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

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Welcome to Working with Rats in Research Settings.

This is the rat module in a course series on use of rats in research. The course will also help with preparation of an animal use protocol involving rats.

Objectives

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

- Summaries of 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 or practical tips.

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 the 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 our institution receives funding from the PHS and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC), your 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.
Lesson 2: Occupational Health Issues

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 staffs are 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 guideline, Occupational Health and Safety in the Care and Use of Research Animals published by the National Research Council.

Potential Hazards

Injuries

Personnel handling rats can be injured by bites from the incisor teeth. Generally, these are caused by a lack of knowledge in how to handle, transport and restrain a rat. Generally, a rat will bite only when frightened or in pain. Likewise, poor technique in handling can cause injury to the rat. Training staff to work effectively and humanely with rats is essential to prevent injuries to people and rats.

Allergies

People can develop an allergy to rats after having contact with them for some time. Persons who develop allergy symptoms should seek medical counseling and may have to discontinue working with rats.

Zoonoses

In general, transmission of zoonotic disease from naturally infected laboratory animals is uncommon because of the ongoing efforts, by vendors of specific pathogen free animals and by facility staff, to improve the health status of animals. Experimentally infected animals are a source of zoonotic transmission to humans. Also, contact with wild rats in field research may expose humans to zoonotic agents carried by these animals. Health surveillance programs, routine sanitation, and personal protective equipment have important roles in preventing zoonoses.

Rats can be a reservoir of the following infectious agents that are transmissible to people:

Viruses

Hantavirus

Hantavirus is a bunyavirus carried by wild mice and rats. 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 rodents (e.g., the wharf rat or cotton rat). 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. Viral
strains originating in Asia produce a hemorrhagic fever and nephropathy. Strains originating in northern Europe generally produce renal symptoms of less severity. Researchers performing field studies on wild-caught rodents are at particular risk.

**Bacteria**

*Leptospira spp.*
Rats 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. Click here for information on human leptospirosis from the CDC website.

*Salmonella spp.*
Rats 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 or neoplasia, immunosupression therapy, and due to treatment of antibiotics.

*Streptobacillus moniliformis* and *Spirillum minus*
These organisms cause rat bite fever. Rats may carry *Streptobacillus moniliformis* and *Spirillum minus* as commensal organisms living in the nasopharynx. Transmission to humans occurs by rat bite. Symptoms may include wound inflammation, lymphadenopathy, fever, headache, malaise, myalgia, joint pain, and arthritis. Click here for discussion of a case study of rat bite fever in a boy.

**Fungi**

*Sporothrix schenckii*
*Sporothrix schenckii* is a fungus associated with multiple species, including rats. Humans acquire *Sporothrix schenckii* via bites or direct contact. Disease manifests as a nodule on the hand or arm, which may progress with additional nodules forming along the path of lymphatic vessels. Deep visceral infections may also occur.

*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. Extended therapy with antifungal medication may be needed to eliminate infection in animals and humans.
Lesson 3: Humane Standards and Alternatives Searches

Humane Standards
All of the federal laws, regulations, policies and guidelines applicable to animal research have a core intent 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.

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

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

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

When performing any procedure, such as a blood collection, 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 to the minimum that may be taken safely. That amount may vary depending upon whether a single sample is taken or multiple collections are done. And, with any venipuncture, you should be prepared to care for the animal in the event of trauma to the vein or excess hemorrhage.

Blood collection is covered in Lesson 7. Consult the animal facility for more information.

Alternative Searches
Your protocol form should ask 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 a mandate 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 which 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

**Polyclonal Antibody Production**

If rats are being used in production of polyclonal antibodies, consideration should be given to alternatives procedures and chemicals (adjuvant) to reduce distress in animals and hazards to technicians. A separate training module dealing with antibody production is required.
Genetics
Inbred strains and outbred stocks of rats 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.
  
  Example: Lewis rats, LEW/CrlBR

- **Outbred rats** are used when outbred vigor is desirable, e.g., when genetic heterogeneity and phenotypic variability are not a concern.
  
  Example: Wistar rats, Hsd:WI

Please check with the animal facility for information on vendor choices as animal source affects animal health status.

Biological Features
Though rats share many anatomical and physiological features with humans, rats have many unique biological characteristics. Knowledge of rat-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 rat anatomy and biology.

