

POLICY ON ASEPTIC RECOVERY SURGERY ON RODENTS AND BIRDS

Adopted by the University Committee on Animal Resources – November 19, 2014

The U.S. Public Health Service Guide for the Care and Use of Laboratory Animals states that “In general, unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery should be conducted in dedicated facilities or spaces. When determining the appropriate site for conducting a surgical procedure (either a dedicated operating room/suite or an area that simply provides separation from other activities), the choice may depend on species, the nature of the procedure (major, minor or emergency), and the potential for physical impairment or postoperative complications, such as infection” (1). As required by the United States Public Health Service (PHS), United States Department of Agriculture (USDA) and the University Committee on Animal Resources (UCAR), all vertebrate animal-use protocols, regardless of the funding source, must comply with the guidelines stated in the Guide and Animal Welfare regulations.

The “tips-only” surgery technique is a modified approach to rodent surgery that is especially useful for multiple-surgery sessions. This technique allows the surgeon to wear non-sterile exam gloves because it relies on the surgeon’s ability to only use the sterile tips of the instruments for all surgical manipulations without touching the animal. While the “tips only” technique does not strictly meet the Guide’s requirements specific to use of sterile gloves, NIH has supported this approach for rodent aseptic recovery surgery. Investigators must identify the intent to use the “tips only” technique in the protocol. The “tips only” approach requires attention to detail and must fulfill the requirements for this approach below.

For USDA regulated rodents (e.g. hamsters, gerbils, guinea pigs, mole rats and voles), a new set of sterile surgical gloves and sterile instruments must be used for each animal. The “tips-only” surgery technique is not applicable to these species.

Investigators who feel that their vertebrate animal experiments require exceptions to the guidelines should contact UCAR for assistance. Otherwise, investigators will be expected to follow:

1. Surgery must be conducted on a clean, uncluttered lab bench or table surface. Wipe the surface with a disinfectant before and after use and/or cover with a clean drape.
2. Remove hair or feathers from the surgical site with clippers or a medical depilatory or by plucking. Disinfect the surgical site with at least a two-minute total contact time using the following two-step process:
 - a. Gross contamination should be removed by using a surgical scrub at the surgical site (chlorhexidine or povidone iodine scrub and solution).
 - b. The surgical site should then be treated with 70% ethyl alcohol, povidone iodine solution or chlorhexidine solution (2).
3. Apply bland ophthalmic ointment to eyes to prevent corneal drying.
4. A sterile drape is recommended to avoid sterile instruments, sterile gloves or exposed viscera from coming in contact with unprepped areas.
5. The temperature in the surgery room should be increased and/or the animal placed on a covered warming device (e.g. circulating warm water blanket, warm water bottle, slide warmer or chemical hand warmer) to prevent hypothermia. The use of heating pads is prohibited due to the potential for thermal burns.
6. All instruments must be sterilized **for both standard and “tips only” aseptic technique**, but the method of choice may vary depending upon the surgical instruments or devices used. Acceptable sterilization techniques include autoclaving using steam under pressure or cold sterilization. Approved cold sterilization methods include: soaking instruments in 2.5-3.5% glutaraldehyde (e.g. Cidex Plus for 10 hrs. at 20-25° C) or 7.5% hydrogen peroxide (e.g. Sporox Sterilizing and Disinfection Solution for 6 hours at 20° C) according to manufacturer’s instructions (3). U.S. Food and Drug Administration, (March 2009) FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices.
<http://www.fda.gov/cdrh/ode/germlab.html>
7. The surgeon should wash his/her hands with an antiseptic surgical scrub preparation and then aseptically put on sterile gloves. If working alone, the surgeon should have the animal anesthetized and positioned and have the first layer of the double-wrapped instrument pack or any individually wrapped items opened before donning sterile gloves.

