TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER
EL PASO

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
Policy #8
RODENT SURGERY

In concurrence with
TTUHSC El Paso Assurance #D19-01056
and Federal Regulations and Guidelines

Purpose:
The Guide for the Care and Use of Laboratory Animals states that "Successful surgical outcomes require appropriate attention to pre-surgical planning, personnel training, anesthesia, aseptic and surgical technique, assessment of animal well-being, appropriate use of analgesics, and animal physiologic status during all phases of a protocol involving surgery and postoperative care." This policy defines the requirements for rodent surgeries performed under IACUC-approved protocols at TTUHSC El Paso. Investigators performing surgeries on non-rodent species should contact the Institutional Veterinarian for guidance.

1. Facility

A. A dedicated facility for rodent surgery is not required. A rodent surgical area can be a room or portion of a room that is easily sanitized. The immediate surgical area must not be used for other purposes during the time of surgery.

B. Surgery must be conducted on a clean, uncluttered lab bench or table. The surface of the lab bench or table must be impervious to liquids. The work surface must be wiped with disinfectant before and after use, or covered with a clean drape.

C. The surgery area MUST be separate from the prep area where hair is removed from the animal.

2. Training

Professional and technical personnel and students who perform anesthesia, analgesia, and surgery must be trained to accomplish these tasks in a humane and scientifically acceptable manner before any surgery can take place. The LARC veterinary staff is available to provide assistance with, or training in, aseptic and surgical techniques and the proper administration of anesthesia and analgesia.

3. Instruments

A. Instrument Preparation

All instruments must be cleaned and sterilized prior to use. First, all instruments must be cleaned of any debris by hand washing or by mechanical washer/sterilizer. Then, prior to surgery, the instruments must be sterilized using one of the following methods. The method of choice may be determined by the procedure, the delicacy of the surgical instruments or the devices being used. Steam autoclaving is the preferred method.

1) Heat Sterilization

a) Steam Autoclave: The instruments must be placed in a specially designed pack or wrapped in sterile drapes or cloths, and secured with a thermo-sensitive tape. The use of such tape provides some indication that the autoclave procedure was effective. Instruments must be autoclaved at 121ºC for 21 minutes in a vacuum autoclave. Different times are required for gravity autoclaves. Once autoclaved, packs or wrapped instruments must be stored in closed cabinets or plastic

bags. Autoclaved items must have a standard indicator to prove complete sterilization. Wrapped autoclaved items must be clearly labeled with the date of sterilization or expiration date. Items that are autoclaved in cloth wraps expire 6 months after autoclaving. Items that are autoclaved in plastic packs expire 1 year from the date of autoclaving.

b) Flash Steam: Used to sterilize supplies intended to be used immediately. The temperature must reach 132°C for three to five minutes.

2) Cold (Chemical) Sterilization
Effective and proper use of chemical sterilization depends on many factors, including the use of chemicals classified as sterilants (not disinfectants), physical properties of the item(s) being sterilized (i.e., smooth, impervious to moisture, clean) and assurance of proper exposure. Chemical sterilants have finite shelf lives and must be used, depending on the agent, within one to four weeks depending on the agent (follow label directions).

Furthermore, the solutions must be protected from contamination. Effective cold sterilization requires thorough cleaning of instruments prior to processing because blood and organic debris may inactivate chemical germicides and/or shield microorganisms from the sterilization process. Clean Rubbermaid-type containers with secure lids or stainless steel instrument trays and lids are recommended for cold sterilization procedures and instrument storage. Sterile water or saline must be used to rinse the instruments, implants and tubing (inside and outside) prior to use to avoid tissue damage to the animals. The following are acceptable chemical sterilants:

a) Alcide® - Active ingredient: Sodium Chlorite 1.37%. Exposure time must exceed 6 hours.Shelf life is 14 days.

b) Cetylcide-G: Active ingredient: 3.2% denatured glutaraldehyde. Exposure time of 20 minutes will kill bacteria, fungi and many viruses, but requires extensive incubation to kill bacterial spores. Full sterilization requires 10 hours. Shelf life is 28 days.

c) Cidex® - Active ingredient: 2% glutaraldehyde. Exposure time must exceed 10 hours for sterilization. Cidex comes in two formulations, Cidex and Cidex-7 (long-life). The shelf life of activated Cidex is 14 days and of activated Cidex-7 is 28 days.

d) Endospore® - Active ingredient: stabilized hydrogen peroxide 6%. Not acceptable for metallic items.

e) Sporicidin® - Active ingredient (activator + buffer): phenol 7.05%, glutaraldehyde 2%, and sodium phenate 1.2%. Exposure time must exceed 6.75 hours for sterilization. Shelf life is 28 days.

f) Ethylene Oxide Gas: This is only used for instruments that will be damaged by heat or steam sterilization. This process is toxic, expensive and is regulated by federal law. Plastic, silicon and polyethylene catheters may be sterilized with ethylene oxide gas on the cool cycle.

g) Volatile Hydrogen Peroxide (VHP): safe and ideal for most applications. Requires an expensive generator.

