GUIDELINES FOR SMALL ANIMAL SURGERY

Two sets of guidelines have been adopted by the United States Federal Government to regulate vertebrate animal use in research. The Public Health Service (PHS), which includes the National Institutes of Health, requires all research on animals that it funds to comply with the Guide for the Care and Use of Laboratory Animal (Guide). PHS expects that all animal-related research conducted at institutions that receive its funding, including work that is not directly supported by PHS, to comply with the Guide. In addition, the Animal Welfare Act (AWA) regulates care and use of most warm-blooded species employed in research. However, some species, including birds, mice of the genus Mus and rats of the genus Rattus that are bred for use in research are exempt from the provisions of AWA. The United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA/APHIS) administers the AWA, its standards, and its regulations. The University of Texas at El Paso (UTEP) has adopted the policy that any experimentation or surgery performed by investigators who employ rats and mice in research must comply with the provisions of the Guide. This guideline will address the requirements for investigators to perform small animal rodent surgery at UTEP.

The Guide: Pages 60-66 of the Guide (7th ed.) provide general surgical guidelines for both large and small animals. Since UTEP is a PHS-assured institution, all surgical procedures conducted on vertebrate animals at this institution (both large and small animals) must comply with the Guide. It is prudent for all investigators conducting surgery to be familiar with the relevant section of the Guide, which can be accessed online at the following address: http://www.nap.edu/readingroom/books/labrats/contents.html

USDA Policy 3: Surgical standards for AWA-regulated species are described in USDA Policy 3. AWA-regulated species include all large animals as well as some small animals. Although the use of most rats and mice at UTEP is not technically regulated by USDA Policy 3, most surgical standards for AWA-regulated small animals stipulated in this policy are generally applicable across rodent species.

Guidelines from The Academy of Surgeons (qualifications for experimental surgeons): The Academy of Surgical Research has formulated a number of guidelines for training investigators who conduct surgery on animals (Guidelines for Training in Surgical Research with Animals, Journal of Investigative Surgery, 22, 218–225, 2009). A principal investigator with an IACUC-approved protocol must take the responsibility for assuring that any personnel performing surgical procedures on animals under that protocol are adequately trained. It is highly suggested that all investigators and their staff read and understand the Guidelines for Training in Surgical Research with Animals. The following is from the recommendations from the Academy of Surgeons:
“It is the responsibility of the institution to ensure that all personnel performing survival or terminal surgical procedures on research animals be qualified by experience, education, and training to perform such procedures. The institution is responsible for supporting the training and providing the necessary resources for the program. If personnel are not qualified to perform these procedures, either education and training must be provided or collaborative arrangements be made with qualified personnel before performing the surgery.”

Other regulations:
Both the Guide and USDA policies recognize that unique conditions exist at every institution, and stipulate some purview for the Institutional Animal Care and Use Committee (IACUC) in the local interpretation and application of the law. UTEP IACUC is responsible for establishing guidelines and policies regulating animal use. All investigators must be familiar with IACUC guidelines and policies, which can be accessed at the following address: https://research.utep.edu/Default.aspx?tabid=58972

SUMMARY

Surgical training opportunities at UTEP:
The UTEP Attending Veterinarian (AV) is available for consultation in designing and reviewing protocols with surgical components. The AV should assist in advising on USDA Pain/Distress categories, surgical approaches, aseptic technique, anesthesia/analgesia selection, and post-operative care and analgesia.

Assessment of qualifications by UTEP IACUC:
As part of the UTEP IACUC functions, the committee reviews surgical practices or surgical outcomes for ongoing animal use protocols. If deficiencies are noted, the IACUC may require further training for personnel or other changes in procedures before the project can continue. The UTEP AV routinely assesses postsurgical outcomes in all animals.

The AV and Veterinary Services Department staff provide training in surgical procedures to investigators, staff, and qualified students on approved animal protocols. This training consists of both a didactic component (classroom setting-1.25 hours) and hands-on component (practicum-4.5 hours). Training and surgical certification will be conducted using either the Veterinary Services Department approved training protocol (“Education, Teaching, Training and Bi méthodology in Animal Care and Research”), or by observation and evaluation by the AV of the surgical procedures conducted as outlined on an approved protocol.

