

# NIAAA Office of Laboratory Animal Science

## RODENT SURVIVAL SURGERY HANDBOOK

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### RODENT SURVIVAL SURGERY OVERVIEW

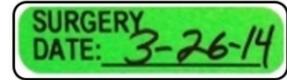
Survival surgery is one of the areas of animal use that receives particular attention during animal care and use program review. The [Guide](#) prescribes extensive recordkeeping requirements for personnel training, pre-surgical planning, animal monitoring, and post-surgical care. To ensure compliance, investigators planning to perform survival surgery are identified as surgeons on the applicable ASP and must demonstrate proficiency in aseptic technique, surgical competence, and cognizance of pre-operative and post-operative care requirements prior to performing survival surgery. NIAAA veterinarians provide guidance on development of Laboratory Animal Surgery Records and surgery plans to ensure that planned care is compliant with the Guide and the expectations stated in the ASP. Suggestions for improvement in the Rodent Survival Surgery Orientation, the Rodent Survival Surgery Handbook, or Laboratory Animal Surgery Record (Surgery Record) are welcomed.

Depending upon the procedure to be performed and the level of experience/expertise of the surgeon, training may also include policy requirements, procedure room set up, recirculating warm water blanket use, gas anesthesia machine use, anesthesia induction and maintenance, waste anesthetic gas scavenging, sterile instrument preparation, steam sterilizer use, surgical attire, surgical site preparation, aseptic gloving, and pain scoring. NIAAA veterinarians are available for training and consultation on specific surgical procedures and surgical model development. Rodent Survival Surgery training may also be necessitated for the Primary Contact if the Primary Contact is not the Surgeon and for the Investigator Responsible after Day 2 post-op if this investigator is not the Primary Contact or the Surgeon.

Unless an exception is approved by the Facility Veterinarian in advance, investigators must limit survival surgeries to Monday through Wednesday to allow sufficient post-operative assessment and care during the critical first 72 hours (Day of surgery = Day 0, Day 1, and Day 2 post-op). **Investigators that must conduct survival surgery on days other than Monday through Wednesday must ensure that they will be available to provide post-operative assessment and care on weekend days or holidays that occur during the first 72 hours post-op. Investigators that conduct survival surgery that causes the animal to require more than 72 hours of post-operative care must receive training on and be available to provide post-operative assessment and care.**



The surgeon must ensure the record reflects the actual surgical procedure performed, pre-operative body weight, actual anesthesia and analgesia dosage used if different from the Analgesia and Anesthesia Recipes, time of completion of surgery and/or time anesthesia was terminated and any other post-operative medications that have been administered. The surgeon must also ensure that any changes in emergency contact information are provided to OLAS. Following surgery, the cage is placed half on and half off of a recirculating warm water blanket with the pump set on the mid temperature range (90 -100°F). No more than four recirculating warm water blankets should be supplied by one pump. Should the pump beep or alarm, the Facility Manager should be notified. The Facility Manager should also be notified of any pump set on the high temperature range. The completed Surgery Record is placed in the wire basket in the animal holding room. The investigator will record the date of surgery (MM/DD/YY) on the **green** "Surgery" sticker.



The cages are monitored by the surgeon until all animals have awakened from anesthesia and are able to walk.

For the first and second days following the day of surgery, OLAS staff will assess the animal(s), record observations, and assign a Pain Score. A laminated copy of SOP 3610D – PAIN SCORING in Rodents is maintained in each Room Book for reference. It is critical that the animal's card number correlate with the pre-operative body weight. Even if the animal is individually identified, its card number must be recorded on the Surgery Record to avoid mix up. All pertinent information, including weight, is to be entered on the record and initialed. Pain Scores are typically assigned for postoperative days 1-2. Animals that have not returned to pre-operative weight on Day 2 or have a Pain Score greater than 0.5 must be monitored daily by the investigator and the period for provision of supplemental care extended as necessary until the Facility Veterinarian determines that body weight has stabilized. Animals are observed each day until cases are closed. Nutritional supplements are typically offered until the animal returns to its pre-surgical body weight. Unless an alternative supplement is stipulated within the approved ASP, bacon treats and feed on the floor of the cage is the standard peri-operative nutritional supplement (See Bacon Softies™ for content). Sutures or wound clips should typically be removed on or before Day 10. Surgical cases are monitored as for clinical cases by the Facility Veterinarian through Day 2. Investigators are requested to record their observations and care provided as well to include annotation of the record when euthanasia is performed or animals are otherwise used before Post-operative Day 3. **Investigators performing weekend or holiday care or care after Day 2 post-op must annotate Surgery Records daily;** e.g. record date, post-operative medication administration, animal health observations/assessments, and initials. Cases may not be closed until sutures and/or wound clips have been removed or the animal has been euthanized.

