

Office of Research Support and Compliance

Vice President for Research, Scholarship and Creative Endeavors

Guidelines for Surgical and Anesthetic Procedures in Rodents

The University of Texas at Austin
Institutional Animal Care and Use Committee

These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements

This document provides information to be used when planning and performing survival surgical and anesthetic procedures in rodents used for research, teaching, or other purposes at The University of Texas at Austin. It is organized into five sections:

Section A – Definitions

Section B – Requirements

Section C – Specific Considerations for Surgical Procedures

Section D – Additional Sources of Information on Disinfectant, Sterilants, Sterilization Guidelines and Wound Closure Materials

Section E – References and Acknowledgements

Section A – Definitions

Non-survival surgery is defined as a surgical procedure after which the animal will not regain consciousness prior to euthanasia.

Survival surgery is defined as a surgical procedure after which the animal will be allowed to recover consciousness, even for a short time.

Minor surgery is a procedure that involves surgical manipulation but does not meet the definition of major surgery. Typical examples include operative procedures in which only skin or mucous membranes are incised, e.g., vascular cutdown for catheter placement or implantation of minipumps in subcutaneous tissue. Also included are minimally invasive means of accessing a body cavity, such as needle biopsy or the introduction of instruments using a trocar.

Major survival surgery is defined as a survival surgical procedure that involves penetration and exposure of a body cavity (abdomen, thorax or cranium) or will produce a substantial physical or physiologic impairment.

Multiple major survival surgery occurs when two or more separate major survival surgical procedures are performed on a single animal. It is allowable only under certain circumstances, the most common being a situation in which each surgical manipulation is an essential and related part of a single study. Cost alone is not an adequate justification for performing multiple survival surgeries on the same animal. Multiple major survival surgeries must be specifically justified by the PI and approved by the IACUC as part of an approved protocol.

Aseptic (sterile) surgical techniques are well-established methods used to avoid the introduction of microbial contamination into tissues exposed and/or manipulated during surgery. More details are provided below.

Non-invasive procedures under anesthesia are those where animals are immobilized but not undergoing a painful procedure. Examples include injections, imaging, suture removal, etc.

Section B – Requirements

1. Survival surgical procedures must be performed in either a dedicated animal procedure room or in a suitably located and dedicated laboratory area, subject to approval by the IACUC. These procedures cannot be done appropriately within an animal holding room. The bench or tabletop areas used for animal prep, surgery, and recovery should have only the ancillary laboratory, diagnostic, or clinical equipment and supplies needed to support the procedure being performed. Equipment used on an infrequent basis and bulk supplies should be stored elsewhere. The area should be free of physical or chemical hazards potentially arising from splashes, spills, etc. A low-traffic area of the laboratory away from doorways, hoods, etc. is optimal. For most procedures a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery.
2. Aseptic (sterile) technique for survival surgery is required for ALL species. However, some modifications are allowed when rodents are involved. For example, the use of surgical drapes in small species may not always be necessary because a) the incisions are very small, and organs are rarely exteriorized during the procedures and b) drapes may impair the ability to assess breathing patterns or other monitoring criteria used to assure proper anesthetic depth; however, the use of a sterile drape is best practice. Glad Press and Seal (Emmer et al., 2019) or other clear sterile drapes allow good visualization of the patient during surgery while also allowing for optimum sterility during surgery. The need for sterile gowning of the surgeon is also considered optional for surgery involving rodents. However, the surgeon should wear a surgical mask and clean laboratory coat. Unless a strict “Tips Only” method is being used (as described below), sterile surgical gloves ARE required.
3. A proper method of anesthesia and analgesia must be selected, and protocol approval must be obtained prior to initiation of anesthesia. Anesthesia should be complete, i.e., a single drug or a combination of agents must be used to induce a loss of consciousness, hyporeflexia, muscle relaxation and analgesia. For consultation on best-practice anesthesia protocols, contact the ARC clinical veterinary staff or refer to the Rodent Analgesic Guidance document that can be found here (<https://research.utexas.edu/arc/arc-guidance/rodent-anesthesia-and-post-op-monitoring-guidance/>). Surgery or potentially painful procedures must not be performed on non-anesthetized animals paralyzed by chemical agents. When gas anesthetics are used, appropriate gas scavenging methods must be employed to prevent hazardous exposure of personnel. SOPs should be developed per EHS guidelines to handle, scavenge and dispose of gas anesthetics.
4. Non-survival surgery is a procedure during which the animal is euthanized before recovery from anesthesia. It may not be necessary to follow all the requirements outlined in this guideline if non-survival surgery is performed, but at a minimum the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. NOTE: As an alternative to clipping the fur, wetting down the hair with alcohol prior to incising the chest is sufficient when rapid transcatheter perfusion prior to tissue harvest is required by the study.
5. Expired anesthetics, analgesics and drugs used for euthanasia may not be used in any animals without explicit IACUC approval (See Guidelines for the Use of Drugs and Chemicals in Animal Research). Expired medical materials (e.g., suture, fluids, etc.) may be used for non-survival procedures, provided that their usage does not adversely affect the animal’s well-being or compromise the validity of the scientific study. Expired materials must be clearly labeled, e.g.: “Expired: For use in terminal procedures only” and stored in such a way that they can be readily distinguished from unexpired materials (e.g., in a separate cabinet or in a separately labeled box).

