Research, Discovery & Innovation

GUIDELINES FOR SURVIVAL AND NON-SURVIVAL RODENT SURGERY

I-IC-GU-304

A. PURPOSE

To standardize approved methods for survival and non-survival rodent surgeries. This document outlines the regulatory requirements for conducting survival or non-survival surgery in rodents, only. This document also serves as a reference when writing a protocol or amendment involving surgery.

B. GUIDELINES

A good surgical outcome requires:

- Sufficient pre-operative preparation and surgical support
- Asepsis, or sterile technique, which reduces clinical or subclinical infection
  - Includes surgery set-up, instrument/tool sterilization, and surgeon preparation
- Appropriate anesthesia
  - Includes selection of drugs and anesthetic monitoring
- Good surgical technique
  - Includes gentle tissue handling, correct use of surgical instruments and adequate knowledge of anatomy and procedure being performed
- Adequate post-operative care and pain management
- Consistent recordkeeping
  - Identifies trends that can be used for surgical refinements
  - Necessary for documentation of scientific data and analgesia to address welfare concerns

IACUC expectations:

- All surgical procedures must be reviewed and approved by the IACUC.
  - Any modifications to the procedures, drugs, and time lines must be reviewed and approved by the IACUC before they are implemented. This includes the use of the "tips only" method.
  - If you have to ask whether a change needs to be reviewed and approved, then you need to submit an amendment.
  - **If you are not sure what is approved, read the protocol**.
- Anyone participating in pre-op, surgery, or post-op care of animals must:
  - Be appropriately trained (includes CITI; GRST; UAC veterinarian; experienced protocol participant).
  - Be familiar with the approved procedures.
  - Perform every aspect of the procedure as approved by the IACUC.
- Adverse outcomes such as anesthetic failure, death under anesthesia, failure to recover, infection, or poor outcome must be reported to the IACUC Office immediately using an Adverse Event Report.
  - Reporting to a UAC staff member is not reporting to the IACUC Office.
- Use of Expired Medical Materials **requires prior** IACUC approval (see IACUC Guidance 212):
  - Prior IACUC Approval is required in all cases, even for non-survival procedures/surgery
  - Expired anesthesia, analgesia and/or euthanasia drugs MUST NEVER be used, even for acute (terminal, non-recovery, non-survival procedures).
  - For all other materials, they must be specifically listed, and approved, on a protocol.

Surgery location and set-up:

- The ideal location is a dedicated room or alcove.

- If an entire room/alcove is not available, the back of the lab, away from foot traffic, is recommended. Avoid areas of high foot traffic and potential disturbances.
- If a permanent space is not possible, reserve the area at least one day prior to the surgery so the area can be cleaned prior to and the day of the surgery, remove unnecessary equipment and materials including anything hanging over the surgical area and disinfect the area.

- Separate the 1) pre-op, 2) surgery and 3) post-op areas for survival surgery.
  - Make sure the surgical area has sufficient lighting for the task.
  - Separation of areas is not required for non-survival surgery.
- Ensure all instruments are sterilized, that sufficient sterile drapes, suture material, and sterile surgical gloves are available and that all equipment, reagents, and drugs are prepared and are in the surgical location.
  - Sterile instruments and supplies should be stored in covered or closed cabinets away from plumbing.
    - All packages containing sterile items must be inspected before use to verify that the package is not torn or damaged in any way and is not currently wet or displaying indication of past wetting.
    - Instruments must be recleaned, packaged in new wrap, and resterilized when outer packaging is damaged.
  - All injectable drugs must be sterile.
- If using the "tips only" method, turn on the glass bead sterilizer prior to the first surgery.
- Pre-heat warming pads (Isothermal, etc.) heat pad(s), for surgery and post-op recovery.
- If using isoflurane, check the vaporizer set up for leaks and assure the calibration date is not expired, add isoflurane, and position it in the surgical area. Vaporizer calibration must occur at least every 3 years as per IACUC Guidance 607.
  - Medical grade O2 gas must be used with the vaporizer (i.e. no room air).
    - Assure there is sufficient O_2 gas in the cylinder for the duration of the surgery.
  - Room air may be used with the rodent SomnoSuite anesthetic machine or an oxygen concentrator device.
    - Manufacture guidelines must be followed.
- Disinfect the surgical area and all devices with commercial disinfectant: 10% bleach solution, Versaclean, Clidox, MB-10, etc. Devices include items such as a stereotaxic device, microscope knobs, etc. to be used in the surgery.
  - 70% alcohol alone is not sufficient for disinfection for survival surgery.
- Once gloved for surgery, the surgeon should cover any knobs that will be operated by the surgeon with a sterile drape, gauze, glove, foil, or Press-N-Seal.
- A source of heat must be provided to prevent hypothermia during surgery.
  - The safest methods for providing thermal support are circulating water blankets or instant heat devices, such as isothermal heating pads.
    - Electric heating blankets and heat lamps are discouraged because of the increased potential to cause burns.
  - Animals must never be placed directly on a heat source as this could cause thermal burns. Use of a clean drape (surgical set up and recovery) and a sterile drape (during surgery) over the heat source will prevent this issue.
  - Heat sources are recommended, but not required for non-survival surgery.
- Cover the surgical area, including the heat source, with a clean drape or absorbent pad.