- a. **Use of the tips only technique (for non USDA regulated rodents) does not require the use of sterile gloves; however, the surgeon should still surgically scrub his or her hands prior to use of exam gloves. The tips only technique allows the surgeon to anesthetize and position the animal between surgeries.**
8. The surgeon must wear a face mask, sterile gloves and a clean lab coat. A cap and sterile gown are recommended, but not required as part of the surgeon's attire.
 - a. **Sterile gloves are not required for the tips only aseptic technique. A sterile field must be prepared on which to place instruments.**
9. Surgery performed on multiple rodents and birds in a series presents special challenges. After the first surgery, the sterilized instruments may be kept in a sterile tray containing 70 – 90% ethyl or isopropyl alcohol (4) for (Contact time for instruments is 2 minutes and finger tips is 30 seconds) and no more than a total of five rodents (5). The alcohol must be replaced when contaminated with blood or other body fluids. Alternatively, a glass bead sterilizer can be used. It is important to remove any gross debris prior to placement of instruments in the sterilizer as well as allowing the instruments to cool sufficiently prior to reuse. Sterile gloves should be changed between surgeries if the surgeon touches nonsterile surfaces; alternatively, surgeons may wipe their sterile gloves for 30 seconds with sterile gauze pads soaked in 70 – 90% ethyl or isopropyl alcohol (4) or nonsterile surfaces may be handled aseptically with sterile gauze pads.
 - a. **TIPS ONLY (for non USDA regulated rodents) – Only handle instruments by the handles, and do not allow the tips of instruments to touch non-sterile surfaces. Sutures, catheters, and other sterile materials to be used in the surgery must only be handled with the instrument tips. Tissues must only be touched with instrument tips.**
 - i. **Instrument tips must be sterilized between surgeries utilizing the same techniques described in #9 for standard aseptic technique.**
10. Monitoring of anesthesia in rodents and birds may be accomplished by observation of color, respiratory rate and pattern, body temperature and observation for the loss of pedal, corneal and pinna (external ear) reflexes. More sophisticated methods of patient monitoring include EKG and heart rate, pulse oximetry, blood pressure measurements, blood gas measurements, etc.
11. The abdominal or thoracic body wall should be closed with absorbable suture material in a simple interrupted pattern. The skin must be closed with staples or with a nonabsorbable suture material in a simple interrupted pattern or absorbable sutures in a simple interrupted subcuticular pattern. Avoid using braided non absorbable material (silk) to close skin or muscle as it has the tendency to wick bacteria into skin and muscle causing an inflammatory response. Absorbable sutures placed in a subcuticular pattern to close the skin need not be removed postoperatively since they are buried under the skin. All other skin sutures or staples must be removed seven to ten days after surgery.
 - a. **When using the tips only technique, it is important to only handle suture with the tips of the surgical instruments.**
12. Rodents and birds should be recovered from anesthesia in a warmed environment. Warm fluids (lactated Ringer's or normal saline solutions) may be administered subcutaneously to improve postoperative hydration and enhance recovery (rats: 5 – 10 mls, mice: 1 – 3 mls and birds: 0.5 ml of 50% PlasmaLyte/50% D5W given subcutaneously or warm LRS 10-15 ml/kg and up to 25 ml/kg if over a 5-7 minute period, SQ). Antibiotics should not be given routinely after surgery unless justified by the investigator and DLAM Veterinary staff. Post procedural or anesthetized animals may not be left unattended or returned to housing until their righting reflex has returned and they are sternal with pink mucous membranes and stable respirations.
13. Monitoring of autoclave equipment —Heat sensitive chemical indicators must be used to verify that surgical instruments and other materials are appropriately sterilized. Investigators must use one autoclave integrator strip in each pack to be autoclaved. The strip should be placed in a location considered to be the hardest for the steam to reach. Autoclave tape must be placed on the pack surface. Contact DLAM for more information about these methods.
14. Systemic analgesics must be given to all species experiencing recovery surgical procedures as well as those undergoing minor procedures that may result in post-op discomfort. Analgesics must be administered prior to the

surgical manipulation and must continue for at least three days for major invasive surgery, or longer if the animal demonstrates signs of pain. Analgesics must be given at the dosing frequency stated in the UCAR protocol*. The decision to discontinue analgesic therapy should be made based on the observation that the animal does not appear painful at the end of the previous dosing interval (when the next analgesic treatment is due). All rodents must be observed at least daily for 3 days post-surgery for signs of pain or distress. Your observations must be documented on the post-operative card affixed to the cage.

