

Guidelines for Surgical and Anesthetic Procedures in Non-Rodent Mammals

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. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.

This document provides information to be used when planning and performing surgical and anesthetic procedures on non-rodent vertebrate animals 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
- Section D – References
- Section E – 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 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 physiological 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 component 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. **Non-survival surgery** is an invasive procedure during which the animal is euthanized before recovery from anesthesia. It may be necessary to follow all the techniques 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 transcardial perfusion prior to tissue harvest is required by the study.*

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).

For non-survival procedures of extended duration, attention to aseptic technique may be more important in order 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.

2. **Minor surgical procedures** may be performed in a suitably located and equipped laboratory area, subject to approval by the IACUC. Appropriate aseptic technique for these procedures include a clean uncluttered work area, preparation of the surgical field including clipping of hair, disinfection of skin and draping of the surgical site with sterile drapes; use of sterile instruments and supplies; and the use of sterile gloves and a surgical mask by the surgeon and any assistant working in the surgical field. Long hair of surgeons and assistants should be covered and/or restrained to keep it away from the surgical field.
3. **Major survival surgical procedures** on mammals other than rodents (including bats) must be conducted in surgical facilities intended for that purpose only, following the basic principles of aseptic techniques as described below. The operating room(s) and surgical support area should be designed and managed to ensure a level of sanitation appropriate for aseptic surgery. The actual surgical procedure must be performed in closed, single- purpose operating rooms. The operating room should contain only the ancillary laboratory, diagnostic, or clinical equipment and supplies required to support the procedure being performed. Equipment used on an infrequent basis and bulk supplies should be stored elsewhere. Permanently installed furniture or fixtures such as laboratory benches impede sanitation and therefore should be minimized in an operating room.

Activities associated with surgery generally include surgeons' preparation, animal preparation, the surgical procedure, surgical support, and post-operative care. In high-volume programs, each activity may require a separate and distinct room. However, for less intensively used areas, it may be acceptable to have a single dedicated operating room and to perform the other activities in nearby procedural rooms or laboratory areas. This is acceptable only if scheduling can be established (and access controlled) to

assure there will be no other ongoing activities in those areas that could compromise the surgical procedure during the peri-operative period.

4. **Non-invasive procedures under anesthesia** may consist of imaging or entering the external body or a preexisting orifice with a catheter, needle, biopsy instrument or similar tool to obtain small samples or inject liquids/medications without disruption of function to an animal. Animals that undergo these non-invasive procedures are expected to return to normal function after recovery from anesthesia. Anesthesia and recovery from anesthesia must be monitored and recorded per the guidance below.

Section C – Specific Considerations

1. Pre-anesthesia or operative preparation

See [ARC Anesthesia Recommendations](#) for specific recommendations on fasting for species. Some non-rodent mammals should generally be fasted overnight prior to anesthesia and surgery to prevent vomiting, aspiration, and problems associated with a distended gastrointestinal (GI) tract. Fasting for 12-16 hours is adequate for most animals, and water should be available at all times. However, the gastrointestinal characteristics of each species should be taken into consideration. For example, rabbits and ferrets may not require a fasting period, while an extended 16-24 hour fast may be indicated for some ruminant species. The veterinary staff can provide guidance on appropriate fasting for specific species.

2. Anesthesia

A proper method of anesthesia must be selected, based on veterinary consultation and protocol approval must be obtained. 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. 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. See [IACUC Guidelines for Safe Anesthetic Use](#) for additional details on appropriate gas scavenging methods.

3. Animal preparation for survival surgery

The hair should be clipped from the surgical site for at least 1-2 inches beyond the extent of the planned incision. If chemical depilatory agents are used, follow the guidance within the IACUC [Guidelines for the Use of Chemical Depilatory Agents on Laboratory Animals](#).

The clipped skin should then be cleaned to reduce bacterial contamination. It is recommended to disinfect the skin with three applications of a surgical scrub product based on chlorhexidine or povidone iodine. Each application step should begin along the incision site and then radiate outward toward the margins of the clipped area in a circular fashion. Each application can be alternated with an alcohol cleansing, but that is not required. A final “painting” of the area with a chlorhexidine or povidone iodine antiseptic solution (NOT a soap-containing surgical scrub) is done to complete the process.

After being prepped, the surgical site should be draped with sterile disposable or sterile reusable cloth drapes.

4. Surgeon preparation for survival surgery

The surgical procedure itself must be performed or directly supervised by a trained and experienced

individual. Personnel unfamiliar with aseptic surgical procedures should contact the ARC veterinary staff for information or training.