Anatomy

Ocular
Rats and mice may develop red staining around the eyes and nostrils when they are distressed, e.g., by disease or 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
Rats 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. Staff must be alert to detect any signs of this condition and to provide appropriate treatment.

Rat with overgrowth of the incisors (upper and lower) and jaw misalignment.
Gastrointestinal

Inability to vomit
Like mice and most rodents, rats do not vomit because they lack the neurophysiological mechanisms for emesis. Therefore, presurgical fasting is rarely necessary in rats because the risk of lung pathology from inhaled vomitus is extremely low.

Gall Bladder
Unlike mice, rats do not have a gall bladder. Bile passes from the liver through a bile duct directly to the duodenum.

Coprophagy
In rats, herbaceous foodstuffs are broken down by microbial action in the cecum, which is a large organ in the rat. To assimilate the microbial byproducts of digestion, the rat regularly eats its own feces, a habit known as coprophagy. Stomach digestion and intestinal absorption of this fecal material yields nutrients that are essential to the rat.

Metabolism

Albinism
Most rats used in research, such as the Sprague Dawley stock, are albinos. Albinism in rats 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.

High rate of metabolism: impact on drug clearance
The rat'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) would reach lower blood concentrations and exert less pharmacological effect in the rat. As a result, rats should receive drug doses that have been scaled to the rat's metabolism. Through a discipline known as allometry, mathematical formulas have been developed to adjust dose rates between species of disparate size.

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

High surface area; impact on hypothermia
Rats have a large body surface area (relative to body volume) plus many hairless body parts (tail, ears, feet). As a result, rats are vulnerable to a 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. Rats should have a source of warmth throughout a procedure that lowers their body temperatures (e.g., anesthesia, surgery) and afterward until they recover the ability to thermoregulate themselves.
Lesson 5: Housing, Acclimation and Quarantine

Housing
Your protocol form may ask you which type of housing you may need for your rats. 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. Typical rodent cage mesh has two to four wires per inch (2.5 cm). This type of flooring has been associated with foot injury in rats. Inserting a solid resting board into the cage provides the rat with a comfortable resting area and may reduce foot injuries. 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 your institution's IACUC.

Acclimation and Quarantine
Upon arrival at your facility, your rats 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. Contact a veterinary medical consultant for recommendations that are appropriate for your project. Typically, acclimation periods range from 4 days to 1 week.

Routine quarantine procedures may prolong the holding of your animals in special facilities. Quarantine aims to prevent transmission of diseases between new animals and established colonies. Acclimation and quarantine periods run concurrently, although they serve different purposes. Most institutions do not allow experiments on animals while quarantined.
Lesson 6: Detecting Pain and Distress

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

Assessing pain and distress in rats is difficult at times because rats, like many other species, commonly conceal outward signs of moderate pain and distress. In this case, the behavioral changes that reveal a rat's pain and distress may be subtle and detectable only with specialized behavioral methods.

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

- Activity levels; e.g., hypoactivity, hyperactivity, restlessness.
- Behaviors; e.g., vocalization, self-trauma, isolation from cage mates, aggressiveness, ataxia.
- Appearance; e.g., unkempt greasy fur, porphyrin staining around eyes and nostrils, hunched posture, cyanosis, pale mucous membranes, soiled anogenital area.
- Vital Signs; e.g., respiratory distress.
- Body Condition; e.g., weight loss, emaciation, dehydration.
- Intake; e.g., 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.

For methods of 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.*

Porphyrin staining and encrustation on a rat's nose. This is a nonspecific sign of pain or distress.
Lesson 7: Injections and Blood Collections

Injections
The following are volume recommendations for intravenous fluid administration and blood collection in adult rats:

<table>
<thead>
<tr>
<th>IV Fluid Volume (ml) max. acute admin.</th>
<th>Total Blood Volume (ml)</th>
<th>Safe Bleed Volume (ml)(^a)</th>
<th>Bleed-out Volume (ml)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 - 1.0 ml</td>
<td>64 ml/kg</td>
<td>5.5 ml/kg</td>
<td>6-14 ml</td>
</tr>
</tbody>
</table>

\(^a\)Removing greater quantities of blood (exceeding 10% of total blood volume) can produce hypovolemic shock. Repeated collections of smaller amounts of blood will have the same effect. In such procedures, animals should receive warmed, physiological fluids to replace the volume of blood collected. In addition, monitor the animal's hematocrit for anemia.

