



Guidelines for Surgical and Anesthetic Procedures in Rodents, Birds, and Cold-Blooded Vertebrates

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

This document provides information to be used when planning and performing survival surgical and anesthetic procedures in rodents, birds, and cold-blooded vertebrates 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 – Additional Sources of Information on Disinfectant, Sterilants, 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 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 not 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. 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. **Survival surgical procedures** may be performed in either a dedicated animal procedural room or in a suitably located and dedicated laboratory area, subject to approval by the IACUC. These procedures generally cannot be done appropriately within an animal holding room. The bench or tabletop areas used for animal prep, surgery, and recovery 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. 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 rodent, bird, and cold-blooded vertebrate surgeries, 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.
3. **Aseptic (sterile) techniques for survival surgery** are required for ALL species. However, some modifications are allowed when rodents, birds and cold-blooded vertebrates 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 actually impair the ability to assess breathing patterns or other monitoring criteria used to assure proper anesthetic depth. Another difference is that chemical disinfection of the surgical site may not be appropriate for the slime-coated and absorptive skin of amphibians and fish. The need for gowning of the surgeon is also considered optional for surgery involving rodents, birds, and cold-blooded vertebrates. However, the surgeon should wear a surgical mask. Unless a strict “Tips Only” method is being used (as described below), sterile surgical gloves ARE required.
4. A proper method of **anesthesia** 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. 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.
5. **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. Preparation of Surgical Facilities and Instruments

a. **Surgery Facilities**

i. Prior to surgery:

- 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 soap and water to clean the area if visibly soiled, followed by the use of a hard surface disinfectant that is appropriately- 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.

b. **Surgical Instrument**

i. 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/or an expiration date (6 months after sterilization). Methods used for sterilization may vary, but all must conform to established medical standards for complete sterilization. Options include:

- Steam sterilization at proper pressure 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 (alcohol immersion is NOT acceptable)
- Hot bead sterilizer (acceptable for use in procedures where a “tips-only” method of aseptic technique is appropriate)

ii. When performing surgeries on multiple animals and re-using instruments during a single session, repeated full sterilization is NOT needed, but instruments must be disinfected between animals. This will require thoroughly removing all organic material (e.g., blood) from instruments and then immersing the instruments in an appropriate disinfectant (e.g., 60-90% ethyl or isopropyl alcohol or a product labeled as an instrument disinfectant) between animals. One set of surgical instruments may be used on five animals before moving to a new set of sterilized instruments.

NOTE: Disinfectant residue must be removed prior to use by allowing adequate drying time (for alcohols) or a including a sterile water rinse (for disinfectants).

2. Preparation of the Animal

- a. Clean and disinfect the surgical site using an appropriate surgical prep technique and an effective antiseptic (e.g., scrubbing in a gradually enlarging circular pattern from the interior of the shaved area towards the exterior for rodents and birds). Chlorhexidine or povidone iodine-based antiseptic solutions are considered the best practice for surgical-site preparation.

RODENTS

- b. 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.
- c. Drying and irritation of the eye during anesthesia in rodents should be prevented by placing a lubricating ophthalmic ointment (e.g., Lacrilube) in the anesthetized animal’s eyes.

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- d. Small feathers may be gently plucked around the surgical site. Avoid removing large feathers. Masking tape or stockinet material may be used to retract feathers.

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- e. Rinse gross debris from the amphibian or fish surgical site using sterile saline or another sterile isotonic fluid and throughout the surgical procedure keep the skin moist.
- f. Fish and amphibians must remain wet during procedures out of the water. Water can be dripped, poured, or sprayed on exposed areas of the animal. Use water from the home tank wherever possible; clean non-chlorinated water is also acceptable.

3. Preparation of the Surgeon

- a. 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.
 - i. A clean lab coat and face mask are required for surgeons.
 - ii. Wash hands with a soap (minimal) or an antiseptic surgical scrub solution (optimal).
 - iii. Sterile surgical gloves must be donned prior to handling sterile instruments or touching surgically-exposed animal tissues.
 - Wetted gloves should be used for aquatics or semiaquatics.
 - iv. When performing surgeries on multiple animals during a single session, one pair of sterile gloves can be used provided that the gloves remain free of tears or punctures and are disinfected (by wiping with an appropriate disinfectant) between animals.
 - v. Long hair of surgeons and assistants should be covered and/or restrained to keep it away from the surgical field.