- The sterile surgical area is encompassed by the area between the lab bench surface and ~mid chest height of the surgeon and the width of the draped area, which includes space for sterile instruments.
- A sterile surgical field is recommended, but not required for non-survival surgery.

Instrument/tool sterilization:

- Sterile disposable drapes are to be used for all survival surgery and may be standard opaque drapes, rodent clear drapes or Glad Press-N-Seal (a food grade quality product that has specifically been tested for this application).
- Surgical scissors must be maintained to remain sharp, and new disposable sterile scalpel blades must be used for each animal.
- Instrument Sterilization: Wrap in appropriate cloth drape or place in a plastic "peel open" pouch.
  - Instruments may be singly wrapped or wrapped as a complete sets.
  - Include in the pack to be sterilized, an extra drape or tray to place instruments on once opened.
  - If autoclaving or using gas sterilization, the wrapping material must be intended for that purpose. Steam or gas cannot permeate a tightly sealed metal container, or something wrapped in aluminum foil.
  - Place appropriate indicator, such as autoclave tape or biological indicator on pack:
    - Appropriate monitoring of sterilizer effectiveness is required.
    - Only sterilized instruments with successful indicators can be used.
- Acceptable methods of sterilization are as follows:
  - Autoclaving/Steam sterilization:
    - Some materials such as PE-10 tubing can be autoclaved by placing in a small container of water.
    - Cloth wrapped 135 °C/5min + 30min dry time; cloth wrapped/plastic pouch 121 °C/30 min + 30min dry time; Liquid 121 °C/30min + 0min dry time (OR follow recommended manufacture guidelines for specific autoclave)
  - Dry heat sterilization:
    - 340°F/170°C for 1 hour; 320°F/160°C for 2 hours; 300°F/150°C for 3 hours
    - Instruments must be dry and must be held at the correct temperature for the entire period.
  - Sterilization with ethylene oxide gas (ETO; performed by UAC for a fee).
  - Vaporized hydrogen peroxide (VHP; performed by UAC for a fee).
  - Ionizing radiation (usually only performed by a commercial service).
- Some devices, such as those with electronic components cannot be sterilized by the above methods. Use of a commercial cold sterilant solution may be considered in these cases:
    - Cold sterilants are not recommended for general use as there is no reliable way to determine sterilization efficacy.
    - Use only the cold sterilant that is IACUC approved in the IACUC protocol.
    - Acceptable commercial cold sterilants include Cidex OPA, SporexII and Sterilox.
    - Follow the manufacturer's recommendations about dilution and duration. Document solution change times, duration of soak, etc.
    - Make sure to adequately rinse the device in sterile water or sterile saline before use.
- After surgery, soak instruments in an appropriate detergent, scrub thoroughly to remove all debris, and rinse thoroughly in clean water.
  - Use of an ultrasonic cleaner provides superior cleaning but is not required.

- Sterilization of instruments is recommended, but not required for non-survival surgery.

Surgeon preparation:

- Surgeon preparation is required for all survival surgery, or for non-survival surgery that opens a body cavity **and** lasts for more than 3 hours.
    - For other types of non-survival surgery, surgeon preparation is highly recommended, but is not required.
- Remove jewelry from the hands and wrists, tie back long hair and roll up long sleeves.
- Don a clean lab coat or scrub top, hair bonnet, and surgical face mask.
- Wash and scrub hands with disinfectant soap or a surgical scrub brush. Dry hands with a sterile towel.
- Don a sterile gown
    - If sterile surgical gowns are not available, a fresh laundered lab coat, or similar cover should be worn.
- Don sterile surgical gloves.
    - Gloves are sterile from the finger tips to the top of the gloves.
    - Gloves lose their sterility if they touch anything that is not sterile.
    - If a sterile surgical gown is worn, pull sterile surgical gloves over the top of the gown sleeve.