If at any time, surgical problems occur, or post-surgical complications are apparent, investigators should feel comfortable in turning to the AV and Veterinary Services Department staff for assistance in resolving problems. Often some fairly minor adjustments to procedures can vastly improve surgical outcomes, and thus increase the success rate for experiments. The goal of both the IACUC and the AV is to facilitate the accomplishment of science while minimizing animal pain and distress.
Didactic Training:

The following will be the topics covered in the didactic portion of the training:

1. Pain and Distress
   a) Define pain
   b) Define distress
   c) Discuss categories of pain
   d) Discuss sources of pain/distress
   e) Discuss methods to minimize pain/distress
2. Anesthesia, Analgesics, Tranquilizers, Neuromuscular Blocking Agents
   a) Define and compare
   b) Discuss factors to consider in their selection
   c) Describe signs to define their effects
   d) Discuss drug selection, administration, and monitoring
   e) Discuss record keeping
3. Surgery and Post Surgical Care
   a) Define survival, non-survival, major/minor surgery, rodent/other surgery
   b) Discuss pre-surgical care
   c) Discuss aseptic surgery
   d) Discuss post-surgical care
   e) Discuss record keeping

Normally, this training will be offered on a quarterly basis, starting on the University’s academic year (August, November, February, and May). However provisions can be made to accommodate an investigator’s protocol requirements. The training will be based on the National Institutes of Health (NIH) “Training in Survival Rodent Surgery” CD. This CD was developed by a subcommittee of the NIH Animal Research Advisory Committee to assist in the development of proper surgical skills when performing survival surgery on rodents. It illustrates the most common practices used in the NIH intramural research program and survival rodent surgery standards established by the NIH "Guidelines for Survival Rodent Surgery." This training normally will be a 1.25 hour classroom session using the CD as the foundation with the AV giving instruction on all aspects of the certification as listed above. A roster with the attendees will be forwarded to the IACUC office to document participation and attendance. The didactic portion is a prerequisite for participation on the hands-on/practicum, or observation and evaluation by the AV.
Surgical Practicum:

The “Surgical Training Checklist” (attachment 1, pages 6-7) will be used by the AV (or qualified representative) during the hands-on/practicum component.

An attendance roster for the hands-on portion will be forwarded to the IACUC office as certification that the individual(s) have completed the didactic and hands-on/practicum or observational training. This roster must include the following certification statement signed by the Attending Veterinarian:

“These individuals, upon successful completion of the didactic and the hands-on practicum are certified to perform the stated surgical procedures as stated on their approved UTEP animal use protocol.”

X____________________________________________  ___________
(UTEP AV or qualified representative)             Date

Glossary of Surgical Definitions

**Major surgery:** Major surgery is a surgical intervention that penetrates and exposes a body cavity or any procedure that produces substantial or permanent impairment of physical or physiological functions.

**Minor surgery:** Any operative procedure in which only skin, mucous membranes and/or connective tissue is resected, such as simple vascular cut-down for catheter placement or implanting pumps in subcutaneous tissue. Non-surgical procedures that might require comparable levels of anesthesia and sterility include CSF collection, intracerebral inoculations and joint fluid collection.

**Survival surgery:** Survival surgery is any surgical procedure from which the animal recovers consciousness. Aseptic technique must be used for all survival surgical procedures.

**Multiple survival surgery:** The *Guide for the Care and Use of Laboratory Animals* provides the following guidance regarding multiple survival surgeries: “Use of one animal in multiple major survival surgeries is allowed only when such procedures are related components of a protocol; they must be scientifically justified in the protocol and approved by the IACUC. Cost savings is not an acceptable justification for multiple survival surgeries on any animal.” Determination that a procedure constitutes major surgery on any animal is usually made during the IACUC review process. However, development of “permanent physical impairment” may not be recognized until after the procedure is performed. If such impairment develops after surgery, that animal cannot be used for another recovery procedure.
Non-survival (terminal) Surgery: Any surgery or procedure conducted on animals that are not allowed to regain consciousness. Non-survival surgery procedures require similar record keeping as survival surgery.

Anesthetic and Analgesia Definitions:

1. **Anesthetic**—a drug that causes a reversible loss of conscious awareness and sensation, including pain.

