



## Surgical Policy

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### SCOPE

This policy is intended to provide specific and detailed descriptions of acceptable methods and considerations for surgical procedures performed on laboratory animals.

### GENERAL INFORMATION

- A. Surgery is conducted in accordance with applicable regulatory documents (e.g., Animal Welfare Act & Regulations (AWA&R), the *Guide for the Care and Use of Laboratory Animals*) and consistent with current veterinary medical standards and practices.
- B. Surgical procedures performed to support animal studies are justified and described in IACUC-approved animal protocols. Clinically indicated surgical procedures to ensure animal health or well-being may be performed as approved by the DLAR, SNI, or MWRI facility veterinarian and do not require an IACUC-approved protocol.
- C. Surgery and anesthesia may only be performed by properly trained personnel. DLAR veterinarians are available to provide training in surgical procedures and anesthetic techniques. Anesthesia may be accomplished using injectable agents, inhalant agents, or a combination of both. An infusion pump is recommended to deliver total intravenous anesthesia.
- D. Pain relief must be provided by the appropriate use of sedatives, analgesics, or anesthetics, unless otherwise scientifically justified in the IACUC-approved protocol. An [Analgesic Policy Chart](#) and a [drug formulary](#) maintained on the IACUC website provides guidance for the expected minimum provision of analgesia based on the type of surgical procedure and species-specific recommendations for certain analgesic drugs. Postoperative pain control is best achieved with the use of preemptive analgesia when analgesics are provided prior to the initial surgical incision.
- E. General categorization of surgery into major or minor procedures is defined below, but some procedures, such as laparoscopic and selective neuroscience procedures, may be classified as either major or minor surgery depending on the impact to the animal. The IACUC and veterinarians consider the potential for pain and other postoperative complications; the nature of the procedure as well as the size and location of the incision(s); the duration of the procedure; and the species, health status, and age of the animal when determining procedure categorization.

### RESPONSIBILITIES

- A. The Principal Investigator (PI) is responsible for:
  1. ensuring that all research personnel performing surgery or anesthesia 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 that all postoperative monitoring and substance administration are performed unless other arrangements have been made with DLAR personnel through the DLAR Service Request process, and
4. reporting peri-operative complications to the facility veterinarian.

B. DLAR veterinarians are responsible for:

1. ensuring that all DLAR personnel performing surgery or anesthesia on animals are adequately trained, and
2. ensuring that DLAR-managed surgical areas and equipment are properly maintained.

## **DEFINITIONS**

A. Aseptic technique: Practices and procedures used to reduce microbial contamination to the lowest practical level. Aseptic technique in animal surgery includes but is not limited to patient preparation; surgeon preparation; the use of appropriate PPE and surgical attire; sterilization of instruments, supplies, and implanted materials; and appropriate surgical techniques.

B. Major surgical procedure: Any procedure that penetrates or exposes a major body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection.

1. Examples include, but are not limited to, head cap placement with dissection in the occipital and/or temporal areas of the skull, burr hole placement with dura penetration and brain exposure over 10% of the frontal and parietal surface area of the skull, thoracotomy, laparotomy, limb amputation, nerve/muscle transection, and biopsies which involve entering a body cavity. Surgical classification of endoscopic procedure (e.g., laparoscopy, laparotomy and arthroscopy) and other unique situations will be managed on a case-by-case basis during IACUC protocol review. Depending on the degree of impairment of physical or physiological function, some non-penetrating surgeries may also be considered Major Operative Procedures on a case-by-case basis.

C. Minor surgical procedure: Any procedure that does not expose a body cavity and causes minimal to no physical or physiological impairment.

1. Examples include, but are not limited to, wound suturing, peripheral vessel cannulation, head cap placement with dissection limited to the frontal and/or parietal areas of the skull, burr hole placement without dura penetration of up to 10% of the frontal and parietal surface area of the skull (cumulative if there are multiple small burr holes), eye coil placement, skin/subcutaneous implants, oral surgery/tooth extractions with no bony involvement, tissue biopsy without body cavity exposure, transcutaneous "Tru-cut" biopsy needles or similar methods of tissue collection within a body cavity and closed castration. Surgical classification of endoscopic procedures (e.g., laparoscopy, laparotomy and arthroscopy) and other unique situations will be managed on a case-by-case basis during IACUC protocol review.