4. Intraoperative and Non-Invasive Anesthetic Monitoring

- a. The animal's physical condition and anesthetic depth must be continually monitored during the surgical or non-invasive under anesthesia procedure and frequently assessed during the recovery period until animal is fully ambulatory. Animals should not be left alone during surgery. All needed materials, (instruments, drugs, etc.) should be easily accessible from the surgery location.
- b. The animal must be maintained in a surgical plane of anesthesia throughout the procedure. Drugs, including dosages, routes of administration, and times given should be recorded before and during surgery. Administer analgesic drugs before surgery as appropriate and approved in your animal use protocol to provide pre-emptive analgesia. See the IACUC [Guidelines for Analgesia Use in Rodents](#) for more information.
- c. Anesthesia monitoring must include a periodic assessment of anesthetic depth recorded at the start of the procedure and then no less than every 15 minutes.
- d. Notation of any variation 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.

- e. 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.
- f. See the ARC Surgical Guidance website for additional record guidance, templates, and examples: <https://research.utexas.edu/arc/arc-guidance/>

RODENTS

- g. The following parameters may be used to check anesthetic depth in rodents.
 - i. If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw. 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.
 - ii. 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 feasible or compatible with the goals of the study
 - iii. Depth, rate, and character of respiration should also be monitored, and can be used in place of pedal withdrawal for non-invasive procedures.
 - iv. Other useful parameters to monitor in anesthetized rodents include: mucus membrane color and body temperature.

BIRDS

- h. The following parameters may be used to check anesthetic depth in birds.
 - i. Testing an animal's reflexes is perhaps the simplest method for assessing the depth of anesthesia. In birds, testing corneal reflexes using a lubricated cotton swab can be an effective reflex test. Another simple test is the toe pinch; foot withdrawal following the pinch can be interpreted as a pain reflex, which indicates an insufficient depth of anesthesia. In addition to monitoring reflexes, investigators also need to monitor heart rate and respiration, both as indicators of depth of anesthesia and adequate tissue perfusion.

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- i. The following parameters may be used to check anesthetic depth in fish.
 - i. Activity decreases and the righting reflex is lost as fish become anesthetized. Opercular movement (respiratory rate) progressively decreases with deepening anesthesia. The heart rate can be directly monitored using a Doppler blood flow probe or ECG leads. Surgical planes of anesthesia can be confirmed by a lack of response to a firm squeeze at the base of the tail.
- j. The following parameters may be used to check anesthetic depth in amphibians.
 - i. At the surgical plane of anesthesia, respiratory movements are greatly depressed to non-existent and there is no response to painful stimuli. However, cutaneous respiration is sufficient to maintain appropriate blood oxygen levels for most short procedures. Cardiac pulsation becomes more obvious as the muscles relax. Deeper anesthesia is associated with reduction in heart rate and then reduction in strength of the contraction. At deep planes of anesthesia which may indicate an anesthetic overdose, cardiac pulsation may be in-apparent.

5. Post-operative and Post-Anesthesia Care

- a. Postoperative analgesia or other supportive care must be recorded on the surgical/ post-procedural record.

- b. Post-surgical and any post-anesthesia care must include continuous observation of the animal to ensure uneventful recovery from anesthesia until the animal is awake. This includes anesthesia used for surgical and non-invasive under anesthesia procedures. Animals must be observed continuously until they are ambulatory 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 time notations and is left with the animal during recovery so that it is clear to others that observation is occurring. A cage-level indication 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.
- c. 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.
- d. 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 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.
- e. Monitoring 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, or if the surgical wound appears abnormal, the veterinary group should be contacted for consultation.

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- f. During recovery, animals must be kept warm and dry in an environment that doesn't pose a risk of injury as they regain muscular control. Warm-blooded animals (or ectotherms subjected to hypothermia) 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.
 - i. 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. Heating devices should not come into direct contact with the animal and the animal must be monitored to ensure overheating does not take place. The use of safer equipment such as a circulating water blanket or isothermic pad is recommended whenever possible.
- g. 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. Separate recovered animals from those that are still under the influence of anesthesia to avoid wound licking or cagemate-induced trauma. Avoid placing rodents directly on wood chip bedding when recovering, because the altered breathing patterns and lack of respiratory reflexes may result in the inhalation of wood dust or fibers.

