POLICY

TITLE:	**Survival Surgery on Non-USDA Covered Laboratory Animals**
Policy Number:	**2014-025**
Responsible Department:	**Institutional Animal Care and Use Committee**
Policy Contact: **Designation:** **E-Mail:**	**Donald E. Walters, Ph.D.** **Chair, Institutional Animal Care and Use Committee** **dewalters@westernu.edu**
Approval Date:	**9/10/14**
Reviewed:	**8/9/17; 4/8/2020**
Revised:	**8/14/17 (Refined procedures throughout); 4/8/2020 (Retitled to specify non-USDA covered animals; refined sterilization of instruments procedure, expiration of autoclaved items, surface disinfectants, preemptive analgesia & plane of anesthesia)**
Legislation:	**Animal Welfare Act (Title 9 CFR Subchapter A, Part 2, Subpart C, § 2.31(d)(ix)**

Purpose of Policy: To ensure that animals undergoing survival surgery do not experience unnecessary pain or distress as a result of the procedure.

Policy Information: The performance of survival surgery on laboratory animals must comply with the guidelines set forth in the Animal Welfare Act and Regulations, the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the *Guide for the Care and Use of Laboratory Animals*. Multiple survival surgeries are not permitted without rigorous scientific justification. For more information on multiple survival surgeries, refer to the Institutional Animal Care and Use Committee's (IACUC) Policy No. 2014-023 on Multiple Survival Surgeries.

Location: Each vivarium has either a dedicated surgery room or a procedure room in which surgeries may be performed. Surgeries may be performed in some other location (such as in a research lab) only if the location has been approved by the IACUC. The surgery location must be appropriate for the species, easily sanitized, and cannot be used for any other purpose during the time of surgery as, per the *Guide* cited above, "the number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of postoperative wound infection."

The surgery location should be sufficiently large to include separate areas within the site for

- animal preparation (weighing, hair or feather removal, skin disinfection)
- the surgical procedure, from skin incision to wound closure

- recovery from anesthesia

Surgical Instruments Preparation: All surgical instruments must be initially sterilized in a steam autoclave held at 121^{O}C (250^{O}F) for 15 minutes. If an instrument becomes contaminated during a procedure, it may be subjected to immediate-use ("flash") sterilization (unwrapped) at 132-135^{O}C (270-275^{O}F) for 3-5 minutes. The tips of rodent surgical instruments that may become contaminated during or between procedures may be sterilized by quick exposure (15-20 sec) to dry heat in the form of a glass bead sterilizer. However, instruments sterilized in this manner must be confirmed to have returned to room temperature prior to using in or on an animal. This can be done by touching the tip of the instrument with a sterile gloved finger to confirm. **If an instrument is used on more than one animal, it must be carefully cleaned free of debris and sterilized between animals** using either immediate-use ("flash") sterilization or a hot glass bead sterilizer. Given that 70% isopropyl alcohol is neither a sterilant nor a high-level disinfectant, and requires prolonged contact time, use of alcohol for surgical instrument preparation is not acceptable. **Sodium hypochlorite (bleach), peracetic acid/hydrogen peroxide or glutaraldehyde are harsh and corrosive on instrument surfaces and are therefore not recommended**.

Monitoring Sterility: When using a steam autoclave, commercially available strips that change color to indicate that the inside of the pack containing the items to be sterilized has achieved the required temperature for sterilization must be placed inside each pack of items. The date of sterilization must be written on the outside of the pack, generally on autoclave indicator tape, along with an expiration date as autoclaved items do not remain sterile indefinitely. Follow manufacturers' recommendations for appropriate expiration dates which are usually six months to one year post autoclave date. In addition, a log must be maintained showing the dates the instruments were autoclaved. The temperature color strip used to verify sterilization must be attached to this log. The autoclave itself must also be monitored for effective function using a spore test (e.g., Self-Contained Biological Indicator (SCBI), NAMSA®) at least weekly (with regular autoclave use) or less frequently (e.g., monthly), if the sterilizer is used less often.

Disinfectants: The surgical table and immediate surrounding areas must be made of materials that are easily disinfected. The immediate surgical areas should be disinfected prior to and between surgeries to reduce cross-contamination and may not be used for any other purpose during the time of surgery.

Chlorhexidine (e.g., Nolvasan, Hibiclens) is an effective surface disinfectant for decontaminating the surgical table and surrounding areas provided that the manufacturer's contact time recommendations are followed. Alternatives include Quatricide PV-15 which requires 1-2 minutes contact time and hydrogen peroxide which disinfects most surfaces within seconds and degrades to water making it the safest to use around animals and people alike. Ethyl alcohols and isopropyl alcohol are generally not recommended for surface disinfection as they evaporate quickly before reaching their optimum wet-contact time. Because of their toxic and corrosive properties, sodium hypochlorite (bleach, Clorox®) and glutaraldehyde (Cidex®, Cide Wipes®) disinfectants are not recommended.

