UC SANTA BARBARA

Institutional Animal Care and Use Committee

Santa Barbara CA 93106-5062

Tel: (805) 893-5855 Fax: (805) 893-2005

Email: iacuc@lifesci.ucsb.edu

https://www.research.ucsb.edu/compliance/animal-subjects/

IACUC GUIDELINE: ASEPTIC RODENT SURGERY AND POSTOPERATIVE CARE

DATE IMPLEMENTED: March 19, 2010

REVISION APPROVED: June 10, 2011; May 17, 2013; July 9, 2014; May 26, 2016;

September 20, 2016; April 6, 2017; June 19, 2017; June 12, 2019; November 13, 2019; January 10, 2020; January 24, 2020; May 19, 2020; February 19, 2021; June 29, 2021; August 29, 2022; October 27, 2022; December 9, 2022; January 19, 2023; March 1, 2023;

April 5, 2023; November 13, 2023

Introduction:

The Federal Animal Welfare Act Regulations and the Guide for the Care and Use of Laboratory Animals 8th Edition¹ (*Guide*) both set standards that are obligatory for biomedical research involving live, vertebrate animals. These documents have been adopted by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and virtually all public and private funding sources, and are therefore pertinent to all research projects, regardless of funding source. The following guidelines augment statements contained in the Guide and further define rodent surgery standards at UCSB. Failure to abide by the standards contained in this guide may result in a revocation of an individual researcher's privilege to perform surgical procedures on laboratory rodents in a research setting.

I. Surgery Facilities

The specific location of the surgical area within the laboratory or vivarium should promote the proper conduct of sterile techniques, and to the extent possible, it should be isolated from other activities in the laboratory or vivarium. The surgical area should be dedicated for that purpose while surgery is performed.²

The area where surgeries are performed should be uncluttered to minimize the potential for contamination and facilitate ease of cleaning. The surfaces (e.g., tables) in the surgical area

should be impervious to moisture (e.g., stainless steel). The surgical area should be routinely cleaned and disinfected.

The area where surgeries are performed should be identified in the animal use protocol and will be inspected by the IACUC on a semi-annual basis to ensure that the area is meeting the expectations set forth in the preceding points.

II. Surgeon Training and Qualifications

Personnel conducting surgical procedures must be appropriately qualified and trained in good surgical technique including asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective homeostasis, and correct use of suture materials and pattern.^{1,3}

Before the hands-on training of any surgical procedure can begin, personnel (e.g., graduates, research assistants/associates, post-docs and PIs) must first complete the Citiprogram.org training course "Aseptic Surgery".

Personnel that are performing survival surgical procedures are required to demonstrate competency, to the Attending Veterinarian (AV), for each different surgical procedure that they perform, unless exempted by the IACUC, and must NOT perform any surgical procedure without the assistance and direct supervision of the surgical trainer or AV until they have successfully completed their competency assessment. Personnel will be deemed competent if they can demonstrate to the AV that they possess the necessary knowledge and technical skills to successfully complete the surgical procedure. Anyone that cannot competently perform the surgical procedure (i.e., fails their evaluation) should receive additional training under the direct supervision of a trained and qualified individual.

The Campus Veterinarian must be consulted for questions regarding animal health, surgical wound care, animal anesthesia techniques, surgical procedures, and provision of postoperative care. Additionally, surgical outcomes should be continually and thoroughly assessed to ensure that appropriate procedures are followed and timely corrective changes are instituted. The Campus Veterinarian must be consulted when there is an increased incidence of anesthetic or perioperative deaths, or increased incidence of post-surgical complications.

III. Preparation of Surgical Instruments

Surgical instruments must be sterilized for both survival and non-survival rodent surgery. Several methods (steam, dry heat, or chemical agents) can be used to sterilize instruments and other materials that will come in contact with the animal's tissues. The sterilization method selected will depend on the physical characteristics of the materials to be sterilized. For example, autoclaving is the preferred method to sterilize common surgical instruments. By contrast,

delicate implants (e.g., cannulas) may be damaged by steam or dry heat sterilization procedures. Alternative sterilization procedures, for example immersion in a liquid chemical sterilant or high-level disinfectant, should be used in these cases. If a liquid chemical sterilant is used, then the manufacturer's recommendations on contact times must be followed, and the sterilized material must be rinsed in sterile water before use to remove any disinfectant residue.