### Post-Surgical

**All post-surgical animals will:**

- have a Laboratory Animal Surgery Record
- be weighed prior to surgery and on at least post-operative days 1 & 2
- be given regular pelleted food and any other supplements listed on the Surgery Record on the floor of the cage until pre-surgical weight is reached, exceeded, or proven to be stable over time
- be given analgesics and/or antibiotics per Surgery Record and/or veterinary direction
- have any non-absorbable sutures/wound clips removed 7-14 days after surgery
- be cared for by the surgeon after post-operative day 2
- have a green Post-Surgical Card in the cage card holder and an active Surgery Record until the wound closure materials are removed and all peri-operative medications are discontinued
- have the date of surgery recorded on the animal's card

SOP 3600B Revision date: 4-17-18

**Green** Post-Surgical cards should not be removed from the cages and Surgery Records must remain in the animal holding room with the cages until the Facility Veterinarian has determined that the cases are closed. Records for cages/animals missing from the room for three days in a row are closed.

Surgically modified animals received from a commercial vendor will have a "Post-Surgical" card placed in the card holder and the date of surgery indicated on the packing list transcribed to the **green** "Surgery Date\_\_\_\_\_" sticker by OLAS staff upon receipt. One Surgery Record is generated

for the animal shipment. **Green** Post-Surgical cards are removed from the cages when sutures and/or wound clips have been removed and the Facility Veterinarian has determined that the cases are closed.

# SURGERY PLAN

ASP#:

Date:

Procedure:

Surgeon:

## **Mark the boxes as you review this plan with OLAS staff.**

Note items where assistance from OLAS staff is desired. Using this check list, plan your schedule and identify supplies and equipment that you will need for your specific surgical procedure. Edit and customize the plan and develop your own checklist that works best for you. As you gain experience with your specific surgical procedure and post-surgical care requirements, please provide suggested additions and clarifications to improve the Surgery Plan template and Surgery Supply Checklist for future rodent surgeons.

## **At least one week before surgery:**

- Review the ASP to ensure that the surgical procedure to be performed is described and the investigator intending to perform the procedure is listed as a surgeon and has received training on survival surgery by the Facility Veterinarian. Amend the ASP and update Training & Experience Forms as appropriate if:
  - An investigator is to perform survival surgery that is not named as a surgeon in Section G of the ASP and/or on their T&E Form
  - An investigator is to provide post-operative care that is not listed as an individual that will provide post-operative care in Section G of the ASP and/or on their T&E Form
- Review the Surgery Plan. Ensure that the Surgery Record generated is concurrent with the Surgery Plan, ASP, and adequately communicates planned pre-operative and post-operative care requirements. Suggest revisions to the Surgery Record as appropriate. For example:
  - Addition of Names of Surgeon and/or Names of Investigator(s) responsible for provision of post-operative care after Day 2
  - Addition of a Surgical Procedure
  - NSAIDS contra-indicated, substitute Buprenorphine for Ketoprofen as analgesic
  - No sucrose containing treats. Substitute softened pelleted feed for bacon treats
  - Animals will be housed on a reversed diurnal cycle (Surgery Record must have no items printed in red font).
  - Review any Special Care requirements and flag post-operative cages and add them to the Special Care Log as appropriate.
- Handle and manipulate animals to be used in surgery. Assess body weight and condition. Mice should weigh enough to withstand the rigors of general anesthesia and the survival surgery planned. Provide them with the supplementation that will be provided during the immediate post-operative period. This will acclimate them to the frequent handling and nutritional supplementation associated with post-operative care, increase body weight and condition if necessary, minimize stress, and speed their post-operative return to normalcy. Similarly, if single housing will be required following surgery, individually house the animals in advance of surgery to minimize the impact of the stress of social isolation on post-operative recovery. Note: According to ARAC [Guidelines for Social Housing of Rodents and Aquatic Species](#), the frequency, duration, and justification for single housing outside of the perioperative period, e.g. one week before and two weeks after surgery, must be approved in advance by the ACUC.
- Reserve procedure room by annotating calendar on the door of desired procedure room with the surgeon's entire last name.

- Review the Surgery Supply checklists.  
Ensure that all items that will be necessary are or will be available (e.g. any Controlled Substances) in adequate supply, not expired, and in good repair.
- Complete an [NIAAA ASP Transfer Form](#) if necessary.
- Ensure that OLAS staff is aware of planned survival surgery by completing a '[Technical Support and Equipment Request Form](#)' > 3 working days in advance.  
If the surgeries will be performed by more than one surgeon, ensure that all surgeons are named in the request.
- Perform survival surgery Monday-Wednesday unless otherwise approved in advance by the Facility Veterinarian.  
Approval to perform survival surgery on days other than Monday through Wednesday is based upon the clinical outcome of prior surgeries performed.
- Steam autoclave instruments.  
A table top Midmark M7 SpeedClave Steam Sterilizer mounted on a portable supply cart is available for investigator use its use is encouraged. Steam Sterilizer Use Instructions are included in this handbook and in the top drawer of the autoclave cart and summarized on the top of the sterilizer to include suggested time and temperature settings. Instruction by the Facility Veterinarian at first use is recommended.

