PURPOSE: This document details the minimum standards for survival (recovery) surgery in all laboratory rodent species.

RESPONSIBILITY: Prior to undertaking ANY surgical procedure, it is the responsibility of the principal investigator to ensure that staff carrying out those procedures are adequately trained and that provisions are made for post-operative care.

All surgery must be performed by qualified trained individuals having completed the requisite courses that apply to surgery, including Rodent Surgery and Rodent Anesthesia. The following is the ACC Policy for rodent surgery.

POLICY STATEMENTS:

1. All rodent recovery surgery must be performed within the animal facility in a suite especially designated for this purpose, unless justified as determined by the Animal Care Committee.

2. All species undergoing surgery should receive a similar level of care and attention.

3. Survival surgery should be performed using aseptic surgical technique; using clean and sterilized (autoclaved, gas or chemically sterilized) instruments and equipment. Only chemical sterilants are considered acceptable based on the manufacturer instructions for sterilization. Note that alcohol is not considered a chemical sterilant, but only a disinfectant. The use of a hot glass bead sterilizer for sterile tip surgery is acceptable for sterilizing instruments in between animals. However, hot glass bead sterilization can be used for no more than 6 animals at which time instruments must be re-autoclaved. Instruments must be washed between each use of the hot bead sterilizer.

4. The animal will be fully anesthetized; when a toe is pinched the animal will not exhibit a toe pinch reflex (withdraw or respond in any way to the pinch) prior to and throughout the duration of the surgical procedure. It is suggested you confirm surgery plane of anesthesia a minimum of every ten minutes. Increased frequency may be needed if there are changes in anesthetic depth, respiration and other vital parameters. Animals will be monitored and attended to throughout the duration of surgery and anesthetic recovery.

5. The surgical site(s) will be prepared by removing the fur appropriate to the size of the surgical site and cleaned with a triple scrub of Chlorhexidine or Betadine Scrub alternated with alcohol (or sterile saline in some cases). Repeat three times to achieve sufficient contact time.

6. The surgeon will wear at minimum a closed, clean lab coat or scrub top, sterile surgical gloves (for full sterile technique) or clean gloves (for sterile tip) over scrubbed hands, a face mask and head cover.

7. Animals should receive supplemental heat during the pre-operative, intra-operative and post-operative periods.

8. Animals undergoing anesthesia should have their corneas protected with ophthalmic lubricant and hydration should be maintained.

9. Animals undergoing recovery surgery will be administered suitable analgesic agents as per the approved Animal Care protocol.
10. Rodents will be recovered from surgery in a quiet area away from the immediate surgical area and they
    will be continually monitored until fully recovered. Animals should not be mixed with conscious animals
    until fully recovered.

11. During recovery, animals should receive adjunct nursing care such as wound care, fluids and
    analgesics.

12. Animals will continue to be monitored at regular intervals on the day of surgery and in the following days
    to ensure full recovery. Adjunctive nursing care may be required. This may include administration of
    analgesics.

13. Animals with abnormal wound healing/closure or morbidity following surgery beyond the morbidity or
    mortality estimated in the Animal Care protocol require consultation with a Clinical Veterinarian unless
    specified in protocol. The veterinarian will, in consultation with the Principal Investigator, decide the best
    course of action for the animal and these actions are the responsibility of the research team.

14. A written record of the surgical details and condition of the animals following surgery will be maintained
    in the unit and be readily accessible throughout the post-operative recovery period.

    Please see the attached guidelines for more details related to these policy requirements.
GUIDELINES ACCOMPANYING POLICY

POLICY 1: All rodent recovery surgery must be performed within the animal facility in a suite especially designed for this purpose, unless scientifically justified as determined by the Animal Care Committee.

The Canadian Council on Animal Care recommends that all survival surgery be performed in areas specifically designed for this purpose. A rodent surgical area can be a room or part of a room that is easily sanitized and not used for other activities when rodent surgery is in progress. These areas will be kept neat and orderly and all contamination including bedding, feces, blood, etc. will be cleaned prior to and after each surgery. This area should not be a high traffic area or immediately next to room ventilation.

POLICY 2: All species undergoing surgery should receive a similar level of care and attention.

Appropriate level of care includes use of aseptic surgical technique, appropriate monitoring and nursing care. The primary objective of aseptic surgical technique is to reduce microbial contamination of the incision site and exposed tissues to the lowest possible practical level. The argument is still made that aseptic technique is not necessary for rodent surgery because mice or rats often survive surgical procedures performed using poor aseptic technique. However, survival alone is not a valid criterion for judgement of the acceptability of a rodent surgical technique. The criterion for acceptability should be the absence of untoward, unplanned alteration of physiological functions or behavior due to peri-operative infection.