Selection of anesthetic drugs and use of anesthetic gas vaporizers:

- Selection of the appropriate anesthetic regimen depends of the species, the type and duration of the procedure, and is made in consultation with a UAC veterinarian.
    - Only the anesthetic regimen approved in the IACUC protocol can be used.
- An inhalant anesthetic, such as isoflurane, should be the first choice as animals are anesthetized quickly, maintain a smoother plane of anesthesia, and recover within several minutes after the gas is discontinued.
    - Use of an inhalant anesthetic requires a vaporizer and nose cone to administer the drug and a method to capture the waste anesthetic gas.
    - No more than two lines must actively deliver gas from a single precision vaporizer (also known as “splitting” the vaporizer line), as the amount of gas going to each anesthetized animal cannot be controlled separately with this modification. With more than two lines active, animals will not receive the appropriate amount of anesthetic gas, unless a commercially designed multi-port device is used.
    - F/AIR charcoal canisters used for waste anesthetic gas scavenging must be weighed, and weights recorded before each surgical session. Canisters must be discarded when they increase in weight by 50g.
- Injectable anesthetics require no specialized equipment; however, the commonly used agents may be DEA controlled substances or non-pharmaceutical grade.
    - The effects of injectable anesthetics are more variable and may be strain dependent.
    - For long procedures, booster doses will be required, and recovery from anesthesia will not occur until sufficient amounts of the drug has been metabolized. Booster doses must be listed in the protocol.
    - If a reversal agent is used, this must be included as part of the anesthetic regimen in the IACUC protocol.
    - The use of non-pharmaceutical anesthetic agents must be scientifically justified.
    - Expired anesthetics may not be used at any time.

Anesthetic monitoring:

- Anesthesia must be monitored a minimum of every 15 minutes throughout the surgical procedures, to assure the appropriate depth of anesthesia is maintained.
    - Whenever possible, anesthesia should be monitored and supplemented, if necessary, by the surgical assistant.
- If there is **insufficient anesthetic depth**, anesthesia can be supplemented by adjusting the isoflurane or providing a booster injection, as approved in the IACUC protocol.
- There is a possibility that **too much anesthesia** can suppress respiration to an extent that death occurs.
    - If using isoflurane, the amount can be reduced, and the depth of anesthesia can be reassessed.
    - If using an injectable anesthetic, the animal should be monitored, and a UAC veterinarian consulted, if possible.
    - If using a Ketamine cocktail, only the Ketamine should be given as a booster at a greatly reduced dosage. This must be detailed in the protocol.
    - If death under anesthesia occurs, **this must be reported to the IACUC as an adverse event.**
- Response to a toe pinch (withdraw reflex) is a commonly used method to assess anesthetic depth. Using two fingers, the animal's foot is squeezed hard enough to cause slight blanching of your fingernail, but not hard enough to cause damage to the foot. If there is no withdrawal reaction, the anesthesia is judged deep enough to commence surgery.
    - This should be performed directly through the drape to prevent sterile gloves from being contaminated.
    - If a standard opaque drape is used, the assistant should perform this test. If the surgeon is performing the test, a sterile gauze pad or glove should be placed over the animal's foot to prevent contamination of the surgeon's glove.
- There are also several visual methods of anesthesia monitoring that can be performed easily and frequently:
    - Respiratory rate and pattern: Anesthesia slows the respiratory rate, which can be seen as the chest is rising and falling. The respiratory rate increases when there is insufficient anesthetic depth.
    - Movement: If anesthesia is not deep enough, movement of the whiskers may be observed. If the animal moves in response to surgical procedures, then there is insufficient anesthetic depth.
    - Mucous membranes: Mucous membranes are evaluated by the color of gums, the pinna (ears) and the toes, which should be pink. A bluish color indicates insufficient oxygen due to a decreased respiratory rate from too much anesthesia. A darker pink to red color indicates that the animal is overheating, most likely from the thermoregulatory support.
    - Instrumentation: Animals may be attached to a device which provides a read out of respiration rate, as well as other parameters.

