The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) is charged with ensuring that all surgical facilities and procedures meet the criteria set by the federal regulations, including the Animal Welfare Act (AWA), the Animal Welfare Regulations (AWR), and the Public Health Service Policy (PHS). The PHS requires institutions to comply with the performance-based standards in the Guide for the Care and Use of Laboratory Animals (Guide).

AWR 2.31 (d)(ix)
“Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques.”

AWR 2.33 (b)(4-5)
“Each research facility shall establish and maintain programs of adequate veterinary care that includes: (4) Guidance to principal investigators and other personnel involved in the care and use of animals regarding handling, immobilization, anesthesia, analgesia, tranquilization, and euthanasia; and (5) Adequate pre-procedural and post procedural care in accordance with current established veterinary medical and nursing procedures.”

PHS Policy VI.C.1.
“...the IACUC shall confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the Guide unless acceptable justification for a departure is presented.”

Guide 2011
“The attending veterinarian should provide guidance or oversight to surgery programs and oversight of postsurgical care involving animals.”

The purpose of this guideline is to clarify the requirements of the Principal Investigator (PI) and the Institution concerning surgical procedures performed on rodents. All investigators, laboratories, and facilities performing surgery (survival and terminal) on rodent species must adhere to the minimum standards addressed in this IACUC guideline. Species typically studied at Penn that are relevant to this guideline include: mice, rats, gerbils, guinea pigs, and hamsters. Surgery involving USDA rodents (hamsters, gerbils, guinea pigs and other non-Mus and non-Rattus rodent species) may follow most procedures as for mice and rats (e.g. facilities), but there are specific requirements that are more consistent with large animal species (e.g. instruments, documentation); these specific requirements are highlighted below in each section.

This guideline offers direction on the following topics:
- Pre-surgical approval and assessment
- Requirements of a surgical area
- Preparation of the surgeon, animal, and surgical instruments
- Aseptic technique
- Anesthesia

APPROVED: 09/25/12
Monitoring the anesthetized patient
Anesthetic and postoperative recovery
Recordkeeping

**PRE-SURGICAL APPROVAL AND ASSESSMENT**

Before performing surgery on any species, the PI must obtain IACUC approval of the research activity. Attaining approval to perform surgery is at least a three-step process:

1. The Institution, during the IACUC review process, evaluates the **surgical training qualifications** of all participants on protocols involving surgical procedures. The PI assures and describes personnel qualifications.
2. The IACUC provides **protocol approval** of the surgical project.
3. IACUC members evaluate the location and provide **surgical location approval** for rodent survival surgeries. Rodent terminal surgery locations are evaluated by the Office of Animal Welfare compliance staff.

**Surgical Training Qualifications**

“Researchers conducting surgical procedures must have appropriate training to ensure that good surgical technique is practiced – that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns” ([Guide](http://example.com)).

Prior to IACUC approval of protocols and/or amendments to add personnel, the “**Regulations and the IACUC**” and “**Species Specific Training**” and “**Aseptic Technique**” must be completed and documented for personnel listed. As additional surgical training opportunities become available they may be required by the Institution. The PI is responsible for ensuring that personnel involved with anesthesia induction, monitoring of an anesthetized animal, direct surgical manipulation, or other advanced techniques receive additional training if necessary. If there are any questions about surgical training or scheduling, please contact ULAR Training ([ULAR-tr@pobox.upenn.edu](mailto:ULAR-tr@pobox.upenn.edu)).

**Protocol Approval**

Any surgical protocol is specifically assigned to a veterinarian for review, in addition to the primary IACUC-designated review. This “vet review” emphasizes the appropriateness of the species used as the surgical model, the surgical procedure itself, anesthesia and analgesia regimen, and completeness of pre- and post-surgical care plan.

