

WVU IACUC Guidelines: Surgery Guidelines for Rodents

Purpose

This document provides guidelines and considerations when performing anesthetic and surgical procedures on rodent species of laboratory animals, such as mice and rats.

The *Guide for the Care and Use of Laboratory Animals*, 8th ed (2011, *Guide*) states (p115): “Successful surgical outcomes require appropriate attention to presurgical planning, personnel training, anesthesia, aseptic and surgical technique, assessment of animal well-being, appropriate use of analgesics, and animal physiologic status during all phases of a protocol involving surgery and postoperative care.”

Definitions

Anesthesia: A state of controlled, temporary loss of sensation or awareness that is induced for medical purposes.

Analgesia: Any member of the group of drugs used to achieve relief from pain.

Major Surgery: Surgery which penetrates and exposes a body cavity, produces substantial impairment of physical or physiological function or involves extensive tissue dissection or transection.

Minor Surgery: Surgery which does not expose a body cavity and causes little or no physical impairment.

Survival Surgery: Animal recovers from anesthesia after the surgical procedure.

Non-survival Surgery: Animal is euthanized before recovery from anesthesia.

Aseptic Technique: The use of practices that restrict microorganisms in the environment and prevent contamination of the surgical site.

OLAR: Office of Laboratory Animal Resources

Related IACUC Policies and Guidelines (<https://animal.research.wvu.edu/policies-and-guidelines>)

IACUC #11-005 Certification, Maintenance and Use of Equipment Used for Inhalation Anesthesia in Animals

IACUC #21-006 Administration of Substances to Research and Teaching Animals

IACUC #19-002 Veterinary Recommendations for Anesthesia and Analgesia

IACUC #14-002 Multiple Survival Surgeries in the Same Animal

IACUC #20-002 Autoclave Validation and Sterile Pack Processing

General Information

- A. The objectives of pre- and post-operative care are to minimize pain, discomfort and/or distress in animals that undergo anesthetic and/or surgical procedures.
- B. Surgery is conducted in accordance with applicable regulatory documents (e.g. the *Guide for the Care and Use of Laboratory animals*) and current veterinary medical standards and practices, where applicable.
- C. Anesthetic and surgical procedures, along with all medications to be given, should be justified and described in IACUC-approved animal protocols. Clinically indicated surgical procedures to ensure animal health or well-being may be performed by a WVU veterinarian or designee (at a veterinarian’s direction) and do not require an IACUC-approved protocol.
- D. Anesthetic and surgical procedures may be performed only by properly trained personnel. WVU veterinary staff is available to provide training upon request.
- E. Pain relief **must** be provided to animals undergoing surgical procedures unless otherwise scientifically justified in the IACUC-approved animal protocol. WVU veterinary staff should be consulted for species-specific recommendations for analgesic drugs. Post-operative pain control is best achieved with the use of preemptive analgesia, when analgesics are provided while the animal is under anesthesia.

- F. Categorization of surgery into major and minor procedures is defined above and in the WVU IACUC protocol form, but some procedures may be classified as either major or minor surgery depending on the impact to the animal (e.g. laparoscopic and some neurological procedures). The IACUC and WVU veterinarians will provide guidance as needed, when determining procedure categorization.

Responsibilities

A. The Principal Investigator (PI) is responsible for:

1. Ensuring that all research personnel under their supervision who are performing anesthetic and/or surgical procedures on animals are adequately trained and familiar with the IACUC-approved protocol(s).
2. Ensuring that PI-managed surgical areas and equipment are properly maintained.
3. Ensuring all post-operative monitoring and substance administration is done, either by trained laboratory personnel or by making arrangements with WVU veterinary staff.
4. Reporting any peri- or post-operative complications to the facility veterinarian.

B. WVU veterinarians are responsible for:

1. Ensuring that WVU personnel under their supervision who are performing anesthetic and/or surgical procedures on animals are adequately trained.
2. Ensuring that WVU-managed surgical areas and equipment are properly maintained.

