

GUIDELINES FOR ASEPTIC RECOVERY SURGERY ON RODENTS

Adopted by the University Committee on Animal Resources

The U.S. Public Health Service Guide for the Care and Use of Laboratory Animals states that “Survival surgery on rodents does not require a special facility but should be performed using sterile instruments, surgical gloves and aseptic procedures to prevent clinical infections”(1). As required by the U.S. Public Health Service 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.

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 these guidelines:

1. Surgery should be conducted on a clean, uncluttered lab bench or table surface. The surface should be wiped with a disinfectant before and after use and/or covered with a clean drape.
2. Hair should be removed from the surgical site with clippers or a medical depilatory. The surgical site should be disinfected 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. A sterile drape is recommended to avoid sterile instruments, sterile gloves or exposed viscera from coming in contact with unprepped areas.
4. 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.**
5. All instruments should be sterilized, but the method of choice may vary depending upon the surgical instruments or devices used. Materials Processing, Prep and Pack Area (X7-0065), located in room B-3540 will sterilize instruments that are double wrapped in blue drape material. 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)
6. 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.
7. The surgeon should 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.
8. Surgery performed on multiple rodents in a series presents special problems. After the first surgery, the sterilized instruments may be kept in a sterile tray containing 70 – 90% ethyl or isopropyl alcohol (4). The alcohol should 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 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.

9. Monitoring of anesthesia in rodents may be accomplished by observation of color, respiratory rate and pattern, body temperature and observation for the loss of pedal, corneal and pinnal (external ear) reflexes. More sophisticated methods of patient monitoring include EKG and heart rate, pulse oximetry, blood pressure measurements, blood gas measurements, etc. The abdominal or thoracic body wall should be closed with absorbable suture material in a simple interrupted pattern. The skin should be closed with staples or with a nonabsorbable suture material in a simple interrupted pattern or absorbable sutures in a simple interrupted subcuticular pattern. 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 should be removed seven to ten days after surgery.
10. Rodents 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 and mice: 1 – 3 mls). 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 until their righting reflex has returned and are sternal with pink mucous membranes and stable respirations. They may not be returned to the animal room until fully recovered (walking normally, able to eat and drink).
11. Systemic analgesics should be considered for all species experiencing major survival surgical procedures as well as for animals undergoing minor procedures that may result in significant post-op discomfort. Analgesics **must be** administered prior to the surgical manipulation **and** are beneficial for pain relief in laboratory animals. If there is potential for postoperative pain, the animal is given the benefit of the doubt and analgesic therapy should be initiated. (1). It is necessary that drugs be given at the dosing interval stated in the UCAR protocol. The decision to discontinue analgesic therapy should be made based on the observation that the animal appears to be comfortable at the end of the previous dosing interval (i.e. when the next analgesic treatment is due).

Pain in rodents 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, increased respiration, respiratory sounds (chattering in mice), vocalization with handling and/or self mutilation.

Commonly Used Analgesic and Anesthetic Agents in Rats and Mice

Analgesics in mice and rats

Systemic analgesics must be considered for all species experiencing major survival surgical procedures as well as for animals undergoing minor procedures that may result in significant post-operative pain or discomfort.

Drug	Mouse dose range	Route of administration	Frequency	Comments
NSAIDs *				Prolonged use may cause gastrointestinal, renal or other problems
Flunixin meglumine (Banamine®)	2.5 mg/kg	SC, IM	Every 12-24 hours	
Carprofen (Rimadyl®)	5 mg/kg	SC	Once every 24 hours	
Ibuprofen (Children's Advil®; Children's Motrin®)	40 mg/kg diluted in drinking water	PO	Daily in fresh water**	