The scenario where an animal on a protocol is used for multiple survival surgeries must be specifically reviewed and approved by the IACUC ([Guide](http://example.com)) ([AWR 2.31 (d)(x)](http://example.com)). As part of the “Survival Surgery” procedure in ARIES, please select “yes” for “Multiple survival surgery?” in the associated questions if at least one animal on the protocol is surgicated, recovered, surgicated again, and recovered again, i.e. 2+ survival surgeries. Then provide an explanation or justification why multiple survival surgeries are necessary. If all animals assigned to the protocol will undergo just one survival surgery procedure, to be eventually followed by a terminal surgery or euthanasia, then select “no” for “Multiple survival surgery?” in the associated questions.

**Surgical Location Approval**

Approval of the IACUC protocol covers the “act” of the surgery. If the pre-, peri-, and post-surgical manipulation and care of the animal occur in a ULAR-operated facility, approval of the protocol is all that is required.
If the procedures are planned to be performed in a non-ULAR operated facility, then (a) the protocol must include a justification as to why a ULAR procedure room is unsuitable for the surgical procedure, and (b) the location must first be evaluated and approved by IACUC members. This site evaluation occurs independently of the IACUC protocol review process and must be specifically scheduled by the PI or senior research staff through contacting the Animal Welfare Compliance Staff at 215-746-6271 or the IACUC office at 215-898-2615.

No surgical procedures may be performed on an animal until the IACUC protocol and the surgical location have been approved. All personnel must be properly trained and listed on the IACUC protocol.

REQUIREMENTS OF A SURGICAL AREA

“For most surgical programs, functional components of aseptic surgery include surgical support, animal preparation, surgeon’s scrub, operating room, and postoperative recovery. The areas that support those functions should be designed to minimize traffic flow and separate the related, nonsurgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers but might also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities” (Guide).

Separated Facilities

“When determining the appropriate location for a surgical procedure (either a dedicated operating room/suite or an area that provides separation from other activities), the choice may depend on the species, the nature of the procedure (major, minor, or emergency), and the potential for physical impairment or post-operative complications, such as infections” (Guide).

PIs and research staff are strongly encouraged to perform survival surgery in the main animal facility’s procedure rooms. If performing survival surgery in a vivarium procedure room is not possible, then the following items should be considered for your laboratory space.

- The working surface (e.g. bench top, hood, etc.) and surrounding equipment (e.g. shelves, chairs, etc.) should be constructed of materials that are easily sanitizable.
- The specific surgical area should not be located under air vents to minimize contamination and reduce the risk of postoperative infection.
- The immediate area should not be cluttered or used for storage of equipment unrelated to the surgical procedure. Storage containers should be easily sanitizable. Cardboard and Styrofoam should be avoided.
- “All surgery on rodents [does] not require a dedicated facility” (AWR 2.31). Survival surgery involving USDA rodents may be performed in adequately organized and maintained laboratory areas, separated from other laboratory activities. This area should not be adjacent to a high traffic through way—an alcove or otherwise limited use area should be considered.

Surgical Support

“The surgical support area should be designed for washing and sterilizing instruments and for storing instruments and supplies. Autoclaves are commonly placed in this area” (Guide). Sink areas should clean and free of rust. Instruments should be stored in suitable containers to keep them clean and in good condition. Laboratories must have the capability to sterilize surgical equipment, e.g. access to autoclaves, plasma sterilizers, etc.
Animal Preparation
Attention should be given to maintain a clean operating area at all times. Therefore, the preparation of the animal (e.g. clipping of fur, intubation, etc.) should not be done in the immediate operating area. There should be a separate but adjacent area where the animal will be physically prepared to undergo a surgical procedure. This area may double as a recovery area after conducting proper cleaning procedures.

Surgeon’s Scrub
Surgeons and surgical assistants must wash their hands with an antibacterial soap prior to initiating the surgical procedure. To avoid contamination with aerosols released during scrubbing, this surgeon prep area should be separated from the operating area (Guide).

Operating Area
Operating areas must be kept clean; items that are not easily sanitized (e.g. rusty equipment, cardboard boxes, etc.) should not be in this area. The area should be sanitized between animals.

