IACUC has requirements for the proper care of your animals prior to, during and after a research procedure.

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.

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.

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, for example, is useful only for small volume collections.

Contact the Veterinary Medical Unit (VMU) staff or attending veterinarian for specific guidelines.

Alternatives Search
The Animal Component of Research Protocol (ACORP) form asks you for an assurance that you have considered alternatives to the use of animals if painful or distressing procedures are proposed. This is to satisfy mandates by the Animal Welfare Act and PHS Policy to avoid or minimize discomfort, pain, and distress consistent with sound scientific practices. Alternative procedures are those that may replace animals with non-animal methods, reduce the number of animals used, or refine the methodology to minimize animal pain or distress. For more information on what is meant by alternatives to the use of animals, please refer to the course Working with the IACUC, which is part of this series.

The assurance often takes the form of a written narrative that describes which sources were used to determine that alternatives were not available. Typically, you may be asked to provide the results of a database search including information on:

1. The databases searched.
2. The date the search was performed.
3. The years of citations covered by database searches.
4. The key words and/or search strategy used when searching a database.

It is strongly recommended that this information be sought during development of a protocol. Organizations that can assist you in performing an alternatives search are:

- ALTWEB, Center for Alternatives to Animal Testing, Johns Hopkins University
- Animal Welfare Information Center, National Agricultural Library

**Monoclonal Antibody Production**

The *in vivo* method of monoclonal antibody production uses mice to grow hybridoma cells on the peritoneal lining of histocompatible animals. Monoclonal antibodies are then collected from the antibody-rich ascites fluid.

Over the past years, there have been a number of *in vitro* techniques introduced that can replace the use of animals for expanding hybridoma cell lines and collecting purified monoclonal antibody. Consequently, **non-animal alternatives** for generating purified monoclonal antibodies should be considered. The *in vitro* method should be considered and deemed unsuitable on scientific grounds before the IACUC approves animal use for the *in vivo* method.

When requesting approval to use animals for expanding hybridoma cell lines, **be prepared to explain why *in vitro* techniques will not work.** In 1999, The Committee on Methods of Producing Monoclonal Antibodies suggested the guidelines for IACUCs to use when evaluating the need for using animals for hybridoma expansion. (See Recommendation 4 of the committee report.)

If your research will involve antibody production, refer to the training module on antibody production for additional information and training.
Lesson 4: Genetics and Biological Features

Genetics
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 isogenicity (animals characterized by essentially identical genes). 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. Check with the VMU for information on vendor choices as animal source affects animal health status.

Biological Features
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 and Physiology

Ocular System
Rats and mice may develop red staining around the eyes and nostrils when they are distressed, e.g., by disease, trauma. This staining is due to the accumulation of porphyrins produced by the Harderian gland, a lacrimal gland. Though a normal constituent of tears in rodents, lacrimal porphyrin is produced in limited amounts and 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. Transgenic or knock-out mice may have unintended genetic anomalies that cause jaw mal-alignment and result in tooth overgrowth. Staff must be alert to detect any signs of this condition and to provide appropriate treatment.

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.

Albinism

Most 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, that 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, the animal care staff is 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.

In general, mouse-specific dose rates have been determined and are widely published for drugs that are commonly used in animal research, such as anesthetics, analgesics, sedatives, and antibiotics. Investigators are advised to obtain mouse dose rates from laboratory animal references or from the VMU staff.

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 may 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 temperatures (e.g., anesthesia, surgery) and afterwards until they recover the ability to thermoregulate.
Lesson 5: Housing and Acclimation and Quarantine

**Housing**

Your protocol form may ask you which type of housing you may need for your mice. There are important considerations in the selection of animal housing that affect the welfare of your animals.

Rodent caging has two types of flooring: solid and wire mesh.

The solid flooring of shoebox cages is covered with a bedding material that absorbs liquid wastes. Bedding has been shown to be preferred by rodents for resting, and it is considered to provide them with comfort, warmth, and the opportunity to burrow. This type of flooring is well suited to breeding because pups are better protected from chilling.

Wire mesh flooring has long been used for rodent caging because of advantages in sanitation. However, the use of wire bottom cages is discouraged for rodents, especially on long-term studies. Use of wire bottom cages should be scientifically justified and approved by the IACUC.

Because of data on rodent preferences for solid flooring and the risks for animal injury on wire mesh flooring, the use of wire bottom cages should be scientifically justified and approved by the IACUC.

**Acclimation and Quarantine**

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.