- h. Non-absorbable sutures or wound clips should be removed in 7-10 days for animals that will be maintained for longer than 14 days after the procedure. If sedation or anesthesia will be required, this must be included and approved in the animal use protocol. NOTE: if there is a need to sedate an animal for suture/clip removal or wound repair, an ARC veterinarian must be consulted to arrange for grant permission to sedate for issues not anticipated in the protocol.

COLD-BLOODED VERTEBRATES

- i. Post-anesthesia monitoring should be performed until the animal is ambulatory. This can be recorded as the time of recovery. No animal should be returned to the housing room tank or enclosure until they are mobile.
- j. Post-operative health monitoring must be done the day following the procedure (and longer if the animal is not deemed to be fully recovered, or if longer monitoring is described in your IACUC protocol). For many animals, this may simply entail evaluating the tank to ensure all animals appear healthy, as animals may not be individually marked to allow for a detailed post-op assessment in a tank setting. Analgesia must be administered as described in the protocol (for each administration).

6. Minimal Requirements for Record Keeping

- a. The surgical, anesthesia, and post-procedural records described above must be readily available to the IACUC, veterinary staff, or representatives of regulatory and accrediting organizations. Records must be maintained for 3 years beyond the expiration of the approved protocol.
- b. Additional recording requirements may be needed depending on the species, but at a minimum, records must contain the following information:
 - i. Date of procedure,
 - ii. Principal investigator,
 - iii. Animal use protocol (AUP) number,
 - iv. Animal tag number/ ID,
 - v. Species,
 - vi. Procedure type/title,
 - vii. Anesthetic, analgesic, or tranquilizing agents used in accordance with the approved IACUC protocol,
 - viii. Doses, frequency, and route of administration,
 - ix. Frequency of monitoring conducted by laboratory or veterinary staff, and
 - x. Date each entry was made and initials of the person making the entry.
- c. NOTE: When DEA/DPS controlled substances are used, the date and drug usage volumes recorded in the controlled substance log and the dates and dosages recorded in the animal surgery records should match.
- d. The procedural and post-procedural care plans must include at a minimum all steps outlined in the approved IACUC animal use protocol.
- e. See the ARC Surgical Guidance website for additional record guidance, templates, and examples: <https://research.utexas.edu/arc/arc-guidance/>
- f. Post-procedure observations should be recorded at an appropriate frequency throughout the recovery period as described under Section 5. *Post-operative and Post-Anesthesia Care* and in accordance with the approved IACUC protocol. They should include at least the following:

- i. Observation of the comfort level of the animal. This can be evaluated by activity, grooming, elimination, food consumption, etc.
 - ii. A specific check of the surgical wounds. Is there any discharge, redness, or swelling? Is the incision intact?
 - iii. Any procedure-specific observations related to potential or unexpected complications such as organ failure, infection, ischemia, etc.
 - iv. The post-operative care plan must include at a minimum all steps outlined in the approved IACUC animal use protocol.
- g. All records must be available when requested, but especially for IACUC semi-annual inspections, protocol reviews, and AAALAC site visits.

7. “Tips Only” Technique

- a. “Tips only” aseptic practices are considered appropriate for survival surgeries in rodents, birds or cold-blooded vertebrates that involve only very small incisions, e.g., embryo transfer, gonadectomy, intraceolomic 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 instrument-working surfaces can be selectively sterilized by immersion in a hot bead sterilizer. Likewise, the instrument tips can be disinfected 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 particular procedure.

Section D – Additional Sources of Information on Disinfectant, Steriliants, 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>

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.

This document contains content that was adapted from materials obtained from Stanford University and University of Michigan.

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

	<p>definition and requirements</p> <ul style="list-style-type: none"> • Section C, #4 updated to include non-invasive anesthetic monitoring. References IACUC Guidelines for Analgesia Use in Rodents • Section C, #5 updated to include post-anesthesia care and requirement for continuous monitoring and documentation. Postoperative monitoring time points updated. • Section C, #6 updated to include anesthesia and post-procedural record keeping requirements. Records should be made available when requested.
11/09/2020	<ul style="list-style-type: none"> • Section C, #4 letters c, d, and e added for rodents, birds, and fish • Section C, #4 parameters to check anesthetic depth added for birds and cold-blooded vertebrates • Section C, #5 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, #5 post-operative complications added
02/08/2021	<ul style="list-style-type: none"> • Section C, #4 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