**In the event that the analgesic described in the UCAR protocol is unavailable, please contact a DLAM veterinarian so they may provide a satisfactory substitute and oversight of the change in analgesic therapy.*

Surgeons must use the green “Be Gentle-Post-Op” cage cards to identify post-surgical animals in the vivarium. The cards must contain all information (PI name, procedure/date, observations, analgesics etc). If an investigator has scientifically justified that analgesics cannot be used pre and post-operatively, it should be noted on the green post-op cards. Remove cards when sutures/wound clips are taken out and maintain with lab notes.

| BE GENTLE – POST-OP | | | |
|--|------|--------------------------------------|----------|
| PI _____ ID # _____ UCAR # _____ | | | |
| Procedure _____ Procedure Date _____ | | | |
| Analgesic _____ | | | |
| Record observations and analgesic administration as described in the UCAR protocol. Observe animals at the appropriate analgesic dosing interval following the last treatment to verify that they no longer need analgesics. Remove card from cage at time of suture/wound clip removal and maintain with lab records. | | | |
| Date | Time | Observation/Analgesic Administration | Initials |
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Pain in rodents and birds may be identified by observing the animal’s reluctance to move about, decreased appetite and/or water consumption, weight loss, listlessness, salivation, hunched posture, favoring of the affected body part, piloerection (rodents), ruffled feathers (birds), increased respiration, respiratory sounds (chattering in mice), vocalization with handling and/or self-mutilation.

Commonly Used Analgesic and Anesthetic Agents

Other anesthetic combinations are available. Please consult a DLAM veterinarian to discuss an anesthetic protocol to fit your research needs.

Analgesics for mice

| Drug | Mouse dose range | Route of administration | Frequency | Comments |
|--------------------|------------------|-------------------------|-------------------|--|
| NSAIDs * | | | | Prolonged use may cause gastrointestinal, renal or other problems. NSAIDs may be used as the sole analgesic agent or they may be combined to provide multi-modal analgesia. Please contact a DLAM veterinarian for more information. |
| Flunixin meglumine | 2.5 mg/kg | SC, IM | Every 12-24 hours | |

| | | | | |
|---|----------------|----------|------------------------|---|
| (Banamine®) | | | | |
| Carprofen (Rimadyl®) | 5 mg/kg | SC | Once every 24 hours | |
| Ketoprofen (Ketofen®) | 5 mg/kg | SC | Once every 24 hours | |
| Meloxicam (Metacam®) | 1-2 mg/kg | PO, SC | Once every 12-24 hours | |
| OPIOIDS | | | | |
| Buprenorphine (Buprenex®) | 0.05-0.1 mg/kg | SC or IP | Once every 6-12 hours | or for moderate to severe pain, consider multi-modal analgesia with a NSAID (e.g. Meloxicam). |
| Buprenorphine (Animalgesics®) Extended Release for mice | 3.25 mg/kg | SC | every 48 – 72 hours | |

Injectable Anesthetics in Mice
(remember to provide supplemental heat to anesthetized rodents)

| Drug | Mouse dose range | Route of Administration | Comments |
|------------------------------------|--|-------------------------|--|
| Sodium Pentobarbital (Nembutal®) | 30-90 mg/kg | IP | Useful for immobilization, not surgical anesthesia, when used alone. |
| Ketamine/xylazine | 100 mg/kg ketamine + 10 mg/kg xylazine | IP | Anesthesia; only redose with ketamine if needed |
| Ketamine/midazolam | 100 mg/kg ketamine + 5 mg/kg midazolam | IP | Anesthesia; only redose with ketamine if needed |
| Ketamine/diazepam | 100 mg/kg ketamine + 5 mg/kg diazepam IP | IP | Anesthesia; only redose with ketamine if needed |
| Ketamine & dexmedetomidine | ketamine 50-75 mg/kg + dexmedetomidine 0.5-1.0 mg/kg | IP | |
| Ketamine & xylazine & acepromazine | ketamine 100 mg/kg + xylazine 20 mg/kg + ace 3 mg/kg | IP | Prolonged anesthesia – can partially reverse with atipamezole or yohimbine |