### Day of surgery:

- Ensure availability to provide necessary post-operative care after post-operative day 2 and on Saturdays, Sundays, holidays, and during periods of Government closure.
- Prepare surgery area:
  - Confirm OLAS has provided supplies requested and the procedure room is adequately stocked.
  - Bring cage(s) to the procedure room where surgery will be performed to acclimate.
  - Pre-heat recirculating warm water blanket (90-100°F) and glass bead sterilizer. Confirm the blanket is heating and no leaks are evident.
  - Pre-warm analgesic fluids by placing them on the recirculating warm water blanket.
  - Check isoflurane level in machine and supply on hand and refill as necessary.
  - Check oxygen supply and replace E tank or transfer regulator on H tank as necessary. **Notify the Facility Manager if a tank is empty.**
  - Test the gas anesthesia delivery system for leaks. Tighten connections where necessary. Instruction by the Facility Veterinarian at first use is recommended.
  - Clean and disinfect surgery area
  - Ensure induction chamber is clean and lined with a clean paper towel and **ensure ethanol or Isoflurane does NOT come into contact with the chamber.** It's acrylic and will be permanently damaged.
- Weigh animal and complete the Day 0 portion of the Surgery Record and all shaded sections as appropriate. Record also the Card Number in the upper left corner. Circle which Surgical Procedure will be performed and by whom. Circle or list the name or names of all investigators that will be providing post-op care for the animal.
- Anesthetize rat/mouse with:
  - Isoflurane – primary drug of choice – induce @ 3-5%, maintain @ 1-2%
  - Ketamine/ Xylazine
  - Lidocaine jelly – instill into ear canals prior to placing ear bars of a stereotaxic frame.
  - Other:
- Instill bland ophthalmic ointment into each eye, e.g. Tears renewed or Puralube, to protect corneas from trauma from drying while the animal is under anesthesia and unable to blink.
- Administer analgesic containing fluids **before** making the surgical incision.
- Clip hair from surgery site. Ensure clipped area extends several millimeters beyond largest potential incision.
- Prep surgery site with three alternating scrubs with surgical antiseptic, e.g. Betadine and 70% alcohol.
- Drape surgery site with sterile drape or plastic wrap.
- Open sterile instrument wrapper or other sterile barrier for instruments.

- If hemorrhage is anticipated, add a sterile eppendorf tube of pre-cut pieces of Gelfoam to the sterile field.
- Ensure hair cover and mask are comfortably arranged and then, using aseptic technique, put on surgery gloves.

The outside of the gloves must not come into contact with any part of the ungloved hands. For example, the ungloved left hand pulls the right glove over the right hand, touching only the inside of the glove. The gloved right hand then reaches inside the rolled cuff of the left glove to pull it over the left hand. Finally, the gloved fingers of each hand are tucked under the glove cuffs of the other hand, and the cuffs are pulled up over the sleeves of the lab coat or surgical gown. Once gloved, it is helpful to have an assistant to handle non-sterile surfaces.

Approach:

- Dorsal midline (head)     Ventral midline cervical     Paralumbar
- Ventral midline abdominal     Inguinal     Other: \_\_\_\_\_

Consider use of a groove director when performing laparotomy

Intra-op:

- Gelfoam will be used as necessary for hemostasis.

Use sterile forceps to grasp the pre-cut piece with one hand. With the other hand, use gentle compression with a cotton tipped applicator to stop hemorrhage. Remove the applicator and immediately insert the gelfoam into the wound and close the wound over the gelfoam. Gelfoam is resorbed with the resulting clot and does not require removal.

Closure:

- Two layer cervical    or     Two layer abdominal    or     Scalp
- Close peritoneum/ muscle and/or subcutis with simple continuous absorbable suture  
Select a 3-0 to 6-0 gauge suture material with a taper needle.

Skin:

- Wound clips

or

- Skin sutures

Close skin with simple interrupted absorbable sutures. Select a 3-0 to 6-0 gauge with a cutting needle and/or

- Tissue Adhesive

Use a product with a fine applicator tip or use a 30 gauge needle and syringe to apply. To avoid pruritus and scratching as the fur grows, adhesive must be limited to the incision line and not spill onto the skin adjacent to the incision line.

and/or

- Other (e.g. Euthanasia prior to Day 14 post-op):