Procedures

A. Prior to an Anesthetic or Surgical Procedure

1. SURGICAL FACILITIES

- a. Rodent aseptic surgery **must** be performed in a location dedicated to this purpose, on a clean and uncluttered surface. This location cannot be used concurrently for any other activities or procedures, unless an exception is justified and approved by the IACUC.
- b. Surfaces should be cleaned and sanitized prior to use.
- c. Areas above the surgical field **must** be clear and uncluttered (i.e. cannot have material stored above the sterile surgical field).
- d. Traffic should be minimized in the surgical area, and those entering the area should wear appropriate personal protective equipment (PPE).

2. PRE-ANESTHETIC EVALUATION

- a. A visual assessment of the animal's health **must** be made prior to anesthesia and any abnormalities noted that may affect outcome. If there are concerns about the suitability of an animal for anesthesia or surgery, the OLAR veterinary staff should be consulted before starting the procedure.
- b. A pre-operative weight should be obtained to provide accurate dosing of any injectable drugs.
- c. Any drugs used during survival surgery **must** be in date (i.e. not expired).

3. INHALATION ANESTHESIA EQUIPMENT

- a. Anesthesia equipment should be operated according to manufacturer's directions.
- b. Anesthesia machines and precision vaporizers should be checked prior to use to ensure proper function and to verify that sufficient volume of anesthetic agent and delivery gas are present to complete scheduled procedures.
- c. Waste anesthetic gas **must** be actively or passively scavenged, as covered in IACUC #11-005 Certification, Maintenance and Use of Equipment Used for Inhalation Anesthesia in Animals.

4. ANESTHESIA AND ANALGESIA

- a. A WVU veterinarian should be consulted prior to initiating the project to ensure the anesthetic plan is appropriate for the species and procedure.
- b. IACUC #19-002 Veterinary Recommendations for Anesthesia and Analgesia should be reviewed when developing anesthesia and analgesic treatment plans.

- c. The anesthetic and analgesic regimen described in the protocol **must** be adhered to. Any changes to anesthetic or analgesic regimens **must** be based on veterinary recommendations (direct discussion and consultation with veterinarian) if not described in the IACUC-approved protocol. Veterinary-approved changes should be subsequently submitted in an amendment to the protocol for IACUC-approval.

B. Aseptic Technique

1. SURGICAL INSTRUMENT and DRAPE PREPARATION

- a. Surgical instruments **must** be sterilized prior to surgery using an acceptable sterilization method:
 - Autoclave (steam)
 - Ethylene Oxide gas sterilization
 - Cold sterilization (Liquid/Chemical) for appropriate contact time; instruments should be rinsed with sterile water or saline before use. **NOTE: Ethanol and rubbing alcohol (isopropanol) are NOT a sterilant nor high-level disinfectant and should not be used for sterilization**
- b. Sterilization should be validated, and machines maintained in accordance with the IACUC #20-002 Autoclave Validation and Sterile Pack Processing.
- c. Instruments may be re-sterilized during the surgical procedure and between animals (maximum 5) when performing multiple surgeries within the same day.
 - The “tips only” method involves touching the surgical site only with the tips of instruments that have been re-sterilized in a glass bead sterilizer. 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.
 - Blood and tissue (all organic material) on instrument tips should be removed using alcohol.
 - 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 5 animals.
 - If the instrument tips become contaminated, they **must** be re-sterilized or obtain a new set.
- d. All supplies and implanted materials used in survival surgeries (e.g. sutures, implants, instruments, catheters) **must** be sterile and not expired.
- e. During the surgery the sterile instruments **must** be placed on a sterile drape or in a sterile holder (when not in use) to prevent contamination.
- f. A sterile drape **must** be placed over the surgical site to maintain a surgical field. The drape should contain a pre-cut opening to expose the surgical site. A new sterile drape **must** be used for each animal.