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®) (Controlled)	2 mg/kg	SC	Once every 3-5 hours	If mild to moderate pain of increased duration is anticipated
Butorphanol (Torbugesic®, Torbutrol®, Stadol®) (Controlled)	5 mg/kg	SC	Once every 1-2 hours	If mild pain of short duration is anticipated
Meperidine (Demerol®) (Controlled)	10–20 mg/kg	SC, IM	Once every 2-3 hours	
	or 0.2 mg/ml of Demerol HCl syrup in water	PO	Daily in fresh water**	
Morphine (Controlled)	10 mg/kg	SC	Once every 2-3 hours	If severe post-operative pain is anticipated
Pentazocine (Talwin®) (Controlled)	10 mg/kg	SC	Once every 2-4 hours	Mild to moderate pain; may develop analgesic tolerance with chronic administration
OTHER				
Acetaminophen (Tylenol Pediatric Syrup) – analgesic / antipyretic	1-2 mg/ml drinking water made fresh daily	PO	Daily in fresh water **	May be appropriate for procedures causing mild discomfort only; efficacy has been questioned in rodents

Notes:

* 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.

**Rodents may exhibit “neophobia” – always monitor for acceptance when adding medications to water or food.

Drug	Rat dose range	Route of administration	Frequency	Comments
NSAIDs *				Prolonged use may result in gastrointestinal, renal or other problems.
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
Ibuprofen (Children's Advil)	10-30 mg/kg	PO	Once every 4 hours	
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®) (Controlled)	0.5 mg/kg	SC	Once every 6-8 hours	If mild to moderate pain of increased duration is anticipated
Butorphanol (Torbugesic®, Torbutrol®, Stadol®) (Controlled)	2 mg/kg	SC	Once every 1-2 hours	If mild pain of short duration is anticipated
Meperidine (Demerol®) (Controlled)		IP		
Morphine (Controlled)	10 mg/kg	SC	Once every 2-3 hours	If severe post-operative pain is anticipated
Pentazocine (Talwin®) (Controlled)	10 mg/kg	SC	Once every 2-4 hours	Mild to moderate pain of short duration; may develop analgesic tolerance with chronic administration
OTHER				
Acetaminophen (Tylenol Pediatric Syrup) – analgesic / antipyretic	1-2 mg/ml drinking water made fresh daily	PO	Daily in fresh water**	May be appropriate for procedures causing mild discomfort only

Notes:

* 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.

**Rodents may exhibit “neophobia” – always monitor for acceptance when adding medications to water or food.

Injectable anesthetics in mice (remember to provide 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 acepromazine IP	IP	Anesthesia; only redose with ketamine if needed
<u>Tribromoethanol</u> (Avertin®)	200-300 mg/kg Or 0.2 ml per 10g BW of 1.25% solution	IP	Requires storage in lightproof container under refrigeration; is an irritant, especially at high doses, high concentrations, or with repeated use. Adhesions are sometimes seen in the abdominal cavity after IP injections.
Avertin is no longer commercially available. Strong Memorial Pharmacy (X5-2379) will prepare Avertin solution for investigators upon request.			

Injectable anesthetics in rats (remember to provide 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 acepromazine	IP	Light anesthesia
Chloral hydrate	300 mg/kg	IP	Dilute as much as possible. Concentrations >2% causes ileitis-peritonitis

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

Inhalation anesthesia of mice and rats

Drug/agent	Usage to anesthetize mice and rats
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.

References:

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- ♦Gades, N. M., et al. (2000) The Magnitude and duration of the analgesic effect of morphine, butorphanol, and Buprenorphine in rats and mice. Contemp. Topics Lab. An. Sci. 39: No. 2:8-23.
- ♦Gillingham, M. B., et al. (2001) A comparison of two opioid analgesics for relief of visceral pain induced by intestinal resection in rats. Contemp. Topics Lab. An. Sci. 40: No. 1:21-26.
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- ♦Heard, D.J.Editor (2001). The Veterinary Clinics of North America. Exotic Animal Practice. Analgesia and Anesthesia.
- ♦Fish, R.E.. et al Editors. (2008) Anesthesia and Analgesia in Laboratory Animals. Academic Press, Inc., New York.