**Note:** Moisten Vicryl suture material with sterile isotonic solution to improve handling and decrease tissue drag. For all suture material, place at least 4 throws to make at least two complete square knots. Ensure that all throws just approximate tissue edges with **no tissue compression**. Sutures or wound clips that are too tight **WILL** cause tissue necrosis. The first indication of sutures or clips too tight is scratching at the incision site. Scratching may also result from reactive suture material. Toe nails may be trimmed at the time of surgery to minimize scratching. **Do NOT use silk or chromic gut suture material in the skin.**

- Record the duration of surgery, time of surgery completion, and any unusual events that may have occurred during the surgery, e.g. difficulty maintaining anesthetic depth, hemorrhage, placement of gelfoam.
- Rinse instruments with sterile saline and place tips in pre-heated glass bead sterilizer in preparation for the next surgical procedure if performing multiple surgeries in one session. Similarly, rinse surgical gloves with sterile saline or change gloves as necessary. Allow the sterilized instruments to cool before proceeding to the next animal.

### **Post-op Day 0:**

- Betadine **solution** is not aversive to appetite and does not need to be removed. Betadine **scrub** is aversive to appetite and any residue should be removed from the skin and fur around the surgical site prior to the animal awakening from anesthesia to encourage grooming in the immediate post-operative period and, in turn, appetite.
- Administer analgesic containing fluids subcutaneously prior to awakening from anesthesia if they were not administered prior to making the skin incision.
- Trim rear toe nails prior to awakening from anesthesia to minimize disruption of wound closure materials.
- Place the animal in a clean cage lined with a clean iso-pad to avoid accidental inhalation of bedding particles and airway obstruction. **Anesthetized animals must not be placed directly on their regular corn cob bedding during anesthetic recovery.**
- Ensure that the cage is positioned so that the cage bottom is half on and half off of a recirculating warm water blanket set at a temperature of 90 -100°F. The cage is only placed half on the blanket to allow the animal to choose its preferred environmental temperature and ensure that it does not become overheated.

**Note:** If hand warmers or pocket warmers are used, Do NOT place hand warmer(s) in the cage without first wrapping them, e.g. with paper towel, kimwipe, or other suitable material with the ends securely stapled. If chewing/eating the pocket warmer is observed, remove the pocket warmer from the cage.

- Ensure that a **green** "Post-Surgical" card is in the card holder.
- Ensure that the investigator responsible for ensuring the provision of post-surgical care is either the Primary Contact or is specifically named on the Surgery Record. The investigator responsible for the provision of post-surgical care need not be the surgeon or the Primary Contact. Annotate the surgery record as appropriate.
- Affix a **green** "Surgery Date: \_\_\_\_\_" label to the animal's card and record the date of surgery in the blank.
- Monitor the animal and rotate it from one side to the other every 15 minutes until it can maintain sternal recumbency and is responsive.
- Ensure the cage has a Top Flow lid and not a Seal Safe lid and leave the cage static on a cart or rack positioned half on and half off of a recirculating warm water blanket (For additional guidance, refer to the Special Care Instructions for [Hypothermia](#)).
- Ensure the cage has a water bottle, feed in the feeder and on the floor of the cage. bacon treats should also be on the floor of the cage unless otherwise specified by the ASP or directed by the Facility Veterinarian.
- Record all treatments and observations on the Surgery Record. Leave the Surgery Record either beside or under the recovery cage or in the wire basket with the Room Book. To avoid interruption of air flow, do not leave the Surgery Record on top of the filter top.
- Clean the surgery area and induction chamber using the quaternary ammonium detergent disinfectant spray and paper towels provided. Clean clippers by removing hair from between blades and spraying with alcohol and clipper spray lubricant. If the clippers do not run smoothly, notify the Facility Manager so that the clippers may be disassembled for thorough cleaning or replaced as necessary.
- Clean the area under and surrounding any stereotaxic device that has been identified as not to be moved by OLAS.**
- Report to the Facility Veterinarian: any animal with weight loss  $\geq 15\%$  or that has a Pain Score  $\geq 2$  at any time during the post-operative period.

### **Post-operative Day 1 and Day 2:**

Unless otherwise requested, this care will be provided by OLAS staff for post-op Days 1 and Day 2 that occur Monday through Friday that do not require containment following hazard administration. This care will be provided by the surgeon or designee for surgery where Post-

Operative Days 1 or Day 2 will fall on a weekend or holiday or for any surgery involving containment following hazard administration.

