

Restraint, Handling and Protocols for Lab Animals

Restraint, Handling

General Principles

The use of proper restraint and handling techniques reduces stress to animals and also to the researcher. Handling stress represents an experimental variable and should be minimized whenever possible. Animals can inflict serious injuries to humans and to themselves as a result of improper handling.

- Animals experience stress as a result of shipping. All large animals must be allowed to acclimate to the facility for three days. During this time they may not be experimentally manipulated. Acclimation periods of up to one week are recommended for all animals.
- If a study will involve significant handling of animals it is recommended that the animals be acclimated to the handling. Prior to experimental manipulation, handle the animal on a regular basis in a non-threatening situation, e.g. weighing, petting, giving food treats. Most animals, even rodents will respond positively to handling and will learn to recognize individuals.
- Handle animals gently. Do not make loud noises or sudden movements that may startle them.
- Handle animals firmly. The animal will struggle more if it sees a chance to escape.
- Use an assistant whenever possible.
- Use restraint devices to assist when appropriate.
- Chemical restraint should be considered for any prolonged or potentially painful procedure.

Handling Methods

The methods described below will assist with performing basic manipulations. Alternate techniques may be needed for special procedures. Most of these methods are also demonstrated in video tapes available to investigator. An excellent website containing laboratory bi methodology for rodents and rabbits is also available with descriptions and pictures of drug administration, blood collection and sex determination.

Needle Re-Use Policy

The use of a new sterile needle and syringe for each animal when giving parenteral injections (intraperitoneal, subcutaneous, intravenous, intramuscular, etc.) is the recommended best practice to prevent the horizontal transfer of contamination between animals. However, University ACUC recognizes that there are some instances where it may be justified to use the same needle and syringe for multiple animals, usually in rodents. In those instances the Principal Investigator must provide justification University ACUC and must adhere to the following guidelines. Use of the same needle and syringe may be permitted with justification on animals housed in the same cage. The needle must be assessed for continued sharpness and the presence of barbing or burring of the tip between animals. If dullness or needle deterioration is found, a new needle must be used.

MICE

Tail restraint, as described below is adequate for examining animals and transferring them to another cage.

These methods may be used to perform minor, non-painful procedures such as injections or ear tagging.

HOW TO PICK UP A MOUSE

When you handle mice, all your movements should be slow and gentle. They are lively animals, but they will rarely bite unless they are afraid or mishandled.

Grasp the middle of the tail between your thumb and index finger, and lift the mouse.

NOTE: Frequently, the animal will hang quietly and can be carried in this manner. Occasionally, an individual will turn around and crawl onto the palm of your hand. It should be allowed to do this.



One-handed Method

Follow the above steps.

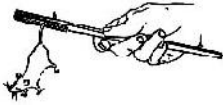
Then, place the mouse's tail between the last two fingers of the hand that is holding the :

HOW TO PICK UP A MOUSE WITH THUMB FORCEPS

The thumb forceps are shaped somewhat like an eyebrow tweezer. The tips must be covered with rubber or polyethylene tubing to prevent damage to the mouse's tail.

Grasp the middle of the tail between the forceps and apply only enough pressure to lift the mouse.

CAUTION: Too much pressure may cause pain or even crush the tail.



Two-handed Method

Place the mouse on a rough surface while holding the tail firmly.

NOTE: Smooth surfaces will frighten the mouse because it cannot get a foothold. This may cause it to turn around and try to bite in its attempt to escape.

Grasp the nape gently and firmly with your free hand and lift the mouse.



RATS may be handled by the tail, with precautions similar to those used for mice, with emphasis on only grasping the tail base. Holding the tail distal to the base can result in a degloving injury to the tail that will require surgical repair or euthanasia.

This method should be used to restrain a rat for injections and other minor procedures.

HOW TO HOLD A RAT FOR TREATMENT

Pick up the rat. Rotate the wrist of your right hand to expose the wrist.

Retain the rat's hind legs with your left hand, grasping one hind leg between your thumb and index finger and the other between your index and second fingers.



HAMSTERS

Because hamsters do not have tails, they must be grasped firmly by the loose skin of its back, or handled in a manner similar to the rat.

GUINEA PIGS rarely bite, but are very easily frightened and will vocalize and squirm to avoid restraint. The hind limbs must be supported at all times to prevent the animal from injuring its back.

HOW TO PICK UP AND CARRY A HAMSTER

There are two acceptable methods for picking up a hamster. You whichever is easier for you.

CAUTION: Always handle hamsters over a bench or table, because are liable to jump from your grasp and injure themselves.



Method 1

Form a cup with your hands it over the hamster. Gently palms against the animal as ;

Method 2

Grasp the loose fold of skin behind the neck with your thumb and index finger. Cup your other hand under the animal's rump and grasp the hind legs between your thumb and index finger.

Carry the hamster in the same position in which you picked him up.



For injecting the animal : section, merely stretch it on

HOW TO PICK UP A GUINEA PIG

Approach the animal in a quiet, confident manner. Place one hand across the shoulders. Place your thumb behind the front legs and the fingers well forward on the opposite side.

NOTE: Do not apply excessive pressure to the animal's body. You can hurt the guinea pig and cause it to squirm needlessly.

Pick up the animal and hold it in an upright position.



HOW TO CARRY A GUINEA PIG

Pick up the guinea pig. Place your free hand palm uppermost under the hindquarters to support the animal's weight. If it struggles, hold the legs to get a more secure grip.

HOW TO HOLD A GUINEA PIG FOR INJECTIONS

Grasp and extend the hind limbs with your free hand. This will enable the investigator to inject the animal in the midsection.



RABBITS are very susceptible to lumbar spinal luxation, resulting in paralysis. It is necessary to support the animal's hindquarter at all times. Although rabbits seldom bite, they can inflict painful scratches with their hind legs. One way of lifting a rabbit is by grasping the skin over the shoulder with one hand and gently lifting it with the other arm cradling the body, the head nestled in the crook of your arm. Rabbits must never be lifted by the ears.

HOW TO REMOVE A RABBIT FROM A CAGE

Approach the cage and open the door slowly so that you will not startle the rabbit. Reach in and grip the loose skin over the shoulders firmly but gently with your left hand. Place your right hand under the rabbit's rump to support its weight.

NOTE: The right hand may be opened and placed flat under the rabbit's stomach instead of under the rump, but this requires more skill. NEVER use the flat-handed method for handling pregnant does. It might injure the unborn young.

Lift the rabbit out of the cage.

HOW TO CARRY A RABBIT

Grasp the loose skin over the neck with your left hand and place your right hand under the animal's rump, as you did in removing the rabbit from its cage.

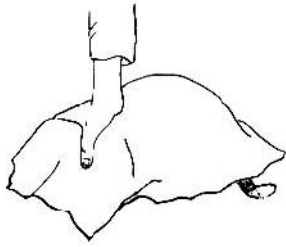
Pick up the rabbit and hold it in an upright position.

CAUTION: Never lift or carry a rabbit by its ears. They aren't handles! Don't carry a rabbit by the neck skin alone. This is painful to the animal and will cause it to struggle. You run the chance of getting scratched by the sharp rear claws.

Carry the rabbit out in front of yourself.



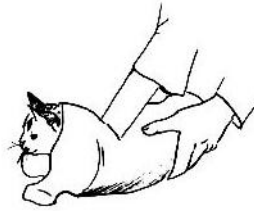
CATS are often cooperative enough to be restrained on a table by the loose skin at the back of the neck and hips, or with one hand restraining the body and the other restraining the head. A fractious cat may have to be wrapped in a heavy towel for restraint with any needed limbs carefully withdrawn for treatment.



How to Use a Blanket (or Sack)

Cover the cat with a blanket or sack. Grab it through the cloth and locate the head. Hold it firmly at the base of the skull.

Turn the blanket back to uncover the cat. Then pick up the animal in the usual manner.



HOW TO REMOVE A CAT FROM A CAGE

When you approach a cat's cage, all movements should be slow and deliberate. Cats are high-strung animals, and sudden movements might frighten them.

CAUTION: Look for warning signs that indicate a cat is on the defensive. These might include hissing or growling, clawing the cage against the head, and arching the back.



Open the cage door just wide enough to insert your left hand. Grasp the cat's neck behind the head to prevent the animal from swamping.

Insert your left hand from behind the head to under the chin. Place your right hand under the cat to support its weight. Lift the cat from the cage and place it on your right hip.



Insert the first two fingers of your right hand between the front legs. Grip firmly over the jaws with your thumb and other two fingers.

Secure the cat's body between the crook of your right arm and hip. Keep your left hand firmly under the chin to prevent the cat from biting.

DOGS

A slip lead is highly recommended for working with dogs. A dog should always be carried with proper support. The dog can be restrained in lateral recumbancy or in a sitting position for injections and minor procedures. For venipuncture, the handler can restrain the dog on a table with one arm around its neck. The other hand is then free to restrain the body if necessary or to occlude the vein for the person with the syringe. A shy or fearful dog may need extra time spent with it to make it more comfortable. Moving slowly and speaking quietly will help to prevent alarming the animal.

An intractable dog may need to be muzzled. A commercial muzzle may be purchased, or a gauze muzzle may be used as described below.

HOW TO LIFT A DOG

The size of the dog determines the method you will use to lift it. The beginning step is the same for all dogs.



Grasp the dog over the neck. Place your other arm around its body, with your hand supporting the dog's chest.

Large Dogs

Stand up straight, placing the weight of the dog on your hip, so you won't strain your back.

NOTE: Lift with your legs to prevent back strain.

Carry the dog in the hip position, leaning away from it for balance.



Small Dogs

Carry the dog in the upright position, shifting it so its is resting on the front of your hip. Lean slightly backwards for balance.

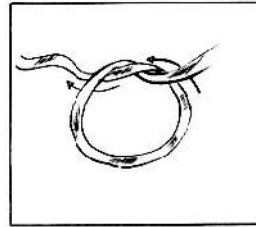


HOW TO MAKE A TEMPORARY MUZZLE

Before you learn how to handle a dog, it's important that you know make and use a temporary muzzle, because you will need it in a few of methods to follow.

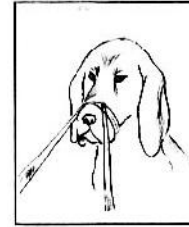
NOTE: Muzzle a short-nosed dog as you would a cat. The direction found on page 19.

Step 1



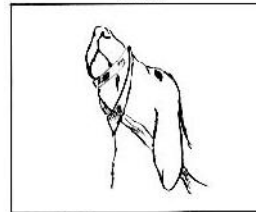
Make a loop in the center of a four-foot cord or double-thickness gauze bandage.

Step 2



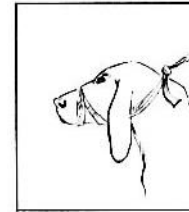
Slip the loop over the muzzle and draw the snugly over the nose.

Step 3



Bring the ends down under the chin and cross them.

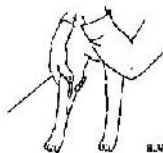
Step 4



Bring them up back to neck and tie a double knot.

HOW TO HOLD A DOG FOR INJECTIONS

This method is the same as the one above, except that your right hand is used for a different purpose.



Reach over the dog's back with your right arm, but instead of grasping the dog's chest, hold the right forelimb firmly at the elbow.

Apply pressure to the top of the leg with your thumb, as shown in the drawing on the left. This will cause the vein to enlarge by stopping the blood flow, making it easier for your investigator to give the injection.

HOW TO RESTRAIN A DOG ON ITS SIDE

It is sometimes necessary to restrain a dog on its side to expose its legs, belly, or side. This position may be used for removing scratches, or for leg, foot, or a dental examination.

Lay the dog on its side on the treatment table. Reach over its back and grasp both hind legs with one hand. Apply pressure with your forearm over the loin.

With your free hand, grasp both forelimbs. Apply pressure on the neck with your forearm. This will prevent the dog from twisting its head to bite you.



Pills are easily administered to most dogs if the proper technique is used.

HOW TO RESTRAIN A DOG IN THE SITTING POSITION

This method of restraint is useful for many different types of treatment.

Seat the dog on a table and approach it from its left side. Place your left arm under the neck. Hook your hand over the top of the dog's head and pull the head close to you to prevent movement.

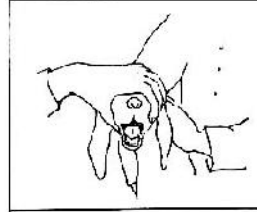
Reach over the dog's back with your right arm and grasp the chest. Pull the dog close to your body.



HOW TO GIVE A DOG A PILL

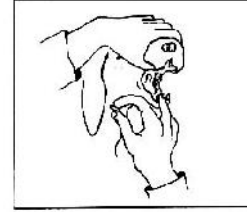
Pills are given to dogs for several reasons, for instance, to add them to worms or to experiment with new drugs.

Step 1



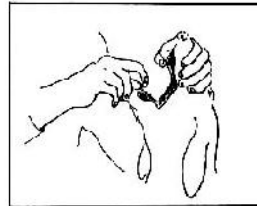
Have someone restrain the dog in the sitting position. Place the dog. Place one hand over the upper jaw, with thumb and fingers applying pressure to roll the lips inside the mouth. This will force the dog's mouth open part way.

Step 2



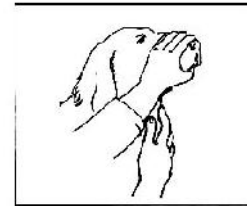
Fix up the pill between your index and thumb. Place the middle finger your five hand on the lower incisor. These are the short teeth in the front end of the lower jaw. Press down, forcing the dog to open its mouth wider.

Step 3



Push the dog's head back, so that its muzzle points up. This will give you a clear view down its throat. Drop the pill on the back of the tongue.

Step 4



Close the dog's mouth and hold it shut. Stroke the throat until the swallows the pill. You will be able to feel the throat move as swallowed.

NONHUMAN PRIMATES, no matter how small, can be dangerous. Chemical immobilization with ketamine is normally used. Injections can be given to a confined animal with the help of a squeeze cage.

Physical restraint of a conscious animal should only be attempted by trained, experienced personnel. Animals may be pole and collar trained if they will be handled frequently. Tether systems are recommended if animals must be administered drugs or if blood must be collected frequently.

Safety:

Absolute requirements for handling of nonhuman primates include attending a **training** module given by RAR (contact 624-9100 to schedule), and wearing appropriate protective clothing. In addition, nonhuman users should be familiar with procedures to follow in case of a bite or scratch and the location of bite kits.

If a nonhuman primate has **escaped**, close all doors and contact RAR at 624-9100. The animal may be recaptured using a net or a dart gun.