Pre-Surgical Animal Management and Evaluation: Withholding food or water is generally not required in rodents unless called for by the experimental design or required by the surgical procedure, e.g. gastrointestinal surgery. However, withholding food or water must be approved by the IACUC. A pre-surgical visual inspection and behavioral evaluation should be performed on each animal to determine if the animal has any pre-existing health condition that might negatively affect the surgery. Physical or behavioral abnormalities must be brought to the attention of the Attending Veterinarian. Surgery must not be performed on animals with pre-existing conditions that may affect the outcome of the surgery.

The first dose of analgesics must be administered preemptively, preferably by injection, e.g., 30 min <u>before</u> making the initial surgical incision. Alternatively, animals may be placed on oral analgesics (e.g., carprofen, ibuprofen, meloxicam) in gel form or in the drinking water at recommended dosing intervals beginning 12 to 24 h prior to painful procedures. It is incumbent on the investigator to verify

that the animals have taken the medication and that it was consumed in a time frame that will ensure maximum action at the time of surgery.

Preparation of the Animal: Hair, fur or feathers should be removed from the surgical site with at least a 1 cm border to avoid contaminating the incision site. The site should then be scrubbed with at least three alternating applications of a povidone-iodine scrub such as Betadine® or a chlorhexidine scrub such as Nolvasan®, and 70% isopropyl alcohol, being careful to scrub from the center of the site toward the periphery. The area should then be covered with sterile drapes to prevent contaminants from entering the surgical field.

Preparation of the Surgeon: A surgical mask and a clean lab coat or sterile gown should be worn. Surgeons must then thoroughly scrub and brush fingers, hands and forearms with a bactericidal scrub (e.g., povidone iodine, chlorhexidine) for at least two minutes prior to donning sterile surgical gloves. Use of gloves dipped in bleach or other disinfectants is unacceptable. For a complete discussion about surgical scrub techniques and surgeon preparation visit How to Perform Surgical Hand Scrubs and Preparation of the Surgeon.

Anesthesia: The anesthetic regimen must be described in the approved animal use protocol. The anesthetic gas isoflurane is generally recommended for long procedures that would otherwise require multiple doses of parenteral anesthesia. It must be determined that the animal is fully anesthetized prior to making an incision and that a consistent and appropriate plane of anesthesia is maintained throughout the duration of surgery. Signs of anesthetic depth may vary according to the species and anesthetic used. However, a proper surgical plane of anesthesia must be monitored and assured throughout the procedure. If an animal shows signs of responsiveness during surgery, all procedures must be halted and the anesthesia increased with procedures not resuming until an appropriate plane of anesthesia is verified. Respiration rate and patterns, muscle tone, and lack of response to toe or tail pinch are the minimum required signs that must be monitored.

Surgical Procedures must be conducted as described in the approved protocol. Normal body temperature must be maintained, as anesthetics induce hypothermia. Water-circulating heating pads or reusable crystal-based heating pouches (e.g., SpaceGels™, Braintree Scientific) are the most frequently recommended warming devices. Electric heating pads can have hot spots and may overheat -- or even burn -- an animal's skin. If an electric heating pad is used, it should be set on low with a light cloth placed between the animal and the pad; the animal must be observed frequently for signs of hyperthermia. **Infrared heat lamps are not permitted as they may cause severe hyperthermia or burns. Ceramic heat lamps (ranging between 60 and 150 watts) are acceptable as long as they are positioned in such a way as to prevent thermal injury or overheating.**

To prevent corneal desiccation, a non-medicated (bland) ophthalmic ointment should be place in the animal's eyes following onset of anesthesia and prior to shaving fur or hair or plucking feathers. If performing survival stereotaxic surgery, blunt ear bars must be used to prevent damage to the tympanic membrane.

Neuromuscular blockers (paralytic agents) may not be used without the benefit of a surgical plane of anesthesia, assisted ventilation and careful monitoring. Refer to the IACUC's Policy No. 2014-018 on the use of neuromuscular blockers during surgery.

Suture Selection: Closure of internal tissues requires an absorbable suture material whereas non-absorbable monofilament suture material should be used for skin closure unless the skin incision is ≤ 1 cm. In this case, the incision may be closed with surgical grade glue without sutures. The smallest practical gauge material should be used. Refer to the tables below for guidelines on suture material and gauges.

Acceptable Suture Materials

Suture	Characteristics and Frequent Uses
Vicryl®, Dexon®	Absorbable; 60-90 days. Suitable for internal wound closure.
PDS®, Maxon®	Absorbable; 6 months. Suitable for internal wound closure where extended wound support is desirable.
Prolene®	Nonabsorbable. Suitable for skin closure.
Nylon	Nonabsorbable. Suitable for skin closure.
Stainless Steel Wound Clips, Staples	Nonabsorbable. Suitable for skin closure. Requires instrument for removal from skin.

