Using Animals in Research at PSU

The Institutional Animal Care and Use Committee

Before you may use animals, the Pennsylvania State University (PSU) Institutional Animal Care and Use Committee (IACUC) must review and approve your experimental plan (protocol). IACUC approval allows a researcher to do only the experiments described in the approved protocol using a specific number of animals. After IACUC approval, you cannot change (modify) any of the animal procedures described in your protocol until you obtain approval for the change(s) from the IACUC. Go to http://www.research.psu.edu/orp/animals for more information on the IACUC.

The Animal Resource Program

The Animal Resource Program (ARP) provides laboratory animal care and housing, veterinary support and research personnel training. Mice and rats are the most commonly used laboratory animals at PSU although campus facilities can house many other species, including rabbits, farm animals and non-human primates.

Investigators order research animals through the Animal Resource Program. ARP employees are available to help investigators select animal suppliers and place orders. Arrangements for obtaining animals from non-approved suppliers or other institutions must be coordinated through the ARP. Investigators who want to send animals to other research institutions must also work with the ARP to prepare and ship animals. Go to the ARP website for information and forms used to acquire animals.

Laboratory Animal Facilities

Laboratory animals are housed in several different buildings on the University Park campus. The ARP operates the animal facilities and determines who is allowed to enter and work in them. Entrance doors to laboratory animal facilities are always locked. No one may enter the animal facilities without approval from the Animal Resource Program. Why is this?

1. Animals need a quiet, safe and secure environment. The presence of loud noise, many people and much activity is stressful for research animals. Stress alters many physiological and psychological measures. Research data accuracy is improved and fewer animals may be needed if the animals are not stressed.

2. Reliable and accurate research requires the use of healthy animals. Humans can carry bacteria and other disease-causing microorganisms into the facility. People handling animals in the facility must also wear protective clothing (gowns or lab coats and gloves) to help prevent disease transmission.
3. Laboratory animal facilities contain specialized and expensive equipment. Damage or theft of this equipment would make it difficult to provide care for the animals and reduce the value of research done in the facility.

Employees and students must complete all required IACUC and ARP facility orientation training before they are allowed to work in a laboratory animal facility. Contact the ARP office (865-1495) for information on facility orientation training.

Working in an Animal Facility

Access to the Facility

The ARP provides research staff and students with access to the specific facility in which they house animals. Entrance to the facility requires a PSU identification card. Contact the ARP office (864-1495) for information on key card access.

Animal Rooms

Temperature and humidity levels in animal rooms are kept within specific ranges that are appropriate for the species. Automatic timers control lights in animal rooms. Animal care technicians observe the animals to ensure they have food, water and clean bedding at least once a day, seven days a week. Cages and other equipment are cleaned and replaced as often as needed to maintain clean, dry housing for each species. Each animal species is provided a nutritionally compete diet. Special diets or sterilized food or water may be used for some animals.

Room entry

Animal rooms within the facility are kept locked when not in use. This provides quiet and safety for the animals, in addition to helping prevent the spread of disease. Staff and students must follow the personnel protective equipment (PPE) instructions for each room. Instructions are displayed on the animal room door. Do not wear the same PPE in more than one animal room.

Procedure Rooms

All PSU animal facilities provide space in the facility that research staff may use to perform animal procedures. Gas anesthetic equipment, CO2 tanks and other equipment is available for use in designated procedure rooms. Research personnel are responsible for room clean-up and disposal of all waste after working in the facility. ARP staff can provide the training required to operate equipment in the procedure rooms. New or untrained personnel should not use this equipment without adequate training and supervision.

Surgical Facilities

ARP facilities include areas and equipment that investigators may use to perform surgery on research animals. Research investigators must have their plans for surgical procedures, anesthetic and analgesic
protocols and postoperative care approved by the IACUC prior to conducting these procedures. Persons involved in surgical procedures must also have completed additional training.

Animal Housing

Most of the rodents in the animal facilities are housed in specially designed plastic cages with wire lids and absorbent bedding. Many of the cages have protective covers to prevent the introduction of dust, dander, dirt (and potential disease causing organisms) into the cage. Most cages (as shown in the photo) fit into specially designed racks that provide clean, filtered air for each cage.