- Assess the animal. Using the key on at the bottom of the Surgery Record, score clinical observations. If the Pain Score is 3, contact the Facility Veterinarian immediately. If the key is not suited to the observation, write a description of the observation.
- Weigh animal on Day 1 post-op. Provide analgesic fluids on Day 1 post-op. If body weight is the same or lower than pre-op weight, repeat analgesic administration daily for up to four total administrations.
- Weigh animal on Day 2 post-op. If the animal has not regained pre-op weight, continue to monitor body weight (e.g. on Day 3, Day 5, or more often as necessary) until body weight has recovered or the animal has otherwise demonstrated its clinical recovery and stability (e.g. nesting, grooming, eating, drinking, urinating, defecating).
- Provide additional bacon treats and feed on the floor of the cage and subcutaneous fluids if necessary until the animal exceeds its pre-op weight.
- Return the animal to regular bedding when the incision is dry and not at risk of collecting bedding or having bedding adhere to the incision. This may usually be done on post-op Day 1, but occasionally will extend to post-op day two. If the incision remains moist or the isopad becomes soiled, change the isopad as necessary.
- Unless contra-indicated, add a TP Roll to the cage.
- Remove the cages from the recirculating warm water blanket when the animal is eating, active, using the side of the cage not on the warm water blanket, and is well hydrated as demonstrated by normal skin turgor.

### **Post-operative Day 3 and thereafter:**

The surgeon must review Day 2 post-operative assessments by the Facility Veterinarian with particular attention to body weight, clinical appearance, and whether or not the animal is still seeking the ½ of the cage on the recirculating warm water blanket to ascertain whether continued monitoring, assessment, and care by the surgeon is necessary. Should there be any question about the level of care required on or after Day 3, the surgeon should consult the Facility Veterinarian.

- If the body weight exceeds pre-operative body weight or has stabilized and the animal is eating, active, and well hydrated as demonstrated by normal skin turgor such that the animal no longer requires thermal support, the cage should be returned to the ventilated rack by the investigator providing post-operative care.**
- As necessary, the surgeon will continue to-
  - Monitor body weight until it exceeds its pre-op weight
  - Assess the animals to ensure that they are eating, active, using the side of the cage not on the warm water blanket, and are well hydrated as demonstrated by normal skin turgor.
  - Provide nutritional and/or fluid and/or heat supplementation as appropriate.
  - Provide topical antibiotic ointment treatment daily for surgical wounds that remain moist (e.g. are healing by second intention).
  - Provide systemic antibiotic treatment as necessary to include shaking trimethoprim sulfa water daily and switching the animals to their normal tap water after a minimum of seven days.
  - Move cages from the recirculating warm water blanket to their regular holding rack as the appetite, hydration, and activity of the animal appears to have returned to normal.
  - Ensure that provision of this care continues if delegated to another
- Remove or replace any wound clips that slip into the incision line and cause poor approximation of wound edges. Dispose of wound clips in a sharps container.
- Remove any remaining skin sutures or wound clips 7-14 days after surgery. The ideal time for removal will depend upon the level of tension on the incision and the degree of pruritis

experienced. Monitor incisions carefully to ensure that wound closure materials are removed or replaced as soon as irritation is noted.

Should an animal require replacement of wound closures, annotate the record with the date that new sutures or clips were placed. The Facility Veterinarian will 'close' the post-surgical record when the animal no longer requires and/or is no longer receiving peri-operative medication, and all wound closure materials have been removed. Should the animal be euthanized before the Facility Veterinarian has 'closed' the surgery case, the post-surgical record should be annotated as such by the animal's user.

## GENERAL SURGERY SUPPLIES PROVIDED BY OLAS

<b>Provided upon Technical Support and Equipment Request for Survival Surgery Preparation</b>	<input type="checkbox"/> Cage w/IsoPad (TP Rolls are <b>not</b> placed) <input type="checkbox"/> NO TP Roll Flag <input type="checkbox"/> NO TP Roll & NO Wire Bar Flag <input type="checkbox"/> Feed pellets and Bacon treats on floor of cage (unless NO Bacon Softies is requested) <input type="checkbox"/> <b>Green</b> Laboratory Animal Surgical Record <input type="checkbox"/> <b>Green</b> Post-Surgical Card and <b>Green</b> Surgery Date labels <input type="checkbox"/> Recirculating warm water blanket(s) and pump(s)
<b>Provided / available upon request</b>	<input type="checkbox"/> Withholding of feed pellets on floor of Post-Op cage <input type="checkbox"/> Dishes for providing softened feed, e.g. when bacon treats contraindicated <input type="checkbox"/> Table top autoclave for sterilizing instruments and supplies mounted on mobile supply cart <input type="checkbox"/> Ordering information for supplies provided by Surgeons
<b>Continuously available</b>	<div style="width: 45%;"> <input type="checkbox"/> Instrument sterilization packet material  <input type="checkbox"/> Autoclave tape  <input type="checkbox"/> Isoflurane  <input type="checkbox"/> Oxygen  <input type="checkbox"/> Disposable face masks  <input type="checkbox"/> Plastic backed absorbent bench pads  <input type="checkbox"/> Bland ophthalmic ointment  <input type="checkbox"/> Betadine solution  <input type="checkbox"/> Sterile cotton tipped applicators  <input type="checkbox"/> Non-sterile gauze  <input type="checkbox"/> Alcohol swabs and prep pads  <input type="checkbox"/> Gelfoam pieces - sterile packaged  <input type="checkbox"/> Kwik Stop Powder  <input type="checkbox"/> Ketoprofen fluids  <input type="checkbox"/> Needles and Syringes  <input type="checkbox"/> Clipper cleaner/spray lubricant  <input type="checkbox"/> Tissue adhesive         </div> <div style="width: 10%; text-align: center; font-weight: bold;"> <b>Equipment provided in designated surgery locations</b> </div> <div style="width: 45%;"> <input type="checkbox"/> Anesthesia machine  <input type="checkbox"/> Fiber optic lamp  <input type="checkbox"/> Glass bead sterilizer  <input type="checkbox"/> Clippers  <input type="checkbox"/> Brush for cleaning clippers  <input type="checkbox"/> Digital Scale         </div>