Pre-operative preparation and surgical support:

- Food and water should not be withheld from rodents prior to surgery (rodents are unable to vomit; thus, aspiration of vomit is not an issue)
    - If food or water must be withheld for any other reason, the duration must be specified in the approved IACUC protocol.
- Whenever possible, a surgical assistant should be responsible for preparing animals for surgery (pre-op), as this is a mostly non-sterile process.

- A surgical record is required to document pre-op, intra-op, and immediate post-op information.
- Evaluate the animal to ensure it is apparently healthy.
- Induce anesthesia as described in the approved IACUC protocol and assure adequate depth of anesthesia.
    - For injectable anesthetics, if adequate depth of anesthesia is not achieved, one or more booster injections may be administered as described in the approved IACUC protocol.
    - If the animal fails to become adequately anesthetized, stop the procedure and consult a UAC veterinarian.
- Apply sterile, bland ophthalmic ointment to the eyes to prevent drying for any anesthetic event; regardless of duration.
    - Use for all anesthetic events, survival and non-survival surgeries.
    - The sterile ointment must be labeled for ophthalmic use.
- Administer pre-op fluids, e.g., warm 0.9% sterile saline, as described in the protocol.
    - Fluids help the animal remain hydrated for longer surgeries.
- Administer analgesia, as described in the protocol.
    - Analgesia should be administered immediately prior to surgery, or up to 1-2 hours before surgery. Remember to request a protocol modification if a change in analgesic dose/route/type is needed.
- Administer any additional pre-op drugs as approved, e.g., antibiotics, topical anesthetic, etc.
- Remove hair at least 2-3 cm around the surgical site using well maintained clippers and/or depilatory cream.
    - When using depilatory cream, apply and remove with minimal contact time necessary to prevent chemical burns. Areas should be cleansed with sterile saline or water to ensure no residual depilatory cream remains.
- Using clean gloves, wipe the shaved skin with gauze soaked in either a chlorohexidine surgical scrub preparation (preferred) or a povidone iodine/betadine solution. Start with the gauze in the center of the shaved area where the surgical incision will be
    - work in concentric circles towards the outer edge of the shaved area
    - or if the area is very small make a center wipe, then wipes to each side (e.g. rodent skull surgery).
- Next wipe the area with gauze soaked with warm sterile saline or 70% (isopropyl) alcohol. Using a new pad each time, repeat these alternating scrubs 2 additional times.
- Finish by covering the area with a gauze and soaking it with chlorhexidine or betadine spray to cover the intended surgical area.
- The animal is moved to the prepared surgical area and is positioned on the surgical field or in a stereotaxic device. If tape is used to secure a position, take care to maintain limb circulation.
- Cover the animal with a sterile drape. Clear drapes are recommended (rodent clear drape, Glad Press-N-Seal (commercial food grade)), leaving an opening around the nose to allow breathing. If using Press-N-Seal, the sticky side should be placed towards the animal and is considered 'non-sterile', so the assistant may place the drape over the animal without touching the non-sticky side.

Surgical technique:

- Perform the surgery <u>exactly</u> as described in the approved IACUC protocol. This includes use of only the approved drugs and techniques.
    - If surgery is planned/in progress and cannot be completed exactly as described, contact the

IACUC Office or a UAC veterinarian to determine whether a modification is possible.

- If performing more than one surgery, or if sterility is broken:
  - Use a new sterile pack between animals or if sterility is broken (preferred).
  - "Sterilize" instruments between animals or if sterility is broken by using a glass bead sterilizer (see below).
    - Instruments must be replaced with a sterilized pack after no more than 7 uses of the glass bead sterilizer, or up to 8 animals total.
- Sterile gloves must be changed:
  - If they become contaminated during the surgery by touching anything non-sterile.
  - If they move out of the sterile area.
  - Between animals, even if using "tips only" technique.
- The specific techniques used will depend on the type of surgery. However, there are a number of common practices that should be incorporated in any surgery.
  - Minimization of the size of incisions to only that necessary for the procedure.
  - Gentle tissue handling to reduce tissue damage around the surgical site.
  - Effective hemostasis to minimize blood loss.
  - Effective wound closure technique to ensure good healing and minimal pain.
  - Appropriate type of wound closure, such as suture type and pattern.
- Consider whether surgical refinements can be made, and if so, amend the protocol.
- Aseptic technique is required for survival procedures, and those non-survival surgeries that open a major body cavity and last more than 3 hours and is recommended for all other surgical procedures.