B. Rubber Tubing

The following methods can be used to sterilize rubber tubing

1) Heat sterilization

2) Ethylene oxide gas

3) 6% hydrogen peroxide solution

4) VHP (volatile hydrogen peroxide)

C. Multiple Surgeries

If multiple surgeries are to be performed on different animals, then previously sterilized instruments can be “quick”-disinfected, using a glass bead sterilizer (at least 15 sec), 70% alcohol (10 minutes), or glutaraldehyde (10 min). However, instruments should be thoroughly clean of blood or tissue prior to sterilization. No more than five successive surgeries can use instruments “quick”-disinfected as described above.

1) Sterile (Hot) Bead Sterilizer: This instrument will sterilize the tips of metal instruments in 15 seconds. However, the beads should be clean. Only clean, cooled instruments may be used on the animals. Instruments must also be of appropriate size for the unit. This type of sterilization is ideal for multiple cage surgeries.

NOTE: Most sterile bead sterilizers take thirty minutes to heat.
NOTE: This method of sterilization may not be used for the initial sterilization of instruments; it is only appropriate when performing 5 or fewer surgeries using a single pack.

2) Chemical disinfectants: Instruments soaked in chemical disinfectants must be rinsed in sterile water or saline before use on animals.

4. Anesthesia and Analgesia Selection

Contact the Institutional Veterinarian for recommendations for appropriate anesthetics and/or analgesics for the species you are using.

The use of a single analgesic agent or combination will depend on the procedure performed. This information below provides some guidelines for determining the expected degree of pain associated with various surgical procedures. For specific advice, please consult the Institutional Veterinarian.

Examples of Surgeries & Degrees of Pain in mice and rats:

1. Mild: subcutaneous incision for implants or device placement, tail clipping or vascular access for port implantation.
2. Moderate: ovariectomy, orchiectomy, craniotomy, open cranium, embryo transfer, or open abdomen.
3. Severe: thoracotomy (open thorax) or musculoskeletal manipulation (e.g., fracture, muscle resection).

SURGERY TYPE	ANALGESIC	MINIMUM DURATION OF TREATMENT
Mild	NSAID or opioid + local anesthetic	Pre-emptive + 1 day post-op
Moderate	NSAID and opioid + local anesthetic	Pre-emptive + 1-2 days post-op
Severe	NSAID and opioid + local anesthetic	Pre-emptive + 2-3 days post-op or as needed

It is important to realize that none of the analgesics work immediately, so it is preferred that analgesics are provided pre-emptively to the painful event. Generally, this can be accomplished by administering the analgesics at the time of anesthetic induction or one – two hours before the surgical procedure. The specific analgesic choice and duration of administration to use is based on the severity of pain expected. These choices listed are not necessarily interchangeable. Please consult with the LARC veterinary staff for additional guidance. Analgesics may be given pre-emptively (preferred), intra-operatively to reduce inhalant requirements and provide additional analgesia, and post-operatively.

Safety NSAID dosing precautions:

NSAIDs must be used with caution after 3 days as it may have deleterious effect on the gastrointestinal mucosa. This may be especially true when using ketoprofen and flunixin.

Please note: a sample of drug calculation according to the body weight (bw) of the animal is provided after the dosages of the medications presented**.**

Local Anesthetics

- Lidocaine: do not exceed 7 mg/kg.
    - 5 min onset and 1 -2 hours duration of action.
- Bupivicaine: do not exceed 2 mg/kg.
    - 20 – 30 min onset and 3 – 5 hours duration of action.

1-2% lidocaine/0.25-0.5% bupivacaine (50/50) mix by volume

May need to dilute, esp. for mice (e.g. 1/10 dilution)
Local: lidocaine, lidocaine/bupivicaine, lidocaine patch, bupivicaine.

