This document provides information to be used when planning and performing survival surgical and anesthetic procedures in Fish and Amphibians used for research, teaching, or other purposes at The University of Texas at Austin. It is organized into five sections:

Section A – Definitions
Section B – Requirements
Section C – Specific Considerations for Surgical Procedures
Section D – Additional Sources of Information on Disinfectant, Sterilants, Sterilization Guidelines and Wound Closure Materials
Section E – References and Acknowledgements

Section A- Definitions

Non-survival surgery is defined as a surgical procedure after which the animal will not regain consciousness prior to euthanasia.

Survival surgery is defined as a surgical procedure after which the animal will be allowed to recover consciousness, even for a short time.

Minor surgery is a procedure that involves surgical manipulation but does not meet the definition of major surgery. Typical examples include operative procedures in which only skin or mucous membranes are incised, e.g., vascular cutdown for catheter placement or implantation of minipumps in subcutaneous tissue. Also included are minimally invasive means of accessing a body cavity, such as needle biopsy or the introduction of instruments using a trocar.

Major survival surgery is defined as a survival surgical procedure that involves penetration and exposure of a body cavity (abdomen, thorax or cranium) or will produce a substantial physical or physiologic impairment.

Multiple major survival surgery occurs when two or more separate major survival surgical procedures are performed on a single animal. It is allowable only under certain circumstances, the most common being a situation in which each surgical manipulation is an essential and related part of a single study. Cost alone is not an adequate justification for performing multiple survival surgeries on the same animal. Multiple major survival surgeries must be specifically justified by the PI and approved by the IACUC as part of an approved protocol.

Aseptic (sterile) surgical techniques are well-established methods used to avoid the introduction of microbial contamination into tissues exposed and/or manipulated during surgery. More details are provided below.

Non-invasive procedures under anesthesia are those where animals are immobilized but not undergoing a painful procedure. Examples include injections, imaging, suture removal, etc.
Section B- Requirements

1. Survival surgical procedures must be performed in either a dedicated animal procedure room or in a suitably located and dedicated laboratory area, subject to approval by the IACUC. The bench or tabletop areas used for animal prep, surgery, and recovery should have only the ancillary laboratory, diagnostic, or clinical equipment and supplies needed to support the procedure being performed. Equipment used on an infrequent basis and bulk supplies should be stored elsewhere. The area should be free of physical or chemical hazards potentially arising from splashes, spills, etc. A low-traffic area of the laboratory away from doorways, hoods, etc. is optimal. For most procedures a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery.

2. Aseptic (sterile) technique for survival surgery is required for ALL species. However, some modifications are allowed when small animals are involved. For example, the use of surgical drapes in small species may not always be necessary because a) the incisions are very small, and organs are rarely exteriorized during the procedures and b) drapes may impair the ability to assess breathing patterns or other monitoring criteria used to assure proper anesthetic depth; however, the use of a sterile drape is best practice. Clear sterile drapes allow good visualization of the patient during surgery while also allowing for optimum sterility during surgery. The need for sterile gowning of the surgeon is also considered optional for surgery involving fish or amphibians. However, the surgeon should wear a clean laboratory coat. Unless a strict “Tips Only” method is being used (as described below), sterile surgical gloves ARE required.

3. A proper method of anesthesia and analgesia (if applicable) must be selected, and protocol approval must be obtained prior to initiation of anesthesia. Anesthesia should be complete, i.e., a single drug or a combination of agents must be used to induce a loss of consciousness, hyporeflexia, muscle relaxation and analgesia. For consultation on best-practice anesthesia protocols, contact the ARC clinical veterinary staff. Surgery or potentially painful procedures must not be performed on non-anesthetized animals paralyzed by chemical agents. When anesthetics are used, appropriate mixing and disposal methods must be employed to prevent hazardous exposure of personnel. SOPs should be developed per EHS guidelines during protocol review to handle, prepare and dispose of MS-222.

4. It may not be necessary to follow all the requirements outlined in this guideline if non-survival surgery is performed, the instruments and surrounding area should be clean.