GOATS, SHEEP and CALVES

- Restrain against a wall or in a corner by placing a knee firmly in the flank.
- Restrain for blood collection by backing the animal into a corner and straddling

- them at the shoulder and firmly restraining the head and neck.
- Use a halter over their head and face.
 - A sheep can be held for bleeding, shearing or hoof trimming by sitting the animal up on its hind end, leaning back against the restrainer.
 - For long term restraint of sheep in the laboratory, a canvas sling and rack is available from several commercial suppliers. Animals are easily acclimated to such slings, and can be comfortable and relaxed enough to fall asleep in them.
 - Additional references on handling of agricultural animals is available from the USDA.
 - Temple Grandin's Website on **Low-Stress Handling of Farm Animals**

RESTRAINT AND HANDLING OF SWINE

By Dr. Jack Risdahl

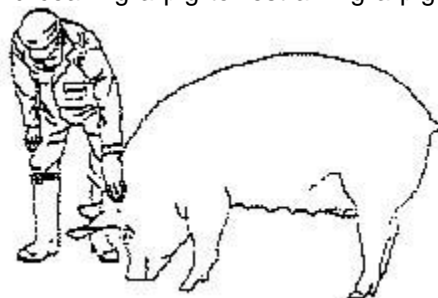
Pigs in general are friendly and docile but will react severely to poor handling or a stressful environment. Pigs can be very vocal. If pigs are chronically stressed they will become skittish and fearful. Handling and restraint in pigs relies greatly on treating the pigs in a humane manner. The benefits of treating pigs well include reducing apprehension, fear and stress in the pigs. There are several levels of restraint and handling, from touching and coaxing a pig to restraining a pig for chronic procedures.

Touch is a very important aid to good husbandry.

Animal-Human Contact

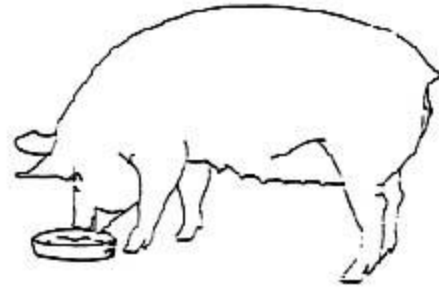
When approaching a pig be sure it is made aware of your presence. If pigs are startled they may cause injury to themselves or others in the pen. The best way to make pigs aware of your presence is to use your voice.

It is important to use a soft soothing voice and not angry, loud, high pitched tone of voice which might startle or stress the animal. Pigs quickly learn to recognize voices, especially if they are associated with food. As pigs become familiar with handlers, the sound of a familiar voice is often calming to the animal. It is important to use touch when developing a rapport with pigs. This applies especially to the researcher who must collect frequent samples or data from pigs. As with voice, gentle petting and hand contact should be associated with feeding time or treats and the pig will become aware of the person in the vicinity and become adjusted to that persons presence. Probably one of the best forms of restraint in pigs is the use of food. Pigs are highly oriented to food and if they are comfortable with the handler will most often stand and eat while minor procedures and examinations are being performed on them. One can often flush catheters,



give injections, treat minor wounds and take temperatures while pigs eat. The use of all three procedures - voice, touch, and food, will be the best investment in reducing stress among research swine and will ultimately reward the researcher with a happy stress free subject.

The giving of food is one of the most effective forms of basic restraint in the pig.



Picking Up Pigs

Pigs best tolerate being picked up in a "horizontal" fashion oriented to the ground. Pigs should not be picked up by the legs or held upside down as this will stress the animal and you will lose their trust. Usually only smaller animals may be picked up while larger animals (>35-40 kg) must be moved by alternative means. Smaller pigs may be easily picked up with their body supported while their legs hang. To perform the procedure in larger pigs place one arm under the chest cranial to the thoracic limbs and the other arm cranial to the pelvic limbs under the abdomen picking up the pig in a "scooping" fashion. Alternatively the arm may be placed caudally just above the pig's hock, hence supporting the animal by the pelvis rather than the abdomen. All handlers must beware to lift with legs and not back as injury can easily result - pigs are usually heavier than they appear! Always avoid picking pigs up by one leg or by the ears as injury may result!

Moving Pigs



The small board used to apply pressure to the side of a pig.

Pigs are best moved in a metal (box style) transport designed for use with large animals. At times this is not possible and pigs must be walked to their destination. When moving a pig always remember pigs will move away from walls toward openings. This is an advantage since one can use a "hog board" to simulate walls. The board is fashioned with a handle so that one can place it to the side, rear or front of the pig to direct them. Excessive force should not be needed to move a pig and is mostly counterproductive as pigs will become excited and belligerent. It should be remembered pigs will refuse to move if the place you wish them to go is dark (e.g. from daylight into a dark room). Sometimes pigs may be coaxed with food along with the use of the board. When pigs are unruly and where control is needed, pigs may be tethered in a harness and controlled by "holder" so that the pig does not run away. Often the use of the hog board may be used to stop pig and slow them down if they are moving too rapidly. The board may also be used to restrain a pig in a corner while minor procedures are performed. The size of the board varies depending on the size of pigs used and application. In general if the board is at least as tall as the pig and 2/3 to about as long as the pig it will usually suffice.



Sling

Several designs for slings to restrain pigs have been described. The most commonly used is that described by Panepinto et al 1983. Here the pig is placed in a hammock with four holes for the limbs. The hammock is supported by a metal frame. These are available in free standing or winch styles (so larger pigs may be raised by winch). The pig is placed in ventral recumbency in the sling with its limbs tied loosely to the frame. It has

been our experience that this form of restraint requires some degree of training for pigs to acclimate to. In general most pigs will become stressed the first several times they are placed in the sling. Positive reinforcement (treats, petting) and repetition usually calms them down so that they may be restrained for extended periods in the sling. We have generally used a training period of two weeks prior to experimental procedures with a minimum of 30 min./day in the sling. In our experience one or two hours is about the most a pig will tolerate.

Acclimation and Socialization

It should be remembered that pigs are social animals and have a rigid dominance hierarchy. If animals are group housed they will generally fight to establish dominance for the first 24-48 hours. Dominance in pigs is almost directly related to size. The largest animals are dominant and smallest are submissive. Be sure to match weights as close as possible when introducing new pigs to each other. Smaller pigs may be injured by larger pigs. Be sure to monitor pigs for the introduction period so that they do not cause major injury to each other - they will fight. Always remember that newly arrived pigs are stressed from transport. Do not initiate experimental procedures in the first few days of arrival. This is just common sense as immune function and physiologic parameters are often altered by stress. We like to see an acclimation period of two weeks so that pigs may adapt to their new environment and establish rapport with handlers.

Drug administration

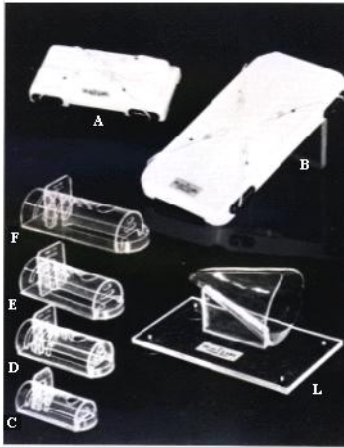
- A butterfly needle can be attached to a syringe for administering injections to swine, allowing them to move during the injection without displacing the needle.
- Intravenous injections may be given in the ear veins.
- Oral drugs may be administered ground or whole mixed with a food treat.
- Pills can be administered orally as with dogs. However, the handler must be sure to get the pill all the way behind the tongue and must avoid being bitten.
- Some drugs may be administered rectally. A literature review should be performed for the drug in question prior to attempting this.
- Indwelling central venous catheters are recommended if animals will be receiving drugs on a regular basis.

Restraint Devices

Restraint devices such as rabbit or rodent restrainers, swine slings or monkey chairs are useful for certain non-painful procedures. However, certain guidelines should be followed when using these devices.

- Animals should be adapted to the restraint devices. This means that for long-term restraint (i.e. more than an hour), it is advisable to "train" the animal to the device by placing it into the device for successively longer intervals until the maximum time of restraint can be achieved without causing distress to the animal.
- Animals in a restraint device be regularly monitored. This means not leaving the area for long intervals unless someone else is available to monitor the animal. Animals have an uncanny ability to attempt escape from devices, if they don't succeed completely, they may end up with a limb or their head entrapped. This could result in ischemia or hypoxia.

- Animals should have access to food or water at appropriate intervals, even when restrained, unless doing so would interfere with the goals of the experiment. Food or water should be offered twice daily. For rabbits and rodents, water should be offered more frequently.
- Animals should be released from restraint devices at least daily and allowed unrestrained activity to prevent muscle atrophy and skin necrosis, unless this interferes with achieving the experimental goals.



Rodent Equipment

Typical rodent restrainers

Ethics and Alternatives

Ethics

The use of animals in research, teaching and testing is an important ethical and political issue. Much of the discussion about this issue revolves around the relative value, often referred to as 'moral value', of humans and animals. When the needs of animals and humans come into conflict, which takes precedence? Today there exists a wide spectrum of views on this subject, ranging from those concerned with animal 'rights' to those who view animals only as a resource to be exploited. All of these viewpoints have contributed to the development of ethical principles of animal use.

Interestingly, advances in biology that began in the 1800's have provided some of the strongest arguments for imbuing animals with an enhanced moral value. By recognizing that the nervous systems of all vertebrate animals are very similar, it is assumed that activities that will cause a human pain or distress, will likewise cause pain or distress to other animals. It is for this reason that current animal use regulations require the use of analgesics, anesthetics and sedatives for any procedures on animals that may cause more than momentary pain or distress.

Animals with advanced nervous systems, such as nonhuman primates, carnivores and marine mammals, have also demonstrated other abilities that humans can relate to and value, such as advanced social behavior, the ability to react to both positive and negative stimuli, intelligence and even self-awareness. Current legislation on animal use emphasizes the idea of replacement of 'higher' animals with 'lower' animals, and requires environmental enrichment or human contact for intelligent, social animals such as nonhuman primates, or dogs and cats, but not for vertebrates like amphibians.

Current legislation also recognizes that there are diverse viewpoints about the moral value of animals. Thus, all live animal use in research, teaching or testing must be reviewed by a

committee (the IACUC) with diverse membership. There is also an emphasis on minimizing the overall use of animals. Proposals for animal use are reviewed based on the potential for learning new information, or for teaching skills or concepts that cannot be obtained using an alternative. There are also provisions for ensuring that animal use is performed in as humane a manner as possible, minimizing pain, distress or discomfort. These provisions include a requirement for a veterinarian to be employed at each institution, so that the needs of the animals are looked after by someone trained in, and sympathetic toward animals' needs. In addition to the requirements for analgesics, anesthetics and sedatives to be used where needed, it is also required that all personnel with animal contact be trained in appropriate handling techniques and that they be skilled in any experimental procedures that will be performed. Finally, basic husbandry requirements are specified, ensuring that an animal's food, water and shelter will be provided for in an optimal manner. Deviations from the numerous requirements are rarely granted by the University ACUC, and then only if adequate justification is given that the proposed experiment is scientifically and socially important, and that any methods to alleviate pain or distress would frustrate the experimental objectives.

Alternatives

An important ethical principle of animal use in biomedical research is that alternatives to live animals should be used whenever possible. Russell and Burch in their book, *The Principles of Humane Animal Experimental Techniques*, Charles Thomas, Springfield, IL, 1959. Promote a definition of alternatives as "the three Rs-replacement, reduction, and refinement" which has become a pervasive theme in biomedical research today.

Replacement means replacing 'higher' animals with 'lower' animals. Microorganisms, plants, eggs, reptiles, amphibians, and invertebrates may be used in some studies to replace warm-blooded animals. Alternately, live animals may be replaced with non-animal models, such as dummies for an introduction to dissection for teaching the structure of the animal or the human body, mechanical or computer models, audiovisual aids, or *in vitro* modeling. This form of alternative is primarily addressed in Appendix A of the Animal Care and Use Protocol (ACUP) form which asks about the alternatives that have been considered, why they were rejected and how the Principal Investigator searched for these alternatives.

Advantages to replacement include utilizing pre-existing knowledge for teaching, applying known principles to new systems to look for similarities, and using less expensive animals or models to screen large numbers of agents for toxicity or mutagenicity. *Disadvantages* to replacement chiefly stem from the fact that any models are dependent on pre-existing information. In a system as complex as a live organism, all of the variables in physiology and pathology are not known. Thus, any research on new biological processes must utilize a living organism at some point.

Reduction means minimizing the number of animals needed to perform an experiment or teach a concept. This alternative is addressed in Appendix A, but also in the ACUP form which asks for justification of the species to be used and the numbers needed for each experimental group. By examining these parameters, the University ACUC can determine if thoughtful experimental design was employed to minimize overall animal use. Methods to achieve this include:

- Performing pilot studies to determine some of the potential problems in an experiment before numerous animals are used
- Designing a study to utilize animals as their own controls

- Gathering a maximum amount of information from each animal, perhaps gathering data for more than one experiment concurrently
- Consulting with a statistician to use only the numbers of animals required to achieve significance *Statistical Approach to Calculating the Minimum Number of Animals Needed in Research*]
- Minimizing variables such as disease, stress, diet, genetics, etc., that may affect experimental results
- Performing appropriate literature searches and consulting with colleagues to ensure that experiments are not duplicated
- Using the appropriate species of animal so that useful data is collected
- Replacement whenever possible.

Refinement means refining experimental protocols to minimize pain or distress whenever possible. This concept is addressed in numerous questions throughout the Animal Usage Form. Examples of refinement include:

- Identifying pain and distress and making plans for preventing or relieving it.
- Setting the earliest possible endpoint for the experiment. That is, if the necessary information can be gathered before the animal experiences any ill effects from the experiment, this should be defined as the endpoint and the animal subsequently euthanized. For example, if measuring toxicity of a compound or survival following implantation of a neoplasm, a pilot study may determine that once certain clinical signs are seen, or a tumor achieves a certain size, the time course until debilitation or death are predictable. Subsequent experiments may then utilize the earlier endpoint of tumor size or clinical signs of toxicity, rather than death as the endpoint.
- Receiving adequate training prior to performing a procedure.
- Using proper handling techniques for animals.
- Ensuring that drug doses are correct and that the drugs used are not expired.
- Ensuring that procedures to be performed on the animal are reasonable for that species.
- Using appropriate analgesics and anesthetics for potentially painful procedures.
- Performing surgeries and procedures aseptically to prevent infection.
- Performing only a single major survival surgery on any one animal, whenever possible.
- Performing appropriate post-surgical care, including thermoregulation and fluid balance.

In some cases, application of one alternative concept may have an adverse effect in another area (i.e using a "lower" animal or minimizing pain or distress may require using more animals.) These issues are discussed by the IACUC and depending on the circumstances different priorities may be set.

Searching for Alternatives

Appendix A of the Animal Usage Form asks for the methods used to search for alternatives to procedures that may cause more than slight pain or distress to animals. Examples of these methods would be a literature search (indexes searched and keywords used should be listed), consultation with peers in the field.

Experiment Guidelines for the Prevention, Assessment and Relief of Pain and Distress in Laboratory Animals

A key aspect of the animal welfare regulations is that pain and distress be minimized whenever possible. Therefore, it is necessary to design and perform experiments in such a way as to prevent the animals from experiencing problems unless it is necessary to achieve the goals of the study (i.e. category "c" studies).