Minimize Contamination
“Inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses affecting surgical success, animal well-being, and research results” (Guide 2011). The entire surgical area must be maintained in such a manner as to reduce risk of subclinical infections in the animal patient. Cleanliness is an essential and integral part of the surgical process. Standard operating procedures should be developed and used to ensure the surgical area is routinely cleaned and sanitized.

Survival surgery must be performed using aseptic technique in an approved surgery area containing space for animal prep, surgeon’s scrub, the operating area, and post-operative recovery. This area must be kept clean before, during, and after surgeries.

Aseptic Technique
“Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. No procedure, piece of equipment, or germicide alone can achieve that objective... Aseptic technique includes preparation of the patient... preparation of the surgeon... [and] sterilization of instruments” (Guide). “All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques” (AWR 2.31).

Patient
How the surgeon or assistants will prepare the animal should be described in the “description” field of the ARIES Survival Surgery procedure. Preparing the animal for surgery is at least a two-step process:

1. Animal Preparation
   - Remove the hair (clip, depilatory cream) in a wide area around the intended surgical site.
   - Clean the hair and any other gross debris from the skin with an alcohol pad.
   - If clippers are used, please ensure any fur is removed and the clippers are cleaned after each day.

2. In the Operating Area
   - The animal must be properly secured to the operating table e.g. tying with gauze, umbilical tape, or other suitable restraint. “Pinning” (use of pins or hypodermic needles) of live animals for survival and non-survival surgeries is PROHIBITED.
Disinfect the area with appropriate surgical scrub. Alcohol alone is NOT an appropriate disinfectant. Iodophors (e.g. Betadine) or chlorhexidines (e.g. Nolvasan) should be used.

Sterile drape should be placed over the animal. Transparent drapes are recommended in order to continuously monitor breathing.

**Surgeon and Surgical Assistant(s)**
All personnel taking part in the surgery must:
- Wear clean lab coat, scrubs, or appropriate disposable gown.
- Wear appropriate face mask.
- Wash hands with antiseptic soap.
- Wear sterile gloves.
- Move carefully to avoid contamination of the surgical location.

Gloves must be replaced if aseptic technique is disrupted, e.g. touching the isoflurane vaporizer with the sterile gloves, moving the animal with sterile gloves, etc. With proper planning, simple survival rodent surgeries may be performed by one person. If this cannot be accomplished because of the complexity of the procedure, then in order to consistently maintain aseptic technique, there must be a surgical assistant or anesthetist who is trained to perform such tasks that would interfere with proper aseptic technique. If it is necessary for the surgeon to leave the surgical area during a procedure, then s/he must re-glove again before resuming surgery.

**Instruments**
It is extremely important to ensure that all instruments are appropriate for surgery
- All instruments must be cleaned and sterilized prior to the beginning of each surgical session. Alcohol alone is NOT a sterilant. Examples of methods of sterilization include steam autoclave, gas (e.g. ethylene oxide), and plasma sterilization.
- Cold sterilization (e.g. Cidex) of surgical instruments must strictly follow manufacturer instructions. The FDA lists specific cold sterilants and the necessary conditions to be considered a sterilant or a disinfectant. Rinse with sterile water or sterile saline before using on an anesthetized animal.
- For mice and rats, if instruments are to be used for multiple surgeries in a single session, they must be sterilized between animals. Hot bead sterilizers are recommended in these cases.
- For USDA rodents, just as in larger USDA species, new sterile instruments must be used for each animal. Hot bead sterilizers are not suitable, but consideration may be given to cold sterilization following the manufacturers’ instructions. New autoclaved, gas sterilized, or plasma sterilized packs are recommended for each animal.
- Do **not** use dull or rusted surgical instruments or those not manufactured for surgical use.

