Anesthesia, survival surgery and post-procedural care of research animals are addressed in the Guide, FASS Guide, PHS Policy, and USDA regulations. These documents specifically require the institutional animal care and use committee (IACUC) to review, and the institutional veterinarian to oversee, anesthesia, surgical procedures and post-procedural care programs. This policy clarifies requirements pertaining to performing anesthetization and/or survival surgery on vertebrate animals. It does not cover minor procedures such as tail clip, ear punch, or neonatal rodent toe clip.

The principal investigator is ultimately responsible for ensuring that care, both appropriate to the species and to the procedure being performed, is provided. In practice, however, appropriate animal care, which conforms to regulatory expectations, requires careful coordination between the principal investigator, surgeon, animal care staff and veterinary staff. Responsibilities of key individuals must be delineated and understood before surgical procedures are performed.

This policy delineates the following 5 major requirements: 1) animals must be appropriately monitored during and after anesthesia, 2) survival surgery must be performed using aseptic technique, 3) analgesia is expected to be used for most procedures, 4) procedures and monitoring must be documented, and 5) animals which have had surgery must be visually identified.

**Anesthesia**

*Animals must be monitored carefully during anesthesia and during recovery, and provided additional physiological support as needed.*

**Monitoring**

Monitoring includes, but is not limited to, checking the anesthetic depth and physiological parameters on a regular basis (minimum every 15 minutes). The specific parameters monitored vary with the species, however, the following list provides recommended methods:

*Anesthetic Depth:* toe pinch reflex (preferred method for rodents), palpebral reflex, corneal reflex, muscle tone

*Circulation:* heart rate, auscultation of heart beat, pulse, ECG, blood pressure.

*Oxygenation:* mucus membrane color, capillary refill time, pulse oximetry, blood gas analysis.

*Ventilation:* respiratory rate, movement of chest or breathing bag, auscultation of breath sounds, respiratory monitor, capnography, blood gas analysis.

*Temperature:* Monitor with a thermometer. Rodent thermometers are available:


**Physiological Support for anesthesia beyond a few minutes with isoflurane**

*Required:* The following physiological supports are required for anesthetized animals:
1. **Eye lubricant:** Eye lubricant is required if anesthesia extends beyond a few minutes. Sterile ophthalmic lubricant/ointment must be applied to each eye. Without this protection, ocular ulceration may occur.

2. **Thermal support:** Thermal support is required if anesthesia extends beyond a few minutes. Hypothermia is the main concern, and small animals are especially susceptible, even after a short time. Methods to prevent hypothermia include: shave the minimum feasible area, rinse disinfectant from the skin with sterile water rather than alcohol, insulate the animal from cold surfaces, provide supplemental heat with a thermal water blanket, discs, gels, heat lamp (electric heating pads without specific temperature control should be used only with extreme care, and with a physical barrier (cloth, cage bottom) between the animal and the pad), provide warm fluids SQ or IV. Other physiological support is recommended, especially for lengthy procedures.

Fluid therapy: Providing fluids may be necessary, especially for a lengthy procedure. Fluids such as sterile saline or LRS may be administered IV, SQ, or IP (5-10ml/kg/hour).

**Recovery**
After surgery, move the animal to a warm, dry location. An animal must be monitored continuously until it is able to hold itself in a normal, upright position and has a normal body temperature.

**Aseptic Technique**

Aseptic surgical procedures must be used for survival (non-terminal) surgery of all vertebrate animals.

**Surgery Space**
Aseptic surgery should be conducted in dedicated facilities or spaces. When determining the appropriate site for conducting a surgical procedure (either a dedicated operating room/suite or a lab area that simply provides separation from other activities), the choice may depend on the species, the nature of the procedure (major, minor, or emergency), and the potential for physical impairment or postoperative complications, such as bacterial infection. Wherever surgery is performed, the area must be clean and uncluttered, and the work surface must be sanitized (wiped with a disinfectant) before surgery begins.

**Surgical Site Preparation**
There must be appropriate preparation of the surgical site, including removal of the fur and adequate, species appropriate disinfection of the skin, such as with dilute chlorhexadine or povidine iodine, followed by sterile saline or 70% alcohol (may contribute to hypothermia), repeated 2-3 times. The skin surrounding the surgical site should be draped with sterile cloths or adherent drapes as appropriate to avoid contamination of the incision, instruments and supplies.