2. **Analgesic**—a drug that causes an absence of pain in response to stimulation that would normally be painful; often, what is actually achieved following administration of an analgesic is hypoalgesia, or diminished pain in response to stimuli.

3. **Sedative**—a drug that produces a state of decreased motor activity, mental calmness, and drowsiness; does not imply analgesia, although most sedatives will increase the pain tolerance threshold by reducing anxiety and fear.

4. **Neuromuscular blocking agents** (paralytic agent)—a drug that blocks transmission at the neuromuscular junction; these drugs lack anesthetic and analgesic properties. It is not acceptable to use a neuromuscular blocking agent without general anesthesia.

5. **General anesthesia**—general anesthesia provides overall insensitivity and unconsciousness. Basic elements include: unconsciousness, amnesia, analgesia, muscle relaxation, diminished motor response to noxious stimuli, reversibility.

6. **Local and regional anesthesia**—local and regional anesthesia can result from topical application or injection of appropriate anesthetics in the region of the surgical incision (local anesthesia); injection in proximity to nerve trunk (nerve block); or injection into the subarachnoid or epidural spaces (regional anesthesia). Examples of agents that produce these types of anesthesia are lidocaine, bupivacaine, and Emla® cream.
Attachment 1—Surgical Training Checklist: The “hands-on” or practicum will be evaluated by the AV (or qualified representative) using the following checklist.

Student/Principal Investigator (PI): _______________________________________

Protocol #_________________________     Date: __________________________

☐ 1. Student/PI on approved current IACUC protocol and has completed Biosafety/Blood-borne Pathogen Training, General Lab Safety, appropriate AALAS training module(s) and Didactic Surgical Training? (NIH Rodent Survival Surgery CD).
   (Notes or observations listed here-Rationale for each item is listed below)

☐ 2. Proper PPE is donned and is clean and serviceable

☐ 3. Appropriate surgical equipment is sterile and serviceable. Clean and serviceable operating field, clean dressings/drapes, surgical sponges (i.e. 4x4’s, 2x2’s), sterile cotton swabs

☐ 4. Surgical area is appropriate for the procedure and is dedicated to surgical procedures; not in vivarium holding area nor in a high foot traffic area

☐ 5. Proper hair clipping, shaving or appellation solution (e.g., Nair) is available and utilized

☐ 6. Surgical area disinfectant is utilized and not-expired; e.g., Povidone/iodine, Chlorhexidine, 70% Isopropyl Alcohol

☐ 7. Animal is healthy and not in distress or has observable signs of disease or injury

☐ 8. Appropriate anesthetic regimen, analgesia drugs are available, non-expired and on approved protocol; e.g., Isoflurane, Nembutal, Ketamine-xylazine

☐ 9. Anesthesia equipment is functional and serviceable-nose cones, anesthesia circuits, vaporizer is certified, scavenging of waste anesthetic gases available
10. Student/PI is knowledgeable on proper animal handling techniques, injection sites, drug dosages and physiological info on species/strain of utilized animal

11. Adequate knowledge of chemical/biological hazards being utilized. Knowledge of drugs being utilized and potential adverse effects

12. Proper induction/maintenance of anesthesia and physiological monitoring, including blood loss and prevention of hypothermia

13. Proper surgical preparation: draping, shaving, and scrubbing of surgical site

14. Follows protocol surgical approach and methodology for procedure, e.g., cannulations, osmotic pump implantation, proper suture placement, laparotomy, etc.

15. Proper incision site closure using appropriate suture/closure material (generally, remove skin closures 7-10 days post-operatively)

16. Proper post-operative monitoring, ensuring animal recovers adequately, i.e., monitors every 10-15 minutes until animal is in sternal recumbency, animal is eating and/or drinking

17. PI/Student has proper surgical logs and post operative monitoring documentation

18. Surgical area is cleaned and disinfected and surgical equipment is cleaned, sterilized and stored. Biohazardous waste is properly contained—blood, needles, syringes, sponges, etc.

19. If procedure is terminal and animal is euthanized, is the animal carcass properly disposed of and euthanasia form filled out?

“This individual, upon successful completion of the didactic and the hands-on practicum is certified to perform the stated surgical procedures as stated on their approved UTEP animal use protocol.”