It is not sufficient to merely address these issues in a protocol. The animals themselves must be monitored, and appropriate actions taken if pain or distress are observed. The problem is that assessing pain and distress in animals is not a simple task. Animals may not show signs of pain as readily as would a human being, and they certainly can not communicate it in the same way. There is even great variability among species in the way pain or distress are expressed. In general, animals whose biological niche is that of a prey species (rodents, rabbits, nonhuman primates, livestock) are less likely to alter their behavior in response to pain than would a predatory animal, as doing so would make them a target for predation. In addition, behaviors are often interpreted by humans in the context of our understanding of what that behavior would mean for our species rather than for the species being assessed.

Research Animal Resources (RAR) animal care and veterinary staff are delegated by the University ACUC to serve in the capacity of monitoring for signs of pain and distress in laboratory animals. They are trained and experienced in this area and are a good resource for monitoring the progress of studies. However, investigators must also be aware of signs that an experimentally induced or spontaneous disease is occurring in their animals. They may have more contact with the animals than RAR, and ultimate responsibility for the ethical use of animals lies with the investigator.

The following guidelines are used by RAR, the University ACUC and by regulatory agencies to determine if an animal is experiencing pain or distress.

It is assumed that for vertebrates, any procedures that would be expected to cause more than slight or momentary pain or distress in a human being will cause similar pain or distress in an animal, unless scientifically demonstrated otherwise.

Distress is defined as a maladaptive response to a stressor. It is recognized that some stress is normal, but if animals react in such a way that their health is being compromised (e.g. anorexia in response to induced disease or self-aggression in response to psychological stress) this is considered distress..

Certain procedures are always assumed to have the potential for causing pain or distress. These are the basis for the numerous University ACUC experimental guidelines that are intended to prevent pain or distress. These include:

- Surgery anesthesia and post-procedural care must be performed in such a way as to prevent pain, infections and other complications
- Repeated use of, large volumes of, or intradermal injections of Freund's complete adjuvant
- Intraperitoneal implantation of ascites-producing hybridomas for monoclonal antibody production
- Prolonged (greater than 1 hour) physical restraint
- Malignant neoplasms
- Prolonged food or water deprivation
- Distal tail biopsy in animals over 3 weeks of age (tail snipping)
- Electrical shock or other adverse stimuli that are not immediately escapable
- Paralysis or immobility in a conscious animal
- Inflammatory disease
- Organ failure resulting in clinical signs
- Non-healing skin lesions
- Whole body irradiation at high doses
- Withdrawal of more than 10% of an animal's blood volume

- Studies that require the animal to reach a moribund state or die spontaneously as the endpoint of the study. The earliest endpoint possible should be used to prevent pain or distress.

Animals subjected to these and similar conditions are expected to receive appropriate monitoring and any necessary supportive care, analgesia, or anesthesia to prevent pain or distress.

Despite these precautions, pain or distress may occur as a consequence of a study or a spontaneous disease.

Assessment of pain or distress may be based on many different criteria including:

- Decreased activity
- Abnormal postures, hunched back, muscle flaccidity or rigidity
- Poor grooming
- Decreased food or water consumption
- Decreased fecal or urine output
- Weight loss (generally 20-25% of baseline), failure to grow, or loss of body condition (cachexia)
- Dehydration
- Decrease or increase in body temperature
- Decrease or increase in pulse or respiratory rate
- Physical response to touch (withdrawal, lameness, abnormal aggression, vocalizing, abdominal splinting, increase in pulse or respiration)
- Teeth grinding (seen in rabbits and farm animals)
- Self-aggression
- Inflammation
- Photophobia
- Vomiting or diarrhea
- Objective criteria of organ failure demonstrated by hematological or blood chemistry values, imaging, biopsy, or gross dysfunction

Relief of Pain or distress

- The RAR veterinarian will consult with the investigator to develop a plan for treatment of animal health problems.
- If an animal is experiencing unrelieved pain or distress, it must be euthanized.

Guidelines for Immunization of Research Animals

Adjuvants

While a variety of adjuvants are available for use in research animals, Freund's adjuvant is perhaps the most commonly used. Complete Freund's adjuvant (CFA) may cause inflammation, induration, pain and necrosis at the injection site in mammals. Despite the common knowledge that CFA is a superior adjuvant, numerous studies have demonstrated comparable results with less irritating adjuvants. It is important to note that complications are still possible with other adjuvants, as well. Investigators should review the USDA website **Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies**. Investigators may wish to use RAR's antibody production service and avoid having to worry about these concerns.

- Keep your eyes open for a developing technology, CpG DNA, which serves as an immunostimulant and can be used as an adjuvant.
- DNA Vaccines

Guidelines

In order to eliminate or reduce discomfort from Complete Freund's adjuvant, the following guidelines have been established by the IACUC. Departures from these guidelines require adequate scientific justification on your Animal Usage Form for approval by the IACUC.

- The use of other adjuvants must be considered.
- CFA may be used only for the first (priming) dose. Subsequent immunizations should be with incomplete Freund's or another adjuvant unless justified. This is rarely warranted and if allowed, an interval of at least three weeks should be given between doses.

Route of Administration

Because of the potential for complications from certain routes of administration of immunizations, the following guidelines have been established.

- The inoculum should be free of extraneous microbial contamination. Millipore filtration of the antigen before mixing with the adjuvant is recommended when possible.
- Injection sites should be cleaned to remove debris that may result in contamination and infection.
- Injections containing Freund's complete adjuvant should be given subcutaneous (SQ), rather than intradermal (ID), intramuscular (IM), intravenous (IV) or intraperitoneal (IP). ID injections frequently result in skin necrosis and sloughing. IM injections can result in temporary or permanent lameness. IV injections can cause pulmonary lipid embolism.
- For subcutaneous injections, the inoculum containing the adjuvant should be divided into fractions so that no more than 0.1 ml is injected per site for rabbits and 0.05 ml per site for mice. If skin necrosis results while following these guidelines, future injections should be spaced farther apart.
- The University ACUC discourages injection of foot pads because animals may develop chronic pain and secondary infections in the inflamed areas. If this procedure is to be used, it must be described and its use scientifically justified, including documentation that injections in other sites do not produce adequate antibody titers for the specific antigen being used. If used, only one hind foot may be injected with a maximum volume of 0.05 ml per site, and injections must be spaced at 10 day to 2 week intervals. Animals that have received foot pad injections must be housed on contact bedding rather than a metal mesh floor.

Birds

Investigators still must specify the details of the immunization procedure in the Animal Usage Form. In addition, investigators should be aware that the University ACUC may request changes in immunization protocols if they result in abscess formation or other painful lesions in birds.

Guidelines for Collection of Blood from Experimental Animals

Guidelines for safe blood withdrawal for laboratory mammals takes into account the fact that each different species has a different blood volume in milliliters of blood to kilogram of body weight. They also assume a 14-21 day cycle for red blood cell renewal. These guidelines are for normal, healthy adult animals. Animals that are aged, stressed, have undergone experimental manipulations, or are suffering from cardiac or respiratory disease may not tolerate this amount of blood loss.

Restraint

Blood collection may be performed adequately in awake animals of some species using the appropriate restraint. Restraint is necessary to prevent movement that may result in laceration of a blood vessel or other organ and serious complications.

Dogs, Cats, Sheep and Calves usually require only physical restraint to collect blood.

Rabbits and Swine - may require only physical restraint if they have been trained to the procedure.

Rabbits, Mice and Rats - may be placed in appropriate restraining devices.

Chemical Restraint - may be used prior to blood collection to minimize distress to the animal and to the person doing the blood collection.

Anesthesia is required to perform blood collection from the orbital sinus or by cardiac puncture because of the pain involved in the procedure and the potential for complications (including cardiac tamponade and death, or injury to the eye), even if performed by experienced personnel. Cardiac puncture is only approved for terminal blood collections unless specifically approved by the University ACUC.

Procedural Guidelines

A table of common blood collection sites is given below. Some sites required anesthesia because the procedures is painful. (Table 1)

Collecting blood by **lacerating ear or tail vessels is prohibited**. There is always the potential that an artery will be lacerated rather than a vein, resulting in severe hemorrhage. In addition, these procedures are more painful than puncture with a needle because of the prolonged time for wound healing. Also, the site of the procedure is very susceptible to infection, hemorrhage and other complications.

Regardless of the method of collection used, **an animal may not be returned to its cage until complete hemostasis has been achieved** (there is no more blood coming from the collection site). Hemostasis should be achieved using gauze and direct pressure. Up to several minutes of pressure may be required following arterial puncture.

Single Blood Draw, Not Repetitive

As a one time large volume blood draw with concomitant IV fluid replacement, a maximum 2% of the body weight of a healthy adult animal can be removed, as long as fluid replacement consists of warmed, isotonic fluids and both the fluid replacement and blood withdrawal are slow and steady.

Single Blood Draw Repeated Multiple Times

For multiple blood draws separated by a period of weeks, **a maximum of 1% of the animal's body weight can be removed**, i.e., 0.15 ml from a 15 gram mouse; 50 ml from a 5 kg cat; 400 ml from a 40 kg dog. A 14 day recovery period is needed for the average healthy adult animal to recover from this blood loss. Although the blood volume is restored within 24 hours after blood withdrawal, two weeks is needed for all constituents of the blood to return to normal. If less than

the maximum amount of blood is withdrawn the animal will replace blood constituents at the rate of 1 ml/kg/day.

Multiple Blood Draws

If blood must be drawn more frequently than once every two weeks, a total of 0.5% of the animal's body weight can be removed each week with this total volume being spread out over the entire week if needed.

Monitoring

By monitoring the hematocrit (or packed cell volume-PCV) and/or hemoglobin of the animal, it is possible to evaluate whether the patient has sufficiently recovered from a single blood draw or multiple blood draws. After a sudden or acute blood loss, it takes up to 24 hours for the hematocrit and hemoglobin to reflect this loss. This means that after a 1% of body weight blood loss without fluid replacement, an animal's hematocrit will not show a measurable drop for several hours, and will not stabilize for 24 hours. After 24 hours although the blood volume will normalize, the number of red blood cells (hematocrit) will be measurably reduced. In general, if the animal's hematocrit is less than 35% or hemoglobin concentration is less than 10 g/dl it is not safe to remove the volume of blood listed above.

Terminal Blood Withdrawal

Terminal bleeds are only allowed on animals under full general anesthesia, and the animal's death must be verified at the end of the bleed. An alternative euthanasia method is recommended after the blood withdrawal.

As a general rule:

An animal's blood volume is 10% of its body weight, and only half of that can be recovered when the animal is bled out. Therefore, as a terminal bleed, 5% of an animal's body weight is the blood volume (in ml) that may be recovered.

Table 1 Common Sites for Blood Collection

Species	Site of collection and permitted conditions
Mouse	Cardiac (terminal only), orbital sinus (anesthetized only), tail vein [note: incisional method not permitted], saphenous vein, facial vein.
Rat	As with mouse, plus subclavian veins [description and pictures]
Guinea Pig	Cardiac (anesthetized only), anterior vena cava/subclavian vein [description]

Table 2 Normal Packed Cell Volume (PCV) for some Laboratory Animals (%)

(Low end of given range is normal in juveniles, but not in adults)

Dog	29-55	Rabbit	30-50
Cat	25-41	Guinea Pig	37-48
Rhesus	26-48	Hamster	40-61
Baboon	33-43	Rat	36-54
Swine	32-50	Mouse	39-49

	and pictures]	Cow	24-48	Gerbil	43-60
Rabbit	Cardiac (anesthetized only), marginal ear vein [description and pictures]	Sheep	24-45		
Dog, Cat & Nonhuman Primate	Cephalic, saphenous, femoral and jugular veins				
Ruminants	Jugular vein				
Swine	Jugular vein, anterior vena cava, ear veins [Link to blood collection techniques]				
Chicken	Brachial wing vein, right jugular vein, cardiac (anesthetized only)				

Guidelines for Maintenance of Tumor Cell Lines and Hybridomas for Ascites Production in Rodents

The implantation of tumors into animals is a procedure that has the potential for causing pain and distress. These guidelines are established by the University of Minnesota Institutional Animal Care and Use Committee to address these concerns.

1. Cell lines should be tested for the presence of murine viruses prior to introduction into the animal colony.
2. Human cell lines should either be certified free of human pathogens. Workers should be offered the hepatitis B vaccine.

3. Solid Tumors

- Mice inoculated with solid tumors should be observed at least three times weekly to assess their physical condition.
- Animals with tumors that have ulcerated or that interfere with the animals normal activity, or animals that become emaciated or debilitated will require euthanasia.
-

4. Intraperitoneal implantation of Hybridomas

There are numerous *in vitro* alternatives to the *in vivo* mouse ascites induction method of producing monoclonal antibodies. These should be investigated prior to making a decision to use the *in vivo* method.

Priming

- 0.5 ml is the preferred maximum volume of pristine. Up to 0.75 ml may be used in large mice.

Monitoring for Distress

- Animals should be assessed at least once daily for clinical condition and food and water intake
- If the animal is in distress, it should be euthanized (refer to Euthanasia Criteria below)

Harvesting Ascites Fluid

- The ascites fluid can be harvested with a 20 gauge or smaller needle. New personnel should be trained using anesthetized mice.
- The animal should be monitored later on the same day it has been tapped

Euthanasia Criteria- An animal must be euthanized if it meets any of the following criteria:

- It is moribund
- It is lethargic, anorexic, dehydrated, or shows other evidence of significant illness or distress
- The skin of its abdomen is gray/green
- It is in respiratory distress

Experimental Surgery

General Issues and Requirements

Surgery is defined as any procedure that exposes tissues normally covered by skin or mucosa. Experimental surgery has great potential for causing pain or distress to animals if not performed properly. Surgery can result in pain, damage to tissue and post-operative infections. Therefore stringent guidelines for training, surgical facilities, asepsis, surgical preparation, anesthesia, intra-operative records, analgesia, surgical technique, and post-operative monitoring have been established.

Surgery is classified in several ways. There are different requirements depending on the type of surgery being performed.

- Surgery is **major** if it enters a body cavity (thorax, abdomen, calvarium), or has the potential for having significant complications. Included would be orthopedic procedures and extensive cannulation procedures.
- Other surgery is classified as **minor**. Minor procedures include peripheral vessel cannulations and skin incisions.
- Surgery is also classified as **survival vs. nonsurgical**. Asepsis and sterility are not required for non-survival procedures, unless the procedures are of sufficient duration to allow bacterial infections to affect the outcome of the study.
- There are also slightly different requirements for surgery performed on large animals such as rabbits, dogs, pigs and monkeys versus rodents and non-mammals.