6. For non-survival procedures of extended duration, attention to aseptic technique may be more important to ensure stability of the model and a successful outcome. A veterinarian should be contacted for a consultation if you are planning acute surgical procedures of more than a few hours duration to determine whether sterile techniques are indicated. Eating, drinking, or smoking is not acceptable in non-survival surgery areas, and locations used for food handling purposes do not qualify as acceptable areas for performing surgeries. Anesthetic records are required for non-survival procedures lasting more than 15 minutes, and euthanasia agents and/or methods should also be documented.

Section C-Specific Considerations for Surgical Procedures

Preparation of the Surgeon: The surgical procedure itself must be performed or directly supervised by a trained and experienced individual. This training must be documented on the IACUC internal training record. Personnel unfamiliar with aseptic surgical procedures or who have questions about surgical techniques should contact the ARC veterinary staff for information or training.

Other requirements for surgeon preparation:

- A clean lab coat and face mask are required for rodent surgeons.
- Wash hands with soap (minimal) or an antiseptic surgical scrub solution (optimal).
- Sterile surgical gloves must be donned prior to handling sterile instruments or touching surgically-exposed animal tissues (unless using tips only method).
- Long hair of surgeons and assistants should be covered and/or restrained to keep it away from the surgical field.

Preparation of Surgical Space: Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery. Clean and disinfect the surface upon which surgery will be performed. Use an appropriate cleaner to clean the area if visibly soiled, followed by a hard surface disinfectant that is labeled for hospital, laboratory, or veterinary use. Use of absorbent “chucks” or bench lining paper can contain liquids and facilitate cleanliness in surgical areas, but these disposable items should be changed and discarded at the end of the surgical procedure.

Preparation of Surgical Instruments: Surgical instruments must be sterilized prior to use for survival surgery. Organic material must be removed by cleaning prior to sterilization. If sterile packs are stored for later use, they must be dated with the preparation date and an expiration date. It is recommended that all sterile supplies be stored in a vermin proof, enclosed cabinet. Methods used for sterilization may vary, but all must conform to established medical standards for complete sterilization. For a full explanation of sterilization requirements please see IACUC sterilization guidelines.

Options for sterilization include:

- Steam sterilization at proper pressure and exposure times (autoclave)
- Prolonged immersion in a hospital-grade peroxide, formaldehyde- or glutaraldehyde-based cold sterilant following label directions (alcohol immersion is NOT acceptable)
- Ethylene oxide gas (ETO) used in a specialty chamber
- Dry heat sterilization (hot bead sterilization) at proper temperature and exposure time (not appropriate for initial sterilization)

Re-sterilization Requirements: Instruments must be re-sterilized between animals (**maximum 4 animals per pack, i.e., instruments cannot be bead-sterilized more than 3 times**) when performing multiple surgeries within the same day. Instruments handles and other parts that are not re-sterilized, as well as gloves that touch these parts, are no longer considered sterile and should not touch the surgical site or contaminate the sterile field. Prior to placement in the hot-bead sterilizer, blood, tissue and all organic material on instrument tips should be removed.