Alcohol (i.e., ethyl alcohol or isopropyl alcohol) is neither a sterilant nor a high-level disinfectant, and therefore should not be used to sterilize surgical instruments. However, alcohol at concentrations between 60-90% is rapidly bactericidal against vegetative forms of bacteria, and a potent virucidal and fungicidal agent. Its use should be restricted to applications were the presence of bacterial spores is not anticipated, for example, decontamination of autoclaved surgical instruments between serial rodent surgeries.

Following sterilization, surgical instruments and medical or surgical devices (e.g., catheters, cannulas, or osmotic mini-pumps) must be handled using aseptic technique to prevent contamination. Further, these sterile instruments and devices must be properly stored. The shelf life of these instruments will depend on how they were prepared and wrapped, how they are stored, and event-related factors contributing to their contamination. Storage of surgical instruments in plastic self-seal sterilization pouches is recommended. Storage in closed or covered cabinets is preferred, but open shelving may be used for storage. Any sterile instrument, device, or sterile package containing these items that has fallen or been dropped to the floor, is exposed to water, or experiences any other event that could contribute to the contamination of the sterile material should be cleaned and re-sterilized.⁴

When performing surgeries on multiple animals (mice or rats), at least 2 sets of instruments should be sterilized by an appropriate method. While the first set of instruments is being cleaned to remove all organic material (i.e., blood) and re-sterilized, the second set is used on a second animal. If only the tips of instruments have contacted the sterile surgical field or the animal's organs/tissues, then the instrument tips may be cleaned to remove organic material (i.e., blood), and placed in a glass bead sterilizer for a few seconds (check instrument manufacturer for recommended contact times). As an alternative to using a glass bead sterilizer in tips-only surgical procedures, surgical instruments may be immersed in a sterile bowl containing 70% isopropyl alcohol for at least 2 minutes. The instruments should be maintained in a sterile holder/container after decontamination (glass bead or alcohol), and until used for the next surgery. Decontamination of surgical instruments with alcohol during serial tips-only surgeries must be limited in use, and after the 5th animal, fresh sterile instruments must be used.

IV. Preparation of the Animals

Prior to taking the animal to the surgery area, remove all hair for at least a centimeter on either side of the incision site. Hair removal should be performed in a location away from the surgery area to minimize the potential for contaminating the surgery area. Hair can be removed by clipping with a #40 clipper blade, shaving with a razor, plucking (in anesthetized mice or similar-sized rodents), or by using a depilatory cream. Vacuum or otherwise remove loose hair.

Clean and aseptically prepare the surgical site. Alternate between a topical antiseptic solution (e.g., Betadine) and 70% alcohol to carefully clean and disinfect the skin surface. Use a surgical sponge, cotton swab, or cotton-tipped applicator to apply the topical antiseptic solution and alcohol. Clean in a gradually enlarging circular pattern from the center of the proposed incision to the periphery. For surgeries in mice, the surgical site is often very small making the above cleaning technique impractical. In these situations, focus on cleaning the surgical area and avoid bringing the sponge or swab back from the contaminated periphery to the clean surgical area. Repeat the skin preparation cycle for a total of three times, each time beginning at the center and proceeding to the periphery. End the preparation of the surgical site with an application of antiseptic solution (i.e., Betadine).

To prevent hypothermia, try not to wet the animal any more than necessary.

Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal. Glad Press'n Seal freezer wrap provides an uncontaminated (i.e., free of microorganisms and organic material), inexpensive and effective method to cover the sterile surgical field.⁶

Place lubricating ophthalmic ointment (such as Lacrilube or Paralube) on the anesthetized animal's eyes to prevent drying of the cornea.

Place the animal on a clean absorbent surface (e.g., disposable underpads) and maintain its body temperature using a water circulating heating pad, or equivalent external heat source, taking care not to overheat the animal. If you're using a homeothermic controller to monitor and control body temperature, make sure that you're familiar with its operation (i.e., read the instruction manual carefully), that you check the over-temperature safety settings, and that you monitor the surface temperature on the heating pad to prevent any thermal injury to the animal.

V. Preparation of the Surgeon

Surgeons should wash their hands before surgery.