**Sterile Instruments and Supplies**
Sterile surgical gloves, a surgical mask, and a clean lab coat, surgical gown or other attire to replace or cover street clothing must be worn by the surgeon and any assistants working in the immediate surgical field. Surgeons should wash and dry their hands before aseptically donning sterile surgical gloves. Gloves must remain intact, clean and disinfected. Gloves should be cleaned with 70% alcohol or disinfectant between animals, and they should be changed anytime they are damaged or become excessively soiled. For rodent surgery, utilizing a ‘tips only’ technique can be employed to further minimize potential contamination of the surgical site. With the ‘tips only’ technique just the tips of sterilized instruments touch the surgical site. Instruments, supplies, implants and wound closure material must be sterile at the beginning of surgery and when used for each animal.*
Aseptic Techniques
Aseptic operative techniques must be utilized to avoid contamination of sterile instruments and gloves and reduce the likelihood of infection. Tips on maintaining asepsis:
1. Gloved hands should be held elevated above the waist – do not drop them below the level of the waist. Gloved hands should touch only sterilized items.
2. Sterile instruments should be placed on a sterile work surface. Do not allow surgical instruments to drop below the level of the surgical area.
3. Keep the sterile areas dry, as moisture can lead to contamination of the area.

*Surgical procedures may be performed on multiple rodents during a single session using one sterile surgical pack, provided that instruments are cleaned and sterilized between animals. For sterilization between animals, instruments may be soaked in an approved chemical sterilant, observing recommended contact times, and rinsed in sterile saline or when using a “tips only” technique, heated in a hot bead sterilizer and cooled. Effective use of these strategies requires rigorous attention to technique.

Analgesia
Analgesia use is the expectation for most surgical procedures.

Expectation for Analgesia
In the absence of evidence to the contrary, it is assumed that something that is painful to a human is also painful to an animal. Therefore, the expectation is that in most cases analgesia will be provided to animals undergoing surgery, and analgesia must be provided as described in the IACUC approved AUP. Because each surgery is different (species, procedure, circumstances) a single method of post-operative analgesia cannot be prescribed for all cases. However, the following concepts can guide the appropriate use of analgesia:

Guidelines for Analgesia
Moderate to Significant Pain
The general recommendation for procedures likely to induce moderate to significant pain is to provide at least 48 hours of post-operative analgesia, and then additional analgesia as needed until the animal does not appear to be experiencing pain. Examples of such procedures would include orthopedic procedures, laparotomy with organ incision or removal, orchidectomy, surgical embryo transfer, thoracotomy, burn or trauma models.

Mild to Moderate Pain
The general recommendation for procedures likely to induce mild to moderate pain is to provide at least 24 hours of post-operative analgesia, and then additional analgesia as needed until the animal does not appear to be experiencing pain. Examples of such procedures would include catheter, cannula or vascular access port placement in a peripheral vessel, superficial lymphadenectomy, thyroidectomy, thymectomy, intracerebral implantation, vasectomy.

Preemptive Analgesia
As well as post-operative analgesia, preemptive analgesia, administered pre-operatively and intro-operatively, should be provided to minimize post-operative pain by inhibiting the initial pain cascade caused by tissue damage during surgery. Thus, the first dose of analgesia is administered prior to surgery.

Local Anesthesia
Local anesthetics (e.g. bupivacaine) may be indicated for some procedures involving disruption of the skin, as these drugs help block the onset of the pain cascade, and minimize post-operative pain. Local
infiltration of the incision site before cutting or dripping dilute anesthetic onto the incision at closing are appropriate methods. However, local anesthesia is not to be used instead of systemic analgesia.

**Multimodal Analgesia**
Multimodal analgesia refers to the use of the 2 major classes of analgesia (NSAID and opioid) simultaneously. This is recommended especially for surgeries expected to cause significant pain (e.g. thoracotomy and orthopedic procedures).

**Non-pharmacological Methods**
Non pharmacological methods of pain control are recommended. Provide, for example, a dark, quiet recovery area; timely wound/bandage maintenance; a soft resting surface; ambient warmth; rehyrdration with fluids; and palatable, easy to reach food.

**Recognizing Pain**
Recognizing pain in traditional laboratory species is challenging due to their masking of signs, and subtleness of signs. Some signs of pain in rodents and rabbits are listed below:

<table>
<thead>
<tr>
<th>APPEARANCE</th>
<th>APPETITE/WASTE</th>
<th>BEHAVIOR</th>
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</thead>
<tbody>
<tr>
<td>Rough fur coat/piloerection</td>
<td>Fewer, small fecal pellets</td>
<td>Lack of normal behavior</td>
</tr>
<tr>
<td>Hunched posture</td>
<td>Eating decreased</td>
<td>Lethargy/lack of movement</td>
</tr>
<tr>
<td>Face “pinched”</td>
<td>Drinking decreased</td>
<td>Restlessness/pacing/circling</td>
</tr>
<tr>
<td>Head/ears down (rabbit)</td>
<td>Drinking increased</td>
<td>Self-isolation from group</td>
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<tr>
<td>Squinted, half-closed eyes, puffy looking eyelids</td>
<td></td>
<td>Abnormal ambulation</td>
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<tr>
<td>Porphyrin (red discharge) on eyes/nose (rats)</td>
<td></td>
<td>Guard painful area</td>
</tr>
<tr>
<td>Abdomen pushed towards floor or tucked up</td>
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<td>Tense when touched</td>
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For further specific guidance on analgesia, contact your Attending Veterinarian, and/or refer to the ‘Formulary for Laboratory Animals’ cited on the URAR and IACUC websites.