\(^b\)Animals should be exsanguinated only under anesthesia; volumes shown refer to adult rats.

From:

Blood Collection
Below are peripheral vessels that are commonly accessed for blood collection or fluid administration. Recommended needle sizes are 23 to 27 gauge. Larger needles may be necessary for injecting large volumes or viscous materials.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail vein</td>
<td>1. Accessing the tail vein and the lateral saphenous vein:</td>
</tr>
<tr>
<td>Lateral saphenous vein</td>
<td>o Does not require anesthesia.</td>
</tr>
<tr>
<td></td>
<td>o May be aided by sedation because vein visibility is enhanced by peripheral vasodilation (drug effect).</td>
</tr>
<tr>
<td></td>
<td>o May be aided by sedation to reduce animal struggling due to distress.</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>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.</td>
</tr>
<tr>
<td>Penile vein (males only)</td>
<td>Commonly performed under anesthesia because of the restraint method and the need for animal immobilization.</td>
</tr>
</tbody>
</table>
Cardiac puncture

1. These two methods require anesthesia.
2. Cardiac puncture is most often allowed only as a terminal procedure.
3. Check with your institution for guidelines on this route of blood collection.

Retroorbital puncture

1. Retroorbital puncture is controversial because of the risk of injury to the optic nerve and other nearby structures.
2. This method is considered to be painful and may cause blindness.
4. Topical ophthalmic anesthetic is recommended post-procedure.

Below are the nonvascular routes of injection that are commonly used in rats. Included are volume recommendations for the safe administration of fluids acutely in adults (average 200 g). Recommended needle sizes are 23 to 27 gauge; larger needles may be necessary for injecting viscous materials.

- **Subcutaneous (SQ or SC)** - 25 ml/kg
- **Intraperitoneal (IP)** - 25 ml/kg
- **Oral (PO)** - 10 ml
- **Intradermal (ID)** - 0.05 ml/site
- **Intramuscular (IM)** - 0.1 ml per site

From:

Lesson 8: Analgesics, Sedatives, and Anesthetics

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

**Analgesics:**
Available in two drug types: the opioids and the nonsteroidal anti-inflammatory drugs (NSAIDs). The rapid clearance of many of these drugs in rats 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.

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

**Anesthetics:**
Because rats 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 is often the most practical method to sustain uniformly adequate levels of anesthesia. Explosive agents such as ether should not be used.

**Hypothermia**
The practice of using hypothermia 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.

An acceptable alternative to hypothermia for neonates may be a non-explosive inhalational agent such as halothane delivered using a non-rebreathing system.
Lesson 9: Surgery, Supportive Care and Monitoring

Surgery
Aseptic technique should be used when performing survival surgery on rats. The standards described here are consistent with the *Guide for the Care and Use of Laboratory Animals*.

Surgery on rats 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.

Surgical procedures in rats should be conducted using aseptic technique. 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 non-aseptic 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
The aims of supportive care are to:

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

Supportive care includes monitoring both physiological parameters and analgesia during anesthetic and surgical procedures. Monitoring of vital signs and pain 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 heating sources that do not pose a risk of thermal injury, 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, rats 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 rats from the onset of anesthesia to the end of anesthetic recovery. Inject sterile physiological fluid (warmed to body temperature) to compensate for blood loss during a procedure and for depressed fluid intake post-procedure.

**Monitoring During Anesthesia:**
Analgesia - test with toe pinch.

Respiration - look for gross changes in rate, character of breathing.

Color of mucous membrane and skin - look for blue (poor oxygenation), pale (poor blood perfusion).

**Monitoring After anesthesia and surgery, the following procedures are recommended.**

**Monitoring Post Anesthesia:**
Rats 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 post procedure if stretching overhead for these items (in the cage wire lid) may be painful.

**Assess wound repair:**
Routine use of antibiotics is not indicated after uncomplicated, aseptic surgery.
Lesson 10: Euthanasia

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

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

Euthanasia: Acceptable Methods

Barbiturates
Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia in many species. Intraperitoneal injection may be used...

