TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER (TTUHSC)
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
POLICY [# 8]

RODENT SURVIVAL SURGERY AND EUTHANASIA

Surgeries on rodents must comply with “PROCUREMENT, HOUSING AND ACCOUNTABILITY OF RESEARCH ANIMALS” policy (# 12) with regard to procedures and housing outside of the local animal facility.

I. FACILITY:

1) 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.
2) 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.
3) The surgery area must be separate from the area where hair is removed from the animal.

II. TRAINING:

Professional and technical personnel and students who perform anesthesia, analgesia, surgery, and euthanasia 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, analgesia and euthanasia.

III. ANESTHESIA AND ANALGESIA SELECTION:

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

IV. PREPARATION OF THE ANIMAL:

1) The animal must be anesthetized with suitable anesthetic using the doses and procedure approved by the IACUC.
2) An ophthalmic lubricant must be applied to the eyes to prevent corneal drying.
3) Hair must be removed from the incision site with clippers, appropriate razor, and/or hair removal product (ie. Nair) applied as directed and thoroughly rinsed off to prevent continual residue action.
4) 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. Using a circular pattern, 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, lead to hypothermia, and therefore must be used with care. These agents may be sprayed or daubed onto the surgical site after the initial third scrub and rinse. The use of cotton tip applicators soaked with scrub/solution materials are ideal during the skin preparation process.

These surgical antiseptic agents may be used:
   a) Povidone iodine: A good choice for a surgical preparation with a broad spectrum of activity, including Mycobacterium. Antiseptic activity is rapid and persistent if not removed.
   b) Chlorhexidine: 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.

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

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

V. PREPARATION BY SURGEON:

1) 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.
2) A surgical mask must be worn to prevent contamination of the surgical field.
3) Gowns and surgical bonnets are optional but recommended in order 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.).
4) A new pair of sterile surgical gloves must be used for each animal. Alternatively, surgeons may wipe their gloves 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.
5) If working alone, the surgeon must have the animal anesthetized and positioned prior to gloving.
6) If the instruments are in a sterile pack, the first layer of the double-wrapped instrument pack must be opened before gloving.

VI. INSTRUMENTS:

1) 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.

a) Heat Sterilization:
   i) 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 121°C
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 (expiration is
one year from date of sterilization as long as the wrapping is not torn or
becomes wet). Note: steam is unsuitable for materials with low melting points.
   ii) Flash Steam: Used to sterilize articles intended to be used immediately. The
temperature must reach 132°C for three to five minutes.
   iii) Sterile (Hot) Bead Sterilizer: This instrument will sterilize the tips of metal
instruments in 15 seconds. However, the beads should be clean and 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.]

b) 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:
   i) Alcide® - Active ingredient: Chlorine Sodium Chlorite 1.37%. Exposure time
must exceed 6 hours. Shelf life is 14 days.
   ii) Cetylcidie-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.
   iii) 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.

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

v) Sporcidin® - 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.

vi) 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.

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

2) Rubber Tubing: The following methods can be used to sterilize rubber tubing:

a) Heat sterilization
b) Ethylene oxide gas
c) 6% hydrogen peroxide solution
d) VHP (volatile hydrogen peroxide)

3) 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 gluteraldehyde (10 min). However, instruments should be thoroughly clean of blood or tissue prior to sterilization. Instruments soaked in chemical disinfectants must be rinsed in sterile water or saline before use on animals. No more than five successive surgeries can use instruments “quick”-disinfected as described above.

VII. INTRAOPERATIVE MONITORING:

1) The animal must be monitored carefully during the surgical procedure. Specifically, the animal's respiratory rate and characteristic response to noxious stimuli (e.g., tail pinch, toe pinch, and when possible the heart rate and body temperature) will be monitored.

2) 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.

VIII. POST-SURGICAL CARE:

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

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

3) To prevent hypothermia, place the animal(s) in a warm room or cage. Do not place the recovering animal directly on the cage bedding. To prevent suffocation of the
animal, place a towel or drape over the bedding until the animal is awake. If necessary, the cage may be placed on a bedded or padded surface and supplied with extra bedding or supplemental heat as required. Water-circulating heating blankets are recommended instead of electrical heat sources. Heating blankets must be covered to avoid direct contact with the animal. Do not place animals directly under a heating lamp, as it may cause thermal burns.

4) 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 (0.9% NaCl or Lactate-Ringers Solution) per 100 gm of body weight by subcutaneous or intraperitoneal injection. If blood loss occurred during the surgical procedure or if the animal is slow to recover from the anesthetic, additional fluids may be necessary.

5) To prevent cannibalism or suffocation, rodents must be housed individually until they are ambulatory.

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

7) 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.

8) Post-surgical care of animals must be evaluated daily for at least five days by a member of the principal investigator's staff or other individuals 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.

9) External sutures, staples, and wound clips must be removed 10-14 days after surgery, unless otherwise approved in the protocol or approved by the LARC vet.

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

IX. SURGICAL RECORDS

1) A "Surgical Record" must be completed immediately after the surgical procedure is performed. Records may be somewhat abbreviated and in composite format and can be included as part of the research data collected, but must also be available for review.

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

1) Close surgical wounds using appropriate techniques and materials. The following table is a guide to the types of sutures that are available:

<table>
<thead>
<tr>
<th>SUTURE</th>
<th>CHARACTERISTICS AND FREQUENT USES</th>
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<tbody>
<tr>
<td>Vicryl®, Dexon®</td>
<td>Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.</td>
</tr>
<tr>
<td>PDS®, Maxon®</td>
<td>Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable</td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Excellent handling. Preferred for cardiovascular procedures. Must not be used to suture skin.</td>
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<tr>
<td>Chromic Gut</td>
<td>Absorbable. Versatile material. Highly reactive to tissues.</td>
</tr>
<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Nonabsorbable. General skin closure.</td>
</tr>
<tr>
<td>Cyanolacrylate surgical glue</td>
<td>Generally used in addition to skin sutures or incisions less than 1 cm in length.</td>
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XI. EUTHANASIA:

See policy # 9: ‘Inhalant Euthanasia’.

XII. EXCEPTIONS:

All planned deviations from this policy must be presented to and 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 iVet and IACUC committee within one week of its occurrence.