Animal Source

PSU Animal Resource Program

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Recognizing Pain in Rodents

Some experimental procedures performed on animals are likely to result in pain that may persist after the procedure ends. Investigators are responsible for recognizing pain in their research animals and providing relief whenever possible. Accurate recognition of pain in rodents requires knowledge of normal behavior and physiology so that relatively subtle changes in either may be noticed. Signs associated with pain in rodents can include the following:

- Decreased activity
- Abnormal posture (hunched back) or gait
- Rough, greasy looking coat
- Weight loss/decreased appetite
- Dehydration
- Increased respiratory rate

In rats:

- Dark, red material around the eyes and nose
- Stretching and back arching
- Abdominal pressing onto the cage floor
- Frequent sudden short movements
- Eating of bedding material

Additional information on pain management may be found on the ARP website.

Making Sense of the Search for Alternatives

Regulatory guidelines require that a principle investigator (PI) provide evidence to the IACUC that the animal use proposed in his/her research is scientifically valid, not unnecessarily duplicative and causes the least possible amount of pain and distress to the animals used in the study. The required search for alternatives included in an IACUC proposal application is the vehicle through which the PI provides this evidence. In reality, it can be presumed that the PI, in development of the proposed research plan, has reviewed the relevant literature in the area of study and determined that the proposed research is scientifically valid and novel.

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Visit the ARP website at www.research.psu.edu/arp for answers to your animal use questions

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Therefore, the goal of the PI when answering the search question should be to adequately address the following three points:

- Are **non-animal alternatives** available (including the use of less sentient species)?
- Can procedures be refined to **reduce the level of pain/distress** experienced by the animal?
- Has the PI considered methods that would **reduce the number of animals** used in painful procedures?

To answer the search question the PI must identify all of the procedures included in the study that may cause pain/distress and address each one. Not only must the PI search for alternative methods for these procedures but he/she must also consider refinements, such as establishing humane endpoints, that minimize animal distress and methods that will reduce the number of animals used (i.e., variations in experimental design).

In addition, the purpose or end goal of a procedure must be considered when searching for alternatives. For example, an investigator may want to perform surgery to implant an osmotic pump for drug delivery. Although it is the surgical procedure that will cause pain, the alternatives search should focus on alternative methods of drug delivery (i.e., intraperitoneal injection) that would not require surgery. If alternative methods are available, the PI must explain why these methods are not acceptable in this study.

While a variety of resources may be used to accomplish the alternatives search, the expectation is that appropriate literature database(s) will be searched. When searching these databases the PI must consider that procedures may be specific for a certain species or research field or they may be quite general. For example, retro-orbital collection of blood is a technique used in many types of research and an alternatives search limited to a specific research field will miss alternative techniques to this procedure.

ARP veterinarians are available to provide assistance to researchers in developing alternatives searches for their research proposals. In addition, online resources are available and several may be accessed through the following website: National Library of Medicine Bibliography on Alternatives to Animal Testing - http://toxnet.nlm.nih.gov/altbib.html.

References:
University of California at Santa Barbara, Institutional Animal Care and Use Committee. 10/18/05. http://research.ucsb.edu/connect/acc/alternvs.html.
Altweb, Johns Hopkins School of Public Health. 10/18/05. http://altweb.jhsph.edu/searchalt.htm.

Facility Fundamentals: Working in the Animal Room

1. Wear appropriate **protective clothing** i.e., shoe covers, lab coat or gown and exam gloves. Protective clothing worn in the animal room must be removed when leaving the room. Do not wear the same protective clothing in more than one animal room.
2. Open rodent cages within a **biosafety cabinet** (if one is in the room). Do not place or open cages on the floor.
3. A limited number of **clean cages** and related equipment may be obtained from the clean cage storage area. If you anticipate needing more than a few cages please call ARP the day before to arrange for delivery.
4. Keep clean and **dirty cages** and related equipment separate. It's also a good idea to have at least two clean cages available in the room for emergency cage changes e.g., water bottle leakage that leads to cage flooding.
5. Make sure **water bottles** are correctly replaced onto the cage lid after opening the cage. Mice/rats will become dehydrated and may die if unable to reach water overnight.

Injectable Anesthetic Mixtures for Rodents

Appropriate general anesthesia is necessary to alleviate pain and distress and facilitate restraint in research animals undergoing many types of experimental procedures. The features of general anesthesia include loss of consciousness, analgesia, suppression of reflex activity and muscle relaxation. Few injectable anesthetic drugs produce all of these features when administered alone. Therefore, mixtures of various drugs are commonly used, each contributing to achieve the overall effect of general anesthesia.