## GENERAL SURGERY SUPPLIES PROVIDED BY SURGEONS

<input type="checkbox"/> Sterile surgical gloves <input type="checkbox"/> Sterile surgical instruments <input type="checkbox"/> Sterile gauze <input type="checkbox"/> Sterile field <input type="checkbox"/> Scalpel blades <input type="checkbox"/> Suture material <input type="checkbox"/> Wound clips and applicator*	<input type="checkbox"/> Plastic wrap for use as surgical drape <input type="checkbox"/> Restraint board <input type="checkbox"/> Stereotaxic apparatus <input type="checkbox"/> Sterile surgical skin marker <input type="checkbox"/> Lidocaine gel for ear bars* <input type="checkbox"/> Acrylic resin <input type="checkbox"/> Controlled substances other than Ketamine, Buprenorphine SR, or Fatal Plus
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\*These items are available to surgeons for training purposes and for emergency use.

# ANALGESIA AND ANESTHESIA RECIPES

For all - Write preparation date and expiration date on bag and on any aliquots placed in vials. Expiration date is one month from date mixed - or sooner if any of the ingredients expire beforehand.

## Analgesia Recipes

### a.) Ketoprofen Fluids:

- Recommended dosage is 5 mg ketoprofen/Kg body weight (BW) or 0.005 mg ketoprofen/g BW once daily.
- Add 0.39 mL stock ketoprofen 100 mg/mL (0.39 mL x 100 mg/ mL = 39 mg ketoprofen) to a 250 mL bag of Lactated Ringers Solution to produce a solution containing 0.156 mg ketoprofen/mL When using a 500 mL bag of Lactated Ringers Solution, add 0.78 mL stock ketoprofen 100 mg/mL to a 500 mL bag of Lactated Ringers Solution.
- Add 0.5 mL Vitamin B complex to a 250 mL bag of Lactated Ringers Solution or 1.0 mL Vitamin B complex to a 500 mL bag of Lactated Ringers Solution.
- Administer 0.032 mL ketoprofen fluids / gram body weight subcutaneously (SC - preferred) or intraperitoneally (IP) as shown in table below to deliver the recommended dosage.
- Discard any unused remaining fluids one month from the date of preparation.

<b>Examples of dosages calculated by body weight in grams for SC or IP administration of Ketoprofen</b>			
<b>Mice</b>		<b>Rats</b>	
Body Weight (BW)	Volume to Administer	Body Weight (BW)	Volume to Administer
7.8 g	0.25 mL	32 g	1 mL
15.6 g	0.50 mL	64 g	2 mL
20 g	0.60 mL	96 g	3 mL
23.4 g	0.75 mL	128 g	4 mL
30 g	1.00 mL	160 g	5 mL
31.2 g	1.00 mL	187 g	6 mL
39.0 g	1.25 mL	218 g	7 mL
40.0 g	1.30 mL	250 g	8 mL
47.0 g	1.50 mL	281 g	9 mL
		312 g	10 mL
		343 g	11 mL
		374 g	12 mL
		405 g	13 mL
		437 g	14 mL
		468 g	15 mL
		512 g	16 mL
		545 g	17 mL
		577 g	18 mL

- **Sample calculations:**

- $5 \text{ mg/Kg} = 0.005 \text{ mg ketoprofen/g BW} \times 0.156 \text{ mg Ketoprofen/mL} = 0.032 \text{ mL ketoprofen solution/g BW}$
- $8 \text{ mL} \times 0.156 \text{ mg/ mL} = 1.248 \text{ mg} \times 1000 \text{ g/} \times 5 \text{ mg} = 250 \text{ g}$
- $20\text{g} = 0.6 \text{ mL ketoprofen solution} \times 0.156 \text{ mg/mL} = 0.0936 \text{ mg/20 g BW} = 4.68 \text{ mg ketoprofen/ Kg BW} - - \text{ which rounds up to } 5 \text{ mg/Kg}$
- $20\text{g} \times 0.032 \text{ mL ketoprofen solution/g BW} = 0.64 \text{ mL} \times 0.156 \text{ mg/ml} = 0.998 \text{ mg ketoprofen /20 gram mouse} = 0.00499 \text{ mg/g BW} = 5 \text{ mg/Kg.}$