*For any surgical procedure to be successful, steps must be taken to ensure that the animal is properly prepared for surgery, the surgeons are outfitted appropriately, and the surgical instruments are sterilized.*

**ANESTHETICS AND ANALGESICS**
"Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals" ([PHS Policy](#)).

When writing a procedure, one must provide the maximum relief of pain/distress possible while maintaining the integrity of the research. Every surgical IACUC protocol **must** describe a clear plan for providing in-date,
pharmaceutical grade injectable or inhalation anesthetics and a description of how and when analgesics will be administered. In order to provide flexibility when performing the procedure, it is recommended to include more than one appropriate option for the anesthetic and analgesic regimens.

In-Date, Pharmaceutical Grade Compounds
“Expired drugs may not be used in any laboratory animals, and expired materials cannot be used, regardless of the species, for survival surgeries.” Pharmaceutical grade anesthetics and analgesics “should be used, when available, for all animal related procedures” (Guide). See the IACUC Policy on the Use of Expired Drugs and Materials in Laboratory Animals for more details. Also see specific guidance from OLAW on this topic, which includes acceptable justifications for using non-pharmaceutical grade drugs.

Injectable Anesthetics
Animals should be weighed prior to surgery to calculate the appropriate dose of anesthetics for the intended route of administration. Contact a ULAR Veterinarian or consult the Rodent Anesthesia and Analgesia Formulary Guideline for suggested drugs, doses, and route of administration for your species and procedure.

Inhalation Anesthetics
Inhalant anesthesia is the preferred method of general anesthesia for rodents. Vaporizers must be used to deliver inhalant anesthetics to rodents. Anesthetics must be scavenged with appropriate devices or methods. Isoflurane vaporizers are available in most ULAR procedure rooms. Contact ULAR Training for assistance if personnel require training in using an anesthesia machine. Also, anesthesia machines should be maintained in good working condition and regularly recertified. Please refer to the IACUC Guideline for Anesthetic Vaporizers & Gas Scavenging for more information. Use of bell jars is prohibited for delivering anesthetics for any surgical procedure.

Analgesics
Surgery is considered a painful procedure; therefore administration of analgesics is required for any animal that undergoes a surgical procedure. Analgesics must be administered before an animal is expected to be painful, not after significant signs of pain are noted. The goal is to have every animal patient maintained post-surgically in a pain-free state. Animals given pre-operative analgesia often require less anesthetic to reach a surgical plane, and thus may be more stable anesthetic patients. Pre-emptive analgesia is recommended. The analgesic options available to investigators are very varied and certain drugs may provide better pain relief for patients undergoing certain procedures. Contact a ULAR Veterinarian for suggested drugs and doses that will best suit the surgical research model.

Animals must be anesthetized during any surgical procedure and be provided appropriate analgesics to be maintained in a pain-free state.

MONITORING THE ANESTHETIZED PATIENT
“Careful surgical monitoring and timely attention to problems increase the likelihood of a successful surgical outcome” (Guide). Careful surgical monitoring includes confirmation of anesthetic depth, maintenance of anesthesia, and monitoring of vital signs.

Confirmation of Anesthesia Depth
The animal must be maintained at an appropriate depth of anesthesia beginning immediately before the surgical procedure is initiated, through the conclusion of the procedure, and until the post-operative analgesics should
IACUC Guideline
RODENT SURVIVAL SURGERY

have taken effect. For most species, the following techniques can be used to ascertain that the animal is appropriately anesthetized.

- **Toe pinch.** Brief clamping of the web of skin between toes or claws with a hemostat or fingers. Firmly pinching multiple toes should not elicit a withdrawal response from an animal at a surgical depth of anesthesia.
- **Palpebral reflex.** Gently tapping the medial canthus of the animal’s eye should not elicit a blink or eye flutter. This technique is not always reliable in all animals.
- **Corneal reflex.** Touching the edge of the cornea with a gauge sponge or cotton swab will produce a good reflex if the patient is too light on anesthesia. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.
- **Vital signs.** Heart rate and respiratory rate may increase if anesthetic depth becomes too light.