5. Expired anesthetics, analgesics and euthanasia drugs may not be used in any animals without explicit IACUC approval (See Guidelines for the Use of Drugs and Chemicals in Animal Research). Expired medical materials (e.g., suture, fluids, etc.) may be used for non-survival procedures provided the use of such items doesn’t compromise the validity of the scientific study. Expired materials must be clearly labeled, e.g.: “Expired: For use in terminal procedures only” and stored in such a way that they can be readily distinguished from unexpired materials (e.g., in a separate cabinet or in a separately labeled box).

6. For non-survival procedures of extended duration, attention to aseptic technique may be more important to ensure stability of the model and a successful outcome. A veterinarian should be contacted for a consultation if you are planning acute surgical procedures of more than a few hours duration to determine whether sterile techniques are indicated. Eating, drinking, or smoking is not acceptable in non-survival surgery areas, and locations used for food handling purposes do not qualify as acceptable areas for performing surgeries. Anesthetic records are required for non-survival procedures lasting more than 15 minutes, and euthanasia agents and/or methods should also be documented. For procedures lasting less than 15 minutes at a minimum the time of start and end of the procedure and the type of anesthetics/euthanasia agent should be recorded.
Section C- Specific Considerations for Surgical Procedures

Preparation of the Surgeon: The surgical procedure itself must be performed or directly supervised by a trained and experienced individual. This training must be documented on the IACUC internal training record. Personnel unfamiliar with aseptic surgical procedures or who have questions about surgical techniques should contact the ARC veterinary staff for information and/or training.

Other requirements for surgeon preparation:

- A clean lab coat is required for fish and amphibian surgeons
- Wash hands thoroughly with a disinfecting soap such as chlorhexidine or iodine based surgical scrubs
- Sterile surgical gloves must be donned prior to handling sterile instruments or touching surgically-exposed animal tissues (unless using tips only method). Latex and powdered gloves should be avoided as they can damage the skin of fish and amphibians.
- Long hair of surgeons and assistants should be covered and/or restrained to keep it away from the surgical field

Preparation of surgical space: According to the Guide for the Care and Use of Laboratory Animals: Eighth Edition, "For most survival surgery performed on rodents and other small species...the space should be dedicated to surgery and related activities when used for this purpose, and managed to minimize contamination from other activities conducted in the room at other times." The surgical area should be a room or a portion of a room that is easily sanitized and not used for any other purpose during the time of surgery. Clean and disinfect the surface upon which the surgery is performed with an approved environmental disinfectant before beginning the surgical procedure.

Preparation of Surgical Instruments: Surgical instruments must be sterilized prior to use for survival surgery. Organic material must be removed by cleaning prior to sterilization. If sterile packs are stored for later use, they must be dated with the preparation date and an expiration date. It is recommended that all sterile supplies be stored in a vermin proof, enclosed cabinet. Methods used for sterilization may vary, but all must conform to established medical standards for complete sterilization. For a full explanation of sterilization requirements please see IACUC sterilization guidelines. insert link

Options for sterilization include:

- Steam sterilization at proper pressure and exposure times (autoclave)
- Prolonged immersion in a hospital-grade peroxide, formaldehyde- or glutaraldehyde-based cold sterilant following label directions (alcohol immersion is NOT acceptable). Please note, cold sterilization is not recommended for amphibian species due to the toxic nature of many of the chemical compounds and the permeable nature of Xenopus skin. (NIH, 2013)
- Ethylene oxide gas (ETO) used in a specialty chamber
- Dry heat sterilization (hot bead sterilization) at proper temperature and exposure time (not appropriate for initial sterilization)

Re-sterilization Requirements: Instruments must be re-sterilized between animals (maximum 4 animals per pack, i.e., instruments cannot be bead-sterilized more than 3 times) when performing multiple surgeries within the same day. 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. Prior to placement in the hot-bead sterilizer, blood, tissue and all organic material on instrument tips should be removed. 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 4 animals. If the instrument tips become contaminated during a procedure, they must be re-sterilized or a new set of sterile instruments should be obtained.