Place in glass bead sterilizer for time recommended by manufacturer. Care should be taken to ensure that the instrument surfaces have cooled sufficiently before touching animal tissues to minimize risk of burns. A new set of sterilized instruments must be opened and used after 4 animals. If the instrument tips become contaminated during a procedure, they must be re-sterilized or a new set of sterile instruments should be obtained.

Tips Only Technique: “Tips only” aseptic practices are considered appropriate for survival surgeries in rodents that involve only very small incisions, e.g., embryo transfer, gonadectomy, intracelomic placement of a pellet or transponder, etc. During these procedures, it is not necessary for the surgeon to directly touch the tissues and the likelihood of inadvertent contact with exposed tissues is minimal. In this case, the emphasis of aseptic surgical practices will be to keep the tips (which enter the body) of the instruments free of contamination during the procedure. “In this situation, sterile gloves are not required, and after gross debris is removed, the instrument tips can be sterilized between animals by immersion in a hot bead sterilizer (or an acceptable disinfectant as described above). If this method is chosen, the surgeon must be very cautious to avoid contact with the surgical site or the working surfaces of the instruments, and to keep the tips free of contamination by having a sterile location to place the instruments if they are put down during the procedure. Animal users can consult with the veterinary staff to determine if the “tips only” method is appropriate for their procedure.

Intra-Operative Considerations (Patient)

Preparation of Patient: During anesthesia, the eyes of any rodent should be thoroughly lubricated with Puralube® or a similar product to prevent corneal desiccation and post-operative ocular complications. The analgesics specified in the approved protocol for relief of pain and/or distress must be readily available for use as described in the approved animal use protocol. Individuals must have been provided training to appropriately administer analgesics. Analgesics should be administered before or very early in the surgical procedure in order to take effect before the animal recovers from anesthesia. Fluids, analgesics, and antibiotics must be administered as indicated in the protocol or as directed by the ARC veterinarians or designee.

Pre-Anesthetic Fasting: Pre-anesthetic fasting is usually not necessary in rodents due to their inability to vomit. However, if fasting is employed (e.g., to stabilize body weights before anesthesia) limit to no more than 2-3 hours due to the high metabolic rate of small rodents. Never restrict water.

Thermal Support: Most anesthetic agents cause vasodilation, and surgical interventions often include opened body cavities, both of which cause significant heat loss. Rodents also have a large surface area and high metabolism which further predispose them to hypothermia during surgical procedures. Hypothermia can prolong recovery and contribute to negative anesthetic outcomes. Thermal support should be supplied for any anesthesia event lasting longer than 5 minutes. Provide support from the time of induction of anesthesia continuously through recovery from anesthesia until the animal is ambulating normally. Thermal support units should be thermostatically controlled (warm water recirculating pump/ pad(s), Braintree Scientific Deltaphase Isothermal Pad, or forced warm air units). Warmed (body temperature) fluid bags and thermal disks can also be used, but the temperature should be verified prior to use. Heating devices should not come into direct contact with the animal and the animal must be monitored to ensure overheating does not take place. Home-use non-thermostatically controlled heating units and heat lamps should not be used during surgery, as they heat inconsistently, are prone to overheating, and are a risk for thermal burns when animals are anesthetized and cannot escape from heat.