POLICY 3: Survival surgery should be performed using aseptic surgical technique; using clean and sterilized (autoclaved, gas or chemically sterilized) instruments and equipment. Only chemical sterilants are considered acceptable based on the manufacturer instructions for sterilization. Note that alcohol is not considered a chemical sterilant, but only a disinfectant. The use of a hot glass bead sterilizer for sterile tip surgery is acceptable for sterilizing instruments in between animals. However, hot glass bead sterilization can be used for no more than 6 animals at which time instruments must be re-autoclaved. Instruments must be washed between each use of the hot bead sterilizer.

Sterilization kills or renders inactive all microbial organisms. The common methods used are steam heat or ethylene oxide gas. Autoclaves should be appropriately maintained and tested for efficacy. Any item used for survival surgery must be sterilized: instruments, materials and devices such as catheters, flow probes, or electrodes, and all fluids used for flushing or injection. Special care is needed to ensure that multi-dose vials of drugs are not contaminated. The surgical site should be draped to isolate the surgical field from the surrounding areas. Drapes are positioned and fixed and should not be dragged across unsterile areas onto the surgical field.

If sterile-tip surgery is performed, all instruments must first be autoclaved. Instruments must be cleaned of contamination in between animals and sterilized in the hot bead sterilizer. Instrument tips placed into a hot-bead sterilizer may be used on up to 6 rodents at which time they are required to be reautoclaved. Ensure that beads are pre-heated to the recommended temperature and instruments exposed for the recommended time. Instruments must be allowed to cool down before touching tissues.

BASIC ASEPTIC PRINCIPLES

1. All articles must be sterilized before use in a procedure.
2. If in doubt about sterility of item or person, assume it is not sterile.
3. Anything that touches sterile articles must also be sterile.
4. Non-sterile persons never reach across a sterile field.
5. Draped tables are sterile only above the table level.
6. Moisture enhances contamination by microbial organisms.

Surgical Techniques: Proper surgical technique is important for preventing wound infection, promoting wound healing, and increasing the likelihood of a satisfactory outcome to the surgical procedure. Good surgical technique includes:
1. Asepsis
2. Gentle tissue handling
3. Effective hemostasis
4. Maintenance of sufficient blood supply to tissues
5. Proper use of surgical instruments to achieve accurate tissue apposition
6. Appropriate use of monitoring equipment
7. Support of vital organ functions
8. Expedient performance of the surgical procedure

Aseptic technique is required at all times and all team members are responsible for monitoring for breaks in aseptic technique. "Asepsis is a chain which is only as strong as its weakest link." Potential sources of contamination include the team members, the patient, articles used in the procedure, the surgical room or area, and other personnel entering the surgical area.

Gentle handling of tissues will help minimize post-surgical pain and maximize healing. Tissues should not be cut or separated without reason and tissue dissection is usually done along fascial planes. Exposed tissue must be protected from drying or contamination. Effective hemostasis and maintenance of blood supply allows visualization of the surgical field while preserving the total blood volume. Hemostasis is achieved by ligation, pressure, electrocoagulation and avoiding damage to major vessels. Only the vessel to be occluded should be incorporated in a ligature or clip. In using electrocoagulation, a high frequency current is applied to a small area of tissue (the bleeding vessel) and electric energy is converted to heat, resulting in coagulation of tissue and sealing of the vessel. To avoid excessive tissue damage, only the vessel to be occluded is in contact with the electrode. Electrocoagulation can be used for minor hemorrhage; large vessels should be ligated. Proper use of surgical instruments minimizes trauma. For example, vascular forceps are used to occlude blood vessels when flow is to be reestablished; hemostatic forceps, which crush, are applied to vessels through which blood will no longer flow.

Accurate tissue apposition enhances healing and promotes rapid return to normal function. Retraction and dissection of tissue can produce pockets known as dead space. Dead space can delay healing and serve as a site for bacterial growth and fluid accumulation. As tissues are returned to their normal positions, dead space needs to be obliterated by careful suture placement to appose tissue and/or placement of drains to prevent fluid accumulation.

As a procedure is concluded, tissues are replaced to their normal anatomic positions. Most tissues should be apposed with minimal amount of tension and sutures must not devitalize the tissue in which they are placed. Type of suture material and pattern to be used will be dictated by the tissue involved and forces applied to those tissues. Behavior of animals also might influence the choices; some animals are more prone to chew or remove certain types of suture materials or devices such as surgical staples. Most animals will be fully mobile within 1 hour after a surgical procedure and the incision line must be able to withstand the twisting, bending and stretching, which will occur.