**Tips Only Technique:** “Tips only” aseptic practices are considered appropriate for survival surgeries in animals that involve only very small incisions, e.g., embryo transfer, gonadectomy, intracelomic placement of a pellet or transponder, etc. During these procedures, it is not necessary for the surgeon to directly touch the tissues and the likelihood of inadvertent contact with exposed tissues is minimal. In this case, the emphasis of aseptic surgical practices will be to keep the tips (which enter the body) of the instruments free of contamination during the procedure. In this situation, sterile gloves are not required, and after gross debris is removed, the instrument tips can be sterilized between animals by immersion in a hot bead sterilizer (or an acceptable disinfectant as described above). If this method is chosen, the surgeon must be very cautious to avoid contact with the surgical site or the working surfaces of the instruments, and to keep the tips free of contamination by having a sterile location to place the instruments if they are put down during the procedure. Animal users can consult with the veterinary staff to determine if the “tips only” method is appropriate for their procedure.

**Intra-Operative Considerations (Patient)**

**Preparation of Patient (general):** Fluids, analgesics, and antibiotics must be administered as indicated in the protocol or as directed by the ARC veterinarians or designee. Due to the protective mucus layer present on fish and amphibian skin, aggressive cleansing is not recommended.

**Fish:** The skin should be prepared with a single wipe using sterile saline or a dilute povidone iodine or chlorhexidine solution immediately before surgery. Removal of scales in fish is generally not recommended and should be avoided or minimized when possible.

**Amphibians:** Rinse gross debris from the surgical site using sterile saline or another sterile isotonic fluid and keep the skin moist throughout the surgical procedure. A 10% providone iodine solution can be used for skin disinfection, but chlorohexidine or scrubs containing soaps or detergents and alcohol (or alcohol containing preparations) may damage the skin.

**Fasting (general):** Consider withholding food for up to 24 hours prior to anesthetic events. Regurgitated materials can contaminate the surgical area. Contact UTARC veterinary personnel for fasting recommendations of specific species.

**Anesthesia**

**Fish:** Anesthetize fish and amphibians during any procedure that is stressful or likely to cause pain and in accordance with the IACUC approved animal use protocol. Ensure animals remain wet during procedures out of the water. Water can be dripped, poured, or sprayed on exposed areas of the animal. Use water from the home tank wherever possible; clean non-chlorinated water is also acceptable.

**Amphibians:** The skin acts as a semipermeable membrane that allows for respiration (cutaneous respiration) and absorption of substances through the skin. After the amphibian reaches the appropriate level of anesthesia for the planned procedure, it should be removed from the anesthetic bath and rinsed with fresh water or the topical preparation removed by rinsing with fresh water. The animal will remain anesthetized for 10-80 minutes, depending on the method and drug concentration used. Amphibians may also show an excitement phase during anesthetic induction. It is important to induce anesthesia in a container that will prevent injury due to the animal jumping or falling out.
Monitoring Anesthesia

Fish: The depth of anesthesia can be monitored by observing the behavior of the animal in water. While appropriate monitoring parameters can vary based upon anesthetics and species, several general guidelines can be used for monitoring anesthetic depth. Activity decreases, the righting reflex is lost, and muscle tone decreases as the animal becomes anesthetized. Opercular movement (respiratory rate) progressively decreases with deepening anesthesia. In fish, Hypoxemia can occur despite good ventilatory efforts and can be recognized by pallor of the gills and the fin margins. Surgical planes of anesthesia can be confirmed by a lack of response to a firm squeeze at the base of the tail or digits.

Amphibians: During the induction stages of anesthesia, they will demonstrate decreased gular movement and diminished withdrawal reflexes. They will then lose their righting reflex. Once they have reached a surgical plane of anesthesia, withdrawal (toe pinch) will be absent, and gular movement will cease. Pulmonary respiration will cease during anesthesia in amphibians and cannot be used to monitor anesthetic depth. Cutaneous respiration is sufficient to prevent clinical hypoxia during anesthesia. Heart rate may be monitored during anesthesia by direct observation.