Surgery Facilities

Surgical facilities used for survival surgery must be designed and maintained in such a way that they help prevent the development of post-procedural infections. Design features include:

- Separation of the preparation areas from the surgery area
- Minimization of personnel traffic flow through the surgery area
- Air flow should be away from the surgery area (e.g. positive room pressure, use of filtered, laminar flow air). It may be desirable to have HEPA filtered air for high-risk procedures
- Room surfaces should be non-porous and easily sanitized
- A regular room cleaning and disinfection schedule should be established (i.e. daily cleaning of floors and work surfaces, weekly to monthly cleaning of walls and cabinets)
- The surgery area should be free of all equipment and materials not necessary for the procedure. Any stored items should be in cabinets or drawers.

How these goals are achieved will vary somewhat depending on the type of surgery.

- An approved surgery suite is needed for large animal major survival surgery, with separate rooms for preparation of the patient, preparation of the surgeon, the operating room and a recovery of the animal from anesthesia.
- Non-survival surgery, minor surgery or rodent/non-mammal surgery may be performed in a dedicated work area. This is a room or bench top which from which all materials are removed at the time of the surgery. The same concepts described above are important for a dedicated area.

The University ACUC will review and approve all surgical areas.

Principles of Asepsis

Asepsis is defined as preventing exposure to microorganisms and prevention of infection. Three things that are extremely important in achieving asepsis are the **reduction of time, trauma and trash**.

- **Time** of surgical procedure is an important factor, as the longer a procedure takes the greater the possibility of contamination and therefore infection.
- **Trauma** that is sustained by the tissue as a result of rough handling, drying out upon exposure to room air, excessive dead space, implants or foreign bodies or non-optimal temperatures will contribute to infections.
- **Trash** refers to contamination by bacteria or foreign matter.

It may be possible to follow slightly different procedures for achieving asepsis when performing surgery on small patients such as rodents, birds, reptiles and amphibians. Typically, surgical times are short, incisions are small and the amount of tissue trauma is minimal. These all minimize the risk of infection.

Preparation: Surgeon, Patient, Instruments and Supplies

- It is essential that anything that will contact the subcutaneous tissues of an animal be appropriately sterilized to prevent post-procedure infections. Other aspects of preparation include pre-operative fasting, if necessary, a decision about prophylactic antibiotics, appropriate anesthesia of the patient, and a plan for post-operative pain control and supportive care.

Preparation of Instruments and Supplies

Surgical instruments and supplies must be sterilized before they are used for survival surgery. There are a number of ways that this can be achieved.

Durable instruments and supplies may be autoclaved. This an extremely reliable and cost-effective method for sterilization. The disadvantage is the time that it takes to perform (from 15 minutes to 1 hour). Normally a wrapped "pack" of instruments is prepared and is opened the day of surgery. Packs may be stored if they are kept away from moisture. A preparation date should be put on each prepared pack and packs should not be used if they are more than six months old.

Instruments and some materials may be sterilized in a **cold sterilant solution**. There are several acceptable commercial sterilants available. Only products classified as sterilants are to be used for sterilizing instruments and implants for surgery and they must be used according to the manufacturer's recommendations for sterilization.

Non-commercial solutions that are acceptable include:

glutaraldehyde 2% for a minimum of 10 hours*
formaldehyde 8% + 70% ethyl alcohol for a minimum of 18 hours*
stabilized hydrogen peroxide 6% for a minimum of 6 hours

***Note:** these cold sterilants should be kept in covered trays and only opened inside a fume hood or externally vented biosafety cabinet.

All surfaces, both interior and exterior, must be exposed to the sterilant. Tubing must be completely filled and the materials to be sterilized must be clean and arranged in the sterilant to assure total immersion. The items being sterilized must be exposed to the sterilant for the prescribed period of time. The sterilant solution must be clean and fresh. Most sterilants come in solutions consisting of two parts that when added together form what is referred to as an "activated" solution. The shelf life of activated solutions is indicated on the instructions for commercial products. Instruments, implants, and tubing (both inside and out) that have been chemically sterilized should be rinsed with sterile saline or sterile water prior to use to avoid tissue damage. **Note: chemicals classified only as disinfectants (for example, 70% alcohol) are not adequate.**

If starting with sterilized instruments, the same instruments can be used for multiple animals (rodent and non-mammal only) if the instruments are re-sterilized between animals, for example with a hot bead sterilizer. However, because only the tips of the instruments are sterilized, the surgeon must constantly be aware of instrument handling and aseptic technique. The pack of instruments must be fully re-sterilized (autoclaved) if the sterile field is broken, e.g. instruments are set down on a table or other non-sterile surface between surgeries. The efficacy of the hot bead sterilization is high and it sterilizes in a very short time (10 sec) but it is necessary to allow the instruments to cool before handling tissue to prevent thermal injury. [Ref: Callahan, et. al, 1995. A comparison of four methods for sterilizing surgical instruments for rodent surgery. Contemp. Top. Lab. Anim. Sci, 34:2, 57-60.]

Instruments and materials are often available pre-sterilized. The packages should have an expiration date on them. Surgical supplies may not be used for survival surgery when they have passed the expiration date.

Use of Expired Materials

Expired medical materials such as drugs, fluids and sutures may not be used on any research animal who is unanesthetized or who is to recover from an anesthetic procedure. The use of such materials under these conditions constitutes inadequate veterinary care under the Animal Welfare Act.

The University ACUC has established the following guidelines for the use of expired medical materials:

1. It is never acceptable to use outdated anesthetics, analgesics, or emergency drugs. Examples of acceptable materials include IV fluid solutions, non-emergency drugs (diuretics, contrast material, antibiotics), IV catheters, bandage materials, surgery gloves and suture materials.
2. Expired materials are only to be used on anesthetized animals in terminal studies (e.g. studies from which the animal does not awaken). Anesthesia for these terminal studies must be induced and maintained using current, non-expired drugs.
3. All expired materials must be clearly and individually labeled as: *Expired--for acute use only*, and are kept together in an area physically separate from all other medical materials and drugs. The area (box, shelf etc.) they are kept in must be labeled: *Expired--for acute use only*.

Preparation of the Patient

The majority of post-procedural infections are the result of contamination of the surgical site with resident or transient skin bacteria from the patient. Therefore, decontamination of the surgical site and prevention of contamination from other areas is the best means of preventing post-procedural infections.

- Normally, the patient's hair should be removed from the surgical site. This should be done with an electric clipper or depilatory rather than a razor. Hair removal should be performed immediately prior to the surgery. Extended time between hair removal and use of razors contributes to post-procedural infections.
- The patient's skin should be scrubbed with a disinfectant such as povidone iodine or chlorhexidine. Scrubbing should start at the center of the surgical site and move to the outside in a linear or circular manner. Scrub the surgical site with a disinfectant and rinse/scrub with alcohol or sterile water or saline to remove debris. Repeat at least three times or until site is free of visible debris. Often a disinfectant solution is then painted onto the surgical site and left to dry. The amount of scrub fluid used should be carefully controlled to prevent hypothermia. It may not be appropriate to scrub the site of some patients. Scrubbing the skin of a fish or amphibian will remove the protective bacterial slime layer, and may actually increase the risk of infection.
- A sterile surgical drape should be used whenever possible to isolate the disinfected area from surrounding areas. To be effective, a drape must fit tightly to the skin and must be impermeable to moisture. Clamps or sutures may be used to fix the drape in place. Self-adhesive drapes are also useful and are particularly recommended for use in small patients. In some cases a drape may

not be practical or necessary. When a drape is not used it places extra responsibility on the surgeon to perform excellent surgical technique.

Preparation of the Surgeon

The patient must be protected from organisms that can be carried and shed by the surgeon. These organisms reside on the surgeon's skin, hair, in the nose or mouth, or may be carried on dust particles from the floor or room surfaces. This route of contamination is minor compared to the patient's own flora, however, it is a significant source of contamination in some types of surgery such as orthopedic and central nervous system procedures.

- Sterile gloves should be used for all procedures. Examination gloves are not sterile. Gloves may be disinfected between surgeries with a cold sterilant for rodent and non-mammal surgeries. Large animal surgeries should be performed with a new pair of gloves for each patient.
- The surgeon's hands and arms should be scrubbed for 3 minutes with a disinfectant such as povidone iodine or chlorhexidine, rinsed with water and dried prior to gloving for any large animal survival surgery. As much as 30% of the time gloves become perforated during surgery, exposing the animal's tissues directly to the surgeon's skin.
- A cap, face mask, shoe covers and sterile gown must be worn for all large animal major survival surgeries.
- A clean smock or lab coat is recommended when performing rodent surgeries. A hair cover and face mask will reduce the risk of gross contamination of the surgical site.
- Minimizing traffic flow and conversation in the operating room significantly reduces the risk of contamination of the surgical site.

Surgical Technique

It has been recognized that one of the greatest influences on the incidence of post-procedural infection rates is the surgeon themselves. Prolonged surgical times expose tissues to contaminants, dry out tissues and compromise the blood flow to tissues. Tissues damaged by crushing or drying, suture and other surgical implants serve as a nidus for infection. There are a number of things that surgeon's can do to prevent post-procedural infections.

- Be aware of instrument and hand position at all times. If an instrument or hand touches something outside of the sterile field (the area delimited by the drape or the inside of the opened instrument pack) the instrument or glove should be replaced immediately.
- Be gentle when handling tissues.
 - Do not use toothed or crushing instruments if it is not necessary.
 - Hold the cut edge rather than grasping in the middle of a tissue layer.
 - When tying off vessels include only a minimum of surrounding tissues.
 - Use electrocautery or electroscalpels sparingly. They cause significant tissue necrosis.
- Use appropriate suture techniques

- Any suture that will be buried in tissues should be either absorbable or monofilament (non-absorbable braided suture is irritating and can harbor bacteria)
- Sutures should be placed evenly and as close to the tissue edge as possible to prevent obstruction of blood flow- typically no more than 1 cm from the edge is necessary in large animals and 0.2 cm in small animals.
- Sutures should only be tightened enough to appose the tissue edges. Any tighter will obstruct blood supply, retard wound healing and may result in dehiscence.
- Skin sutures are often unnecessary. They may cause the animal to chew or scratch at the incision site. Alternatives include use of subcutaneous/intradermal closure techniques or tissue adhesive.
- Ablate all "dead space" during closure. Any pockets or potential space that remains between tissue layers will fill with extracellular fluid or blood. This is an abscess waiting to happen. However, it is important not to place excessive tension on the suture line or the incision may not heal. Tacking down tissue layers can be used. If this is not possible, use of a drain for 3 to 5 days following the procedure is recommended.

Post-procedural Care

Post-procedural care includes the following:

1. Monitoring anesthetic recovery

- **Someone must be present** with any animal recovering from anesthesia until that animal is able to hold itself in a **sternal position** (on its chest, able to hold its head up). Rodents and rabbits must be ambulatory, since even an anesthetized rodent is stable enough to rest on its chests.
- **Endotracheal tubes** should be kept in place as long as possible; they must be removed when the animal begins to chew or swallow
- **The animal must be able to maintain normal physiology.** Heart rate, respiration, and hydration should be stable and within normal limits for the species.

2. Addressing post-procedural complications

- **Provide analgesia** for any procedures with potential for pain or distress. See **standard RAR post-operative analgesic regimens** for pigs, sheep, and dogs.
- **Administer antibiotics** to prevent post-procedural infections
- **Monitor incisions** for swelling, exudate, pain or dehiscence
- **Monitor catheters & devices**
- **Monitor for procedure-related complications** such as organ failure, thrombosis, ischemia

3. Maintaining records

- **Records must include a daily assessment and treatments given.** Other items that could be included in the record are anesthetic agents and time administered, intra-operative assessments and recovery observations.
- **Post-operative records are required on all animals and must be readily available for review.** Records on rats and mice may be somewhat abbreviated, and can be included as part of research data collected, but should also be available for review. **Sample recordkeeping forms** can be viewed and downloaded from the lower right side of the University ACUC points of emphasis and tip sheet page.

Antibiotic Guidelines

General Recommendations

- If antibiotics are being used, they should be administered before surgery so that they are in tissues when the surgeon is.
- An appropriate antibiotic should be administered at an adequate dose at the recommended frequency to minimize the development of resistance.
- Antibiotics should not be used in place of surgical asepsis and good tissue handling techniques. Tissue trauma contributes to post-operative infections.

Selection

If a culture and sensitivity is not available, select antibiotics based on probable organism and probable sensitivity. For example, normal skin flora are usually Gram +, so for a skin incision, select something with a Gram + spectrum, e.g., amoxicillin/clavulanate or a potentiated sulfonamide. If GI surgery is performed, an antibiotic with a Gram - spectrum is more appropriate, e.g. an aminoglycoside or ceftiofur. Indwelling catheters tend to become infected with skin or fecal contaminants, including anaerobes. Thus a broad spectrum and anaerobic spectrum is required, e.g. amoxicillin/clavulanate, ceftiofur or ticarcillin. *Pseudomonas* is an opportunist with a high likelihood of a multiple antibiotic resistance phenotype. An extended spectrum penicillin (ticarcillin), a fluoroquinolone (enrofloxacin) or an aminoglycoside (amikacin) may be necessary.

Combinations

Antibiotic activity is classified as being either bacteriostatic (inhibits cells from dividing) or bacteriocidal (kills bacteria even if they are not dividing). In general, combining two bacteriostatic drugs results in additive effect, combining two cidal drugs results in synergistic effect. Combining cidal and static agents can result in impairment of bacteriocidal activity. If you are treating a specific infection, select two drugs with activity against the organism in question. If you are looking for broad spectrum activity, select drugs with complementary activity, eg. penicillin and an aminoglycoside, or enrofloxacin and clindamycin.

Euthanasia Guidelines

Animals are normally euthanized at the end of a study for the purpose of sample collection or post-mortem examination. Animals may be euthanized because they are experiencing pain or distress. Euthanasia is defined as a pain-free or stress-free death. The University ACUC has approved certain methods for humanely killing animals that meet the definition of euthanasia. The appropriateness of the method may vary from species to species. These guidelines are adapted from the report of the American Veterinary Medical Association Panel on Euthanasia, J Am Vet Med Assoc 2007.

NOTE: You may only use a euthanasia method that is approved in your IACUC Animal Care and Use Protocol. A change in euthanasia method, including dose or route of administration, is a significant change in protocol and must be reviewed and approved by the University ACUC before implementation.

CRITERIA FOR EUTHANASIA

Euthanasia of animals is expected if animals demonstrate the conditions listed below, whether the animal has been manipulated or not. Additional criteria may be specified on the Animal Usage Form. Fulfillment of one criterion can constitute grounds for euthanasia. **Exceptions** are permitted only if approved by the University ACUC as part of the protocol review process (i.e. the clinical signs listed below are expected as part of the experiment and appropriate measures are taken to minimize pain or discomfort in the animals).