Surgeons must wear a mask, sterile gloves, and clean scrub shirt or lab coat to prevent contamination of the sterile surgical field. A new pair of sterile surgical gloves should be used for each animal. Alternatively, if only the tips of the surgical instruments will come into contact with the surgical field or animal's organs/tissues, then the same pair of surgical gloves may be decontaminated between animals and used on up to 5 animals.⁵ To decontaminate the gloves, either spray them with 70% isopropyl alcohol and rub the wet gloved hands together for 30s, or dip the fingertips of the gloves in a sterile bowl containing 70% isopropyl alcohol for 30s.

VI. During Surgery

The surgical field must be kept sterile throughout the procedure. Sterile instruments must be prevented from contacting non-sterile surfaces. Catheters, probes, or cannulas must not contact non-sterile surfaces, for example, the animal's fur or paws. In most cases, sterile drapes are required for maintenance of the sterile field.

Monitor and control the animal's body temperature. Use a lubricated (sterile single-use lubricant; rectal temperature probe or thermometer, of appropriate size for mice or rats, to monitor the animal's body temperature.

Use a water circulating heating pad, or a heating plate/pad, to maintain the animal's normal body temperature: mice (36.5 - 38°C) and rats (35.9 - 37.5°C).

Monitor and control the level of anesthesia. If isoflurane is used to anesthetize the rodent patient, then please make sure that there is an SOP for instruction on how to use and maintain the anesthesia machine. This is the SOP for the isoflurane anesthesia machines in the shared procedure rooms of the ARC vivarium. The exact monitoring procedure and frequency should be based on the invasiveness and complexity of the surgical procedure, and the duration of the surgery. In general, more intensive intraoperative monitoring, both in terms of methodology and frequency, is required for longer duration, technically complex, and invasive surgeries. Similarly, the surgical records should reflect these differences, and will be evaluated by the IACUC to assess the adequacy of the intraoperative monitoring procedures.

The surgeon or the anesthetist must pay continuous and close attention to the animal's anesthetic depth and adjust the anesthetic dose accordingly. At a minimum, the following vital signs should be monitored and controlled during all rodent surgeries:

The animal's response to painful stimuli (incision, toe/tail pinch) - there should be no
response or movement. If there is a response, wait for the anesthetic to take effect, or
increase the anesthetic concentration (isoflurane) gradually.
The animal's respiratory rate (i.e., breaths per minute) and character - it should not be
too slow (<60 breaths per minute) or too shallow. If the respiratory rate is too low,
decrease the anesthetic concentration (isoflurane).
Check the animal's cardiac output and tissue perfusion - the color of mucous membranes
should be pink and moist; the color of the eyes (albino rodents) should be red; and/or
the color of the skin (ears) should not be pale, or blue.
Use a pulse oximeter to monitor heart rate and oxygen saturation of the blood (%SpO2)
is strongly recommended. Saturation levels <90% are of concern; immediately lower the
anesthetic (isoflurane) concentration and the increase oxygen flow rate.

Control any intraoperative bleeding by placing a sterile gauze (e.g., 2×2) or sterile cotton-tipped applicator over the bleeding area and apply gentle pressure. Blot, do not rub or wipe the area to remove or soak up the blood. Alternatively, sterile absorbable gelatin sponges (e.g., Gelfoam) may be used to control bleeding.

Use the appropriate material and method to close the surgical incision. Stainless steel wound clips are recommended for closing skin incisions in rodents. Tissue adhesive (glue; Vetbond™

or DermabondTM) should only be used for a topical application and only to close easily approximated skin edges (i.e., no tension) created by a sterile surgical incision. Tissue adhesive must be used sparingly (only one or two drops), and applied only on the cut edges of the skin, and not on the deeper tissues. The applicator tip should not contact the tissue, and if it does it should be discarded and replaced.

Provide analgesic treatments to alleviate the pain caused by the surgical procedure. The evolving standard of veterinary care for rodent surgical procedures is to provide multimodal analgesic treatments combining a local anesthetic (i.e., 0.5% lidocaine with 1:200,000 epinephrine in a multi-dose vial) administered by irrigation of a surgical opening or local infiltration, a non-steroidal anti-inflammatory analgesics (e.g., meloxicam) administered by SC injection immediately prior to starting the surgical procedure, and an opioid analgesic (e.g., buprenorphine) also administered by SC injection. Additional doses of the non-steroidal and opioid analgesics should be administered at prescribed dosing intervals during the postoperative period as described in the approved IACUC protocol.