Intra-operative Considerations (Surgical)

Aseptic Technique: The goal of aseptic technique is to reduce the normal bacterial burden present on the animal and in the environment before beginning surgery. This includes the use of sterile instruments, disinfection of the animal’s surgical site and surgeon’s hands, and the establishment of a sterile surgical field. To create a sterile field, the working surface should first be disinfected before surgery. All supplies and implanted materials used in survival surgeries (e.g., sutures, implants, instruments, catheters) must be sterile and not expired. During the surgery, a sterile field should be established, and the sterile instruments and materials must be placed within this field when not in use to prevent contamination. The sterile inside-surface of the instrument autoclave pack, a sterile drape, or the sterile interior of the glove packaging can be used as a sterile field for instruments and supplies during the procedure. Unwrap sterile instruments making sure to only touch the outer surface of the wrap. Do not touch the interior of the packaging or instruments, as this will compromise the sterility of the instruments. Maintain sterility of gloves, instruments, suture material and any other items being used intra-operatively during the procedure. To maintain a clean surgical field, surgeons should avoid touching fish with their gloves during surgery, leaving handling of the animal to an assistant. Breaks in sterility occur when the surgeon touches something outside of the sterile field. This may be the surgeon’s face, a light fixture, an unprepared area of the animal, or a non-sterile instrument. Care should be taken to avoid these breaks in sterility, as proper aseptic technique limits the risk of introduction of infectious agents, primarily bacteria, into surgical sites.

Suture Materials and Wound Closure: Use monofilament suture material (e.g., PDS, Maxon, or Ethilon) to minimize tissue reactions and infection that could slow healing time. Braided materials such as Vicryl or silk are generally contraindicated for skin closure in fish despite their historical use. Tie sutures snugly for a watertight seal. Post-operative swelling in is minimal, unlike mammals. Absorbable suture materials have been recommended as a means of avoiding additional handling for removal. Remove all skin sutures (nonabsorbable) by 14 days after surgery if they are still present (provided the skin incision is adequately healed), unless described otherwise in an IACUC approved protocol or as recommended by UTARC veterinary personnel to necessitate complete wound healing. Tissue adhesives (e.g., cyanoacrylate) are not recommended for use in aquatic species (associated with tissue reactions and wound dehiscence). Similarly, surgical staples are regarded as inferior to appropriately placed sutures for wound closure. Routine prophylactic antimicrobial use is generally not necessary if appropriate aseptic technique is utilized. Contact UTARC veterinary personnel for cases that may warrant antibiotic treatment (contaminated wound repair, inadvertent bowel perforation, other break in aseptic technique). Close amphibian laparotomy sites in two layers (muscle and skin) to prevent incisional dehiscence.
Anesthetic Monitoring and Record Keeping

The animal’s physical condition and anesthetic depth must be continually monitored during the surgical or anesthetic procedure and frequently assessed during the recovery period until the animal is fully ambulatory. The animal must be maintained in a surgical plane of anesthesia throughout the procedure. Animals should not be left alone during surgery or recovery from anesthesia. Anesthesia monitoring must include a periodic assessment of anesthetic depth (pinch reflex) recorded at the start of the procedure and then no less than every 15 minutes throughout the duration of the procedure. Monitor animals every 15 minutes during anesthesia and until they resume normal swimming. Return animal to its home tank when normal swimming/activity has resumed. Records must be kept near the animal, either in the animal housing room or in an adjacent procedure area. Monitoring should continue until skin sutures are removed, or if sutures are not placed, animals should be monitored specifically for post-operative complications twice daily for the first 48 hours following surgery and once on day 3 of the post-operative period unless the protocol specifies differently. All post operative observations must be documented on the post operative record. If it is not possible to identify the individual post-op animals after return to a tank environment following surgery, the cohort/tank should be monitored for the duration of the post-operative monitoring period and this approach noted on the record.

Drugs, including dosages, routes of administration, and times given should be recorded before and during surgery. Special attention should be paid to documenting analgesics on the surgical record, paying close attention to the initial dose and subsequent dosing intervals and must strictly follow the approved protocol.