1. **Weight loss:** loss of 20-25% (depending on attitude, weight recorded at time of arrival, and age: growing animals may not lose weight, but may not gain normally) or if not measured, characterized by cachexia and muscle wasting.
2. **Inappetance:** complete anorexia for 24 hours in small rodents, up to 5 days in large animals; partial anorexia (less than 50% of caloric requirement) for 3 days in small rodents, 7 days in large animals.
3. **Weakness/inability to obtain feed or water:** Inability or extreme reluctance to stand which persists for 24 hours, assuming that the animal has recovered from anesthesia.
4. **Moribund state:** In rodents, measured by a lack of sustained purposeful response to gentle stimuli (example of purposeful response- weak attempt to get up; if animal is on its side, attempts should be asymmetrical in nature); in larger animals, measured by F (assuming in either case depression coupled with body temperature below 99 that the animal has recovered from anesthesia).
5. **Infection:** infection involving any organ system (either overt, or indicated by increased body temperature or WBC parameters) which fails to respond to antibiotic therapy within an appropriate time and is accompanied by systemic signs of illness.
6. **Signs of severe organ system dysfunction non-responsive to treatment, or with a poor prognosis as determined by an RAR veterinarian:**
 - Respiratory:** dyspnea, cyanosis.
 - Cardiovascular:** blood loss or anemia resulting in hematocrit below 20%; one transfusion may be performed.
 - Gastrointestinal:** severe vomiting or diarrhea, obstruction, intussusception; peritonitis, evisceration (immediate euthanasia required).
 - Urogenital:** renal failure characterized by elevated BUN, creatinine or uroperitoneum.
 - Nervous:** CNS depression, seizures, paralysis of one or more extremities; pain unresponsive to analgesic therapy.
 - Musculoskeletal:** muscle damage, bone injury, locomotor deficits, etc. resulting in inability to use the limb, unless anticipated as part of the study.
 - Integumentary:** Non-healing wounds, repeated self-trauma, second or third degree heating pad burns.

SURGERY TO CORRECT EXPERIMENTAL COMPLICATIONS

Only one major surgical procedure (involving entry of abdomen or thorax) may be performed per animal, unless indicated on an approved protocol. Therefore, major surgery intended to correct complications arising after a major experimental procedure is not permitted without prior approval. In such cases, euthanasia must be performed. Procedures such as repair of dehiscences and wound cleaning/debridement for treatment of infection may be performed following notification of the RAR veterinary staff.

Acceptable Methods for Euthanasia of Animals

RAR formulary dosages.

Method	Animals under 125 g	Rabbits/Rodents over 125 g under 1 kg	Rabbits/Rodents over 1 kg under 5 kg	Birds	Dogs	Cats	Nonhuman Primates	Farm Animals (e.g. swine, ruminants and horses)	Reptiles	Amphibians ³ /Fish
CO ₂	A	A ⁴	A ⁴	A	UNA	UNA	UNA	A ⁵ UNA	A	A
Barbiturate Overdose/ Euthanasia Solution, Intravenous (100 mg/kg)	A	A	A	A	A	A	A	A	A	A
Barbiturate Overdose/ Euthanasia Solution, Intraperitoneal (100 mg/kg)	A	A	A	A	UNA	A	UNA	A ⁶ UNA	A	A
Anesthesia and Exsanguination	A	A	A	A	A	A	A	A	A	A
Anesthesia and Intravenous KCl (1-2 meq/kg)	A	A	A	A	A	A	A	A	A	A
Anesthesia and Decapitation	A	A	AWJ	A	UNA	UNA	UNA	UNA	A	A
Anesthesia and Cervical Dislocation	A	A	UNA	A	UNA	UNA	UNA	UNA	N/A	N/A
Decapitation of Awake Animal	AWJ ¹	AWJ	AWJ	AWJ ¹	UNA	UNA	UNA	UNA	AWJ	AWJ
Cervical Dislocation of Awake Animal	AWJ ²	UNA	UNA	AWJ ¹	UNA	UNA	UNA	UNA	N/A	N/A
Other								Stunning or electrocution followed by exsanguination may be		A- Immersion in MS-222 (tricaine) or benzocaine at 2 g/L water

								used for on-farm or slaughterhouse euthanasia. Rifle shot may be used for field euthanasia where restraint is not possible.	
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Volatile agents used to euthanize animals should not be stored or used in animal rooms because of improper ventilation, toxicity to laboratory animals, and possible effects on experimental results.

Chloroform is not acceptable for either anesthesia or euthanasia as it is very toxic to many species of mice. Additionally, this compound has been shown to be carcinogenic.

Ether is irritating, flammable and explosive, and should not be used in animal rooms. In addition, animals euthanized with ether must be left in a fume hood for several hours so that the carcasses are not explosive when disposed of.

Chloral hydrate and alpha chloralose used as sole agents are not adequate to reliably achieve euthanasia

Abbreviations:

- A = Acceptable
- AWJ = Acceptable only with scientific justification, in writing, on the Animal Usage Form, that another methods would interfere with the goals of the experiment
- UNA = Unacceptable
- N/A = Not applicable or not specifically addressed by the IACUC
- **Always UNACCEPTABLE** in awake animals: KCl, MgSO₄, strychnine, neuromuscular blocking agents, exsanguination, air embolism, freezing and chloroform (due to its hazards to personnel).

¹⁺² Unless precluded by scientific considerations, it is required that all animals be sedated or anesthetized before decapitation or cervical

³ Amphibians may also be double-pithed

⁴ It is recommended that rabbits not be euthanized by CO₂ inhalation because of difficult induction.

⁵ Swine <40 kg may be euthanized with CO₂ in an appropriate chamber.

⁶ Neonatal swine may be euthanized by IP barbiturate injection.

Standard Euthanasia Methods for Commonly Used Species

Below are a set of standard acceptable euthanasia methods. Please contact the University ACUC or RAR veterinary staff if you have any questions about these methods or would like training in the use of these methods.

Rodents (Mice, Rats, Gerbils, Hamsters, Guinea Pigs, and Voles)

- Carbon dioxide (CO₂) To effect
- Sodium Pentobarbital 100 or > mg/kg IV, IP

- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV, IP
- Decapitation under anesthesia (anesthesia details must be specified in ACUP)
- Cervical dislocation under anesthesia (anesthesia details must be specified in ACUP)

Rabbits

- Sodium Pentobarbital 100 or > mg/kg IV, IP
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV, IP
- Exsanguination under anesthesia (anesthesia details must be specified in ACUP)

Cats and Dogs

- Sodium Pentobarbital 100 or > mg/kg IV
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV
- Potassium chloride under anesthesia to effect (anesthesia details must be specified in ACUP)

Livestock (Cattle, Goats, Horses, Sheep, and Swine)

- Sodium Pentobarbital 100 or > mg/kg IV
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV

Nonhuman Primates

- Sodium Pentobarbital 100 or > mg/kg IV, IP
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV, IP

Amphibians and Fish

- Sodium Pentobarbital 100 or > mg/kg IV, ICL
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV, ICL
- Benzocaine hydrochloride 250 mg/liter (Water bath)
- Tricaine methane sulfonate 3 g/liter (Water bath)

Birds

- Carbon dioxide (CO₂) To effect
- Sodium Pentobarbital 100 mg/kg IV, ICL
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV, ICL

Reptiles

- Sodium Pentobarbital 100 or > mg/kg IV, ICL
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) 0.22 ml/kg IV, ICL

Abbreviations

IC = intracardiac

ICL = intracoelomic

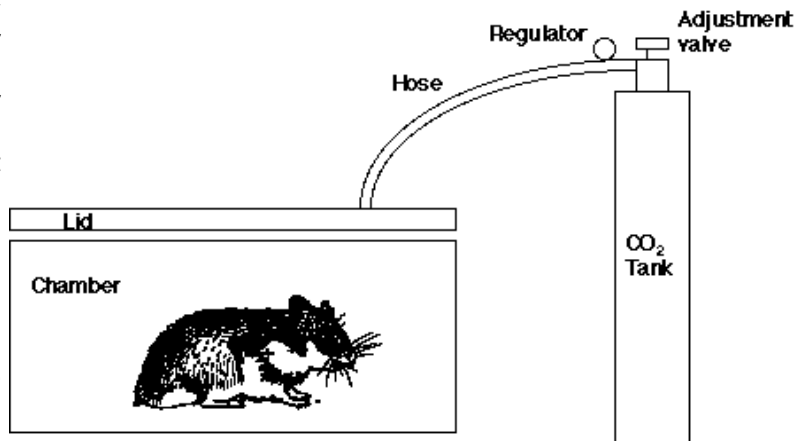
IP = intraperitoneal

IV = intravenous

USE OF THE CO₂ CHAMBER FOR EUTHANASIA OF RODENTS

DIRECTIONS

1. Whenever possible, **euthanize animals in their home cage** rather than transferring them to a new cage or chamber for euthanasia.
2. Do **not** pre-fill the cage or chamber with CO₂.
3. Open the tank and adjust the regulator to read:
 - no higher than 5 psi or
 - 1 liter/minute for small cage (mouse box)
 - 4.5 liters/minute for large cage (rat box)
4. **Fill slowly** to minimize nasal/ocular irritation & aversion to CO₂.
5. Wait approximately 3-5 minutes for animal to stop moving or breathing. Eyes should be fixed and dilated.



VERIFICATION OF COMPLETE EUTHANASIA IS MANDATORY. THE ANIMAL IS NOT DEAD IF:

1. Its heart is beating, check this by feeling the chest between your thumb and forefinger.
2. It blinks when you touch the eyeball.
3. If the animal is not dead, place it back in the chamber, recharge and wait another 5 minutes or, use scissors to open the chest cavity and create a pneumothorax. **MAKE SURE THE ANIMAL IS NOT AWAKE WHEN YOU DO THIS!**

Guidelines for the Use of Anesthetics, Analgesics and Tranquilizers in Laboratory Animals

What is Anesthesia?

Anesthesia is a state of unconsciousness induced in an animal. The three components of anesthesia are *analgesia* (pain relief), *amnesia* (loss of memory) and *immobilization*. The drugs used to achieve anesthesia usually have varying effects in each of these areas. Some drugs may be used individually to achieve all three. Others have only analgesic or sedative properties and may be used individually for these purposes or in combination with other drugs to achieve full anesthesia.

Curariform skeletal muscle relaxants or neuromuscular blockers (e.g. *succinylcholine*, *decamethonium*, *curare*, *gallamine*, *pancuronium*) are not anesthetics and have no analgesic effects. They may only be used in conjunction with general anesthetics. Normally, artificial respiration must be provided. Physiologic monitoring methods must also be used to assess anesthetic depth, as normal reflex methods will not be reliable.

It is important to realize that anesthesia is not a simple thing. It has profound effects on an animal's physiology because of the generalized central nervous system effects as well as specific effects on all other body systems. Thus, while anesthesia is necessary to prevent pain or distress in research animals, it must not be ventured into lightly. It is important to learn about the drugs you will be using and about the physiology of the animal you will be monitoring. Specific anesthetic drugs and their use are detailed below. All drug dosages are listed in RAR's formulary.

Stages of Anesthesia

These stages occur (when using inhalation anesthesia alone; other drugs added will modify these stages).

Stage 1 (Induction, aka voluntary excitement). Excitement and struggling are common. Usually accompanied by epinephrine release with associated rise in respiratory rate and heart rate.

Stage 2 (delirium, involuntary excitement). Voluntary centers and loss of consciousness begin. Exaggerated reflexive responses to stimuli are common, as is vomiting (in species that can vomit). Breath holding may occur. Common hazard: self-injury.

Stage 3 General Anesthesia

- Plane 1—Light anesthesia. Most reflexes (pedal, corneal, palpebral) are still present.
- Plane 2 – Medium anesthesia. Most surgeries are conducted at this level. Muscles are relaxed. Most reflexes (pedal, palpebral, corneal) are absent.
- Plane 3—Deep anesthesia. Intercostal muscles are relaxed; ability to maintain respiration is endangered. Pupillary light reflex may be slow or absent.
- Plane 4—Too Deep. All muscles, including diaphragm & intercostal muscles, are paralyzed.

Stage 4 Irreversible Anesthesia—respiratory arrest, followed by circulatory collapse. Death within 1-5 minutes.

Stages of Anesthetic Recovery (including comments about monitoring)

Recovery Stage 4- Animal is unconscious or semi-conscious and in lateral recumbency. Some reflexes are still diminished or absent. For animals in Stage 4, it is standard procedure to assess body temperature, heart rate & rhythm, pulse, respiratory rate and character, capillary refill time and state of hydration at least every two hours. The condition of the surgical site is monitored, and analgesics are administered when the animal becomes semi-conscious.

Recovery Stage 3- Animal is conscious and all reflexes are present, but may not be able to control its body position. The swallow (gag) reflex is present, and the endotracheal tubes (if used) can be removed. In Post-Op, the animal is still being closely monitored. The parameters listed above are assessed, but less frequently- approximately every 8-12 hours. Analgesics are continued.

Recovery Stage 2- Animal can either maintain itself in a sternal position, or can stand and move about, but may still show some sedation, ataxia, hypothermia or dehydration. In Post-Op, the parameters listed above, as well as attitude, activity, food and water consumption, are assessed at least every 12 hours.

Recovery Stage 1- All functions are normal, unless altered directly by the experimental procedure. In Post-Op, the animal is monitored every 12 hours for the parameters listed above..

Intra-operative and Anesthesia Records

The *Guide for the Care and Use of Laboratory Animals* and the *Institutional Animal Care and Use Committee Guidebook* require that animals under anesthesia be carefully monitored to insure adequate depth of anesthesia, animal homeostasis, timely attention to problems, and support during anesthetic recovery. Monitoring includes, but is not limited to, checking anesthetic depth and physiological parameters (minimum: heart rate and respiratory rate) on a regular basis (minimum every 10 minutes).

Record keeping is an essential component of peri-operative care. For major surgical procedures on non-rodent mammals, an intra-operative anesthetic record must be kept and included with the surgeon's report as part of the animal's records. In addition to the above requirements, the record should include all drugs administered to the animal, noting the dose, time, and route of administration. These records should be available to RAR and any other personnel providing post-operative care. Although it is not required, RAR strongly encourages the use of an intra-operative rodent anesthetic record during surgical procedures.

The required monitoring will vary according to the species and the complexity of the procedure, but should include:

- A pre-surgical assessment;
- Adequate monitoring of anesthetic depth and homeostasis
- Support such as fluid supplementation, external heat, or ventilation
- Monitoring and support during anesthetic recovery
- Post-operative monitoring

The following are suggestions from the American College of Veterinary Anesthesiology for monitoring anesthetized animals:

1. Circulation: to ensure that blood flow to the tissues is adequate.
 - a. Methods: Heart rate, Palpation of peripheral pulses, ECG, auscultation of heartbeat,
2. Oxygenation: to ensure adequate oxygen concentration in the animal's arterial blood.
 - a. Methods: observation of mucous membranes color and CRT, pulse oximetry, blood gas analysis
3. Ventilation: to ensure that the animal's ventilation is adequately maintained.
 - a. Methods: respiratory rate, observation of thoracic wall movement or breathing bag movement if animal is spontaneously breathing, auscultation of breath sounds,

What is Analgesia?