The surgeon and any assistant that will be required to manipulate instruments, tissues, or other components of the sterile surgical field should don a bonnet and mask and then wash their hands and forearms using a chlorhexidine or povidone iodine scrub for 3-5 minutes. A sterile gown and sterile gloves should be put on prior to surgery, following proper technique to maintain sterility. The wearing of shoe covers, double gloving or other additional precautionary steps may be indicated depending on the specifics of the procedure being performed, based on veterinary consultation.

5. Sterilization of instruments and implanted devices for survival surgery

All instruments, supplies, or devices that will need to be handled by the surgeon or will have contact with the tissues of the animal must be sterilized before the surgery and must be handled and placed so that they remain uncontaminated until the surgery is completed. If sterile packs are stored for later use, they must be dated with the preparation date and/or an expiration date (6 months after sterilization). For additional information on shelf life, contact the ARC veterinary staff. Instruments that become contaminated by touching a non-prepped part of the animal, being dropped or placed outside of the sterile field, touched by a non-prepped assistant, etc. must be re-sterilized before they can be reused.

Methods used may vary, but all must conform to established medical standards for complete sterilization. Options include:

- Steam sterilization at proper pressures and exposure times
- Ethylene oxide gas (ETO) used in a specialty chamber
- Dry heat sterilization at proper temperature and exposure time
- Prolonged immersion in a hospital-grade formaldehyde- or glutaraldehyde-based cold sterilant following label directions and include a rinse with sterile water

6. Closure of incision

Multiple layer closure should be performed on thoracic and abdominal incisions and any other significant incision that may result in dead space detrimental to healing. Skin closure should be performed using noncapillary (monofilament) suture material, which may or may not be absorbable. The use of wound clips or staples to close a skin incision is acceptable in some cases but careful attention should be given to placement and spacing to promote proper healing and to prevent the clips from catching on anything in the cage. Wound clips have a higher potential for post-operative infection or adverse tissue responses if used improperly. If non-absorbable sutures or clips/staples are used they should be removed 7-10 days post-op. This should be described in the IACUC protocol if animals will be sedated for suture removal.

7. Post-operative and post-anesthesia care

All IACUC proposals involving survival surgery must provide specific details of procedural and post-procedural care and monitoring, as well as relief of pain and distress. The IACUC must approve the specific details. However, the Attending Veterinarian (or designee) retains the authority to change peri- and post-operative care as necessary to ensure the comfort of the animal. The goal of anesthetic monitoring should be to maintain cardiovascular homeostasis and core body temperature. During anesthesia, animals should be provided with supplemental heat as anesthesia often leads to hypothermia.

Assessment of the animal's physiologic condition and plane of anesthesia must occur at the start of the

procedure and then at least every 15 minutes throughout the procedure and be documented in the anesthesia record. Any animal that is sedated or anesthetized must have an anesthesia record. Parameters that are often monitored during anesthesia include:

- Body temperature
- Heart rate and pulse character
- Respiratory rate and pattern
- Oxygen saturation
- Expired CO₂
- Capillary refill time
- Jaw tone - resistance to opening
- Response to toe pinch - withdrawal or non-withdrawal
- Palpebral response to touching the medial canthus
- Color of mucous membranes at gums or conjunctive
- Examination for other abnormalities
- For more involved procedures, EKG, blood pressure monitoring and blood gas analysis may be indicated.

At a minimum, anesthetic depth, heart rate, and respiratory rate should be recorded (unless collection of such data is not appropriate for that species).

Post-surgical and post-anesthesia care must include continuous observation of the animal to ensure uneventful recovery from anesthesia until the animal is sitting upright unassisted. During this time, animals must be observed continuously. These observations should be recorded every 15 minutes in a written record that includes time notations and is left with the animal during recovery so that it is clear to others that observation is occurring. 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, vomiting), and to ensure that post-procedural pain has been alleviated. A cage-level indication that the 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.

During recovery from surgery and anesthesia, animals must be kept warm and dry in an environment that does not pose a risk of injury as they regain muscular control. The animal may be extubated once the swallowing reflex has returned. In some cases, the animal may be returned to its home cage during recovery, but no food or water should be left in the cage until the animal is fully conscious. Animals should be provided with a heat source (preferable) or be placed on an insulating surface (minimally) until they have fully recovered to prevent post-procedural hypothermia.

Caution: Use of heat lamps and electric heating pads can result in severe burns or hyperthermia in animals that are anesthetized or otherwise unable to escape from the heat. Close observation is required, and the use of safer equipment such as a circulating water blanket or isothermic pad is recommended whenever possible. Items such as heat support and insulating materials must be removed from the cage before the animal regains full consciousness, as they pose ingestion and choking risks for some species.

Post-operative observations should be conducted twice daily for the first 48 hours following the procedure and at least once daily for 1-3 additional days 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 as long as 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.