Microisolator Housing

Mice are housed in microisolator caging systems that help protect against disease transmission if specific handling procedures are followed. Microisolator cages have a plastic, filter-top lid that fits over the wire food hopper. Personnel working with mice should only open the cages inside a biosafety cabinet or changing hood that is turned on.

Opening cages outside of a hood will expose the animals to contaminants that may be present, including disease-causing microorganisms. ARP provides training to investigators, staff and students on how to work with microisolator caging systems. An online tutorial is also available on the ARP website.

Other Rodent Housing

PSU facilities are equipped to house other rodents, including rats, guinea pigs, gerbils and hamsters. A variety of caging types are available for these rodents.

Environmental Enrichment

Various types of environmental enrichment are provided for research animals to reduce stress and improve welfare. Most laboratory animals, including mice and rats, prefer to be with others of their own species. Pair or group housing of animals is used whenever possible as a form of environmental enrichment.

In addition, depending on the species, physical objects such as nesting material or chew toys are provided to allow animals to perform normal behaviors and increase physical and psychological comfort. For example: Rats are provided with toys to gnaw on; mice are given nesting material to shred and build nests; rabbits are provided with various toys to explore and manipulate as well as food treats such as alfalfa cubes.

Prior to use in the animal facility, toys, nesting material and food treats or supplements must be approved by ARP and be free of potential contaminants.
**Rodent Disease Prevention**

Many different bacterial, viral and parasitic organisms can infect rodents. Infected animals may not look sick to us but they are physiologically and behaviorally different from uninfected rodents. These differences may affect research results. For this reason, and because some microorganisms may also infect people, it is important to ensure that rodent research colonies do not harbor infectious organisms. The ARP uses a health surveillance program to monitor rodent colonies for the presence of these organisms.

**Disease Transmission**

Infectious organisms may be brought into an animal facility by:

- Infected animals imported from outside the university
- Contaminated cell lines used in research with rodents
- Contaminated personnel or equipment
- Wild rodents

Infectious organisms may spread (be transmitted) quickly throughout an animal facility. Sometimes these organisms are difficult to detect because they do not cause obvious signs of illness in infected animals. Once they occur, it is often time consuming and costly to eliminate these infections from rodent colonies and facilities.

**Health Surveillance Procedures**

The goal of a health surveillance program is to detect the presence of unwanted infectious organisms in an animal facility. The ARP monitors the health of rodents in PSU animal facilities using ‘sentinel’ animals. At cage changing, dirty bedding taken from the cages of research animals is placed into the cages of the sentinel animals. Many infectious organisms are spread through urine or feces. If research animals in the room are infected, the dirty bedding should expose the sentinel animals to the infectious organisms. Sentinel cages are marked by a yellow cage card and are usually placed on the lower right shelf of the rack. Please do not move or disturb the animals in these sentinel cages.

**Quarantine Procedures**

Most laboratory animals are purchased from commercial suppliers who maintain strict husbandry and health standards to ensure their animals are free from potential disease-causing organisms. However, researchers sometimes acquire animals from other sources whose quality control standards may not be as reliable. Animals imported from other academic institutions or purchased from non-approved commercial suppliers fall into this category.

ARP veterinary staff request and review health records from the supplier before animals from non-approved sources are shipped to PSU. When animals arrive on campus they are placed in ‘quarantine’ in a protected area of the animal facility. This allows the animal(s) to recover from the stress of shipment and undergo testing for health problems and infectious diseases without exposing other animals in the facility.
facility. If the animals are found to carry pathogens (disease causing organisms) or parasites they will not be permitted into the research colonies unless they can be treated to eliminate the organisms.

**Animal Health and Welfare**

Healthy animals make the best research subjects and research animals at PSU are provided with excellent care. However, animals occasionally suffer injury or illness due to a variety of causes. This may include infectious disease, accidental injury, cancer, or sickness related to genetic background, experimental procedures, breeding or old age.