If an emergency evacuation occurs during surgery, and the animal being operated on has an exposed body cavity (e.g., craniotomy), open incision(s), or exposed cannulas or catheters, then that animal should be euthanized by administering an overdose of anesthesia prior to evacuating the area, if time and circumstances permit. If the animal is anesthetized but no or only minor surgical manipulations have been performed, then leave the anesthetized animal to recover in a confined space (e.g., a cage in the surgery room) and evacuate the area. If a live animal was left in a procedure area during an emergency evacuation and the animal's welfare is at risk due to prolonged building access restrictions, notify the AV or ARC staff immediately. Employees should never put themselves at risk during an emergency evacuation to care for animals.

VII. Postoperative Care

The animal should be continuously monitored until it is conscious, and has stable vital signs (e.g., regular breathing pattern and rate).

The animal should be recovered from surgery by itself (i.e., not housed in a cage with other animals) on a clean and dry surface (no bedding), and it should be provided with supplemental heat during the recovery period. Specifically, the animal should be placed on clean absorbent pad in a clean and empty rodent cage that is resting on a heating pad. Be cautious with supplemental heat sources; hyperthermia can be as detrimental as hypothermia.

The animal should be given fluid replacement therapy, as follows: sterile **warmed** physiological fluids (sterile saline, or Lactated Ringer's Solution) should be administered subcutaneously at a volume of 10 ml/kg for rats (e.g., 1 ml per 100 gm) or 25 ml/kg for mice (i.e., 0.25 ml per 10 gm).

The animal should not be returned to the animal room until it can stand and move about the cage. To prevent cannibalism or suffocation, continue to house rodents individually until they

can stand and move around. Additionally, continue to house rodents individually even after they are completely recovered from anesthesia, if they have exposed (i.e., not protected by a metal cover) external catheters or ports.

Once an animal is recovered from anesthesia, it is recommended that it be placed in a home cage with clean bedding. Make sure that the animal has access to food and water when it's returned to the animal room. If necessary, provide food/water on the bottom of the cage in the form of moistened pellets or NutraGel.

A member of the research team or other individual to whom postoperative care has been delegated must monitor each animal at least once a day and document all observations and treatments in the postoperative recovery log.

Each animal must be carefully observed for signs of pain, and surgical complications, including infection or dehiscence of the surgical site(s). Pain assessment guidelines are included below.

All surgical wounds must receive appropriate wound care (e.g., cleaning skin wounds with antiseptic wipes and applying topical antimicrobial gel containing Povidone-Iodine Complex). External wound clips or sutures should be removed 7-14 days after the surgery.

Subcutaneous and cranial implants are generally well-tolerated in mice and rats, but treating any wound or infection that develops can be difficult without removing the implant, so early detection and treatment are critical. In some cases, rodents may cause self-directed (through scratching) skin wounds around their surgically implanted devices, such as vascular access ports or cranial implants (e.g., headplate or windows). Cage mates or the cage environment may also trigger these wounds. Trimming their nails, separating the animal from any cage mates, and daily treatment with topical antiseptics (Vetericyn, or Povidone) are the usual conservative/first-line approaches to preventing infections. If the surgical wounds do not heal (e.g., wound dehiscence) or if there is any swelling or discharge from the implant site that persists after the first week or get worse during the postoperative care period, then please notify the AV.

The Campus Veterinarian must be contacted if there are any unexpected complications.

VIII. Pain Assessment and Alleviation

As defined by the International Association for the Study of Pain (IASP): Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Further, the fourth principle of the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training states: "Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."

Technical proficiency in aseptic surgical technique, especially gentle tissue handling, is very important in reducing pain from surgery, because: "As a rule, pain is likely to occur in proportional terms as a result of tissue injury—more extensive tissue damage results in greater pain and thus a need for a stronger analgesic regimen." Furthermore, "the intensity of acute pain following a tissue insult is greatest within the first 24 to 72 hours after the insult [surgery]."