Aseptic tip technique or "Tips Only":

- Use of this technique must be specifically approved in the IACUC protocol for each surgery in which it will be implemented.
- This is a modified set of procedures used to achieve asepsis in rodent surgeries where the surgeon only utilizes the sterile working ends of the surgical instruments (or "tips") to manipulate the surgical field.
  - This technique is well suited for less invasive surgeries, such as subcutaneous mini-pump implantation, tumor implants, and intracranial cannula/head cap placement.
  - The technique is best suited to situations where an assistant is available to perform pre-op and other non-sterile activities.
- Instruments must be first sterilized as for non-"tips" surgery and sterile gloves must be worn for the first surgery. Therefore, the first surgery follows classical aseptic principles.
- Prior to the subsequent surgeries, the tips of instruments are hot bead sterilized and the instruments are placed on the existing sterile field so the tips remain sterile for the next surgery.
- As the surgeon's hands and instrument handles will become contaminated, only the tips are to be used to manipulate any tissues in the surgical field to maintain asepsis.
  - If the surgeon performs pre-op or other non-sterile activities, new sterile gloves must be worn for the next surgery.
- When suture is used for incision closures while utilizing this technique, additional caution needs to be taken to ensure the suture remains in the narrow sterile field and is not moved through areas contaminated by previous surgeries.
- Instruments must be replaced with a sterilized pack after no more than 7 uses of the glass bead sterilizer, or up to 8 animals total. Fresh, sterile gloves must be worn every time a new instrument pack is opened.

Post-operative care and pain management:

- Post-operative recovery must occur in a separate location to pre-op and surgery.
- Animals must be recovered in a clean cage without bedding to prevent accidental aspiration of bedding; an absorbent pad or paper towel should be place on the bottom of the cage.
    - The recovery area should provide thermal support. The ideal method is to have the recovery cage half on, half off a heat source, with the animal placed fully on the warm side at the onset of recovery. The split allows the animal to move away from the heat as they recover. An incubator may also be used. If using a heat lamp, extreme caution must be used to place the device a sufficient distance to prevent overheating and thermal burns (use of heat lamps is discouraged).
- For prolonged or invasive surgeries, fluid replacement should be administered prior to waking from anesthesia.
    - Warm sterile 0.9% saline can be administered IP or SC, if approved in the IACUC protocol. The usual amounts are 0.5-1.0 ml for mice or 3.0-5.0 ml for rats. Larger rodents may have an indwelling IV catheter placed, if necessary, and approved in the protocol.
    - Use of oral hydration gels (Napa nectar) or water-softened food do not require IACUC approval and can assist in recovery from particularly invasive surgeries or surgeries with long anesthetic periods.
        - If using an oral hydration gel, provide the gel to the rodent at least 2 days in advance to allow acclimation. (Rodents are neophobic and will not touch it if placed in the pan immediately following surgery).
- Monitor the color of the pinna and/or footpad and watch for the respiration rate and movements to increase as the animal recovers from anesthesia.
    - Once the animal has righted itself (becomes sternal) and is ambulating normally, it is considered recovered from anesthesia. At this point, the animals can be transferred to their home cage (which should be a clean cage – no to minimal soiling).
    - It is recommended that the animals be monitored for an additional 30 minutes before being returned to the animal housing room.
    - When returning the animals to the UAC housing room, place a properly completed SURGERY Cage Card on the cage to flag the animal so UAC husbandry and veterinary services are aware the animal is recovering.
- Administer analgesics or other drugs as stipulated in protocol or approved by a UAC veterinarian.
    - The initial dose is recommended to be administered in pre-op. Repeat as necessary.
    - Maintain a record of administration of all drugs, including animal information, dosage, route of administration (SC, IP, etc.) and time of delivery.
- Monitor daily: Appetite, wound healing, energy/normal movement, incision healing, etc. Repeat analgesics as approved in the protocol.
- Wound closure removal: Non-absorbable suture material and wound clips must be removed as described in the protocol, including timing and use of sedation. Wound reclosure may not be performed unless described in the protocol.
- Consult a UAC veterinarian if complications arise.