Analgesia is the relief of pain. Pain is normally defined as an unpleasant sensory and emotional experience associated with potential or actual tissue damage. Pain is difficult to assess in animals because of the inability to communicate directly about what the animal is experiencing. Instead, indirect signs of pain are often used. Because of the difficulty of determining when an animal is in pain, animal welfare regulations require that analgesia be provided whenever a procedure is being performed or a condition is present that is likely to cause pain. In the absence of evidence to the contrary, it is assumed that something that is painful in a human will also be painful in an animal. It is best if analgesia can be provided to animals *preemptively*, or prior to the painful procedure, rather than waiting until after clinical signs of pain are observed. Analgesia is normally provided using one of several types of pharmaceutical preparations.

Drug Selection

Inhalation Anesthetics

General

Inhalation anesthesia is superior to most injectable forms of anesthesia in safety and efficacy. It is easy to adjust the anesthetic depth. Because the anesthetics are eliminated from the blood by exhalation, with less reliance on drug metabolism to remove the drug from the body, there is less chance for drug-induced toxicity. Inhalation anesthetics are always administered to effect, because the dosage can vary greatly among individual animals and different animal species. The disadvantages to inhalant anesthesia are the complexity and cost of the equipment needed to administer the anesthesia, and potential hazards to personnel. All inhalant drugs are volatile liquids. They should not be stored in animal rooms because the vapors are either flammable or toxic to inhale over extended periods of time. In particular, ether must be stored in a proper hood or cabinet for flammable materials.

Inhalant Agents

Drug	MAC	Response	Toxicity	Comments
Ether	3.2	slow	liver	Pre-medication with an anticholinergic may be necessary to reduce

Chloroform				excessive respiratory secretions. Induction and recovery can be rough. Flammable and can become explosive with prolonged storage. Ether must be used according to appropriate safety guidelines.
				A hazardous agent (carcinogenic) and cannot be used at U of M.
Methoxyflurane	0.2	slow	nephrotoxicity-potentiated tetracyclines	is by It has good analgesic activity, but there is significant metabolism, ++ respiratory depression and + cardiac depression. This drug is not currently being manufactured. For a replacement, consider ether or diluted isoflurane.
Halothane	0.9	moderate	hepato- and nephrotoxicity if the animal is hypotensive	++ cardiopulmonary depression, and a risk of malignant hyperthermia in some breeds/strains
Isoflurane	1.5	fast	none	++ respiratory depression and + cardiovascular depression
Enflurane	2.0	fast	none	++ cardiopulmonary depression and minimal respiratory depression
Nitrous Oxide	180	very fast	hepatotoxic	Cannot be used as a sole anesthetic agent. Do not exceed a 50% mix w/ oxygen and other inhalant agent to prevent hypoxia. Moderate analgesia is provided by nitrous. In general, use of nitrous oxide in animals is discouraged.
Carbon Dioxide (CO ₂)	50-70	very fast	cerebral anoxia	Can be used as an anesthetic for brief procedures and as a euthanasia agent. It has antinociceptive activity and causes unconsciousness prior to hypoxia. It is necessary to monitor carefully and work quickly, as animals die quickly (1-2 min) after losing consciousness, and likewise they wake up quickly when exposed to room air. Appropriate for quick procedures such as tail snipping, ear marking and orbital bleeding. Poses minimal hazard to personnel and can be used in laboratories or animal room

MAC. This is the % concentration of the drug needed to anesthetize 50% of animals. It does vary somewhat by species and by individual. 1.2X MAC is an approximate vaporizer setting for maintenance of anesthesia. Induction generally requires 2-3X MAC. MAC listed here is for rats (ether), mice (CO₂), goat (enflurane) and dogs (all others).

Response. This refers to how rapidly concentrations in the blood change when the lung alveolar concentration is changed. Slow anesthetics have slow induction and recovery times.

Toxicity. Drugs that are metabolized by the body can cause toxicity, especially if a pre-existing organ dysfunction exists.

Injectable Anesthetics, Analgesics and Sedatives

1. General
2. Route of Administration
3. Local Anesthetics, and Fish and Amphibian anesthesia
4. Phenothiazine and Buterophenone Sedatives (Acepromazine)
5. Benzodiazapines (Diazepam, midazolam)
6. Thiazines (Xylazine and medetomidine)
7. Opiate analgesics
8. Barbiturates
9. Dissociative Anesthetics (Ketamine and Telazol)
10. Other Anesthetics (Alpha Chloralose, Tribromoethanol, Urethane)
11. Other Analgesics
12. Analgesia in Rats and Mice
13. Anesthetic Drug Combinations

General

Some of the drugs listed here do not possess all three criteria for an anesthetic and must be used in combinations to achieve full anesthesia or may be administered individually for restraint, sedation or analgesia. Dosages for specific animals are linked from RAR's formulary. Often injectable drugs are used in combinations. These drugs tend to have synergistic effects. Mixing them can significantly reduce the dosage needed for any individual drug.

As with inhalation anesthesia, injectables are given to effect. Dosages listed are guidelines. Effects may vary among individuals. If a drug is scheduled by the Controlled Substances Act of 1970, licenses are required to purchase them, and written records must be kept of their use. University policy outlines these requirements. Anesthetic drugs that have exceeded their expiration date may not be used, even for terminal procedures

Injectable anesthetics are, in general, metabolized by the liver and excreted by the kidneys. Animals with liver or kidney disease should not be anesthetized with these agents. Inhalation anesthetics are safer for use in sick or debilitated animals, because there is minimal metabolism, the amount of anesthetic administered can be controlled and one can cease administration as the situation dictates. Injectable anesthetics offer the advantage of requiring less expensive equipment.

Local Anesthetics

The generic and brand names of local anesthetics often have the suffix "*caine*". Common local anesthetics are procaine (Novacaine), bupivacaine, lidocaine (Xylocaine) and proparicaine. Considerable experience and skill are necessary in the administration of local anesthetics to animals, and aseptic techniques must be employed. Some animals must be sedated before local anesthetics are injected.

Local anesthetics may be administered by several techniques. Anesthetic effects are seen within 15 minutes of administration and may last from 45 minutes to several hours, depending on the drug used.

- *Infiltration or infusion*- injection beneath the skin and other tissue layers along the site of an incision before or after a procedure
- *Field block, ring block*- injection into soft tissues distant from the actual incision in a pattern that intersects the nerve supplying the surgical site
- *Nerve conduction block*- infusion of a small amount of drug or directly adjacent to the sheath of a nerve supplying the surgical site
- *Regional or spinal anesthesia*- injection into the vertebral canal, epidurally or into the sub-arachnoid space. To avoid systemic toxicity, care must always be taken not to inject local anesthetics into blood vessels.
- Topical local anesthetics, such as lidocaine jelly, may be useful for some surgical wounds.
- Proparicaine may be used as a local anesthetic during retroorbital blood collection from mice. One drop on the eye, wait 10-15 minutes before performing the procedure.

An interesting use of local anesthetics is for **amphibian and fish anesthesia**. Tricaine and benzocaine can be added to water at a dose of from 25-100 mg/L, depending on the depth of anesthesia required. When the fish loses equilibrium (floats belly up) or an amphibian becomes inactive, it can be handled. For longer procedures, intermittent supplementation of anesthetic treated water to the gills or skin may be required. The animal is recovered in fresh water.

Phenothiazine and Buterophenone Sedatives

These sedatives include acepromazine, chlorpromazine, droperidol (Innovar-Vet) and azaperone (Stresnil). These drugs have excellent sedative properties, as well as muscle relaxation, antiemetic and antiarrhythmic effects. They have no analgesic activity,

but when administered with other anesthetics can potentiate their effect. Acepromazine is the most commonly used. It is recommended as a sole sedative in dogs and as an anesthetic premedication to improve both induction and recovery (it is long acting) in all species. Droperidol is usually only available in combination with the narcotic, fentanyl (Innovar-vet) and has been associated with aggressive behavior in dogs.

Disadvantages of these sedatives are that they are alpha adrenergic blockers and cause peripheral vasodilation which can lead to hypothermia. They may have prolonged activity in sight hounds. Acepromazine and chlorpromazine decrease seizure threshold, and are contraindicated in animals with CNS lesions. Because these sedatives lack analgesic activity it is important to realize that any painful stimulation of the animal may cause it to emerge rapidly from the sedated state.

Benzodiazapines

The benzodiazapines include diazepam (Valium), midazolam (Versed) and zolazepam (Telazol). These drugs are anti-anxiety and anticonvulsant drugs with good muscle relaxation. They have minimal cardiovascular and respiratory effects. Sedation is minimal in most species, except for swine and nonhuman primates. The primary use of these drugs in anesthesia is in combination with other drugs. Ketamine-diazepam, midazolam-narcotic, and tiletamine-zolazepam (Telazol) combinations can be very useful for induction of general anesthesia and for short procedures. These drugs are regulated by the Controlled Substances Act and require special record keeping.

Thiazines

The thiazine derivatives include xylazine and medetomidine. These two drugs are very similar. They are alpha-2 adrenergic agonists. They cause CNS depression resulting in sedation, emesis and mild analgesia. They also cause hypotension, second degree atrio-ventricular block and bradycardia. Occasionally, aggressive behavior changes have been seen in dogs. They are very useful in combination with other drugs, like ketamine for anesthesia in rodents and swine. They are best avoided in dogs, cats and nonhuman primates, primarily because their significant side-effects can be avoided by using other drugs. They can be used alone for minor procedures in ruminants. *It is important to note that the dose for these drugs in ruminants is 1/10 that used in other species.* The effects of the thiazine derivatives can be reversed with yohimbine or atipimazole. Use of these drugs with the reversal agent shortens anesthetic recovery and greatly expands the safety and utility of these drugs. Xylazine is a potent analgesic in frogs appropriate for relief of post-surgical pain.

Opiates

The opiates, sometimes referred to as narcotics, are a large class of drugs that exert their effects on the opiate receptors in the central nervous system. Depending on the receptors a drug is active against, and the type of action it has on the receptor, the effects of narcotics can be primarily analgesic, as with buprenorphine (Buprenex), pentazocine (Talwin) and nalbuphine (Nubain), or a mixture of analgesia and euphoria with sedation as with butorphanol (Torbugesic), fentanyl (Innovar-Vet), morphine, meperidine (Demerol) or oxymorphone. Opiates have little effect on the myocardium. However, there can be significant respiratory depression, as well as other side-effects such as nausea and vomiting, delayed gastric emptying, hypotension, and bradycardia. Some species may develop hyperexcitability if given certain opiates. These side-effects are seen more with the mixed effect opiates than the pure analgesics. Naloxone is a opiate antagonist that can be used to reverse the effects of other narcotics. Other

opiates, like buprenorphine, nalbuphine and nalorphine, have mixed agonist-antagonist effects and may interfere with the effects of concurrently administered narcotics. All opiates are controlled substances and their use requires special record keeping. These drugs can be given alone as a post-procedural analgesic or in combination with other agents to provide balanced anesthesia, restraint with analgesia for minor procedures, or can be used to decrease the dose of an anesthetic that is needed to provide a surgical plane of anesthesia.

Barbiturates

The barbiturates are an acid ring molecule with various ring substitutes that imbue the drug with different properties. Barbiturates are also considered narcotics.

- Phenobarbital is the longest-acting of the barbiturates. Its use is limited primarily to sedation or as an anticonvulsant.
- Pentobarbital is a short-acting oxybarbiturate. It is usually used as a sole anesthetic agent, or is supplemented with an analgesic. When given intravenously, about 50-75% of the calculated dose is administered. Within several minutes the animal will lose consciousness, although it may experience a brief period of excitement. When the jaw muscle tone is relaxed, the animal should be intubated. If given intraperitoneally, usually the entire dose of pentobarbital is given and surgery can be performed when the animal no longer reacts to a toe pinch. Anesthesia from pentobarbital can last from 45-120 min, depending on the dose given. Additional drug can be supplemented as needed, being careful not to overdose as described below under "Precautions".
- Thiopental and Thiamylal are thiobarbiturates that are considered ultra-short acting. Similar to these is methohexital which is an oxybarbiturate. Because of the extremely short duration of activity (up to 10 min with methohexital, up to 15-20 min with thiopental or thiamylal) of these drugs, they are usually used as an intravenous anesthetic induction agent to allow intubation prior to use of inhalant anesthesia. Use is similar to that described for pentobarbital. However, when low doses are given IV, there may only be several minutes of anesthesia before the animal begins to waken. This is desirable as an induction agent. If higher doses are given for longer effect, care must be taken not to overdose as described below under "Precautions". Longer anesthesia may be seen when these drugs are used intraperitoneally in rodents.
- **Effects and Side Effects** In general the barbiturates cause generalized central nervous system depression, which can be dosed to provide sedation or general anesthesia. The drugs also have an anticonvulsant effect. Analgesia provided by the barbiturates is poor and a relatively deep plane of anesthesia is required for surgery, unless used in combination with analgesics. The barbiturates have significant cardiopulmonary depression, with apnea and hypotension commonly seen. Anesthetic death is common in animals that are not receiving supportive care. The barbiturates induce hepatic microsomal enzymes and may increase the metabolic rate of other drugs. Tolerance to the barbiturates develops with repeated use and doses may have to be adjusted accordingly.
- **Precautions**
 - Barbiturates are poorly water soluble and are only available in intravenous preparations, although they are frequently administered intraperitoneally to smaller animals with limited venous access. Because of their acidic properties, barbiturates can be irritating when administered intraperitoneal, or if any leak from the intravenous injection site.

Perivascular barbiturates can result in significant tissue necrosis and skin sloughing. If any barbiturate leaks (a visible swelling is seen during injection), the best thing to do is to infuse the area with sterile saline at several times the volume of the original leak. Some people recommend mixing the saline with 2% lidocaine to prevent pain and subsequent self-trauma.

- The barbiturates redistribute rapidly into all body tissues, including fat. Redistribution is one way that the drug is eliminated from the blood and obese animals may require higher doses of barbiturates to induce anesthesia. However, once the fat becomes saturated with the drug, metabolism becomes the primary means of elimination. Because metabolism is much slower, a common problem in administering barbiturates is overdosing with prolonged anesthetic recoveries (up to several days). Because of this problem it is best to titrate the dose carefully rather than administer large boluses. For obese animals, alternative anesthetics might be considered, although to a greater or lesser extent, most anesthetics share this problem when administered to obese animals. Prolonged anesthetic recovery can also be a problem when barbiturates are used in older animals or other animals with compromised hepatic and renal function which decreases metabolism of the drugs.
- Barbiturates are also controlled substances and their use requires special record keeping].
- Despite these disadvantages, the barbiturates are perhaps the most commonly used anesthetics in laboratory animals. Overall, they are a relatively easy to use anesthetic.