**Maintenance of Anesthesia**

Each animal responds slightly differently when under anesthesia, therefore it may be necessary to modify your use of anesthetics during the procedure. **All routinely used anesthesia options must be described in the IACUC protocol.** Anesthetists must be trained in not only delivering the anesthetic to the patient, but also in identifying anesthetic related problems.

- Increases and decreases in vital signs may require modifications in anesthetic dosing.
- If, at any time, an animal begins to respond to pain or attain an anesthetic depth that is too light, stop the procedure and adjust the inhalant anesthetic level or give a supplemental dose of injectable anesthetics. Reassess the animal before resuming work.
- Animals must be continually monitored by the anesthetist providing appropriate anesthesia and life support for the duration of the procedure. **Anesthetized animals should NEVER be left alone during the procedure.**
- In order to maintain sterility during complex surgical procedures and to properly monitor the animals, it may be necessary to include a second person in the procedure—a surgical assistant or anesthetist.

**Monitoring Vital Signs**

The anesthetist should continuously monitor the animal patient’s basic physiological function for the duration of the procedure—from induction through recovery. The following vital signs should be monitored during all procedures involving mice, rats, hamsters, and gerbils (see Rodent Anesthesia Record Template for a suggested template):

- **Respiratory Rate (RR)** can be assessed by watching the rising and falling of the chest. Subjective changes should be addressed by altering the depth of anesthesia.
- **Body temperature** should be maintained near normal during surgical procedures.
  - Hypothermia often occurs due to anesthesia-induced vasodilation and from surgery via opened body cavities.
  - During any surgical procedure, the animal’s body temperature should be maintained by a heat lamp, a [covered] recirculating water heating pad or forced-air warming (Bair-Hugger).
  - **ULAR Veterinarians** may assist in choosing an appropriate method for your species.

Guinea pigs’ vital signs should be documented using the **ULAR anesthesia sheet,** or a similar template, including heart rate (HR), RR, and body temperature every 5-10 minutes. Pulse oximetry is recommended.
Vital signs for common rodent laboratory animal species (ACLAM 2002).

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>TEMPERATURE</th>
<th>RESPIRATORY RATE</th>
<th>HEART RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICE</td>
<td>96.6 - 99.7 F (35.8 - 37.4 C)</td>
<td>90 - 220 per minute</td>
<td>328 - 780 per minute</td>
</tr>
<tr>
<td>RATS</td>
<td>96.6 - 99.5 F (35.9 - 37.5 C)</td>
<td>66 - 144 per minute</td>
<td>250 - 600 per minute</td>
</tr>
<tr>
<td>GUINEA PIGS</td>
<td>98.6 - 103.1 F (37 - 39.5 C)</td>
<td>42 - 104 per minute</td>
<td>230 - 320 per minute</td>
</tr>
<tr>
<td>HAMSTERS</td>
<td>98.6 - 100.4 F (37 - 38 C)</td>
<td>35 - 120 per minute</td>
<td>250 - 600 per minute</td>
</tr>
<tr>
<td>GERMILS</td>
<td>96.3 - 102.7 F (35.7-39.3 C)</td>
<td>70 - 120 per minute</td>
<td>260 - 600 per minute</td>
</tr>
</tbody>
</table>

A well-trained anesthetist with experience with the species under investigation must continuously monitor the animal's vital signs and maintain the surgical patient at an appropriate plane of anesthesia throughout the surgical procedure.

ANESTHETIC AND POSTOPERATIVE RECOVERY

"An important component of postsurgical care is observation of the animal and intervention as necessary during recovery from anesthesia and surgery. The intensity of monitoring will vary with the species and the procedure and might be greater during the immediate anesthetic recovery period than the postoperative recovery period."

Immediate Recovery Period

"Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and postoperative pain or discomfort during recovery from anesthesia" (Guide). The immediate recovery period may last from minutes to hours.