Everyone working with research animals has a responsibility to ensure that the animals do not suffer unnecessary pain, distress or discomfort. ARP caretakers check all animals every day to ensure that they have food, water and clean caging. ARP veterinarians and veterinary technicians are available to care for ill or injured animals. Research staff and students are responsible for learning how to work with the animals in the most humane manner possible. Research staff and students must also recognize when an animal is showing signs of illness, pain or distress and know what to do when this happens.

**What is normal?**

Normal animals are active, alert and move easily around their cage. They readily eat and drink the food and water offered them. They sleep and groom themselves (and others in the cage) at regular intervals. Their haircoat is full and shiny. An online tutorial ([http://www.ahwla.org.uk/site/tutorials/HW/HW01-Title.html](http://www.ahwla.org.uk/site/tutorials/HW/HW01-Title.html)) produced by Newcastle University in Great Britain gives a good introduction to assessing the health and welfare of laboratory animals.

Animals in pain or discomfort show changes in normal behaviors and physiology. The changes may be slight or subtle in an animal with mild to moderate pain/discomfort or obvious in an animal with moderate to severe pain. Another online tutorial ([http://www.ahwla.org.uk/site/tutorials/RP/RP01-Title.html](http://www.ahwla.org.uk/site/tutorials/RP/RP01-Title.html)) provides an introduction to the recognition of pain in animals after surgery.

Some specific behaviors are associated with ill or injured animals. Research personnel should recognize these behaviors as abnormal.

- Decreased activity or a reluctance to move
- Hunched or other abnormal posture or gait (e.g., lameness)
- Rough, greasy-looking coat (due to lack of normal grooming)
- In rats: dark, red material around the eyes and nose
- Excessive licking or chewing of a body part or area
- Decreased appetite and/or weight loss
- Unusual aggressiveness when handled
In rodents after abdominal surgery:
  - Muscular contractions of the lateral abdomen and back arching
  - Abdominal pressing onto the cage floor
  - Rabbits may show similar behaviors after abdominal surgery
  - Dehydration
  - Fluctuations in body temperature, heart and breathing rates and blood pressure

What do you do with sick or injured animals?

Research personnel are responsible for knowing what signs of illness in animals may be expected due to experimental procedures used in their research. Your IACUC protocol must clearly define what the signs are and provide a plan for how the animals will be treated. The protocol must also clearly define when an animal is to be removed from the experiment and/or euthanized.

Research staff must contact an ARP veterinarian or veterinary technician when an animal shows unexpected signs of illness or injury. If you call the ARP office (865-1495) after normal business hours, you will hear a recorded message with instructions on how to reach a veterinarian.

Health Alert System

The ARP uses a three-part form to identify cages with ill or dead rodents. One part of the form stays with the cage, one part is hung on the animal room door and one part is returned to the ARP office. Do not remove the health alert form from the animal cage. Forms placed on the animal room door are there to inform the investigator of a sick or dead animal and can be removed after viewing.

Euthanasia

Euthanasia is the humane killing of an animal. The IACUC must review and approve all euthanasia methods used in experimental animals. The American Veterinary Medical Association provides guidelines on euthanasia methods for each species. Information on acceptable euthanasia methods is available on the ARP website. Research staff and students must have IACUC approval and receive training to perform animal euthanasia.

Most rodents at PSU are euthanized using carbon dioxide (CO2) gas. CO2 tanks and related euthanasia equipment are available in the procedure rooms of each facility. Personnel must be trained prior to using this equipment. ARP animal caretakers and veterinary technicians are available to train research staff.
Humane Endpoints in Animal Research

A humane endpoint is a physiological or behavioral sign that determines the point at which specific action is taken to relieve an animal’s pain and/or distress. The action taken may include ending the experimental procedure, medical treatment or euthanasia. Humane endpoints are not the same as experimental endpoints (i.e., the point at which scientific aims and objectives have been reached). Humane endpoints are used to prevent animals from experiencing pain and distress unnecessary for completion of the experiment.