**POLICY 4:** The animal will be fully anesthetized; when a toe is pinched the animal will not exhibit a toe pinch reflex (withdraw or respond in any way to the pinch) prior to and throughout the duration of the surgical procedure. It is suggested you confirm surgery plane of anesthesia a minimum of every ten minutes. Increased frequency may be needed if there are changes in anesthetic depth, respiration and other vital parameters. Animals will be monitored and attended to throughout the duration of surgery and anesthetic recovery.

For intra-operative patient support and monitoring accepted medical and veterinary surgical practice requires assessment of the physiologic status of the animal on a regular basis. Vital time for resuscitation can be lost by failure to notice life-threatening physiologic or metabolic problems.

The degree of monitoring sophistication depends on the species, the type of surgery and the extent and duration of the surgical procedure. Monitoring can be qualitative, using the anesthetist's sense of touch, sight and hearing to evaluate the patient; and/or quantitative, using instruments for periodic measurement of specific vital organ performance.
The anesthetic record provides a detailed account of the course of anesthesia and intra-operative events. It is important to record data from the pre-operative, induction, anesthetic/surgical and immediate post-operative periods. Later measurements may be written on the individual animal’s monitoring sheet.

Expeditious performance of the surgical procedure implies that the surgical team will have sufficient training and competence to perform the surgical procedure in the least required amount of time. A rapid response to unplanned or emergency situations is the result of good organization and coordination of team members.

**POLICY 5:** The surgical site(s) will be prepared by removing the fur appropriate to the size of the surgical site and cleaned with a triple scrub of Chlorhexidine or Betadine Scrub alternated with alcohol (or sterile saline in some cases). Repeat three times to achieve sufficient contact time.

Immediately prior to surgery, the surgeon and assistants should thoroughly wash their hands. When an animal is being handled it is advisable to wear clean, non-sterile gloves at all times. To prevent fur and dander contamination of the sterile area and instruments, an animal preparation area where the animal is anesthetized and prepared, should be physically separate from the location where the surgery will be performed. Fur covering the surgical site and surrounding area should be removed and the loose fur taken away. Fur removal should be done carefully to avoid causing small skin abrasions. Fur should be removed from a liberal sized area around the planned incision site in anticipation of any surgical emergency and to minimize wound contamination from adjacent areas. After removing the fur, the skin should be washed with an antiseptic surgical scrub, containing soap (Chlorhexadine or Betadine scrub). This removes any remaining fur or dirt. The surgical area is cleansed two more times with antiseptic scrub. In between each scrub, rinse away with alcohol. Where alcohol cannot be used sterile saline can be substituted. If the animal is transported a long distance to the operating area, the clean surgical site should be covered with clean gauze sponges or cotton, saturated with the antiseptic solution devoid of soap. Care should be taken to avoid soaking the animal in disinfectant, as wetting the fur and skin can contribute to hypothermia. For this reason, a small cotton applicator should be used rather than large gauze sponges for very small animals such as mice.

**POLICY 6:** The surgeon will wear at minimum a closed, clean lab coat or scrub top, sterile surgical gloves (for full sterile technique) or clean gloves (for sterile tip) over scrubbed hands, a face mask and head cover.

Scrubbing hands prior to putting on gloves for surgery (whether using full aseptic or sterile tip technique) is required. There are several acceptable methods but the key components are to use an antiseptic soap and to scrub all surfaces of the fingers, hands, wrists and arms up to the elbows. Rodent survival surgery usually requires a cap, and mask, but a clean scrub top or lab coat may be substituted for a sterile gown. Sterile gloves are required for full aseptic technique. Clean gloves are required for sterile tip technique. Note that in order to keep sterile gloves sterile, the surgeon must keep their hands in the area between their waist and chest, dropping their hands below their waist or below the sterile table edge will render them contaminated. A surgical mask and cap may not be required if using a properly functional Class 2 Biosafety Cabinet (protects animals and personnel).

**POLICY 7:** Animals should receive supplemental heat during the pre-operative, intra-operative and post-operative periods.

Hypothermia is the most common cause of prolonged recovery and post-surgical mortality. When animals are removed from their cages or placed directly on metal surgery tables, body heat is lost, therefore insulating materials or warming devices should be placed between the table and the animal. Circulating warm water heating pads or warm water bottles should be used, since electric heating pads are likely to burn. When heat lamps are used, avoid placing the animal in the direct beam. If possible, reflect the heat lamp off of a metal surface and place the animal in the reflected beam. Prevent heat loss during surgery by placing an insulating material underneath the animal (to minimize heat loss by conduction).

**POLICY 8:** Animals undergoing anesthesia should have their corneas protected with ophthalmic lubricant and hydration should be maintained.