X

(UTEP AV or his/hers representative)
Rationale for Checklist:

1. **Acceptable surgical sites—Rodent surgery**: A separate facility for rodent surgery is not necessary. A room or part of a room that is easily sanitized and not used for other activities during surgery is appropriate. Surgery should be conducted in a disinfected uncluttered area, which promotes asepsis during surgery. The surgical surface must be impervious to water and able to be easily disinfected. Surgical table and instruments, e.g. microscopes, head frames, heating blankets, should be cleaned and wiped with disinfectant and allowed sufficient time to dry.

2. **Pre-surgical assessment of animals**: It is recommended that all animals have a pre-procedural assessment done before every procedure. This assessment can be recorded on the anesthetic form or recorded separately and maintained by the PI as part of the animal’s record.

3. **Aseptic techniques for rodent surgery**: These guidelines apply to all surgical procedures performed on rodents in which the animals are expected to recover from anesthesia. In general survival surgery on rodents should be performed using sterile instruments, sterile surgical gloves, mask and aseptic procedures to reduce microbial contamination of exposed tissues. Surgical equipment is appropriate for the species and is clean, serviceable and sterile.
   - Surgical instruments must be sterilized using ethylene oxide gas or steam sterilization. For multiple surgeries, instruments must be disinfected at the tips between surgeries. Suggested methods include: hot bead sterilization followed by cooling with sterile saline or cold sterilization solutions such as gluteraldehyde.
   - Pre-operative guidelines for rodent surgery: Prepare the animal by removing the hair from the surgical site. Perform this procedure in an area separate from where the surgery is to be conducted. Prepare the surgical site(s) with an appropriate skin disinfectant such as Betadine. Alcohol is not an adequate disinfecting agent. Surgeons should don surgical mask, scrubs or a clean lab coat and wash hands before aseptically donning sterile surgical gloves.

4. **Anesthesia and Analgesia**: Federal law requires pain relief according to the “Guide” and the Animal Welfare Act. The “Guide” on page 64 states that “the proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative”. The Animal Welfare Act (AWA) provides (section 13-3) requirements for animal care, treatment and practices in experimental procedures to ensure that animal pain and distress are minimized. These requirements include adequate veterinary care with the appropriate use of anesthetics, analgesics, or tranquilizing drugs or euthanasia. Care must be taken when choosing the proper dosage and schedule for a particular species, and different analgesics are indicated for different types of pain.

Consultation with the AV is an essential part of the protocol development to assess the requirement for anesthesia and analgesia.
5. Monitoring depth of anesthesia: Monitoring depth of anesthesia is species and individual dependant. An anesthetic text should be consulted for details. The research animal must be maintained at a surgical plane of anesthesia throughout the procedure. Monitoring should be documented every fifteen minutes (for large animals). Specific parameters to be monitored must be specified in the approved IACUC protocol and will depend on species of animal, the procedure, and length of time that the animal is under anesthesia.

6. Post-surgical recovery required before returning to animal facility: Animals moved to a warm, dry area where trained personnel must monitor recovery. Return the animal to routine housing once righting reflexes have returned and the animal can maintain normal body temperature. Do not offer food or water until the animal is fully recovered from anesthetic. Administer analgesics according to the schedule defined in the IACUC approved protocol unless there is scientific justification and approval by the IACUC for not doing so. Maintain surgery, anesthesia and post-operative care records. The Investigator for each rodent procedure or surgery must keep a logbook record. For batches of rodent procedures, a group logbook can be maintained, but for high-risk procedures or procedures with high complication rates (consult with the AV veterinarian with questions), individual information must be maintained for each rodent in the logbook or a separate animal record can be maintained. The Principal Investigator (PI) must have their logbook readily available during normal work hours for review.

7. Post-procedural recovery and care: It is the PI’s responsibility to perform all post-procedural recovery and care unless determined in advance that a second party, such as the Veterinary Service Staff or AV will perform the post-procedural recovery and care services.

8. Documentation: The PI is responsible for documenting all post-procedural recovery and care activities. Administration of post-procedural analgesics and antibiotics and the performance of daily observations of the animal to assess for species-specific pain and distress must be documented.