Effective humane endpoints are clearly defined and based on objective criteria. Non-specific signs of illness such as inactivity, hunched posture or a rough coat are an indication that an animal should be examined more closely. These signs by themselves do not often constitute an endpoint. Familiarity with the animal model is necessary to select endpoints that are both humane and scientifically sound. Humane endpoints can often be refined or modified as an investigator becomes more experienced with the use of an animal model. The following websites provide more information on humane endpoints:

UC Davis Center for Animal Alternatives.
USDA National Agricultural Library
Canadian Council on Animal Care

Investigators should include the following information when describing humane endpoints in an IACUC protocol:

- A precise definition of the humane endpoint(s), including the criteria used to evaluate animals. Examples of potential humane endpoints are listed below.
- The number of times per day animals will be observed for signs that an endpoint has been reached.

  **Frequency of examination:** Experimental animals must be observed at least once a day. More frequent observations are often required to determine when a specific endpoint is reached in studies involving pain and/or distress. An appropriate observation schedule must be described in the IACUC protocol.

- The training of personnel who will observe and evaluate the animals.
- The action(s) taken when an animal reaches a humane endpoint.

Moribund animals

The term moribund refers to an animal that is near death or in the process of dying. Animals in this state are often comatose (unresponsive and unaware of stimuli) and so beyond awareness of suffering. However, an animal may have experienced much pain and distress prior to reaching a moribund state. **Stating that animals will be euthanized when they become moribund is not an appropriate humane**
endpoint as this may not reduce or alleviate any suffering that the animal will experience. The purpose of identifying endpoints is to prevent or minimize animal pain and distress.

**Death as an Endpoint**

While certain types of studies have historically used death of the animal as a scientific endpoint, this is now rarely accepted and investigators must present conclusive evidence to support the use of such an endpoint.

**How to select and use humane endpoints:**

Appropriate endpoints are **objective and relevant** for the assessment of pain/distress in the species used. Examples include:

- Body weight changes
- External physical appearance
- Behavioral changes
- Physiological changes (e.g., body temperature, hormonal fluctuations, clinical pathology, etc...)

Research personnel responsible for observing and evaluating animals must be **adequately trained and experienced in the recognition of these signs for the species being used.** Personnel must know what is “normal” before they can recognize “abnormal”. Investigators are responsible for ensuring that students and employees are trained and have the skill and authority to treat or euthanize animals who have reached an endpoint.

**Pilot studies** (experiments) can be useful in determining endpoints, especially when the effects of an experimental treatment in animals are not well known. They may also be used to evaluate earlier or more sensitive humane endpoints and provide training for personnel in the recognition of endpoints.

**Humane Endpoint Examples:**

**Poor Body Condition Score**

- Body condition scoring is a method used to evaluate animal health when body weight may not be an accurate indicator (e.g., tumor models, ascites production and pregnant or growing animals).
- Scoring methods have been developed for many species. [This link](#) provides a description and example of how to evaluate mice.

**Weight loss**

- Rapid weight loss of 15-20 percent within a few days. This requires frequent monitoring of body weight.
- Gradual weight loss - over an extended period of time leading to emaciation. The degree of weight loss should be specified in terms of % or quantity (grams, pounds, kg).
• Note: Certain debilitating conditions such as tumor growth and ascites may mask true weight loss.

The inability to stand or walk
• Animals will be unable to reach food or water.
• Objective and easy to assess.

Tumor size
• Usually measured as diameter of the mass or percentage of body weight (e.g., greater than 1.5 cm diameter or greater than 10% of body weight)

Abnormal breathing pattern or effort
• The animal shows increased respiratory rate and/or effort.

The presence of large open wounds

Dehydration
• The skin loses its elasticity. In a normal animal, skin pinched between the fingers will immediately return to its normal position when released. In a dehydrated animal, the skin will remain tented.

Your Responsibilities When Working in a Laboratory Animal Facility
• Treat all animals with respect and dignity.
• Use only methods and/or procedures that have been approved for your IACUC protocol.
• Follow facility security and disease prevention instructions.
• Clean up after yourself and dispose of waste appropriately.
• Ask for help if you need it.