Notations of any variations from the normal and expected events during the anesthetic and recovery periods (including the actions taken, the time performed, and the animal’s response to these actions) should be documented. Assessment for pain and distress as well as any action taken to alleviate pain and distress (including pharmacological and non-pharmacologic interventions, and the response to these actions) should be documented on the surgical and post operative records. If pain or distress is observed a veterinarian must be contacted unless the protocol allows for such presentation (Cat. E study). Special attention should occur in Cat. E studies with strict adherence to humane endpoints. ARC veterinarians are available to help determine if humane endpoints have been met.

For animals that undergo non-invasive procedures that are expected to return to normal function after recovery from anesthesia with no more than momentary pain (E.g., blood collection, tail vein injection, tail biopsy for genotyping or intranasal inoculations), when anesthesia is used for immobilization during non-painful procedures lasting no longer than 15 minutes, no anesthesia or post-operative record is required. Instead, an entry describing the time, type of procedure and anesthetic used should be documented in these cases.

Non-Surgical Anesthesia

Anesthesia can be used for immobilization during non-invasive, non-painful procedures. Non-invasive procedures under anesthesia may consist of imaging or entering an external body structure with a catheter, needle, or other instrument (to obtain small samples or inject liquids/medications without disruption of function to an animal). Since animals that undergo these non-invasive procedures are expected to return to normal function after recovery from anesthesia with no more than momentary pain (E.g., blood collection, tail vein injection, tail biopsy for genotyping or oral dosing inoculations), when anesthesia is used for immobilization during non-painful procedures lasting no longer than 15 minutes, anesthesia or post-operative records are not required. Instead, an entry describing the type of procedure, time it began and ended, and the anesthetic used should be documented in for each animal in these cases.
Post-Procedural Care and Record Keeping

**Fish:** Recover fish in aerated, untreated water. During recovery, a reversal of the stages of anesthesia should occur with a gradual increase in opercular movement, return of equilibrium, and eventual resumption of normal swimming. Most fish are fully recovered from immersion anesthetics within 5 minutes of placement in fresh water. Prolonged recoveries (> 10 minutes) indicate excessive anesthetic depth or a compromised animal. Recovery from parenteral anesthetics can be highly variable. Fish may pass through an excitement phase during recovery and may attempt to escape from the recovery tank. Stimulation can exacerbate the excitement phase and once fish are showing progressive signs of recovery (increased opercular and fin movement, increased muscle tone, and a return of equilibrium) it may help to cover the tank with a lid. Occasionally, fish demonstrate vigorous movement and may need to be restrained to prevent self-injury.

**Amphibians:** Recover amphibians partially submerged in fresh water with the head and nares held above water or place in a closed but not airtight container with a moist paper towel on the bottom during recovery. Other methods are also acceptable if the skin is kept moist. DO NOT submerge aquatic amphibians in water until fully ambulatory and recovered from anesthesia as drowning can occur.

**Monitoring (general):** When the approved IACUC protocol specifies that animals be monitored at least twice daily, it is expected that monitoring events will be well-spaced to assure that humane issues can be identified promptly. Monitoring generally occurs in the early morning and late afternoon, but alternative schedules are acceptable if observations occur every 10-14 hours, including weekends and holidays. All observations should be documented in the surgical/post-operative records and available upon request.

Monitoring post procedurally must include close inspection of the surgical site for dehiscence or infection as well as clinical observation of the animal for signs of pain, abnormal behavior, appetite, and excretory functions. While not common, complications may occur post-operatively such as bleeding or clotting. If the animal appears ill postoperatively, experiencing post operative pain or distress or if the surgical wound appears abnormal, the veterinary group must be contacted for consultation unless the protocol specifically complication being exhibited. I.e., Category E studies.

**Amphibians:** do not raise their body temperature above that of normal room temperature in an attempt to speed recovery. Increasing the body temperature will increase metabolism and oxygen requirements. Cutaneous respiration may not be sufficient to maintain adequate oxygenation in this situation.

**Section D- Additional Sources of Information on Disinfectant, Sterilants, Sterilization Guidelines and Wound Closure Materials**


Section E – References and Acknowledgements


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