- Ketoprofen fluids may also be dispensed into empty sterile vials labeled with the abbreviated dosing tables below:

Mouse Keto Analgesic:	Rat Keto Analgesic:
Give 0.6 mL for a 20 g mouse SC	Give 8 mL for a 250–300 g rat SC
0.75 mL for a 23.5 g mouse SC	10 mL for a 301–350 g rat SC
1.0 mL for a 30 g mouse SC	12 mL for a 351–400 g rat SC
1.3 mL for a 40 g mouse SC	14 mL for a 401–450 g rat SC
<b>(Do Not Refrigerate)</b>	15.5 mL for a 451–500 g rat SC
	17 mL for a 501–550 g rat SC
	<b>(Do Not Refrigerate)</b>

**b.) Buprenorphine SR (ZooPharm):**

- If non-steroidal anti-inflammatory drugs (NSAIDS) are contra-indicated, use sustained release (SR) buprenorphine. One injection will provide therapeutic drug levels for 72 hours of analgesia in rats and mice. This is a refinement over the gaps experienced in analgesic coverage between the twelve hour dosing interval required for the regular buprenorphine formulation. There are currently two sustained release Buprenorphine products available. The Animalgesic product is produced at a site that follows Good Manufacturing Practices. However, the Animalgesic product was recalled and is not currently available from the manufacturer and 'will not be available until further notice'. The ZooPharm product is not manufactured under Good Manufacturing Practices (GMP), but it is readily available. One reported clinical finding by an NIH IRP user was a skin reaction that developed into a self-limiting ulcer responsive to topical treatment. It is unclear whether this skin reaction resulted from the injection material or from injection technique. Post-injection monitoring must be provided as appropriate. Should skin lesions develop, this finding will be reported to the ACUC and to the NIH IRP animal care and use community. The concentrations of the two products do differ because they have two different sustained release vehicles and different drug release characteristics and thus require different dosing. In order to provide the subcutaneous fluid supplementation along with the analgesia as is currently provided when ketoprofen is used, preparation for injection of the ZooPharm buprenorphine slow release (SR) analgesia will be as follows:
  - The Animalgesic product concentration is 1.3 mg/mL and the dose for a mouse should be 3.25 mg/kg.
  - The ZooPharm product concentration is 1 mg/mL and the dose for a mouse should be 0.5-1 mg/kg. Based on clinical research experience and formal studies, a starting dose of 0.6 mg/kg has been recommended by the Facility Veterinarian.
  - Using aseptic technique, prepare a total of 10 mL of analgesic solution by adding 1.0 mL of the 1 mg/mL Buprenorphine (SR) to 9 mL of sterile saline in order to generate a 0.1 mg/mL Buprenorphine (SR) solution. In order to deliver a 0.6 mg/kg dose, administer 0.12 mL of the analgesic solution per 20g body weight subcutaneously (SC) once. The label on the vial of mouse Buprenorphine SR will say:

Mouse Post Op Buprenorphine SR
0.10 mg/mL, 0.6 mg/kg SID QD, i.e.
0.12 ml for 20 g mouse, SC
0.18 ml for 30 g mouse, SC
0.24 ml for 40 g mouse, SC

- When the Animalgesic product again becomes available, a similar dosing table will be prepared.

## Anesthesia Recipes

### a.) Ketamine/ Xylazine

- Used when isoflurane anesthesia is contra-indicated. When Xylazine is used, confirm that the Xylazine is the 20 mg/mL concentration and NOT the 100 mg/mL concentration. Consider having yohimbine on hand to reverse Xylazine in the event of respiratory depression and/or prolonged anesthetic recovery.
- For mouse Ketamine/Xylazine anesthesia, mix a total of 6 mL of anesthetic solution by adding:
  - 1.0 mL of 100 mg/mL Ketamine
  - 0.5 mL of 20 mg/mL Xylazine
  - 4.5 mL of sterile saline
- To deliver a 100 mg/kg Ketamine dose combined with a 10 mg/kg Xylazine dose, administer 0.1 mL anesthetic solution / 20 g body weight IP
- The label on the vial of mouse Ketamine/Xylazine Anesthetic mix will say:

Mouse Anesthetic (Ketamine/Xylazine) Give 0.1mL/20g BW IP
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- For rat Ketamine/Xylazine anesthesia Ketamine/Xylazine (40-100 mg Ketamine/kg and 4 - 10 mg Xylazine/kg) IP will generally be used to induce general anesthesia. For example, a combination of 80 mg/kg ketamine / 4 mg/kg xylazine will be used as the starting dose and the combination adjusted as necessary for the size of the rat and duration of injectable anesthesia required prior to maintenance with isoflurane. The starting dose combination of 80 mg/kg ketamine / 4 mg/kg xylazine is typically made as follows:
  - For a total volume of 3 mL combine 0.6 mL of 20 mg/mL Xylazine and 2.4 mL of 100 mg/mL Ketamine
  - For a total volume of 5 mL combine 1.0 mL of 20 mg/mL Xylazine and 4.0 mL of 100 mg/mL Ketamine
  - Dose at 1.0 mL / kg body weight or 0.1 mL/100 g body weight IP
- The label on the vial of rat Ketamine/Xylazine Anesthetic mix will say:

Rat Anesthetic (Ketamine/Xylazine) Give 0.1mL/100g BW IP
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Pentobarbital supplied by OLAS as Fatal Plus euthanasia solution is only used as an anesthetic for euthanasia procedures such as terminal perfusion. (See [Investigator Orientation Handbook](#)).

## REFERENCES

NIH ANIMAL RESEARCH ADVISORY COMMITTEE (ARAC) GUIDELINE:

[https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b6-survival\\_rodent\\_surgery.pdf](https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b6-survival_rodent_surgery.pdf)

OACU Training Resources:

<https://oacu.oir.nih.gov/training-resources>

Columbia University Medical Center Microsurgery Research & Training Laboratory

<http://microsurg.hs.columbia.edu/>

Newcastle University Aseptic Technique in Rodent Surgery

<http://www.procedureswithcare.org.uk/aseptic-technique-in-rodent-surgery/>

VIDEO:

*Ensure that NIH ARAC expectations wrt aseptic technique are given consideration and applied to procedures learned via JOVE.*

1. "Principles of Rodent Surgery for the New Surgeon."  
<http://www.jove.com/details.stp?id=2586>
2. "Survivable Stereotaxic Surgery in Rodents,"  
<http://www.jove.com/details.stp?id=880>  
- Demonstrates Guide cannula placement.
3. "Intracranial Injection of Adeno-associated Viral Vectors,"  
<https://www.jove.com/details.stp?id=2140>
4. Aseptic Technique Training Course, video produced by NINDS:  
[http://ahcs.ninds.nih.gov/ACUC\\_Movies/ATTC.wmv](http://ahcs.ninds.nih.gov/ACUC_Movies/ATTC.wmv) (Windows Media Player)
5. "Training in Survival Rodent Surgery" CD produced by OACU and NHGRI

**BACON SOFTIES™, CERTIFIED**  
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Soft Pellets - 1/2" diameter x 1 1/2" long  
Shelf Life: 6 months, refrigerated

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For additional information:

[http://www.bio-serv.com/Rodent\\_Special\\_Needs/Bacon\\_Softies.html](http://www.bio-serv.com/Rodent_Special_Needs/Bacon_Softies.html)

Catalog # F3580

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# STEAM STERILIZER USE INSTRUCTIONS

## Midmark M7 SpeedClave® Steam Sterilizer



### Step 1



- Refill reservoir.**
- Remove cap and fill reservoir with steam distilled or demineralized water to Full mark, every 15-20 cycles
  - Do **NOT** overfill reservoir

### Step 2



- Open door.**
- Place handle in the unlatched position
  - Push door arm toward the right and pull outward

### Step 3



- Load trays and place into chamber.**
- Do **NOT** layer instruments
  - Pouch items to preserve sterility
  - Include a process monitor strip with each load

### Step 4



- Fill chamber.**
- Press down and hold Fill/Vent lever until water is within 1/2" of chamber rim

### Step 5



- Close and latch door.**
- Swing door to the left until it stops
  - Push door in and to the right
  - Latch door by swinging handle to the right and pressing firmly

### Step 6



- Set timer.**
- Turn timer knob to 15 minutes to start heating the chamber

### Step 7



- Set temperature.**
- Turn temperature control knob fully to the left (counter-clockwise)
  - This is a maximum setting of 270°F (132°C)

### Step 8



- Re-set Temperature control.**
- When temperature gauge reaches the desired temperature, **immediately** turn temperature knob slowly clockwise until pilot light goes out
  - Refer to Instrument Manufacturer Guidelines for appropriate sterilization temperature

### Step 9



- Re-set timer.**
- Re-set timer to desired sterilization time
  - Refer to Instrument Manufacturer Guidelines for appropriate sterilization time

### Step 10



- Vent sterilizer.**
- Turn timer off when buzzer sound
  - Move door handle to vent position by swinging handle to the left
  - Hold Fill/Vent lever down until door "pops" inward
  - Leave door in vent position – do not open door

### Step 11



- Dry Instruments.**
- Keep door handle in vent position for 15 minutes

### Step 12



- Remove trays.**
- Swing door handle to the far left position
  - Push door arm toward the right and pull outward
  - Remove trays
  - Trays may be placed on the racks on top of the sterilizer