Surgical Site Preparation: Remove hair from the surgical site, e.g., with clippers, a razor, or very careful use of depilatory cream (which can quickly injure the skin of small animal; refer to the IACUC Guidelines for the Use of Chemical Depilatory Agents on Laboratory Animals). Whenever possible, perform this procedure in an area separate from where the surgery is to be conducted. Carefully vacuum or otherwise remove loose hair and debris. Do not use scissors only to remove hair. Perform surgical site preparation of the region to be incised utilizing povidone-iodine or chlorhexidine antiseptic products. Note, these products are available in two forms. Solutions contain the antiseptic agent alone, and surgical scrubs, which contain a cleansing agent combined with the antiseptic agent. Apply betadine or chlorhexidine surgical scrub or solution with clean gauze or sterile cotton swabs using a circular motion starting at the center of surgical site and rotating outward. This should be followed

by isopropyl alcohol applied using the same technique. Repeat a minimum of three times with each solution discarding the cotton pad or swab after each use. End with an application of betadine, or chlorhexidine solution (properly diluted antiseptic solutions may be left on the skin during surgery but surgical scrubs can be irritating and require a final rinse with alcohol). Attempts should be made to avoid excessive wetting of non-surgical areas of the animal with alcohol or antiseptic as this can exacerbate hypothermia. If surgical drapes are being used, cover rodent with sterile drape or Glad Press and Seal to avoid contamination of the incision, instruments and supplies (it is recommended to use clear drapes to facilitate observation of the rodent).

Intra-operative Considerations (Surgical)

Aseptic Technique: The goal of aseptic technique is to reduce the normal bacterial burden present on the animal and in the environment before beginning surgery. This includes the use of sterile instruments, disinfection of the animal's surgical site and surgeon's hands, and the establishment of a sterile surgical field. To create a sterile field, the working surface should first be disinfected before surgery. All supplies and implanted materials used in survival surgeries (e.g., sutures, implants, instruments, catheters) must be sterile and not expired. During the surgery, a sterile field should be established, and the sterile instruments and materials must be placed within this field when not in use to prevent contamination. The sterile inside-surface of the instrument autoclave pack, a sterile drape, or the sterile interior of the glove packaging can be used as a sterile field for instruments and supplies during the procedure. Unwrap sterile instruments making sure to only touch the outer surface of the wrap. Do not touch the interior of the packaging or instruments, as this will compromise the sterility of the instruments. Maintain sterility of gloves, instruments, suture material and any other items being used intra-operatively during the procedure. Breaks in sterility occur when the surgeon touches something outside of the sterile field. This may be the surgeon's face, a light fixture, an unprepared area of the animal, or a non-sterile instrument. Care should be taken to avoid these breaks in sterility, as proper aseptic technique limits the risk of introduction of infectious agents, primarily bacteria, into surgical sites.

Closure of Incisions: Incisions should be closed using proper techniques and materials. If more than one anatomic layer was incised (e.g., both skin and muscle/ thoracic/abdominal tissues were cut), a **two-layer closure must** be performed. Staples/wound clips can only be used to close the skin and other layers must be sutured closed. Monofilament suture is recommended. Absorbable suture must be used for the muscle layer and either non-absorbable or absorbable suture can be used for the skin. Using absorbable suture in the skin of rodents eliminates the need for sedation/restraint for removal of sutures. Multifilament suture (e.g., silk) is not recommended as it elicits more intense tissue inflammatory response and has more potential to wick bacteria into the incision. Non-absorbable sutures and staples should be removed 10-14 days following surgery. If sedation or anesthesia will be required for suture or staple removal, this must be included and approved in the animal use protocol. If surgical glue is used, it should be applied at the opposing wound edges (or over the skin sutures) with a ≤ 27 g needle and never placed inside the incision/wound. The ARC vetstaff is available to discuss ideal closure techniques for specific situations or provide hands-on training. Dehiscence repair must be described and approved in your IACUC protocol in order to perform the procedure. If this is not approved, contact the ARC veterinary staff should dehiscence of the surgical site occur to discuss the best options for specific circumstances.