Recommended Suture Gauges/Sizes

Species	Location or Function
Mouse	Abdominal muscle + peritoneal layer: 4-0 to 5-0 Skin (subcuticular): 5-0 to 6-0 Skin (external): 4-0 to 6-0
Rat	Abdominal muscle + peritoneal layer: 4-0 to 5-0 Skin (subcuticular): 4-0 to 6-0 Skin (external): 3-0 to 5-0
Bird	3-0 to 6-0 (depending on animal size) Skin (external): as for mice

Consult the Attending Veterinarian for suture recommendations for microsurgery. **Because they cause tissue inflammation, the use of silk and chromic gut sutures are not recommended for wound closure. Their use is limited to specialized ophthalmic and vascular applications.** Sutures, staples or wound clips must be removed 7-14 days after surgery unless the animals will be euthanized within this time. Intradermal suture patterns that close the skin are particularly useful in larger animals and cannot be seen externally. These require absorbable sutures which should not be removed. The Attending Veterinarian must be consulted when an incision does not appear to be healing properly.

Post-Operative Recovery: Animals must be placed in a clean, un-bedded cage, and observed during post-operative recovery and must be kept warm without overheating them. Unconscious animals are especially vulnerable to overheating, as they cannot escape the heat. The same warming devices and precautions as described above for intraoperative management may be used for the posy-anesthetic phase. Animals must be observed frequently for signs of hyperthermia (intense coloration of paws and tail, rapid respiration rate). Animals recovering from anesthesia should be occasionally turned to prevent burns. Provisions must also be made for a conscious animal to escape the heat source if it becomes too warm, e.g., place a warming device under just one half of the animal's enclosure. However, **hypothermia** is more common post-op and provisions must be made to ensure that animals are maintained normothermic as most post-op anesthesia deaths are from respiratory arrest which is a greater risk in hypothermic animals.

Recovering animals must be watched until they are ambulatory. **Unconscious animals must not be left unattended.** After surgery, to prevent injury or device removal, rodents should be housed singly until the sutures or wound clips are removed or until two weeks post-surgery if the closures are not to be removed.

Post-Operative Analgesia: Unless scientifically justified and approved by the IACUC, or if otherwise required by the Attending Veterinarian, all animals undergoing survival surgery must receive post-operative analgesia. Investigators are referred to the IACUC's Policy No.2014-024 on postoperative analgesia for guidelines on the choice of analgesics.

Antibiotic treatment: Post-operative antibiotic treatment should be discussed with the Attending Veterinarian to determine its need and the choice of antibiotic. The degree of invasiveness of the surgery and the immune status of the animal are some factors that will determine the need for an antibiotic. Administering an antibiotic before the procedure begins can reduce the risk of post-operative infection.

Post-Operative Treatment Cards or stickers must be placed behind or on the back of the regular cage card of each cage housing a post-operative animal. Do **not** attach adhesive labels directly to the cages as they are difficult to remove. The date of the surgery and all treatments, such as administration of analgesics or antibiotics, must be recorded on these cards. A sample form is provided below. Investigators may obtain these forms from the Director of the Office of Animal Resources (x-5610).

POST-OP CARD

Date of procedure_____ **Procedure** _____

Medication:_____ **(Please initial when given)**

DATE					
AM					
PM					

Medication:_____

DATE					
AM					
PM					

Emergency contact: **Phone number**:

Long-Term Recovery and Monitoring: Post-operative animals must be observed at least daily, including weekends and holidays, for state of arousal and signs of pain or discomfort, infection, dehydration or abnormal wound healing. Prolonged monitoring may be required for animals with chronic debilitating diseases, such as diabetes mellitus, or animals that have undergone an organ transplant, immunosuppressive therapy or that have chronically implanted catheters or other devices.

Record-Keeping Requirements: Surgical records must be kept for all animals. The records must include the administration of anesthetics, fluids and any other drugs; details of the procedure, including intra-operative monitoring; daily post-operative recovery observations and treatment, including administration of analgesics and antibiotics; monitoring of incision healing, including suture/staple removal if applicable; and the initials of the person performing these tasks. The name, dose, route and time of administration of all medications must be recorded. Any adverse outcomes must also be recorded.

Definitions:

Surgery: a procedure involving a skin incision or penetration of a body cavity by means other than an injection.

Survival Surgery: a surgical procedure from which an animal is expected to regain consciousness.

Non-survival Surgery: a surgical procedure from which an animal is euthanized before regaining consciousness.

Minor Surgery: a surgical procedure that does not expose a body cavity and causes little or no physical impairment, such as wound suturing, peripheral vessel cannulation and placement of subcutaneous implants.

Major Surgery: a surgical procedure that penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions, such as laparotomy, thoracotomy, craniotomy, joint or bone replacement, spinal transection, limb amputation and enucleation.

Aseptic Technique: surgical methods and precautions used to reduce microbial contamination to the lowest extent practical, such as disinfection of the operative site; provision of decontaminated surgical attire; surgical scrub; sterile surgical gloves; sterilization of instruments, supplies and implanted materials; and the use of operative techniques that reduce the risk of infection.

Related Policies: IACUC Policy No. 2014-018, Use of Neuromuscular Blocking Agents; Policy No. 2014-023, Multiple Survival Surgeries; Policy No. 2014-024, Post-Operative analgesia and Pain Management