D. Survival surgery: Any surgery in which the animal regains consciousness (recovers from anesthesia).

1. Aseptic technique and procedures must be utilized for all survival surgeries.

E. Nonsurvival surgery: Any procedure in which the animal is euthanized before anesthetic recovery.

1. Aseptic technique and procedures are not required for non-survival surgical procedures.
- F. Multiple survival surgeries (MSS): A situation where an animal undergoes two or more survival surgeries involving separate anesthetic events and conducted on separate occasions.
- G. Multiple major survival surgeries (MMSS): A situation where an animal undergoes two or more major survival surgeries involving separate anesthetic events and conducted on separate occasions.
  1. Multiple major procedures performed during a single anesthetic period are not considered multiple major surgeries.
  2. MMSS are acceptable if
    - a) scientifically justified to meet protocol objectives conducted under a single IACUC-approved protocol, or
    - b) the procedures are necessary for clinical reasons as determined by the DLAR, SNI, or MWRI facility veterinarian.
  3. For USDA-regulated species, MMSS performed on the same animal but involving separate IACUC-approved protocols require approval from the USDA-APHIS.
  4. Cost savings alone does not justify MMSS.

## **SURGICAL FACILITIES**

- A. USDA-regulated non-rodent species:
  1. Survival surgery must be performed in a dedicated surgical facility approved by the IACUC.
    - a) The use of a DLAR surgical facility is scheduled through the DLAR website (<http://www.dlar.pitt.edu>) using the Service Request Form.
  2. Nonsurvival surgeries may be performed in another IACUC approved area designated for this purpose.
- B. Mice, rats and aquatic species: A dedicated facility for surgery is not required. All surgical procedures must be performed in an IACUC-approved area designated for this purpose and may include DLAR facility procedure rooms or investigator laboratories appropriately managed to minimize contamination during surgery from other activities.

## **PRE-OPERATIVE FASTING**

- A. USDA regulated species are fasted prior to surgery according to DLAR SOP #306 (*Fasting Procedures in Non-Rodent Mammals*) located on the DLAR website.
- B. Mice, rats, other rodents, and aquatic species are not routinely fasted prior to surgery.

## **PRE-ANESTHETIC EVALUATION**

- A. Observe the general health status and clinical appearance of the animal. Animals with clinical concerns or in poor body condition should not undergo anesthesia or surgery without consultation from a DLAR veterinarian.
- B. Pre-surgical diagnostic screening tests may be appropriate for certain large animal surgical candidates and can be coordinated with the DLAR.

## **SURGICAL INSTRUMENTS**

- A. USDA-regulated species. A sterile set or surgical pack of instruments must be used for each animal undergoing survival surgery. All instruments should be sterilized via autoclave (steam or dry heat) or gas (ethylene oxide) sterilization.

- B. Mice, rats, and aquatic species. Sterile instruments must be used on each animal undergoing survival surgery. All instruments must be initially sterilized via autoclave or gas sterilization. If a separate sterilized set is not available for each animal, instruments between surgeries may be cleaned appropriately and the working tips sterilized in a glass bead sterilizer according to manufacturer's directions. Instruments must be autoclaved or gas sterilized after 5 consecutive surgeries in the same session or a new sterile instrument pack used.
- C. Liquid chemical sterilization of surgical instruments is not recommended. The use of liquid chemical sterilants must be outlined in the IACUC-approved protocol, be used with the appropriate contact time, and the instruments thoroughly rinsed with sterile water or saline before use.

## **INHALATION ANESTHESIA EQUIPMENT**

- A. Anesthesia equipment should be operated according to the manufacturer's directions and consistent with practices outlined in the University's Safety Manual, EH&S Guideline [Number 04-013](#).
- B. Anesthesia machines and precision vaporizers should be checked prior to use to ensure proper function and verify that a sufficient volume of the anesthetic agent and supply of delivery gas (e.g., oxygen, medical grade air) are present to complete scheduled procedures.
- C. Waste anesthetic gas must be actively or passively scavenged.