- Animals should be placed into a clean recovery area (with clean paper towel on bedding) in sternal or lateral recumbency. Sternal recumbency is preferred.
- During anesthetic recovery, the animal’s body temperature should be supported with an appropriate, well-maintained heating device (e.g. recirculating water heating pad, or heat lamp). To avoid burning, be cautious that the heating device is not too hot or too close to the animal. If a heat lamp is used, the rodent cage should be covered solid lid (e.g. microisolator top). There should always be a cooler location in the enclosure to which the animal can escape if they become too warm.
- Recovering animals should not be in the enclosure with awake animals.
- Personnel monitoring recovery of animals must remain in in the same room as the animals at all times. Only when animals have regained all postural reflexes and are ambulatory (can walk well on their own) should they be left alone in their regular, freshly-cleaned housing. Animals must be specifically assessed at least every 10 minutes until they have completely recovered from anesthesia.
- Recovered animals must be returned to the facility housing room when alert and active.

Long Term Recovery Period

"After anesthetic recovery, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and behavioral signs of postoperative pain" (Guide). Depending on the surgical procedure, the postoperative recovery period may last from days to weeks.

- During the post-surgical period, animals must be appropriately monitored for signs of pain and/or distress. In most species, signs of pain include decreased activity, abnormal posture, increased attention to surgical site, and gait abnormalities.
The cardinal signs of infection including heat, swelling, redness, pain, and/or exudation. Consult a ULAR Veterinarian for any abnormal medical condition.

The frequency and length of observation may depend on the degree of invasiveness of the procedure and the individual animal. A written plan of observation must be outlined in the IACUC protocol for each procedure proposed. The IACUC recommends the PI or the lab staff observe animals at least daily for a minimum of three days following major procedures.

The IACUC protocol must fully and clearly describe the clinical signs expected to be observed following the surgical procedure and the humane endpoints that may necessitate euthanasia or other removal from the study. Please refer to the IACUC guideline Humane Intervention and Endpoints for Laboratory Animal Species for more information.

If the health of the animal is questionable, a ULAR veterinarian should be notified for treatment recommendations.

“The attending veterinarian [or designee] retains the authority to change post-operative care as necessary to ensure the comfort of the animal” (USDA Policy #3). The IACUC supports the professional judgment of ULAR veterinarians. The Attending Veterinarian has direct or delegated program authority and responsibility for activities involving all animals at the institution as defined under the Animal Welfare Act and PHS policy. At Penn, the Attending Veterinarian is the Director of ULAR.

The IACUC requires that peri-/preoperative and postoperative analgesics are used for all major invasive procedures, and for procedures that have the potential for causing pain in laboratory animals (USDA Policy 11). Contact a ULAR Veterinarian or consult the Rodent Anesthesia and Analgesia Formulary Guideline for recommended analgesic agents.

After surgery, animals must be monitored and given appropriate veterinary treatment during anesthetic and postoperative recovery.

RECORDKEEPING

The USDA and PHS require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. Surgical records for rodents may be kept in laboratory notebooks, binders, or computer databases. Please ensure the following records are legible, organized, and available upon request. A group of mice, rats, gerbils, and hamsters may be documented on a single surgical record. Guinea pigs should have individual surgical and postoperative records. Associated with this guideline are templates of acceptable logs for surgical and postoperative documentation.

- Surgical or procedural records. These should include the protocol number, animal identification, date of surgery, surgeon’s initials, weight of animal prior to surgery, anesthesia and dose, procedure title or brief description, any deviations from the approved procedure (i.e. supplemental doses of anesthetics), pre- or intra-operative analgesics, and the time returned to the facility.

- Post-operative records. These should include the protocol number, animal identification, and initials of individuals making observations, date of observation, a comment on the general condition and health of animal, analgesics and/or other medications given. The specific date, time, and amount (in mg) of the analgesics administered should be written into the animal’s (or group’s) post-op record.