The drugs 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. Fluids, analgesics, and antibiotics must be administered as indicated in the protocol or as directed by the Attending Veterinarian or designee. Surgical wounds must be kept clean. When bandages or wound dressings are used, they must be changed as frequently as necessary to keep them clean and dry. Subsequent care must consist of daily monitoring of the animal (as consistent with the IACUC-approved protocol) to include daily clinical observation for signs of pain, abnormal behavior, appetite and excretory functions. While not expected, all surgical procedures involve some anesthetic and surgical risk. The most common anesthetic complications include intra-operative hypotension, cardiac arrhythmias, regurgitation, and aspiration pneumonia. The most common surgical complications include incisional dehiscence, post-operative infection and unrelieved pain. Animals should be monitored closely such that veterinary consultation and intervention can be sought for suspected anesthetic complications, post-operative dehiscence, infection, or pain not managed by the described analgesic plan. Depending on the nature of the surgical procedure, animals may also be at risk for additional rare but more serious complications such as excessive bleeding at the surgical site, edema, and embolism.

Supportive fluids, analgesics, and other drugs must be administered as required and must be documented.

8. Recordkeeping

Investigators must maintain accurate records of anesthesia, surgery, and post-procedural care, including analgesic administration. Anesthesia and post-procedural care records must provide documentation of animal evaluation, including a notation for each time the animal is examined during and after the surgery or non-invasive procedure under anesthesia and a description of what parameters were monitored. The ARC veterinary staff can provide guidance on what recordkeeping is appropriate for particular studies. Examples of both large and small animal anesthesia monitoring records are available on the [ARC website](#). Records should also state what procedure was performed, include notation of any complications that occur, and list all drugs or treatments that are administered.

Post-operative observations must be conducted and recorded throughout the recovery period as indicated under Section 7: *Post-operative and post-anesthesia care*. Special attention should be made to physiologic function as well as behavioral signs of post-procedural/operative pain, infection, and wound dehiscence. All records must be available when requested, but especially for IACUC semi-annual inspections, protocol reviews, USDA inspections, and AAALAC site visits.

After the post-operative period, the monitoring records can be filed for future reference, either as a part of the animal's medical file or along with the research data. When research groups take the responsibility for maintaining these records, they must store them in a readily-retrievable manner. NOTE: These files are subject to record retention requirements, so they must not be discarded or destroyed without proper institutional approval.

9. Other

In some cases, there may be additional study-specific requirements that are either specified in the procedure description of the approved IACUC protocol or are prescribed by the Attending Veterinarian

or designee based on a case-by-case determination using professional judgment.

Section D – References

National Research Council. 2011. *Guide for the Care and Use of Laboratory Animals*. National Academies Press, Washington, D.C. 220p.

Animal Welfare Act of 1966 (P.L. 89-544) as amended in 1970 (P.L. 91-579), 1976 (P.L. 94-279) and 1985 (P.L. 99-198).

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care Resource Guide. Policy #3 – Veterinary Care. 2011. http://www.aphis.usda.gov/animal_welfare/policy.php?policy=3

Section E – Acknowledgements

This document contains content that was adapted from materials obtained from East Tennessee State University and The Ohio State University.

Approval Date	Major Change(s) Approved
03/09/2020	<ul style="list-style-type: none"> • Title and guideline description updated to include anesthetic procedures • Sections A & B updated to include non-invasive procedures under anesthesia definition and requirements • Section C, #1 updated to include pre-anesthesia preparation. ARC Anesthesia Recommendations should be referenced for fasting information. • Section C, #2 updated to reference IACUC Guidelines for Safe Anesthetic Use • Section C, #3 updated to reference IACUC Guidelines for the Use of Chemical Depilatory Agents on Laboratory Animals • Section C, #6 updated to include sedated suture removal • Section C, #7 updated to include post-anesthesia care and requirement for continuous monitoring and documentation • Section C, #9 updated to include anesthesia and post-procedural record keeping requirements.
11/09/2020	<ul style="list-style-type: none"> • Section C, #7 clarified that any animal that is sedated or anesthetized must have an anesthesia record • Section C, #7 states investigators who cannot perform post-operative monitoring schedule as stated in this section must “disagree” with this guideline in eProtocol and provide scientific justification • Section C, #7 anesthetic and surgical risks added
02/08/2021	<ul style="list-style-type: none"> • Section C, #7 clarified that anesthetic depth must be monitored and recorded at the start of the procedure and then every 15 minutes. • Section C, #7 clarified timing requirements of twice daily post-operative monitoring checks
09/13/2021	<ul style="list-style-type: none"> • Section B, #1 updated to include the use of expired materials (excluding drugs) for non-survival procedures.