## **ANIMAL PREPARATION AND PROCEDURES**

- A. USDA regulated species
  1. Anesthetize the animal according to procedures outlined in the IACUC approved protocol.
  2. Provide thermal support for the duration of anesthesia to minimize hypothermia. Note: static thermal support (ex. electric heat blankets, warmed bags of fluids, heat lamps) present high risk of burns to anesthetized animals and must not be used. Circulating water blankets, air warming blankets and IV fluid warming devices are safer mechanisms to provide thermal support to anesthetized animals.
  3. Lubricate the eyes with sterile, bland ophthalmic ointment.
  4. Place an endotracheal tube and establish intravenous access according to the IACUC approved protocol.
  5. Remove fur from the surgical site using appropriately sized clean clippers. The area of fur removal should be approximately twice that of the surgical site.
    - a) Depilatory cream should not be necessary but may be appropriate for certain procedures. When used, depilatory cream must not contact the eyes or mucus membranes and must be rinsed off thoroughly with water to avoid skin irritation.
  6. Perform a surgical scrub for all survival surgeries, working from the inside of the clipped area outwards. Alcohol or sterile water should be alternated with either betadine or chlorhexidine scrub/soap (not solution) a total of three times to provide adequate skin disinfection.
  7. Move the animal to the surgical area being careful not to contaminate the scrubbed surgical site.
  8. Connect appropriate anesthetic monitoring equipment and initiate any fluid support.
  9. Perform a final aseptic surgical scrub and drape the surgical area.
  10. Confirm a surgical plane of anesthesia and proceed with the surgical procedure.
    - a) A lack of palpebral reflex, ventromedial pupil position, reduced muscle tone, and a

lack of response to painful stimuli are indicators of surgical anesthesia in non-rodent mammals. A regular but decreased heart rate and respiratory rate should also be noted.

11. Appropriate aseptic techniques must be used at all times during survival surgery.
12. Tissues and organs should be gently manipulated using the appropriate surgical instruments.
13. 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 suture pattern, suture and needle size, and suture and needle type.
14. Administer analgesics, antibiotics, fluids or other supportive care according to the IACUC approved protocol.

#### B. Mice and rats

1. Anesthetize the animal according to procedures outlined the IACUC approved protocol.
2. Provide thermal support for the duration of anesthesia to minimize hypothermia. Note: minimally controllable static thermal support (ex. electric heat blankets, warmed bags of fluids, heat lamps) present high risk of burns to anesthetized animals and should not be used. Circulating water blankets, air warming blankets, and infrared heat pads are safer mechanisms to provide thermal support to anesthetized animals.
3. Lubricate the eyes with sterile, bland ophthalmic ointment.
4. Remove fur from the surgical site using appropriately sized clean clippers. The area of fur removal should be approximately twice that of the surgical site.
  - a) Depilatory cream may be appropriate for certain procedures and when used must not contact the eyes or mucus membranes and must be rinsed off thoroughly with water to avoid skin irritation.
5. Move the animal to the surgical area.
6. Perform a surgical scrub for all survival surgeries, working from the inside of the clipped area outwards. Alcohol should be alternated with either betadine or chlorhexidine scrub/soap (not solution) a total of three times to provide adequate skin.
7. Drape the surgical area.
8. Confirm a surgical plane of anesthesia and proceed with the surgical procedure.
  - a) A toe or tail pinch test may be used to indicate anesthetic depth in mice and rats. A lack of blink reflex should also be noted, as well as a regular, slightly slowed respiration rate. A lack of response to surgical stimulation confirms a surgical plane of anesthesia.
9. Appropriate aseptic techniques must be used at all times during survival surgery.
10. Tissues and organs should be gently manipulated using the appropriate surgical instruments.
11. 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 suture pattern, suture and needle size, and suture and needle type.
12. Administer analgesics, antibiotics, fluids or other supportive care according to the IACUC-approved protocol.

#### C. Aquatic species

13. Anesthetize the animal according to the IACUC-approved protocol.
14. Prepare the surgical site by gentle removal of gross debris followed by thorough rinsing with sterile saline.

15. Confirm a surgical plane of anesthesia and proceed with the surgical procedure.
  - a) Finfish: A surgical plane of anesthesia is characterized by the inability to swim, shallow respiration and loss of response to stimuli.
  - b) Amphibians: A surgical plane of anesthesia is characterized by loss of the righting and withdrawal reflexes.
16. Appropriate aseptic techniques must be used at all times during survival surgery.
17. Administer analgesics, antibiotics, or other supportive care according to the IACUC-approved protocol.