Dissociative Anesthetics

The dissociative anesthetics include ketamine (Vetalar, Ketaset) and tiletamine (Telazol). These drugs are easy to use and have a wide margin of safety for most laboratory species. They are cyclohexamine compounds, chemically related to piperazine and phencyclidine (PCP). The dissociative anesthetics uncouple sensory, motor, integrative, memory and emotional activities in the brain, providing there is a functional cerebral cortex. The state induced by high doses of ketamine is best described as catalepsy and is not accompanied by central nervous system depression. There is depression of respiratory function, but cardiovascular function is maintained. Muscle relaxation is very poor.

Ketamine and Telazol are supplied in a solution of 100 mg/ml. Telazol is a 50-50 mixture of tiletamine and zolezepam, a benzodiazepine. These drugs can be injected intramuscularly, intraperitoneally or intravenously; however, the subcutaneous route is discouraged. IP and IM injections of the dissociative anesthetics can be painful, as the drug is very acidic. Induction time for IM administration is three to five minutes; peak effect lasts about 20 min in most laboratory species. IP induction times are longer than with IM administration and recovery may be prolonged. Because the volumes needed are very small, in small animals there is no real advantage to IP injection and IM injection should be used whenever possible. Induction time following IV administration is rapid with only about 10 min of anesthesia provided. Approximately 1/2 of the dose should be given when dosing IV. The drug can be supplemented as needed.

The swallowing reflex is often preserved in animals receiving dissociative anesthetics. This may help prevent aspiration pneumonia if the animal regurgitates. However, this is not 100% and fasting and intubation are still recommended when using these anesthetics. The animal's eyes will usually remain open and the corneas should be

protected with a layer of ophthalmic petrolatum or other suitable ointment. These drugs have poor analgesic activity, especially for visceral pain, and should be used in conjunction with an analgesic for abdominal, intracranial, orthopedic, ophthalmic or thoracic surgery.

Other Anesthetics

Propofol- is a sedative/hypnotic that can be used for induction or maintenance of general anesthesia. Analgesic effect is poor and addition of an analgesic to the anesthetic regimen is necessary for surgery. The drug comes as an emulsion that must be mixed and used within several days. The advantages of propofol are that it has rapid induction and recovery times. It can be easily titrated and given to effect for prolonged periods without resulting in prolonged recovery. The disadvantages are that it must be given intravenously, it is expensive, it may result in apnea and it can cause bradycardia and hypotension.

Alpha Chloralose- or chloral hydrate is a mild hypnotic drug that does not produce complete anesthesia because of its poor analgesic properties. Chloral hydrate is shorter acting (1-2 h) than alpha chloralose (8-10 h). The primary advantage of these drugs is the minimal cardiopulmonary depression seen at the normal doses (high doses can cause severe respiratory depression). The disadvantage is that they can only be used alone for non-painful procedures. In addition, the drugs are very irritating to the GI tract, causing adynamic ileus if given IP and ulcers if given orally. Therefore IV use is the only route recommended. These drugs should not be used if any other alternative is available.

Tribromoethanol-is a short-acting anesthetic used in rodents for surgeries. The drug has rapid induction and recovery (15 min of surgical anesthesia and up to 90 min for complete recovery). The effect on animals is reported to be quite variable. Tribromoethanol was commonly used in the past but its use is now discouraged. Abdominal adhesions caused by IP administration have been reported to cause high post-procedural mortality, however, other studies have not demonstrated this. Tribromoethanol is not available commercially and must be prepared. Sterile preparation procedures are essential. The drug must be stored in the dark at 4°C to prevent degradation. Avertin Guidelines.

Urethane- is a long-acting (8-10h) anesthetic with minimal cardiopulmonary depression. The drug is used for long procedures in rodents. However, it is carcinogenic and is only allowed to be used with special justification and only for terminal (acute) procedures.

Other Analgesics

Analgesics are pain relievers most often given after a surgery. Narcotic analgesics have already been described above. Nonsteroidal antiinflammatory drugs (NSAIDs) may also be used for their analgesic effect. The NSAIDs consist of drugs like aspirin, ketoprofen, acetaminophen, flunixin and ketorolac. There are a large number of these drugs available, however, relatively few are used in animals. NSAIDs are, in general, less potent analgesics than are the narcotics. However, in specific instances they can have similar activity.

The advantages of the NSAIDs are that they do not cause sedation nor are they addictive as are the narcotic analgesics. There are no special recordkeeping requirements. In addition, they are more effective against pain caused by inflammation, such as is seen with tissue repair, orthopedic surgery, infection and injury.

One disadvantage of the NSAIDs (or any other analgesics) when given to the animal for oral self-administration (e.g. in drinking water, juice, treat food, etc) is that the physiological disturbances caused by an anesthetic episode may significantly decrease an animal's willingness to eat or drink during the immediate post-procedural period. This effect is independent of the level of invasiveness of any surgical procedure. To alleviate pain from surgical procedures, some form of parenteral analgesia should be given prior to anesthetic recovery and should be continued for a minimum of 12-24 hours after the animal has regained consciousness.

The NSAIDs have several side-effects related to their pronounced anti-prostaglandin (anti-cyclooxygenase and in some cases lipooxygenase) activity. This is peripheral with most drugs, but is primarily central with acetaminophen. These effects can alter immune function, platelet function and can cause gastrointestinal ulceration. In addition, the NSAIDs all have the potential to cause nephro- and hepatotoxicity. This is variable among species. Cats, in particular, are sensitive to the NSAIDs. Acetaminophen is contraindicated in cats due to risk of methemoglobinemia.

Acetaminophen- mild analgesic, antipyretic, no effect on platelet function/bleeding time

Aspirin- mild analgesic, antipyretic, antiinflammatory, affects platelet function/bleeding time

Carprofen is a nonsteroidal antiinflammatory drug with antiinflammatory and analgesic effects and lower risk for toxicity in animals than other NSAIDS.

Flunixin meglumine (Banamine)- potent analgesic, antiinflammatory, antipyretic. Has potential for GI ulceration, hepato- and nephrotoxicity.

Ketoprofen- moderate potency analgesic, antiinflammatory, antipyretic. Has potential for GI ulceration, hepato- and nephrotoxicity, affects platelet function/bleeding time.

Ketorolac (Toradol)- potent analgesic, antiinflammatory, antipyretic. Has potential for hepato- and nephrotoxicity, less potential for GI ulceration than other NSAIDs, affects platelet function/bleeding time.

Acetaminophen

Update

Alternatives to acetaminophen in rats and mice

Anesthetic Drug Combinations

In general, by mixing anesthetic and analgesic drugs, the dose required for each individual drug is reduced, sometimes quite dramatically. Start at the low end of the dose range listed; you can always give more if needed! Drugs not listed below can be mixed using the same concepts, mix a sedative or hypnotic with an analgesic. Do not mix drugs in the syringe until you have determined that they are compatible when mixed. If in doubt administer separately.

Determining expiration dates for mixed/diluted anesthetic or pain relieving drugs:

In the absence of empirical evidence, expiration dates of diluted or mixed drugs will be determined as follows:

- I. Manufacturer's dating of the drugs to be mixed together will determine the expiration date if shorter than the timelines given below.

- m. Mixed anesthetic drugs (ketamine, xylazine, acepromazine, butorphanol, telazol): Expiration date is 30 days after mixing, based on Minnesota Board of Pharmacy recommendations.
- n. Diluted drugs (buprenorphine) - Expiration date is 30 days from dilution date.
- o. Avertin - All solutions should be discarded 4 months after mixing, including stock solutions. pH should be tested prior to every use. Solution should only be used if pH is greater than 5. See <http://www.ahc.umn.edu/rar/avertin.html>
- p. Any substance which shows signs of precipitation, change of color, change in transparency or other signs of transformation should be immediately discarded.
- q. All diluted/ mixed substances must be combined into sterile containers (unless the combination is for a single acute procedure and the mixture will not be stored).

Ketamine/Diazepam: Mix drugs 1:1 by volume and administer 0.1 ml/kg IV for restraint, anesthetic induction or for non-painful procedures. This gives excellent muscle relaxation, has minimal respiratory or cardiovascular depression and the animals wake up smoothly and quickly (within 10-15 min). Visually, these drugs do not appear to mix completely. When combined and administered as described, the dose is 5 mg/kg ketamine and 0.25 mg/kg diazepam.

Ketamine/Acepromazine: Mix 10 mg acepromazine (1 ml) with 1 g (10 ml) ketamine and give 0.1-0.3 ml/kg mixture IM or IV (up to 0.6 ml/kg in rodents and rabbits). Good for restraint, but not for painful procedures. When combined and administered as described, the dose is 0.09-0.27 mg/kg acepromazine and 9-27 mg/kg ketamine.

Acepromazine/Butorphanol: Mix drugs 1:1 by volume (using 10 mg/ml butorphanol) and administer at 0.01-0.02 ml/kg IV or IM. Creates a hypnotic state that is good for restraint and minor procedures that cause some pain. When combined and administered as described, the dose is 0.05-0.1 mg/kg butorphanol and 0.05-0.1 mg/kg acepromazine.

Ketamine/Acepromazine/Butorphanol: Mix 10 mg acepromazine (1 ml), 10 mg butorphanol (1 ml) with 1 g (10 ml) ketamine and give 0.1-0.3 ml/kg of mixture IM or IV (up to 0.6-0.8 ml/kg in rodents and rabbits). Good for restraint and moderately painful procedures. More cardiac and respiratory depression will be seen with this mixture than with ketamine alone. When combined and administered as described, the dose is 8-25 mg/kg ketamine, 0.08-0.25 mg/kg acepromazine, and 0.08-0.25 mg/kg butorphanol. For rodents & rabbits, the dose is 50-67 mg/kg ketamine, 0.5-0.7 mg/kg acepromazine, and 0.5-0.7 mg/kg butorphanol.

Ketamine/Xylazine: Good for restraint and painful procedures. Administer IM, IP, or IV. More cardiac and respiratory depression will be seen with this mixture than with ketamine alone. Use 100 mg/ml ketamine and 100 mg/ml xylazine to create any of the mixtures listed below.

CAUTION: DO NOT USE this cocktail of ketamine-xylazine for cattle, sheep, goats, or other ruminants. Giving ketamine and xylazine simultaneously is not recommended for horses.

Species	Recipe by volume (ket:xyl)	Vol to give (ml/kg)	Dose (per kg body weight)	Sedation insufficient? May redose with ketamine alone...
Mouse	mix 10:1	1.1	100 mg Ket + 10 mg Xyl	At 1/3 to 1/2 original volume

Rat	mix 15:2	0.85	75 mg Ket + 10 mg Xyl	At 1/3 original volume
Rabbit	mix 20:3	0.40	34 mg Ket + 5.2 mg Xyl	At 1/3 to 1/2 original volume

Ketamine/Midazolam/Butorphanol: Mix 0.4 ml each ketamine and midazolam with 0.01 ml of 10 mg/ml butorphanol and administer 0.8 ml/kg. This provides good muscle relaxation and surgical anesthesia in rodents. When combined and administered as described, the dose is 40 mg/kg ketamine, 2 mg/kg midazolam, and 0.1 mg/kg butorphanol.

Telazol/Xylazine: For pigs: reconstitute powdered Telazol (tiletamine & zolazepam) with 5 ml of xylazine instead of saline. For pigs < 50 kg, use 20 mg/ml xylazine to make the cocktail. For pigs > 50 kg, use 100 mg/ml xylazine. Administer at 0.05-0.1 ml/kg IV or IM. When combined and administered as described, the dose is 2.5-5 mg/kg tiletamine, 2.5-5 mg/kg zolazepam, and either 1-2 mg/kg xylazine (if 20 mg/ml xylazine was used) or 5-10 mg/kg xylazine (100 mg/ml xylazine). For rats, use 20 mg/ml xylazine and administer up to 0.4 ml/kg IM. Here, the dose can be as high as 8 mg/kg xylazine, 20 mg/kg tiletamine, and 20 mg/kg zolazepam. More cardiac and respiratory depression will be seen with this mixture than with Telazol alone.

Reversal with yohimbine 0.1-0.15 mg/kg (IM or IV) or atipamezole at 0.25 (IM) or 0.2 (IV) mg/kg is recommended to shorten recovery times. CAUTION: DO NOT USE this cocktail of Telazol-xylazine for mice, rabbits, or ruminants such as cattle, sheep, or goats. Giving Telazol and xylazine simultaneously is not recommended for horses (contact an RAR veterinarian for more information).

Anesthetic Induction and Maintenance Injectable Anesthesia

Anesthetic induction using injectable anesthetics is fairly simple. It involves administration of the drug and monitoring the depth of anesthesia. Supportive care may be needed. Maintenance of injectable anesthesia can be through repeated bolus doses of the drug or through a constant infusion. Infusion rates are calculated based on the clearance time of the drug. Bolus dosing is simpler. Typically, 1/2 of the original dose is given for repeat doses.

Injectable anesthetics can be administered by various routes depending upon the specific compound. The most frequently used routes of administration in laboratory animals are intraperitoneal, intramuscular and intravenous. Less frequently used routes, among others, are intrathoracic, oral and rectal. Techniques are described below. Contact RAR at 624-9100 for training materials on handling animals and administering injections.

- Intravenous(IV)

Method- An appropriate vein must be selected. For large animals, the saphenous, cephalic or jugular veins are best. For rodents, the tail veins are best. For rabbits and swine, ear veins may be used. The vein is held off proximal to the venipuncture site. The vessel may be stroked with a finger to stimulate blood flow into it. The needle is inserted at a 30-45° angle to the vessel. Then the needle is lowered to align with the longitudinal axis of the vessel and advanced slightly. Draw back. If blood appears in the hub of the needle, the drug may be injected. If not, try redirecting the needle (before you pull it out of the skin) and repeat. You may need to try several times while learning. Using a new,

sharp needle for each stick, even if it is the same animal, will improve your chances for success. Once the needle is withdrawn, it is necessary to put pressure on the vessel to prevent bleeding.

Advantages- rapid delivery of drug, ability to titrate dose, irritating substances may be given IV

Disadvantages- small veins are hard to access (i.e. small animals), restraint is critical, developing skill in venipuncture takes experience

- Intramuscular (IM)

Method- Insert the needle into a large muscle mass. Draw back slightly. If blood is aspirated, you are in a blood vessel. Redirect the needle. When the needle is placed correctly, inject the drug. The best muscle masses to use are for small animals, the caudal thigh muscles. For larger animals, the lateral dorsal spinal muscles or the cranial or caudal thigh muscles may be used. When administering into thigh muscles, inject from the lateral aspect, or if from the caudal aspect, direct the needle slightly lateral. This will help avoid injecting into the sciatic nerve.

Advantages-- Fairly rapid absorption, technique is simple

Disadvantages- IM injections are painful, small volumes are necessary, the animal may try to bite or escape

- Intraperitoneal (IP)

Method- The animal is usually restrained in dorsal recumbency. The drug may be injected anywhere in the caudal 2/3 of the abdomen. However, it is best to try to avoid the left side in rodents and rabbits because of the presence of the cecum. After the needle is inserted, draw back. If anything is aspirated, you have likely hit the viscera. Withdraw and get a new needle before trying again. If the needle is placed correctly the drug may be injected.