Anesthetic Monitoring and Record Keeping

The animal's physical condition and anesthetic depth must be continually monitored during the surgical or anesthetic procedure and frequently assessed during the recovery period until the animal is fully ambulatory. The animal **must be maintained in a surgical plane of anesthesia throughout the procedure**. Animals should not be left alone during surgery or recovery from anesthesia. Anesthesia monitoring must include a periodic assessment of anesthetic depth (toe-pinch reflex) recorded at the start of the procedure and then no less than every 15 minutes throughout the duration of the procedure. The following parameters may be used to check anesthetic depth in rodents:

- a) If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw, though testing on multiple paws is best practice. Pedal withdrawal must be used to monitor anesthetic depth for invasive procedures whenever possible. If an alternate stimulus/response

will be used, this must be described in the IACUC protocol.

- b) Depth, rate, and character of respiration should also be monitored, and can be used in place of pedal withdrawal for non-invasive procedures.
- c) Other useful parameters to monitor in anesthetized rodents include mucus membrane color and body temperature.
- d) Other parameters may be used to evaluate anesthetic depth if the procedure is non-invasive (e.g., MRI, CT, DEXA scanning, etc.) and pedal withdrawal is not possible or compatible with the goals of the study

The identification of the animals involved (animal ID), date of the procedure, protocol number, description or title of the procedure, and date performed are important to include on the surgical record. Drugs, including dosages, routes of administration, person administering, and times given should be recorded before and during surgery. Special attention should be paid to documenting analgesics on the surgical record, paying close attention to the initial dose and subsequent dosing intervals and must strictly follow the approved protocol. It is best practice to administer analgesic drugs before invasive procedures begin (as appropriate and approved in your animal use protocol) to provide pre-emptive analgesia before painful stimuli are perceived. See the IACUC Guidelines for Analgesia Use in Rodents or contact ARC veterinary staff for more information on analgesics.

Notations of any variations from the normal and expected events during the anesthetic and recovery periods (including the actions taken, the time performed, and the animal's response to these actions) should be documented. Assessment for pain and distress as well as any action taken to alleviate pain and distress (including pharmacological and non-pharmacologic interventions, and the response to these actions) should be documented on the surgical and post-operative records. If pain or distress is observed a veterinarian must be contacted unless the protocol allows for such presentation (Cat. E study). Special attention should occur in Cat. E studies with strict adherence to humane endpoints. ARC veterinarians are available to help determine if humane endpoints have been met. Surgical records should be readily available for IACUC, veterinary staff, or representatives of accrediting organizations. Records must be maintained for 3 years beyond the expiration of the approved protocol.

Anesthesia can be used for immobilization during non-invasive, non-painful procedures. Non-invasive procedures under anesthesia may consist of imaging or entering an external body structure with a catheter, needle, or other instrument (to obtain small samples or inject liquids/medications without disruption of function to an animal). Since animals that undergo these non-invasive procedures are expected to return to normal function after recovery from anesthesia with no more than momentary pain (E.g., blood collection, tail vein injection, tail biopsy for genotyping or intranasal inoculations), when inhalant anesthesia is used for immobilization during non-painful procedures lasting no longer than 15 minutes, no anesthesia or post-operative record is required. Instead, an entry describing the type of procedure and anesthetic used should be documented in these cases. For all other procedures using inhalant anesthesia, or any procedure using injectable anesthesia, an anesthesia record is required.

Post-Procedural Care and Record Keeping

Post-surgical and post-anesthesia care must include continuous observation of the animal to ensure uneventful recovery from anesthesia until the animal is awake and moving normally in the cage. Following anesthesia, all animals must be observed continuously during recovery until they are ambulating normally in the cage. This is necessary to ensure that the investigator can intervene if there are problems with the surgical site (i.e., incisions open when the animal starts moving), if there are problems with recovering from anesthesia (i.e., seizures), and to ensure that post-procedural pain has been alleviated. These observations should be recorded in a written record that includes the date, time, and initials of the person performing the evaluations and this record should be with the animal during recovery so that it is clear to others that observation is occurring.

- a) Separate recovered animals from those that are still under the influence of anesthesia to avoid wound licking or cage mate-induced trauma.