Intracardiac injection must only be used if the animal is heavily sedated, unconscious, or anesthetized.

Pentobarbital Combinations. Several euthanasia products are formulated to include a barbituric acid derivative (usually sodium pentobarbital), with added local anesthetic agents or agents that metabolize to pentobarbital. Although some of these additives are slowly cardiotoxic, this pharmacologic effect is inconsequential. These combination products are listed by the DEA as Schedule III drugs, making them somewhat simpler to obtain, store, and administer than Schedule II drugs such as sodium pentobarbital. The pharmacologic properties and recommended use of combination products that combine sodium pentobarbital with lidocaine or phenytoin are interchangeable with those of pure barbituric acid derivatives. A combination of pentobarbital with a neuromuscular blocking agent is not an acceptable euthanasia agent.

Inhalant anesthetics
With inhalant anesthetics, the animal can be placed in a closed receptacle and the anesthetic can be introduced from a vaporizer. The latter method may be associated with a longer induction time. Vapors are inhaled until respiration ceases and death ensues. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Also, sufficient air or O₂ must be provided during the induction period to prevent hypoxemia.

In order of preference, enflurane, isoflurane, sevoflurane, methoxyflurane, and desflurane, with or without nitrous oxide, are acceptable for euthanasia of small animals (< 7 kg).

Nitrous oxide (N₂O) may be used with other inhalants to speed the onset of anesthesia, but alone it does not induce anesthesia in animals, even at 100% concentration. When used by itself, N₂O produces hypoxemia before respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness.
Carbon dioxide (compressed tanks only)
Compressed CO\textsubscript{2} gas in cylinders is the only recommended 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 (eg. antacids) is unacceptable.

Species should be separated and chambers should not be overcrowded.

With an animal in the chamber, an optimal flow rate should displace at least 20% of the chamber volume per minute. Loss of consciousness may be induced more rapidly by exposing animals to a CO\textsubscript{2} concentration of 70% or more by pre-filling the chamber for species in which this has not been shown to cause distress.

Gas flow should be maintained for at least 1 minute after apparent clinical death. It is important to verify that an animal is dead before removing it from the chamber. If an animal is not dead, CO\textsubscript{2} narcosis must be followed with another method of euthanasia. Adding O\textsubscript{2} to the CO\textsubscript{2} may or may not preclude signs of distress. Additional O\textsubscript{2} will, however, prolong time to death and may complicate determination of consciousness.

There appears to be no advantage to combining O\textsubscript{2} with carbon dioxide for euthanasia.

Potassium chloride in conjunction with general anesthesia
"Saturated potassium chloride solutions are effective in causing cardiac arrest following rapid intracardiac or intravenous injection.

"It is of utmost importance that personnel performing this technique are trained and knowledgeable in anesthetic techniques, and are competent in assessing anesthetic depth appropriate for administration of potassium chloride intravenously.

"Administration of potassium chloride intravenously requires animals to be in a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli."

Euthanasia: Conditionally Acceptable Methods*

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

Cervical dislocation (rats < 200 g)
Manual cervical dislocation is a humane technique for euthanasia of...[rats]...when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be sedated or anesthetized prior to cervical dislocation. The need for technical competency is greater in heavy rats and rabbits, in which the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult.

In research settings, this technique should be used only when scientifically justified by the user and approved by the Institutional Animal Care and Use Committee. Those responsible for the use of this technique must ensure that personnel performing cervical dislocation techniques have been properly trained and consistently apply it humanely and effectively.
Decapitation
Decapitation is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental design and approved by the Institutional Animal Care and Use Committee. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so.

Euthanasia: Ensuring Death

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

To ensure death in rodents euthanatized with carbon dioxide, the chest cavity may be opened with scissors, or the rodents 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.

Unintended recovery of animals after apparent death from CO₂ (e.g., in necropsy coolers) is a documented occurrence. Institutions are reminded that such incidents constitute serious noncompliance with the PHS Policy and serious deviation from the provisions of the Guide. As such, the IACUC, through the Institutional Official, must promptly provide ORO and OLAW with a full explanation of the circumstances and actions taken. Prompt reporting in accordance with ORO and PHS Policies requirements is an essential component of the formal relationship between OLAW and PHS-Assured institutions.
References


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