The response of animals to pain, and hence any behavioral signs of pain, will vary depending on the animal species and the surgical procedure. There is no simple cage-side evaluation of pain in rodents. A nuanced interpretation of animal behavior is required to make a reasonable assessment of animal pain and distress. The best way to identify signs of pain is to closely observe the appearance and behavior of the animal for at least two days prior to surgery, and note any changes after surgery. However, this behavioral assessment only works well if the animals have a varied repertoire of normal behaviors, which only exists when animals are housed in an enriched environment.

The following signs are associated with persistent pain in rodents.⁷⁻¹⁰

Reluctance to move or reduced level of spontaneous activity (e.g., vertical rise in rats)
Absence or reduced frequency of normal behaviors (e.g., nest building in mice)
Increased grooming or licking directed at the injured area
Partially closed or squinted eyes
Piloerection (hair standing up)
Increased aggressiveness or vocalization when handled (in rats)
Abnormal posture (hunched posture in mice; arched backed and/or tucked-in abdomen
in rats)
Reduced food and water intake resulting in rapid weight loss

IX. Guidance on the Selection and Use of Non-Pharmacological and Pharmacological Postoperative Support¹⁰⁻¹³

The following categorical examples of the potential pain caused by different surgical procedures may be useful to investigators in determining the appropriate postoperative support for their animals.

The specific anesthetic and analgesic plans for the rodent surgical procedure(s) described in approved IACUC protocols were developed in consultation with the Campus Veterinarian, and were based on the predicted pain intensity of the surgical procedure and the expectation that the surgical procedure will be performed by a member of the research team that is technically competent in the surgical technique.

Members of the research team are expected to assess, at appropriate intervals, the effectiveness of analgesic treatments, and must notify the Campus Veterinarian if the analgesic treatments are not effectively alleviating the animal's pain.

Mild to Moderate Pain	Moderate Pain	Moderate to Severe Pain
Catheter implantation	Laparotomy incision and visceral organ manipulation	Major laparotomy and organ incision - Heterotopic organ transplantation
Superficial tumor implantation		Vertebral procedures
Intracerebral injection or implantation		Trauma models
Vascular access port implantation		Orthopedic procedures

Non-pharmacologic postoperative support for mice and rats.

Mild to Moderate Pain	Moderate Pain	Moderate to Severe Pain
Wound care House singly until ambulatory Supplemental heat	Wound Care House singly until ambulatory Supplemental heat Soft, absorbent bedding, nest material Modified food and water access DietGel® or moistened food pellets placed in a dish/cup on the bottom of the cage	Wound Care House singly until ambulatory Supplemental heat Supplemental hydration, warmed fluids (saline, LRS), 1 ml/100 g BW for rats or 0.5 ml/mouse, SC or IP Soft, absorbent bedding, nest material Modified food and water access DietGel® or moistened food pellets placed in a dish/cup on the bottom of the cage

Pharmacologic postoperative support for mice¹⁵⁻¹⁸.

Mild to Moderate Pain	Moderate Pain	Moderate to Severe Pain
	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (≤0.05 ml), irrigation of a surgical opening or local infiltration	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (≤0.05 ml), irrigation of a surgical opening or local infiltration
	AND	AND
Local anesthesia*: 0.5% lidocaine with 1:200,000	Buprenorphine, 0.1-2 mg/kg, SC, 3-4 times per day, 1-3 days	Buprenorphine, 0.1-2 mg/kg, SC, 3-4 times per day, ≥3 days
epinephrine (≤0.05 ml), irrigation of a surgical	OR	OR
opening or local infiltration AND	Buprenorphine extended- release (Ethiqa XR) 3.25 mg/kg, single dose.	Buprenorphine extended- release (Ethiqa XR) 3.25 mg/kg, single dose, two doses 48-72
Meloxicam, 2-5 mg/kg, SC, twice a day, 1-2 days	AND	hrs apart
	Meloxicam, 2-5 mg/kg, SC, twice day, 1-3 days	AND Meloxicam, 2-5 mg/kg, SC, twice day, at least 3 days
	OR	OR
	Carprofen (MediGel CPF)**, 10- 25 mg/kg, PO, 1-3 days	Carprofen (MediGel CPF)**, 25 mg/kg, PO, at least 3 days

^{*}Care should be taken to calculate and use an appropriate safe dose of the local anesthetics in mice due to their small size. The dose (% x volume of administration) provided here is the maximum dose for a 25g mouse (10 mg/kg).¹¹ Use only as much as need to irrigate/infiltrate the incision site.