Advantages- relatively large volumes may be injected (0.5 ml in mice, 2 ml in rats, etc.)

Disadvantages- technique is more difficult than IM injections, drug may be administered into the viscera resulting in no effect or in a complication.

- Subcutaneous (SQ)

Method- Pinch an area of loose skin. Inject into the center of the "tent" created by pinching.

Advantages- Technique is the simplest of any, large volumes may be given (basically as much as the tent of skin will hold that doesn't cause discomfort to the animal)

Disadvantages- Irritating substances cannot be given this way, absorption is slow

Inhalant Anesthesia

Induction of inhalation anesthesia can be difficult. Anesthetic gases are irritating to eyes and nasal passages. Animals may resist as they begin to lose consciousness or they may stop breathing temporarily. For this reason induction using a mask or nose cone held over the animal's nose can only be performed on smaller or non-fractious animals. In smaller animals gas can be delivered into an induction chamber large enough to contain the entire animal. Induction via a nose cone or chamber requires delivery of the anesthetic gas at 2-3x MAC. Frequently an injectable anesthetic is used to induce anesthesia and the inhalation agent is used for maintenance.

Maintenance of inhalation anesthesia is normally accomplished by delivering approximately 1.2 MAC to an animal via a mask or nose cone, or directly into the lungs via an endotracheal tube. Intubation is recommended whenever possible, particularly when a procedure will be prolonged. Endotracheal access is essential to provide ventilation support.

Gas Delivery Systems

The most complicated aspect of using inhalant anesthesia is the delivery system. A delivery system must provide the anesthetic gas to the animal at a known and constant rate. It must also ensure that animals receive adequate oxygen. There are several types of delivery systems typically used in laboratory animals.

Anesthetic Machine

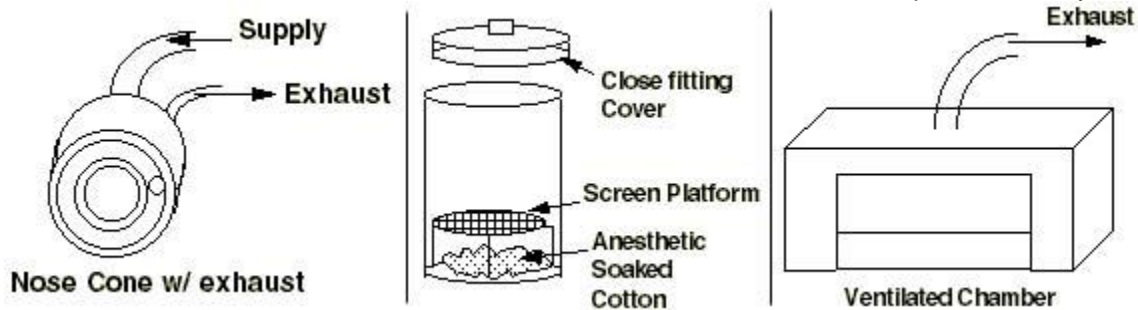
The best method of delivering an inhalant anesthetic is with an anesthetic machine. These machines precisely mix the gas with air or oxygen and can be easily adjusted. Machines can vary in construction and design. Anesthetic machines typically require more training to learn to operate.

- Anesthetic concentration is accomplished by sets of mixing valves or a precision vaporizer. Vaporizers are easier to use but are very expensive. Vaporizers are calibrated for the specific anesthetic gas to be used.
- Anesthesia circuits can be re-breathing or non-rebreathing.
 - Re-breathing circuits include typical circle systems used in large animals. The gas/oxygen mixture is delivered to the animal via a one-way valve. When the animal breathes out the gas passes out another valve attached to a y-piece. This is passed over a carbon dioxide absorbent and then back into the system. Additional gas and oxygen are continuously delivered to replace that lost.
 - Re-breathing circuits conserve anesthetic gas and the animal's body heat. The CO₂ absorbent must be replaced regularly.
 - Non-rebreathing circuits are primarily used for smaller animals that cannot cycle the valves in a re-breathing system. With newer machines non-rebreathing circuits are normally only necessary for rodents and birds. In older machines with metal valves a non-rebreathing circuit may be necessary for rabbits and cats as well. A Bain system is the most common non-rebreathing circuit available.
 - The non-rebreathing circuit is attached to the same anesthetic supply as used for a re-breathing system. However, the exhaust line is connected directly to the waste gas scavenging system.
 - Non-rebreathing circuits depend on gas and oxygen being delivered at a higher pressure than is present in the exhaust line. This tends to increase anesthetic usage and can increase body heat loss in the patient.
- Anesthesia machines must have a waste gas scavenging system. Normally the exhaust line on a non-rebreating system or the pop-off valve on a re-breathing system is connected to a vacuum line or to the building exhaust.

- Low-flow anesthetic techniques in large animals.
- Anesthesia breathing circuits.

Apparatus for Rodent Anesthesia

Left: a non-re-breathing nose cone that can be used with a large animal anesthetic machine; Middle: a typical drop system closed anesthetic chamber; Right: a gas scavenging system that can be used with a drop system.



Preparation, Monitoring and Maintenance of Normal Physiology

A variety of things must be done to prepare for anesthesia. Once animals are under anesthesia they must be monitored closely while they are anesthetized to ensure that they do not become too deep and die, and to ensure that they do not become too light and experience pain from the surgical procedure. Normal physiologic functions such as body temperature, respiration and cardiovascular function must also be monitored and supported while the animal is anesthetized. For all major surgical procedures on non-rodent mammals, an intra-operative anesthesia record must be kept and included with the surgeon's reports as part of the animal's record. The anesthetist must be prepared to handle emergencies if they occur.

Preparation

- **Withhold food and water** from large animals for 12 h prior to anesthesia and from small animals for 2 h to prevent regurgitation and aspiration. It is not necessary to withhold food and water from rodents prior to anesthesia. Prolonged food or water deprivation are distressful to animals and are rarely necessary.
- **Have all drugs and equipment ready** before the animal is anesthetized. You may not have time to look for things once the animal is under.
- **Have an assistant.** Anesthesia takes time to perform and monitor. A person should be available to assist so the surgeon does not have to break sterility to monitor the animal or administer medications.
- **Premedication** with atropine or glycopyrrolate (anticholinergics) may reduce the respiratory tract secretions in some animals
- **Protect** the eyes from drying out using an ophthalmic ointment and protect them from being contaminated with surgical scrub solutions. Also protect pressure points, such as bony protrusions, from pressure necrosis or peripheral nerve damage by providing padding between the animal and the table.

Respiration

Most anesthetics cause direct depression of the respiratory center in the brain and reduce ventilation. This is complicated by other factors that may interfere with respiration. When an animal is in lateral recumbency the lung that is down is being compressed by the rest of the body.

Likewise, animals in dorsal recumbency may experience compression of the diaphragm by abdominal viscera. The airway may be compromised by regurgitated food or pharyngeal and tracheal secretions that normally would be removed by reflex swallowing or coughing. These reflexes are lost during anesthesia. There are several ways to monitor and support the ventilation of an anesthetized animal.

- **Intubate** the trachea whenever possible, even if injectable anesthetics are being used. Intubation can be achieved on animals as small as a rat. This will prevent aspiration pneumonia and allow you to assist respiration if the animal stops breathing.
- **Assist respiration** during the procedure. This can be done with a mechanical ventilator. However, mechanical ventilation is rarely needed (unless a thoracotomy or diaphragmectomy is being performed) and can be detrimental to the animal if over-done. Attaching an AMBU bag to the endotracheal tube or using an anesthetic machine's rebreathing bag will allow you to administer a deep breath every 2-5 min during the procedure. This will inflate all areas of the lungs and improve gas exchange. If the animal is not intubated, ventilation can be performed using a nose cone or face mask.
- **Monitor** respiratory function throughout the procedure and recovery.
 - Monitor respiratory rate and depth (compare to normal for your species. You can expect them to be slightly decreased). Observe chest movement, or use a stethoscope or esophageal stethoscope.
 - Monitor the color of the mucous membranes (gums, conjunctiva, vulvar mucosa). A bluish color means the animal is not getting enough oxygen- ventilate!
 - Red-tinged foam present in the airway along with dyspnea (difficulty breathing) may indicate pulmonary edema. This can result from overventilation or overhydration. A diuretic like furosemide can be administered, but prognosis is poor.
 - Sophisticated respiratory monitoring can be achieved by measuring blood gasses, or expired oxygen and carbon dioxide concentration or by use of a pulse oximeter.

Fluid Therapy/Cardiovascular Support

Many anesthetics have direct effects on the heart or vasculature, decreasing cardiac output and blood pressure. This is further complicated by increased fluid requirements during anesthesia and surgery that may result in hypovolemia. Fluid requirements are increased because: breathing dry, cold oxygen (if inhalant anesthesia is used) increases respiratory fluid loss; the animal has not received its normal fluid intake since it was fasted; fluid may be lost through hemorrhage or exposure of moist viscera to room air; many anesthetics are metabolized in the kidney (creating a slight diuresis minimizes renal toxicity).

To minimize the effects of surgery and anesthesia on hydration:

- Place an **intravenous catheter** whenever possible to provide access for fluids and medications
- **Supplement fluids**, intravenously if possible; otherwise intraperitoneally or subcutaneously
 - Fluid should be supplemented at the rate of 5-10 ml/kg/hour during anesthesia
 - **Monitor** hydration status- **Overhydration** results in frequent urination and pulmonary edema, **underhydration** results in sticky mucous membranes, loss of skin elasticity, the eyes sinking into the orbit, decrease in blood pressure and increase in heart rate
 - **To replace blood loss** with saline or lactated ringers, administer 3X the volume of blood lost by slow IV drip. Monitor the hematocrit. If it drops below 20%, whole blood replacement may be necessary.

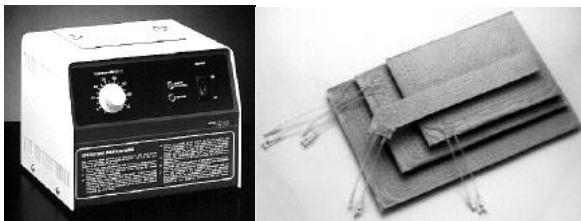
- Click here for clinical case studies in fluid management.
- **Monitor cardiovascular function** by monitoring one or more of the following:
 - Mucous membrane color and capillary refill time (the time it takes for the mucous membranes to regain their normal color after pressure is applied)
 - Heart rate and rhythm- stethoscope or esophageal stethoscope
 - Pulse rate and pressure- using your fingers

If the animal has pale mucous membranes, the capillary refill time is greater than 2 seconds, or if the other cardiovascular parameters are out of normal range (determine normal for the species you are using!) you may have a cardiovascular emergency. Increasing the rate of intravenous fluid administration will improve cardiac output temporarily. However the depth of anesthesia will need to be reduced and if there is a primary cardiac problem it will require specific treatment.

Thermoregulation

Animals frequently become hypothermic during anesthesia because of inhalation of cold gases, exposure of body cavities to the room air, and loss of normal thermoregulatory mechanisms and behaviors. Hypothermia depresses all physiologic functions, including respiration and cardiac function, slows the metabolism of anesthetics and results in prolonged recoveries. All of these can contribute to anesthetic death. Hyperthermia is less common, but may occur because of excessive application of heat, hot surgery lights or malignant hyperthermia in genetically predisposed animals. To thermoregulate your patient:

- **Monitor the body temperature** frequently using a thermometer during the procedure and during anesthetic recovery. While animal normals vary from species- to-species, in general, when body temperature drops below 99° F, an animal is considered hypothermic. Below 95-96° F an animal cannot regain normal body temperature without supplementation.
- **Prevent heat loss** by insulating cold surfaces with a blanket
- **Prevent heat loss** during gas anesthesia by utilizing low flow techniques that conserve heat
- **Supplement heat** with a thermal blanket (keep blanket temperature below 40 C to prevent burns!) or with pre-warmed fluids
- **Treat hyperthermia** by administering intravenous fluids or applying water to foot pads or exposed skin. Only use an ice bath as a last resort, as it may cause cardiovascular shock.



Water blanket and heater

Monitoring Anesthesia

The depth of anesthesia must be monitored carefully. Animals that are too light will experience pain and may move during the procedure. Animals that are too deep run the risk of experiencing cardiopulmonary arrest. If an animal is too light the anesthesia should be supplemented, if too deep, animals on gas anesthesia can be turned down. Animals given injectable anesthetics can not be lightened directly. Instead respiratory and cardiovascular support must be administered until the anesthetic is metabolized and the animal begins to lighten on its own.

To monitor the depth of anesthesia, perform the following:

- **Reflexes**- these reflexes disappear as the animal becomes deeper in the following order:
 - *Palpebral reflex*- touching the eyelids causes blinking. The animal is light if it is blinking.
 - *Toe pinch reflex*- pinching the toe or foot web will cause a pain response. If the animal withdraws the toe it is not deep enough. If it doesn't, it is not sensing pain.
 - *Corneal reflex*- touching the cornea of the eye with a tuft of cotton results in a blink. Once the animal has lost its corneal reflex, it is too deep.
- **Muscle tone** increases as the depth of anesthesia decreases, unless the animal is receiving a cataleptic drug like ketamine in the absence of a sedative. Test muscle tone by pulling on the lower jaw or a limb. Rigid tone indicates inadequate depth of anesthesia.
- **Monitor cardiopulmonary function and body temperature**- As an animal becomes too deeply anesthetized, respiration and cardiac output decrease, resulting in poor blood oxygenation and tissue perfusion and decreased blood pressure and temperature. Likewise, elevations in heart rate and blood pressure may be indications that an animal may be feeling pain and is anesthetized too lightly. Monitor as previously described.

Anesthetic Emergency Drugs	Dose (mg/kg)	Indications
Doxopram (Dopram)	1-5 IV (10x in farm animals)	Respiratory stimulant, for complete respiratory arrest only, use with CPR
Furosemide (Lasix)	2- IV, IM	For pulmonary edema. Administer as needed
Naloxone (Narcan)	0.04 IV	For reversal of narcotic sedation or respiratory depression
Yohimbine	0.1-0.15 IV	Reversal of xylazine or detomidine sedation
Atropine	0.02-0.04 IV	For bradycardia
Epinephrine (1:1000)	0.1 ml/kg IV, IT, IC, IM	For cardiac arrest only. Administer IV, intratracheal or intracardiac and perform cardiac massage
Lidocaine	2, IV (0.5 mg/kg in cats)	For diagnosed ventricular tachycardia only. Administer to effect and monitor

Recovery

Monitoring and support must continue until the animal is completely recovered from anesthesia. Complete recovery means the animal is able to hold itself in a normal upright position, has returned to normal body temperature and all physiological indices are within normal limits. Anesthetic recovery can be rapid for gas agents and short anesthetic episodes. Recovery time can be prolonged if animals were under for a long time or if injectable agents were used.