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.

Contact the VMU for additional information on quarantine requirements in place at this institution.
Lesson 6: Detecting Pain and Distress

If your proposed study involves a painful procedure, the protocol form asked for details concerning your 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.

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.

**Appearance:** unkempt or greasy fur, porphyrin staining around eyes and nostrils, hunched posture, cyanosis, pale mucous membranes, soiled ano-genital area.

**Vital Signs:** e.g., respiratory distress.

**Body Condition:** weight loss, emaciation, dehydration.

**Intake:** reduced intake of food and water.

The mouse shown above 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) or hypoperfusion (abnormally low levels of blood in tissue). This mouse is showing signs of severe pain and/or distress.

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. For methods on assessing and alleviating pain and distress in rodents, refer to another course in this series, *Post Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress*. 
## Lesson 7: Injections, Blood Collection

**Injections and Blood Collections**

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)(^a)</th>
<th>Tot. Bleed-out Volume (ml)(^b)</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.

\(^b\)Animals should be exsanguinated only under anesthesia.


**Blood collection sites**

Below are peripheral vessels that are commonly accessed for blood collection or fluid administration. Recommended needle sizes are 25 to 29 gauge. Larger needles may be necessary for injecting large volumes or viscous materials.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Comment</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).  
| Lateral saphenous vein  |   o May be aided by sedation to reduce animal struggling due to distress.  
|                         | 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. |
### Mice in Research

#### Vessel

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jugular vein</td>
<td>Jugular venipuncture is commonly performed under anesthesia because of the restraint method and the need for animal immobilization.</td>
</tr>
<tr>
<td>Tail tip amputation</td>
<td>1. These three methods generally require anesthesia, but institutions may allow tail tip amputations (for genotyping) without anesthesia prior to weaning.</td>
</tr>
<tr>
<td>Cardiac puncture</td>
<td>2. Cardiac puncture is generally allowed only as a terminal procedure.</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>3. Check with your institution for guidelines on the carotid route of blood collection.</td>
</tr>
<tr>
<td>Retroorbital puncture</td>
<td>1. Retroorbital puncture must be performed by skilled personnel or the risk of injury to the eye and surrounding structures is high.</td>
</tr>
<tr>
<td></td>
<td>2. This method is considered to be painful and may cause blindness. Generally requires anesthesia.</td>
</tr>
<tr>
<td></td>
<td>3. Topical ophthalmic anesthetic may provide pain relief after the procedure.</td>
</tr>
<tr>
<td></td>
<td>4. This technique is gradually being replaced by the lateral saphenous bleeding technique for small volume collections at many institutions.</td>
</tr>
</tbody>
</table>

#### Injection Sites

Below are the nonvascular 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 27 gauge; larger needles may be necessary for injecting viscous materials.

<table>
<thead>
<tr>
<th>Injection Site</th>
<th>Volume Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous (SQ or SC)</td>
<td>2-3 ml total; maximum of 0.5 ml per site.</td>
</tr>
<tr>
<td>Intraperitoneal (IP)</td>
<td>2-3 ml</td>
</tr>
<tr>
<td>Oral (PO)</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Intradermal (ID)</td>
<td>0.05 ml/site</td>
</tr>
</tbody>
</table>
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. If an IM injection were necessary, the volume administered should not exceed 0.05 ml per site.
Lesson 8: Analgesics, Sedatives and Anesthetics

Because mice have high rates of metabolism, drugs are rapidly eliminated from their bodies. Dose rates appropriate for larger species produce ineffective drug concentrations when used in mice. This section includes mouse dose rates for the common drugs and drug regimens. If you need to use other drug agents, check with the VMU staff for assistance in determining a dose rate appropriate for use 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.

**Opioid**
Buprenorphine, 0.05 – 0.1 mg/kg SQ twice daily.

**NSAID**
Ketoprofen, 1.0 – 2.0 mg/kg SQ, once daily.

**Sedatives:**
Sedatives may obtund consciousness, but in normal doses they 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. Examples include acepromazine, diazepam, midazolam and zolazepam. These agents are commonly mixed with other drugs in an anesthetic cocktail. Refer to “Anesthetics” below.

**Sedatives + Analgesia:**
Some sedatives also have analgesic effects. When combined with general anesthetics, these sedatives enhance analgesia and a "balanced" anesthesia is attained.

Sedatives with analgesic effect include medetomidine and xylazine. These agents are commonly mixed with other drugs in an anesthetic cocktail. Refer to “Anesthetics” below.