- b) Avoid placing rodents directly on wood chip bedding when recovering. Rodents are obligate nasal breathers and should be recovered in an empty cage with paper towels or a blue pad to avoid aspiration of bedding into the nasal cavity restricting normal respiration.
- c) Thermal support should continue during the post-operative period to prevent hypothermia. The cage should be placed with ½ of the cage bottom on a thermal supportive device as described above.
- d) A cage-level sign that an animal has had surgery is strongly recommended so that individuals who observe the animal are aware that the animal in question has undergone an anesthetic event.

A critical component of a proper analgesic dosing strategy is timing of post procedural administration. For all analgesics, the dosing interval is strict and cannot be exceeded. For example, if a drug is to be given every 12 hours and the previous dose was at 7 p.m., the next dose **MUST** be given by 7 a.m. the following morning. Although dosing intervals must not be exceeded, in some situations it may be acceptable to administer an analgesic dose somewhat earlier, for example to help synchronize and standardize analgesic dosing times over a multi- day treatment schedule. The ARC veterinarians should be consulted to determine if this is appropriate, and it must be remembered that this will reset the timing interval for subsequent dosing. All analgesics **MUST** be documented on surgical and post-operative records and will be subject to review by the IACUC and other regulatory agencies. The drug name, route of administration, dose, concentration, time and date of administration, and initials of the person administering the treatment should be included on the record.

Surgical wounds must be kept clean. If bandages or wound dressings are used, they must be changed as frequently as necessary to keep them clean and dry. Postoperative monitoring should be performed twice daily for the first 48-hours and daily for the next 1-3 days after that depending on the invasiveness of the procedure (if this monitoring schedule cannot be followed, the investigator must disagree with this guideline within the animal use protocol and provide scientific justification). When the approved IACUC protocol specifies that animals be monitored at least twice daily, it is expected that monitoring events will be well-spaced to assure that humane issues can be identified promptly. Monitoring generally occurs in the early morning and late afternoon, but alternative schedules are acceptable if observations occur every 10-14 hours, including weekends and holidays. All observations should be documented in the surgical/post-operative records and available upon request. Although observation schedules have some flexibility to accommodate alternative schedules, analgesic dosing windows do not. Analgesic dosing intervals must be stated in the protocol, and analgesics must be administered as described without allowing the dosing interval to be exceeded (generally every 8, 12, or 24 hours depending on the protocol-specific analgesic plan). The time of each dose administration must be written down (using AM or PM notations) on the surgical record.

Monitoring post procedurally must include close inspection of the surgical site for dehiscence or infection as well as clinical observation of the animal for signs of pain, abnormal behavior, appetite, and excretory functions. While not common, complications may occur post-operatively such as bleeding or clotting. If the animal appears ill postoperatively, experiencing post-operative pain or distress or if the surgical wound appears abnormal, the veterinary group must be contacted for consultation unless the protocol specifically complication being exhibited. I.e., Category E studies.

Section D – Additional Sources of Information on Disinfectant, Sterilants, Sterilization Guidelines and Wound Closure Materials

The NIH intramural animal research program has compiled tables listing appropriate antiseptics, disinfectants and wound closure materials at https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_surgery.pdf

National Research Council. 2011. Guide for the Care and Use of Laboratory Animals. National Academies Press, Washington, D.C. 220p. <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>

https://research.utexas.edu/wp-content/uploads/sites/3/2023/01/GUIDELINE_23-Sterilization_of_equipment_and_supplies_for_Sterile_Procedures_and_Surgery_2022_0.pdf

Section E – References and Acknowledgements

Fair, J., E. Paul, and J. Jones, Eds. 2010. Guidelines to the Use of Wild Birds in Research. Washington, D.C.: Ornithological Council.

Approval Date	Major Change(s) Approved
01/10/2022	<ul style="list-style-type: none">• Section C, #1 revised to state surgical instruments used on multiple animals must be sterilized between animals• Section C, #5 revised to include details about analgesic dosing interval requirements
11/13/2023	<ul style="list-style-type: none">• Guideline was revised to address rodent procedures only. Fish and Amphibians have been broken out into a new separate guideline.
	<ul style="list-style-type: none">•
	<ul style="list-style-type: none">•