Analgesics for Rats

| Drug | Rat dose range | Route of administration | Frequency | Comments |
|--------------------------------|------------------------------------|-------------------------|------------------------|--|
| NSAIDs * | | | | Prolonged use may result in gastrointestinal, renal or other problems. NSAIDs may be used as the sole analgesic agent or they may be combined to provide multi-modal analgesia. Please contact a DLAM veterinarian for more information. |
| Flunixin meglumine (Banamine®) | 2.5 mg/kg SC, IM every 12-24 hours | SC, IM | Once every 12-24 hours | |
| Carprofen | 5 mg/kg | SC, PO | Once every 24 hours | Oral doses may need to be increased |
| Ketoprofen (Ketofen®) | 5 mg/kg | IM, SC, PO | Once every 24 hours | Oral doses may need to be increased |
| Meloxicam (Metacam®) | 1-2 mg/kg | SC, PO | Once every 12-24 hours | |
| OPIOIDS | | | | |
| Buprenorphine (Buprenex®) | 0.05 -0.5 mg/kg | SC | Once every 6-8 hours | If mild to moderate pain of increased duration is anticipated |

Injectable Anesthetics in Rats
(remember to provide supplemental heat to anesthetized rodents)

| Drug | Rat Dose range | Route of Administration | Comments |
|----------------------------------|---|-------------------------|---------------------|
| Sodium Pentobarbital (Nembutal®) | 40-50 mg/kg | IP | Light anesthesia |
| Ketamine/xylazine | 40-80 mg/kg ketamine + 5-10 mg/kg xylazine | IP | Surgical anesthesia |
| Ketamine/midazolam | 75 mg/kg ketamine + 5 mg/kg midazolam | IP | Light anesthesia |
| Ketamine/diazepam | 75 mg/kg ketamine + 5 mg/kg diazepam | IP | Light anesthesia |
| Ketamine & dexmedetomidine | ketamine 50-75 mg/kg + dexmedetomidine 0.25 mg/kg | IP | |

Analgesics for Other Rodents

| Drug | Species | Dose Range | Route of Adminis- tration | Frequency | Comments |
|---------------|----------------|-----------------|------------------------------|--------------------------|---|
| Flunixin | Guinea Pig | 2.5-5mg/kg | SC | Once every 12 – 24 hours | |
| | Hamster | 2.5mg/kg | SC | Once every 12 hours | |
| Carprofen | Guinea Pig | 5mg/kg | SC | Once every 12 hours | |
| | Guinea Pig | 1-2 mg/kg | PO | Once every 12 hours | |
| | Hamster | 5mg/kg | SC | Once every 24 hours | |
| Ketoprofen | Guinea Pig | 1mg/kg | SC, IM | Once every 12 – 24 hours | |
| Meloxicam | Guinea Pig | 0.1-0.3mg/kg | PO | Once every 24 hours | |
| | Mole Rat | 1mg/kg | PO | Once every 24 hours | |
| OPIOIDS | | | | | |
| Buprenorphine | Rodents | 0.05 – 0.1mg/kg | SC, PO | Once every 6-12 hours | |
| Morphine | Naked Mole Rat | | | | AVOID – causes hyperalgesia and severe aggression |