Recordkeeping:

- Records must be kept for survival and non-survival surgery.
- Records must contain:

- Date of procedure
- The protocol number, PI, Surgeon, and assistant if applicable
- Location – building and room #
- The species and animal or cage identifier
- Brief description of the procedure
- Pre-surgery
  - Assessment:
    - Weight (not required for gas anesthesia)
    - General physical condition/readiness for surgery, such as:
      - Within Normal Limits (WNL)
      - Bright Alert Responsive (BAR)
  - Pre-op Preparation:
    - Sterile Ophthalmic ointment applied to all anesthetized animals
    - Hair/fur removal completed
    - Surgical scrub completed
- Listing of all drugs/compounds, including anesthesia, analgesia, fluids, topical anesthetic, antibiotics, etc.
  - Name, route, dose, volume
- Event times:
  - Anesthesia: Start/stop time
  - Surgery: Start/stop
  - Drug administration: When all drugs/compounds are given (Pre-Op, Surgery, Recovery and Post-operatively (per protocol or via consultation with UAC veterinarian))
  - Recovery: When animal is sternal and returned to home cage
- Depth of anesthesia (intra-op) monitoring. This must occur at least every 15 minutes; the following methods may be used, dependent upon individual species and procedures:
  - Toe/paw/ear withdraw (from pinch)
  - Whisker movement
  - Jaw tone
  - Temperature
  - Respiration rate
  - Heart rate
  - Reflexes and muscle tone
  - Eye position and pupillary reflex activity
  - Swallowing reflex
  - Mucous membrane color
  - Blood pressure
  - Muscle movements/voluntary movement especially in response to noxious stimuli
- Post-op monitoring for survival surgery
  - Must occur at least every 15 minutes until sternal recumbency and normal ambulation is established
    - Document progress throughout recovery (including subsequent days of post-op drug administration per approved protocol or via consultation with UAC veterinarian).
  - Removal of skin closure materials, suture or staples, 7-14 days posts-surgery, or as

approved in protocol
    - Records must be initialed and dated.
- Records must be kept in a central location and available to all protocol participants and IACUC or other regulatory inspectors.
    - Copies should be placed in individual lab books.
    - Records must be maintained for the duration of the protocol approval period and for any additional time required for research records, as applicable.

C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

DEFINITIONS:

- **Aseptic technique:** A combination of techniques used to create and maintain a controlled environment free of microbial contaminants (bacteria, viruses, and fungi).
- **Aseptic tip technique or "Tips-only" technique:** A modified combination of techniques wherein the surgeon only uses the sterile working ends of the surgical instruments (or "tips") to manipulate the surgical field in order to achieve asepsis. The gloved, but non-sterile, hand never contacts the instrument tips, suture, suture needle, or any other part of the surgical field during use.
- **Disinfection:** Also known as sanitization. Disinfection removes the majority of microorganisms. Some bacteria and viruses are resistant to use of alcohol as a disinfectant. Most bacterial spores and protozoan parasites are resistant to many disinfectants.
- **Drape:** A sterile drape is a porous (cloth, paper) or non-porous (plastic) sheet that is used to provide a temporary sterile barrier. A dedicated roll of Saran Wrap or Glad wrap, especially the Press n' Seal variety, can be used as a see-through, non-porous drape. Due to the way the plastic is formed at extremely high heat, the inner surface of the roll is sterile, as long as it is untouched.
- **Glass bead sterilizer:** Glass bead "sterilization" uses ~1.5mm glass beads and high temperature (217-232°C) for brief exposure times to inactivate microorganisms. The instruments must be placed about halfway into the beads for at least 45 seconds. Only the portion of the instrument placed in contact with the beads will be sterilized. All instruments must be cleaned of blood and tissue debris prior to being placed in the bead sterilizer. Remove and place instruments on the sterile instrument drape to cool before touching the animal again.
- **Non-survival surgery**: An animal is subjected to surgical anesthesia and surgical penetration of the body occurs or invasive measurements are performed over a long period and the animal is euthanized without waking from anesthesia. Aseptic technique is recommended but may or may not be required.
- **Rodent:** Rodents are characterized by a pair of unremittingly growing incisors in the upper and lower jaws, and include mice, rats, guinea pigs, hamsters, gerbils, and squirrels.
- **Sterile:** Free of all forms of life and biological agents, such as bacteria, viruses, and fungi.
- **Survival surgery:** Surgery in which the animal is subjected to surgical anesthesia and surgical penetration of the body occurs or invasive measurements are performed, and the animals is revived from anesthesia. Aseptic technique must be used for all survival surgeries.