An advantage to the use of anesthetic mixtures is the minimization of undesirable side effects from individual drugs due to the relatively low dose of each used in the mixture. A disadvantage is that it can be somewhat confusing to calculate appropriate dose amounts.

The following are some commonly used anesthetic mixtures provided in cookbook format to facilitate accurate preparation. Additional mixtures are available at www.research.psu.edu/arp/anesthesia.shtml. As with any injectable anesthetic regimen, individual animal response will vary. When using a new anesthetic regimen we recommend testing it on non-experimental animals of the same strain prior to use in your experimental subjects.

Mice:

Ketamine/Xylazine

Dosage:	Ketamine	100 mg/kg
	Xylazine***	10 mg/kg
Dose Rate:	0.1 ml/10 gm body weight IP*	
Volumes:	Ketamine	0.50 ml
	Xylazine	0.25 ml
	WFI**	4.25 ml
	Total Volume	5.0 ml = 50 doses

Ketamine/Medetomidine

Dosage:	Ketamine	75.0 mg/kg
	Medetomidine	1.0 mg/kg
Dose Rate:	0.1 ml/10 gm body weight IP*	
Volumes:	Ketamine	0.375 ml
	Medetomidine	0.50 ml
	WFI**	4.125 ml
	Total Volume	5.0 ml = 50 doses

*IP = intraperitoneal injection
**WFI = sterile water for injection
***Xylazine = 20 mg/ml concentration

Rat:

Ketamine/Xylazine

Dosage:	Ketamine	100 mg/kg
	Xylazine***	10 mg/kg
Dose Rate:	0.2 ml/100 gm body weight IP*	
Volumes:	Ketamine	4.0 ml
	Xylazine	1.0 ml
	WFI**	3.0 ml
	Total Volume	8.0 ml = 40 doses

Ketamine/Xylazine/Acepromazine

Dosage:	Ketamine	50 mg/kg
	Xylazine‡	5 mg/kg (100 mg/ml)
	Acepromazine	1 mg/kg
Dose Rate:	0.1 ml/100 gm body weight IP*	
Volumes:	Ketamine	5.0 ml
	Xylazine	0.5 ml
	Acepromazine	1.0 ml
	WFI**	3.5 ml
	Total volume	10.0 ml = 100 doses

‡Note that Xylazine used in this mixture is at a concentration of 100 mg/ml. Xylazine may be purchased at concentrations of either 20 mg/ml or 100 mg/ml.

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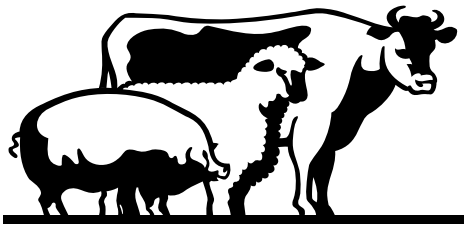
The Animal Resource Program (ARP) is committed to providing PSU research personnel with high quality animal care services and facilities, to facilitate and improve animal research, and to ensure the health, well-being and humane treatment of all animals at PSU. ARP provides veterinary and diagnostic services, personnel training and expertise in laboratory animal, agricultural and wildlife technology and medicine. ARP veterinarians have specialized training and are available to assist with animal model development, experimental design, budget projections and grant preparation. Participation in collaborative research projects is welcomed.

Remember: Isoflurane anesthetic machines must be used within an anesthetic hood to reduce operator exposure to waste gas.

Beyond the Mouse: Livestock and Wildlife in Biomedical Research



Although rodents comprise greater than 90% of the animals used in biomedical research the contribution of other nontraditional species should not be overlooked. Many livestock and wildlife species may be utilized as effective animal models. For example, calves are used for development and preclinical evaluation of cardiac assist or prosthetic devices. Sheep are widely used as models for basic and applied fetal and reproductive research. Genetically engineered ruminants have been developed and proposed for use in the production of proteins that will be secreted in milk. Swine are important models for research of the cardiovascular system because of their anatomical and physiological resemblance to the human system. Woodchucks are used as models of viral hepatitis and the zebrafish is a primary model of vertebrate embryonic development, gene function analysis and mutagenesis.

To provide assistance for researchers using these nontraditional models the Animal Resource Program offers training in animal handling, procedural techniques such as oral gavage, blood and other specimen collection, surgery, and anesthesia. ARP is also equipped to perform radiology. There is a veterinarian on staff skilled in many different aspects of working with these species and offers services including surgery, assisting with IV and urinary catheterization, performing biopsies and other procedures, and is available for collaboration on research projects. Contact the ARP office for more information on training, protocol review and experimental models within this exciting area of research.