Injectable Anesthetics for Other Rodents

| Drug | Species | Dose Range/Species | Route of Administration | Comments |
|------------------------------------|----------------|--|-------------------------|--|
| Sodium Pentobarbital (Nembutal®) | Guinea Pig | 45mg/kg + xylazine 7mg/kg | IP | |
| | Hamster | NOT RECOMMENDED | | |
| Ketamine/xylazine | Guinea Pig | 30 - 87 mg/kg ketamine + 5 -13 mg/kg xylazine | IP | Higher dose induces surgical plane of anesthesia |
| | Hamster | 200mg/kg ketamine + 10 mg/kg xylazine | IP | |
| | Gerbil | 50-70mg/kg ketamine + 2-3mg/kg xylazine | SC, IP IM | |
| | Blind mole rat | 27mg/kg ketamine + 0.6mg/kg xylazine | | |
| | Prairie vole | 67mg/kg ketamine + 13mg/kg xylazine | SC | |
| Ketamine/midazolam | Guinea Pig | 5-10mg/kg ketamine + 0.5 – 1.0 mg/kg | IM | Anesthesia; only redose with ketamine if needed |
| Ketamine/diazepam | Guinea Pig | 30-100 mg/kg ketamine + 2-5 mg/kg diazepam | IM | Anesthesia; only redose with ketamine if needed |
| | Hamster | 70mg/kg ketamine + 2mg/kg diazepam | IP | |
| | Gerbil | 100mg/kg ketamine + 5mg/kg diazepam | SC, IP | |
| Ketamine & dexmedetomidine | | ketamine 50-75 mg/kg + dexmedetomidine 0.5-1.0 mg/kg | IP | |
| Ketamine & xylazine & acepromazine | | ketamine 100 mg/kg + xylazine 20 mg/kg + ace 3 mg/kg | IP | Prolonged anesthesia – can partially reverse with atipamezole or yohimbine |
| Propofol | Guinea Pig | 10mg/kg | IV | Administer to effect |

Inhalation Anesthesia of Rodents and Birds

| Drug/agent | Usage to Anesthetize Rodents |
|---|---|
| Isoflurane | Maintain at 1-3% to effect (5% for induction). If survival surgery, analgesics should be used. Use precision vaporizer. DLAM has rodent anesthetic machines available for use for a small fee. Contact DLAM for reservations and questions. |
| Isoflurane in a jar in fume hood (no vaporizer) | ♦Jar needs a perforated platform in the bottom to prevent animal contact with anesthetic. ♦Moisten gauze with isoflurane and place it below platform. ♦After animal is anesthetized, use a nose cone with isoflurane-wetted cotton ball in a beaker /syringe case to sustain anesthesia. Distance from nose controls depth of anesthesia. Contact DLAM with any questions or to schedule a training session. |

Analgesics in Birds

| Drug | Bird dose range | Route of administration | Frequency | Comments |
|--------------------------------|-----------------|-------------------------|-----------------|--|
| OPIOIDS | | | | |
| Butorphanol | 3-4 mg/kg | IM, PO | Every 4-6 hours | |
| Carprofen | 2-4 mg/kg | PO | 2-3 times daily | |
| NSAIDs * | | | | |
| Flunixin meglumine (Banamine®) | 1-10 mg/kg | IM | Once a day | Good hydration need for short term use |
| Ibuprofen | 5-10 mg/kg | PO | 2-3 times a day | Pediatric suspension |
| Meloxicam | 0.2 mg/kg | PO | Once a day | |

Injectable Anesthesia in Birds

| Drug | Bird Dose range | Route of Administration | Comments |
|----------------------------------|--|-------------------------|---|
| Sodium Pentobarbital (Nembutal®) | 30 mg/kg | IP | |
| Ketamine/Diazepam | 10-50 mg/kg ketamine 0.5-2.0 mg/kg diazepam | IM | |
| Ketamine/Acepromazine | 25-50 mg/kg ketamine 0.5-1.0 mg/kg acepromazine | IM | |
| Ketamine/Xylazine | 10-30 mg/kg ketamine 2-6 mg/kg xylazine | IM | Use the higher ranges for birds less than 100 grams (finches, canaries) |

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