2. ANIMAL PREPARATION AND PROCEDURES

- a. Animal preparation **must** be performed in a designated location separate from the location of aseptic surgery.
- b. The animal should be anesthetized according to procedures outlined in the IACUC-approved protocol.
- c. Thermal support (preferably circulating warm water blanket) should be provided for the duration of anesthesia to minimize hypothermia. Caution should be taken if using heating pads or heat lamps, as these devices can lead to overheating and burns due to high temperatures and inconsistent heating. The animal should never have direct contact with external heat source.
- d. For procedures lasting more than 10 minutes, sterile ophthalmic ointment **must** be applied to the eyes to prevent corneal damage.
- e. Hair/fur should be removed from the surgical site using clean, appropriately sized clippers. The area of hair/fur removal should be approximately twice that of the surgical site.
 - Use of depilatory cream may be appropriate for certain procedures. When used, it **must not** contact the eyes or mucous membranes and **must** be rinsed off thoroughly with water to avoid skin irritation. Maximum contact time should be 2-3 minutes (rats) and < 30 seconds in mice; the minimal amount of contact time should be used.

- Some mouse strains/backgrounds (CD-1) have an increased sensitivity to depilatory cream regardless of contact time. Depilatory cream application in these strains can lead to gross and histopathological skin changes.
 - If using depilatory cream, unscented facial formulations are recommended.
- f. The surgical site **must** be disinfected with three alternating applications of a disinfectant (e.g. chlorhexidine or iodine solution) and 70% alcohol or sterile water.
 - g. After preparation, the animal should be moved to the surgery location and covered with a sterile drape with an opening over the surgical site.
 - h. Appropriate aseptic technique **must** be used at all times during survival surgery.
 - i. Tissues and organs should be gently manipulated using the appropriate surgical instruments.
 - j. Incision closure of organs, tissues and skin should be accomplished using material appropriate for the species and procedure. When sutures are used, consideration should be given to the type of suture, suture pattern, and needle size and type.
 - Closure of body cavities **must** be done in two layers, body wall and skin.
 - To reduce risk of dehiscence, a simple interrupted suture pattern is recommended.
 - Recommended material for body wall closure: absorbable suture material
 - Recommended material for skin closure: non-absorbable monofilament suture material (nylon), wound clips, or skin glue for incisions < 0.5cm

3. SURGEON PREPARATION AND PROCEDURES

- a. Surgeon should wash hands and forearms thoroughly with soap and warm water.
- b. Surgeon **must** don clean gown, scrub top, or lab coat, as well as surgical mask, hair cover and sterile surgical gloves.
- c. Surgical assistant should don PPE appropriate to their activities. Assistance with or over the sterile field requires the same PPE as the surgeon. Assistance with anesthesia and other activities outside the sterile field requires clean but not sterile PPE.
- d. The surgical pack/instruments should not be opened until surgery is ready to begin.

C. Anesthetic Monitoring and Records

1. Prior to initiation of the surgical procedure (incision) a surgical plane of anesthesia **must** be verified. Methods of verification include: lack of response to toe or tail pinch, lack of palpebral reflex.
2. The animal **must** be monitored throughout the surgical/anesthetic procedure, maintained at a surgical plane of anesthesia. Anesthesia should be appropriately adjusted throughout the procedure to maintain these expectations.
3. During anesthesia the animal **must** be observed for anesthetic depth and physiological parameters (e.g. body temperature, respiratory rate/pattern, tissue perfusion, etc.) as described in the IACUC-approved protocol, and should be assessed at least every 15 minutes. These observations may be recorded contemporaneously (if surgical assistant is present) or summarized after completion (if surgeon is working alone).
4. Records **must** be maintained for all surgeries performed. Records **must** include:
 - a. Date of procedure
 - b. Name of procedure
 - c. Anesthesia used (name, dose, route, time)
 - d. Analgesic(s) administered (name, dose, route, time of administration)
 - e. Drug(s) administered (e.g. fluids, antibiotics, etc.)
 - f. Initials of research personnel
5. A pink "Rodent Anesthesia / Surgery Record" card ([see Appendix 1](#)) **must** be filled out and placed on the animal's cage after a surgery or anesthetic event to alert OLAR staff. These cards are required of PI's using OLAR vivaria, and cards will be filled out and updated by the PI/research staff. The card should remain on the cage until post-procedure analgesia and monitoring are complete (usually 7-10 days).