Mouse

Opioids

- Buprenorphine 0.05-1.0mg/kg every 6-8 hours SQ, IP
- Buprenorphine ER-LAB(Zoopharm)0.5 -1 mg/kg q48-72hrsSQ
- Ethiqa XR extended release buprenorphine 3.25 mg/kg q48-72h SQ

NSAIDS

- Carprofen 5mg/kg q12-24 hours SQ
- Meloxicam 2-5mg/kg q24 hours SQ, PO
- Meloxicam ER Lab(Zoopharm) 4 mg/kg q72hours SQ

Rat

Opioids

- Buprenorphine 0.01-0.05mg/kg SQ q 6-8 hours
- Buprenorphine ER-LAB(Zoopharm) 1-1.2 mg/kg q48-72hoursSQ
- Ethiqa XR extended release buprenorphine 0.65 mg/kg q48-72hSQ

NSAIDS

- Carprofen 5mg/kg SQ q 12-24 hours; can be combined with opioids
- Meloxicam, 2.0mg/kg q12-24 hours PO, SQ,
- Meloxicam ER Lab(Zoopharm) 4 mg/kg q96hours SQ

Example of drug dosage calculation in accordance to the body weight (bw) of the animal:

- How much Meloxicam (2 mg/kg) should be given to a rat that weighs 400 gm (bw)?
- Convert bw gm → kg
  - 400 gm = 0.4 kg
- Calculation:
  - 0.4 kg x 2 mg/kg = 0.8 mg per dose

4. Preparation of Animal

A. The animal must be anesthetized with a suitable anesthetic using the doses and procedure approved by the IACUC.

B. An ophthalmic lubricant must be applied to the eyes to prevent corneal drying.

C. Hair must be removed from the incision site with clippers, appropriate razor, and/or hair removal product (i.e., Nair) applied as directed and thoroughly rinsed off to prevent continual residue action.

D. Skin Preparation: The bare skin at the incision site must be thoroughly scrubbed with a surgical antiseptic agent to disinfect the skin and create a sterile field around the incision site. Starting in the middle, and continuing in an outward spiral, apply the scrub at least three times alternating each scrub with 70% isopropyl or ethyl alcohol, sterile water or saline.

Note: Copious application of topical alcohol in rodents will soak the animal and lead to hypothermia. The use of cotton tip applicators are ideal during the skin preparation process.

These surgical antiseptic agents may be used:

i. Povidone iodine scrub: A good choice for a surgical preparation with a broad spectrum of activity, including Mycobacterium. Antiseptic activity is rapid and persistent if not removed.

ii. Chlorhexidine scrub: The 4% aqueous preparation effectively cleans the skin with a rapid onset of activity and a broad spectrum of activity with minimal loss of antiseptic activity.

NOTE: A scrub is different than a solution. A scrub contains a soap, and therefore has cleaning properties that a solution does not have. Scrubs are not to be mixed or diluted with water.

Antiseptic agents must be rinsed from the skin with sterile water, sterile saline or alcohol prior to surgery.

5. Preparation of the Surgeon

a) Hands must be washed with an antiseptic soap or a surgical detergent/scrub (iodophors or chlorhexidine)and rinsed with water. Sterile surgical gloves must be worn.

b) A surgical mask must be worn to prevent contamination of the surgical field.

c) Gowns and surgical bonnets are required to maintain a sterile surgical field. The sleeves of garments must not be allowed to come in contact with sterile surfaces (e.g., gloves, the animal, etc.).

d) A new pair of sterile surgical gloves must be used for each animal. Alternatively, surgeons may wipe theirgloves for 30 seconds with sterile gauze pads soaked in 70% alcohol, or with chlorine

dioxide for 3 minutes. Gloves must be wiped with 70% alcohol after the 3-minute chlorine dioxide application.

e) If working alone, the surgeon must have the animal anesthetized and positioned prior to gloving.

f) If the instruments are in a sterile pack, the first layer of the double-wrapped instrument pack must be opened before gloving.

E. For survival surgery, the surgical site must be covered with a sterile drape after the surgeon has donned sterile gloves.

6. Intraoperative Monitoring

a) The animal must be continually monitored carefully during the surgical procedure. Specifically, the animal's respiratory rate and characteristic response to noxious stimuli (e.g., toe pinch, and when possible the heart rate and body temperature) will be monitored. Recommended to add at least two methods of monitoring as part of the surgical procedures on research protocol.

b) The surgical team must be trained by LARC personnel to be able to respond to the most common emergencies associated with the type of procedure being performed.

7. Post-Surgical Care

a) Post-surgical care must include observing the animal to ensure uneventful recovery from anesthesia andsurgery, administering analgesics, providing adequate care to surgical incisions and maintaining appropriate medical records.

b) Administration of analgesia is required, except when specific IACUC approval has been granted.

c) To prevent hypothermia, place the animal(s) in a warm room or cage. Do not place the recovering animaldirectly on the cage bedding. To prevent suffocation of the animal, it is required to maintain the animal in a cage without bedding until the animal has regained the righting reflex. The cage may be placed on a bedded or padded surface and suppliedwith extra bedding or supplemental heat. Water-circulating heating and/or warming blankets are recommended instead of electrical heat sources. Electrical heating blankets must be covered to avoid direct contact with the animal and justification must be provided. Do not place animals directly under a heating lamp, as it may cause thermal burns. Heat lamps are not allowed for use with rodents.