**Documentation of Surgery and Monitoring**
*Surgeries and post-anesthesia and post-operative monitoring records must be maintained for all survival surgeries and most non-surgical procedures which require anesthesia.*

**Responsibility**
It is the responsibility of the principal investigator to assure accurate records regarding anesthesia, surgical procedures and post-anesthesia/post-operative care are maintained.

**Exception**
Exception: Procedures for which animals are briefly anesthetized with inhalant anesthesia for minor procedures (e.g. tail clip, gavage, microchip implant) and wake up within a few minutes do not require
documentation of anesthesia or post-anesthesia monitoring. However, as with any other procedure conducted on research animals, this should be documented as part of scientific data collection.

**Group vs. Individual Records**

For rodents and non-mammalian vertebrate species, group records are acceptable. For non-rodent mammals, individual records are required.

**Required Contents of Record**

All records must list the PI, AUP #, date of the procedure, identification of each animal, anesthesia and analgesia provided, surgeon, an emergency contact phone number, a description of the procedure with any complications and post-anesthesia/post-operative monitoring. ‘A Template Anesthesia/ Surgery Form’ and a ‘Template Post-Procedure Monitoring Form’ are provided for this purpose. These forms, the Veterinary Teaching Hospital forms, or a lab generated document may be used, as long as they contain the information required. Note that 1 template with the required information may be copied, into which only variable information must be added at the time of the procedure (e.g. animals ID, date, surgeons, complications).

**Immediate Post Anesthesia Period**

During the immediate post-anesthesia period (until the last animal is able to move purposefully, right itself, and maintain balance), continuous postoperative monitoring is required and must be documented.

**Post Operative Period**

During the post-operative period (until the sutures are removed, surgical wounds are adequately healed, or 10-14 days) a minimum of daily-recorded observations is required. All surgery related medications administered (e.g., analgesia) must be documented.

**Location of Records**

The surgical and post-operative monitoring records must remain in the animal holding room with the animals during their post-operative period. These documents are to be added to the individual medical record for animals with individual records, such as USDA covered species.

**Identification of Animals during the Post-operative Period**

*Animals which have had surgery must be identified by a physical method approved by URAR.*

For example, tags or special cage cards may be placed on the cage/pen. The identification method should be determined in consultation with the URAR Supervisor. This identification is to alert animal care technicians and the veterinary staff that an animal has had surgery. The physical identification should be removed when the post-operative recovery period is at an end.

**Reference(s):**

- Animal Welfare Regulations, 9 CFR, chapter I, subchapter A.
- Standford School of Medicine Veterinary Service Center-Comparative Medicine. Veterinary Guidelines for Anesthetics: Mice.
• Stony Brook University, Division of Laboratory Animal Resources. Rodent Anesthesia and Analgesia
• Stony Brook University, Division of Laboratory Animal Resources. Guidelines for Survival Rodent Surgery
• ILAR. Recognition and Alleviation of Pain in Laboratory Animals. 2009. Lab Animal.

Resources:

• AALAS Learning Library UGA Rodent Surgery track courses (Anesthesia and Analgesia; Aseptic Technique for Rodent Survival Surgery; Facilities, Supplies, and Materials.)
Template Anesthesia/Surgery Form (page 1)

- Written documentation MUST be completed for every anesthesia and or surgery (or 1 day session of surgeries) and kept with the animal/s during the post-operative period. This form is one option for documenting. Documentation may also be in the animal's individual medical record for species with medical records.
- You MUST leave documentation of a surgical procedure in the animal room until sutures/clips are removed, or ~10-14 days
- You MUST identify all cages with animals that have had surgery-such as by tag or card

<table>
<thead>
<tr>
<th>PI:</th>
<th>AUP:</th>
<th>Date of surgery:</th>
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<tr>
<td>Person performing anesthesia/procedure:</td>
<td>Animal Identification/s (each animal):</td>
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<tr>
<td>Emergency contact #:</td>
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Procedure name:________________________________________________________________________

Pre-operative Exam and Therapy

Physical Exam (note any abnormalities for each animal):

Name and dose of pre-operative medications (sedatives, analgesics):

Any other therapy: fluids, heat, etc

Anesthesia monitoring and immediate recovery from anesthesia
For each animal, note anesthesia administration and recovery. Include initial dose and any re-dosing.
Template Anesthesia/Surgery Form (page 2)

Must be attached to page 1-- printed on the back of page 1 or stapled to page 1

**Description of procedure if applicable:** This information must be provided for surgery or complicated procedures (e.g., repeated dosing and blood collections of anesthetized animal). It is not required for non-invasive procedures that (e.g., imaging, mosquito feeding).

<table>
<thead>
<tr>
<th>Description: General procedure method. Also note any complications, findings (for each animal)</th>
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• Note that you can make a template form with the standard information such as PI/AUP, anesthesia drug/dose, surgery general procedure description, then fill in only the details for each surgery by hand.