**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 be 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 is often the most practical method to sustain uniformly adequate levels of anesthesia. Potentially explosive agents, such as ether, are not recommended.

**Gaseous anesthetics** provide the best means for long periods of anesthesia due to the continuous administration via inhalation. Since methoxyflurane is no longer available, and the use of ether is not recommended, researchers have access only to those agents that are best administered via a vaporizer. The recommended agent is isoflurane.
Injectable anesthetics are generally used in a cocktail mixed with one or more sedatives and analgesics. The anesthetic cocktails most commonly used on mice in the USA contain ketamine or tiletamine as the anesthetic agent. Bolus injections of anesthetic cocktail may produce a surgical level of anesthesia for periods ranging from 20 to 45 minutes, which is ideal for many surgical procedures commonly performed in mice, such as embryo implantation. The duration of surgical anesthesia depends on the drug agents used as well as the strain/stock of mouse. Repeated dosage of injectable agents to provide a long term anesthesia is not recommended because of the resulting fluctuations in systemic blood concentration and therefore in anesthetic effect. In such situations, the repeat doses of anesthetic cocktail are administered only when the animal begins to show evidence of pain. This is a poor practice of anesthesia because a surgical plane of anesthesia is not continuous throughout the procedure. Animal welfare mandates require an avoidance of such unnecessary animal pain and distress.

Examples (administered IP):

1. Ketamine 75 mg/kg + Medetomidine 1 mg/kg
2. Ketamine 100 mg/kg + Xylazine 10 mg/kg
3. Ketamine 200 mg/kg + Diazepam 5 mg/kg
4. Ketamine 40 mg/kg + Midazolam 2 mg/kg + Butorphanol 0.1 mg/kg

Hypothermia used as an anesthetic for neonates is generally discouraged. 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 administered using a non-rebreathing system may be an acceptable alternative to hypothermia in neonatal rodents.
Surgery
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, nonsterile 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.

Surgical procedures in mice should be conducted using aseptic technique. Nonaseptic 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.

Supportive Care and Monitoring: Overview
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.

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.

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.
- Inject sterile physiological fluid (warmed to body temperature) to compensate for blood loss during a procedure and depressed fluid intake post-procedure.

**Monitoring**

**Monitoring During Anesthesia:**
- Analgesia - toe pinch.
- Respiration - gross changes in rate, character of breathing.
- Color of mucous membrane and skin – blue (poor oxygenation), pale (poor blood perfusion).

**Monitoring Post Anesthesia:**
- Mice must be monitored until fully recovered from anesthesia as indicated by the ability to ambulate and maintain core body temperature. Routine use of antibiotics is not indicated after uncomplicated, aseptic surgery.

**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 post procedure if stretching overhead for these items (in the cage wirelid) may be painful.
- Assess wound repair.
Lesson 10: Euthanasia

The term *euthanasia* is derived from Greek and means "god death." Animals should be euthanatized when killed for any purpose, including research. To euthanatize a mouse, you must be trained in the concepts of euthanasia, the method to be used, and the proper handling of mice.

Methods are classified as **acceptable** or **conditionally acceptable**, as set by the American Veterinary Medical Association publication [AVMA Guidelines on Euthanasia](#). The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.

**Acceptable Methods:**
- Barbiturates
- Inhalant anesthetics
- Carbon dioxide (compressed tanks only)
- Carbon monoxide
- Microwave irradiation
- Potassium chloride in conjunction with general anesthesia

**Conditionally Acceptable Methods***:
- Ether
- Nitrogen
- Argon
- Cervical dislocation
- Decapitation

* The inclusion of conditionally acceptable methods in your protocol may require scientific justification and IACUC approval.

**Very Important!** Before placing euthanized rodents in a bag and placing the bag in a necropsy refrigerator or freezer, **you must make sure the mice are really dead!** Mice can stop breathing for a minute or more then *regain respiratory function and survive*. This is particularly true of younger mice, which are somewhat resistant to carbon dioxide asphyxiation and take longer to succumb than adult mice.

To ensure death in mice euthanatized with carbon dioxide, the chest cavity may be opened with scissors, or the mice may be observed for an extended period of time to make sure they are dead.

The Office of Laboratory Animal Welfare (responsible for enforcing PHS policy) has made it clear that rodents remaining alive in bags after ineffective euthanasia is a serious breach of PHS policy, and must be reported to regulatory officials.


For general references, see the Animal Studies webpage of the Research Service website.