Pharmacologic postoperative support for rats 17-18.

Mild to Moderate Pain	Moderate Pain	Moderate to Severe Pain
lidocaine with 1:200,000	·	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (≤0.5 ml), irrigation

^{**} When analgesics are provided PO (e.g., MediGel CPF), start offering it to the animal 2 days before surgery.

irrigation of a surgical opening or local infiltration	of a surgical opening or local infiltration	of a surgical opening or local infiltration
AND	AND	AND
Meloxicam, 1-2 mg/kg, SC, once a day, 1-2 days	Buprenorphine, 0.05 mg/kg, SC, 2-3 times per day, 1-3 days	Buprenorphine, 0.05 mg/kg, SC, 2-3 times per day, ≥3 days
	OR	OR
	Buprenorphine extended- release (Ethiqa XR) 0.65 mg/kg, single dose.	Buprenorphine extended- release (Ethiqa XR) 0.65 mg/kg, single dose, two doses 72 hrs apart
	AND Meloxicam, 2 mg/kg, SC, once a	AND
	day, 1-3 days OR	Meloxicam, 2 mg/kg, SC, once a day, ≥3 days
	Flunixin meglumine, 2.5 mg/kg,	OR
	SC, twice a day, 1-3 days	Flunixin meglumine, 2.5 mg/kg, SC, twice a day, ≥3 days

^{*}Care should be taken to calculate and use an appropriate safe dose of the local anesthetics in mice due to their small size. The dose (% x volume of administration) provided here is the maximum dose for a 250g rat (10 mg/kg).¹¹ Use only as much as need to irrigate/infiltrate the incision site.

IX. Records^{2,14}

Anesthesia, surgery, and postoperative care records are required for all surgical procedures in order to document the appropriate performance of anesthesia and surgery, and to demonstrate compliance with the surgical, anesthetic/analgesic, and post-operative plan in the approved protocol. These records will be regularly reviewed by the Campus Veterinarian, or designee. The records should be kept in the room where the animals are housed. Having the records in the room accomplishes several functions. 1) It explains the condition of the animals to animal care staff (a sedated animal may otherwise be thought to be ill). 2) It assures animal care staff and USDA Animal Welfare inspectors that the animal is being cared for. 3) It informs animal care staff how recently the investigator has seen the animal; this knowledge helps them decide whether or not there is a need to contact the investigator to inform him or her of the present condition of the animal.

Although individual records are desirable, a composite record may be used for a group of rodents. The record (individual or composite) should include the surgery date, name of the surgeon(s), a brief description of the surgical procedure, any drugs or treatments that were

administered, and a note of any complications or previous surgical procedures that may have been performed on the animal. The latter is required so that the IACUC or other regulatory agency (e.g., USDA, AAALAC) can assess whether or not an animal has undergone more than one survival surgical procedure. Repeated failure to maintain clear, concise, and complete surgery or postoperative records may lead to a revocation of an individual researcher's privilege to perform surgical procedures on laboratory rodents in a research setting. If a researcher did not perform the surgical procedures, but is responsible for providing postoperative care for that animal, their repeated failure to maintain clear, concise, and complete records may lead to a revocation of an individual researcher's privilege for working with animals.

The record should document that the animal was appropriately anesthetized prior to making the first surgical incision, and it should identify the intraoperative assessments of the animal's physiological status that were performed at regular intervals (e.g., every 15 minutes). Additionally, it should include notations of any variations in vital signs and anesthetic depth that were observed and actions that were taken to correct such variations.

The record should include a notation for each time the animal was examined postoperatively. After all wounds have healed and all sutures/wound clips have been removed, the postoperative record requires no further entries, but should continue to be kept in the area where the animals are housed.

Templates for a rodent anesthesia and surgery record and a postoperative care record recommended by the Campus Veterinarian are available at this OR/IACUC <u>website</u>. Alternatively, the PI may use a different template for their lab, but it should capture and document important information with comparable detail. Please consult with the Campus Veterinarian to ensure that the level of surgical and postoperative documentation in your template is appropriate for your surgical procedure and species.

Reference:

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