d) Dehydration can be ameliorated by the administration of appropriate fluid therapy. Initially this may be done by giving 1 to 2 ml of warm (approximately 37°C) sterile fluids (Saline solution, 0.9% NaCl, or Lactate-Ringers Solution) 0.5-1 ml SC or IP for mice and 5-10 ml SC or IP for rats. If blood loss occurredduring the surgical procedure or if the animal is slow to recover from the anesthetic, additional fluids maybe necessary.

e) During the recovery process, animals must be monitored continually until they gain the righting reflex.

f) If recovery from the anesthetic will be prolonged (i.e., over one hour), the animal must be rotated from side to side every 15-30 minutes to minimize atelectasis (collapse) of the lungs. This practice must be continued until the animal regains the righting reflex and/or call the LARC/Institutional Veterinarian for guidance

g) After surgery, animals must be evaluated daily for at least five days by a member of the principal investigator's staff to whom post-operative care has been delegated. Animals must be monitored

for evidence of excessive inflammation at the incision site, suture dehiscence (incision line failure or separation), infection, behavioral abnormalities indicative of illness (anorexia, listlessness, lethargy, dehydration, ruffled coating, lack of movement, weight loss greater than 10%). If evidence of wound infection or illness is noted, then LARC vet services must be contacted for evaluation and treatment, or the animal must be euthanized as soon as possible.

h) External sutures, staples, and wound clips must be removed 10-14 days after surgery, unless otherwiseapproved in the protocol or approved by the Institutional Vet.

i) If infections or complications occur, the LARC veterinary staff must be notified immediately.

8. Surgical Records

a) A Surgical Record must be completed immediately after the surgical procedure is performed. Recordsmay be somewhat abbreviated and in composite format and can be included as part of the research datacollected, but must also be available for review. Please see the example of the surgical record that is available for your use in the LARC, at the end of this document.

b) Records must identify the type of surgical procedure performed, the date of the procedure, the person who performed the procedure (or initials), information on all drug administration (including anesthesia and analgesia), and peri-operative monitoring, and must be maintained by the laboratory. This information must be available for review by regulatory bodies, including the IACUC.

9. Suture Selection

a) Close surgical wounds using appropriate techniques and materials. The following table is a guide to the types of sutures and other methods of skin closure available.

Suture	**Characteristics and Frequent Uses**
Vicryl®, Dexon®	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
PDS®, Maxon®	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Prolene®	Non-absorbable. Inert. General skin closure.
Nylon	Non-absorbable. Inert. General skin closure.
Silk	Non-absorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Excellent handling. Preferred for cardiovascular procedures. Must not be used to suture skin.
Chromic Gut	Absorbable. Versatile material. Because gut is highly reactive to tissues, its use is discouraged.

Other Methods of Skin Closure	Characteristics and Frequent Uses
Stainless Steel Wound Clips, Staples	Non-absorbable. General skin closure. Sizes may vary; choose appropriately according to wound size. Place them appropriately 5-8 mm apart.
Tissue Adhesives - surgical glue (2-Octyl or n-butyl Cyanoacrylate)	Generally used in addition to skin sutures or incisions less than 1 cm in length. Note that many rodents will rip the glue out, along with large areas of skin, making it difficult to close the incision. Thus, surgical glue is contraindicated as the primary method of closure when the wound is under tension.

10. Exceptions

All planned deviations from this policy must be approved by the IACUC prior to the performance of the surgical procedure. Emergency situations that involve deviations from IACUC-approved procedures must be reported to the Institutional Vet and IACUC committee within one week of its occurrence.

11. Example of surgical record

Date of SX Procedure _____ Procedure _____
Protocol # _____ PI _____ ID Info _____

Emergency Contact: _____ Phone Number _____

Anesthesia: _____

DRUG	DOSE	ROUTE	TIME WHEN GIVEN

Analgesia: _____ (drug, dose, route)

DATE					
AM					
PM					

Antibiotic: _____ (drug, dose, route)

DATE					
AM					
PM					

POST-OPERATIVE MONITORING

Post-operative monitoring must be continued for at least 5 days after surgery

DATE/TIME	REMARKS (Incision, Pain, Add'l Medications)	INITIALS

Related policies

Investigators must comply with all other institutional policies at TTUHSC El Paso and Federal Guidelines. This list includes, but is not limited to, the following:

IACUC Policy #2: Veterinary Care
IACUC Policy #4: Pain Categories for Experimental Protocols
IACUC Policy #9: Rodent Euthanasia
IACUC Policy #15: Survival Surgery
IACUC Policy #19: Humane Endpoints Regarding Severe or Chronic Distress
IACUC Policy #21: Use of Non-Pharmaceutical Grade Compounds
IACUC Policy #24: Expired Drugs