## **SURGEON PREPARATION AND PROCEDURES**

- A. Survival surgical procedures require the surgeon to wash and scrub hands and arms appropriately for the species used and the specific surgical procedure.
- B. Wear the appropriate personal protective equipment (PPE) based on the location and biohazard level of the work area.
  1. USDA-regulated species
    - a) Major surgical procedures. Surgical personnel must wear clean scrubs, sterile gown, shoe covers, surgical mask, hair cover, and sterile gloves.
    - b) Minor surgical procedures. Surgical personnel must wear clean scrubs and/or a clean gown over street clothes, shoe covers, surgical mask, hair cover, and sterile gloves.
  2. Mice, rats and aquatic species
    - a) Major and minor surgical procedures. Surgical personnel must wear a clean lab coat or disposable gown, surgical mask, hair cover, and sterile gloves. Shoe covers are required in DLAR rodent facilities.
- C. Additional PPE (e.g., eye protection) may be required for surgeries involving the use of biological and chemical hazards, nonhuman primates, or specific procedures.

## **ANESTHETIC MONITORING AND RECORDS**

- A. Anesthetic depth should be assessed at least every 15 minutes throughout the procedure and supportive drugs and fluids administered as needed and described in the IACUC approved protocol.
- B. USDA regulated species
  1. During anesthesia monitor vital parameters (e.g., heart rate, respiratory rate, mucous membrane color, oxygen saturation) continuously as described in the IACUC approved protocol and record them at least every 15 minutes.
    - a) The DLAR Anesthetic Monitoring Form located on the DLAR website may be utilized for recording vital parameters.
    - b) Record the dose, route and time of substances administered including anesthetics, analgesics, antibiotics, and fluids.
    - c) Describe and record the time of all significant peri-operative events (e.g., beginning of surgery, organ manipulation, end of surgery, endotracheal tube removal) and any complications that occur during the procedure.
  2. Complete medical records according to IACUC Policy "Medical Record Keeping Requirements – Large Animals" located on the IACUC website.
  3. Provide copies of anesthesia logs, procedure forms, and other peri-operative documentation to the DLAR in a timely manner, typically within 3 business days, for inclusion in the animal's clinical veterinary medical record.
- C. Mice and rats
  1. Anesthetic depth should be assessed at least every 15 minutes throughout the

procedure and additional drugs and substances administered as needed and described in the IACUC approved protocol.

2. During anesthesia monitor vital parameters (e.g., respiratory rate) continuously as described in the IACUC approved protocol.
3. Complete medical records according to IACUC Policy “Medical Record Keeping Requirements – Rodents” located on the IACUC website.

D. Aquatic species

1. Anesthetic depth should be assessed at least every 15 minutes throughout the procedure and additional drugs administered as needed and described in the IACUC approved protocol.
2. During anesthesia monitor vital parameters (e.g., respiratory rate) as described in the IACUC approved protocol.
3. Aquatics surgical records are maintained by the PI and reviewed by the IACUC during the semi-annual review (SAR) process.

## **POSTOPERATIVE MONITORING AND CARE**

A. Animals must be monitored from anesthetic induction through anesthetic recovery.

1. USDA-regulated species. Anesthetized animals cannot be left unattended until the endotracheal tube is removed and sternal recumbency regained.
  - a) After sternal recumbency is achieved, animals must continue to be monitored at an appropriate frequency until fully recovered from anesthesia (e.g., return to normal level of alertness and responsiveness).
2. Mice and rats. Animals must be monitored at least every 15 minutes until sternal recumbency is regained and then at an appropriate frequency until fully recovered from anesthesia (e.g., return to normal level of alertness and responsiveness).
  - a) Sternal recumbency in rodents can be identified using the “righting reflex” which is the animal’s correction of its body orientation when taken out of its normal upright position.
3. Aquatic species. Animals must be monitored at least every 15 minutes until the animal has regained sternal recumbency and is able to surface for air (amphibians), or is able to maintain equilibrium/position in the water column (fish).
4. The use of anesthetic reversal agents may hasten anesthetic recovery.

B. Return the animal to its primary enclosure (cage, pen, or tank).

5. The animal should not be placed in the same primary enclosure with other animals until fully recovered from anesthesia.
6. Social species may be single housed up to 14 days for postoperative recovery and observation in accordance with the IACUC’s social housing policy. Single housing beyond 14 days must be outlined in the IACUC approved protocol or granted by the DLAR, SNI, or MWRI facility veterinarian for animal health or welfare concerns.

C. Administer postoperative analgesics, antibiotics, and other substances according the IACUC approved protocol.

D. Remove staples or sutures used for skin closure 7-14 days postoperatively for mammals and 2-4 weeks postoperatively for aquatic species.