6. Anesthetic or surgical complications, responses, and outcomes **must** be documented in surgical records and discussed with veterinary staff.
7. Any unexpected morbidity or mortality **must** be reported to the AV or clinical veterinarian.
8. All records shall be maintained for the duration of the activity (protocol, procedure, or life of the animal) and for an additional three years after completion of the activity.

D. Post-operative Monitoring and Care

1. Animals **must** be monitored from anesthetic induction through anesthetic recovery.
2. Anesthetized animals cannot be left unattended until they have regained consciousness and are ambulatory. Supplemental oxygen and/or continued heat support may be beneficial during this time, and if utilized it should be recorded in surgical record.
3. Animals should be placed in a clean cage with minimal soft bedding or no bedding (or a paper towel can be used) to avoid irritation of the surgical site and ensure the airway does not become obstructed. Animals should not be placed in the same primary enclosure with other animals until fully recovered from anesthesia.
4. Social species may be singly housed for post-operative recovery or for scientific (if in the IACUC-approved protocol) or clinical reasons (based on veterinary discretion).
5. Post-operative analgesics, antibiotics and other substances should be administered according to the IACUC-approved protocol. If an animal continues to show signs of pain, contact veterinary staff immediately for guidance.
6. Animals should be evaluated daily based on the length of time indicated in the IACUC-approved protocol. In general, animals should be monitored daily until sutures or wound clips are removed (usually 7-10 days after surgery). This includes weekends and holidays.
7. Research staff should ensure animals are eating, drinking, ambulating, and surgical incisions are closed/dry/not red/no discharge. Animals should also be evaluated for signs of pain.
8. If a post-operative complication is observed (e.g. incision dehiscence, infection, pain) veterinary staff **must** be contacted to discuss treatment options. **Treatments and procedures that are NOT in the IACUC-approved protocol cannot be initiated without veterinary guidance.**
9. Recommended supportive care during the post-operative period, which can improve outcomes:
 - a. Heat support – circulating warm water blankets can be placed under ½ of rodent cage to provide additional heat. OLAR vet staff can assist with setting this up in the animal housing room.
 - b. Nutritional support – soft chow (moistened rodent diet in petri dish), diet gel (Clear H₂O), bacon softies (Bio-Serv[®]) and liquid rodent diet (Bio-Serv[®]) are products that can be used to provide additional nutritional +/- hydration support. These products are recommended to maintain weight/hydration. Food may be placed on the cage floor in a receptacle (e.g. petri dish) to reduce need for animal to rear up during the post-operative period.
 - c. Hydration – if animals appear dehydrated (e.g. walking on toes, inactive, loss of elasticity of skin between shoulder blades / delayed skin tenting) veterinary staff should be contacted to advise on fluid support. Nutritional support may provide some additional oral rehydration (e.g. diet gel, soft chow, liquid diet). Depending on the degree of dehydration, subcutaneous (SQ) fluids may be necessary. Depending on length of surgery, SQ fluids could be provided post-operatively (e.g. 1mL SQ sterile saline) to provide extra support and replace losses during the surgical period.
 - d. Special bedding – depending on location of incision, soft paper bedding should be considered. OLAR husbandry staff can assist with this change.

E. Non-survival procedures

1. Non-survival rodent surgery should be performed as described above with the following exceptions:
 - a. Non-survival surgery does not require aseptic technique.
 - b. The animal should be anesthetized according to procedures outlined in the IACUC-approved protocol.