When anesthetized, animals should have their corneas protected with an ophthalmic lubricant. To avoid contamination of the lubricant, do not touch the tip of the tube to the skin or eye surface.
If anesthesia is expected to last more than 10 minutes, to maintain hydration, warmed subcutaneous fluids (i.e. sterile 0.9% saline or sterile lactated ringer's solution) will likely need to be administered.

**POLICY 9:** Animals undergoing survival surgery will be administered suitable analgesic agents as per the approved Animal Care protocol.

Where protocols include the use of narcotic or other analgesics, it is the responsibility of the Principal Investigator to ensure that analgesics are purchased, used, disposed properly and that the records are maintained in the laboratory for viewing by the ACC or the Clinical Veterinarians. Analgesics should be administered pre- or peri-operatively to achieve maximally effective pain relief in the post-operative period. For many surgeries, repeated doses of analgesics may be needed in the following days. This requires appropriate monitoring of individual animals in order to evaluate for evidence of pain. Typically pain relief is given for 3 days following major surgeries or procedures involving moderate to severe pain whether signs of pain are evident or not. Pain relief may be required beyond the 3rd day depending on the study and individual.

**POLICY 10:** Rodents will be recovered from surgery in a quiet area away from the immediate surgical area and they will be continually monitored until fully recovered. Animals should not be mixed with conscious animals until fully recovered.

The post-operative period consists of 3 overlapping phases: anesthetic recovery, acute and long-term post-operative care. Adequate post-operative care enhances the animal's recovery by improving its physiologic status and minimizing pain and distress. Recovery from anesthesia is a critical time because it is a period of physiologic disturbance during which crises can arise. Frequent observation and monitoring is required, thus animals should be placed in a quiet area where they can be easily monitored. Animals should be individually caged during this time period.

Post-operative care should include an external heat source while the rodents recover from anesthesia. Rodents who are still anesthetized should be protected from breathing in small particles of bedding by placing them on a clean cloth substrate such as a paper towel or surgical towel. Rodents should not be returned to group cages until each and every individual in the cage has regained normal mobility. Rodents should be returned to clean cages with fresh bedding to prevent fecal contamination of the fresh surgical incision. If there is danger of injury from other animals, animals should be individually housed.

**POLICY 11:** During recovery animals should receive adjunct nursing care such as wound care, fluids and analgesics.

Fluids should be provided to replace losses from respiration, blood loss, increased urination, etc. A rodent undergoing major surgery may need continued fluid support if they are not consuming appropriate amounts of fluid. Supplemental fluid sources, such as Transgel®, should be provided to animals that cannot or will not reach their water source.

In addition to intra-operative analgesia it may be necessary in some cases to provide post-operative analgesia. Acute post-operative care includes the time the animal is maintained in the recovery area until it is ready to be returned to standard housing. Analgesics, antibiotics and additional fluids might be needed during this time period.

Wounds will be cleaned from gross contamination from blood, bedding, prep solutions, etc.

There is no requirement for post-operative antibiotics, if proper aseptic technique is followed.

**POLICY 12:** Animals will continue to be monitored at regular intervals on the day of surgery and in the following days to ensure full recovery. Adjunctive nursing care may be required. This may include administration of analgesics.

Long-term post-operative care requires careful observation of body temperature, food intake, activity level, locomotion, attitude, appearance, behaviour, and signs indicating pain. Research staff must examine the
surgical site daily and remove sutures or other devices at the correct time (generally 7-10 days). The surgical site should be observed for signs of infection, incision breakdown (dehiscence), or self-inflicted trauma. At least once a day, catheters should be examined and they may need daily cleaning and flushing. Using body weight measurements is a useful indicator of food and water intake, which is important for successful recovery. Oral or parenteral supplementation may be necessary to maintain normal hydration and anabolic state. Special diets and/or water supplements may be indicated during the recovery period. The quantity and character of urine and feces should be monitored, because changes could indicate complications such as paralytic ileus, acute renal failure, or intestinal hypermotility caused by irritation.

**POLICY 13**: Animals with abnormal wound healing/closure or morbidity following surgery beyond the morbidity or mortality estimated in the Animal Care protocol require consultation with a Clinical Veterinarian unless specified in protocol. The veterinarian will, in consultation with the Principal Investigator, decide the best course of action for the animal and these actions are the responsibility of the research team.

**POLICY 14**: A written record of the surgical details and condition of the animals following surgery will be maintained in the unit and be readily accessible throughout the post-operative recovery period.

All supportive care and experimental procedures should be recorded with the date performed. Administration of all drugs (including anesthetic drugs) must be documented with volume and route of administration. For more details see UBC ANIMAL CARE COMMITTEE POLICY 017 Policy on Monitoring and Medical Records of Animals used for Research, Teaching and Testing.