



# STANDARD OPERATING PROCEDURE No. 1

## ANESTHESIA AND ANALGESIA IN RODENTS

General anesthesia is needed for surgery, for any procedures that could potentially cause more than minor discomfort, and for terminal blood withdrawals. Analgesics are required after any major surgery (e.g., exposure of a body cavity such as laparotomy, thoracotomy or cranial procedure, orthopedic procedures) unless justified to the IACUC. Analgesics are suggested for minor surgeries (e.g., skin incision, cannulation).

### Anesthetics:

Weigh the animal and calculate the dosage depending on the anesthetic used.

- If the dose is in mg/kg, divide the weight of the animal in grams by 1000 to calculate weight in kg then multiply the dose in mg times the weight in grams. For example, to dose a 400 gm rat with a drug that is 40 mg/kg. A 400 gm rat is 0.4 kg (400 g/1000 g). The dose would be 16 mg (40 mg x 0.4 g). If the drug's concentration is 4 mg/ml, the amount injected would be 4 ml (16 mg/4 mg/ml).
- Because rodents are smaller, often dosages are given in mg or ml per 100 grams. If so, divide the animal's weight in grams by 100 and then multiply by the number of ml. For example, a drug is dosed at 0.05 ml/100 gm. A 400 gm rat would receive a dose of 0.2 ml (400 g/100 g x 0.5 ml).

There is a logbook located in N110E for use with both injectable and inhalation anesthetics.

### Injectable:

- **Pentobarbital** - Pentobarbital solution is made from reagent grade pentobarbital powder according to SOP No. 6 Preparation of Sterile Non-Pharmaceutical Grade Compounds.
- **Ketamine/Xylazine/Acepromazine ("Cocktail")** - A balanced anesthesia cocktail consisting of a combination of Ketamine (anesthetic dissociative agent with analgesic properties and no CNS depression), Xylazine (sedative and analgesic), and Acepromazine (muscle relaxant and sedative). Ketamine is a controlled substance and use must be logged. Onset of anesthesia (demonstrated by loss of pedal withdrawal reflex) is approximately 10 minutes and duration of anesthesia is approximately 60 minutes. Different concentrations of the cocktail are used for mice and rats and the amounts are shown in the table below:

Drug (concentration)	Mouse cocktail volume (ml)	Mouse concentration (mg/ml)	Rat cocktail volume (ml)	Rat concentration (mg/ml)
Ketamine (100 mg/ml)	0.75	7.5	2.5	25
Xylazine (20 mg/ml)	0.75	1.5	2.5	5
Acepromazine (10 mg/ml)	0.25	0.25	1.0	1
Water (sterile for injection)	8.25		4.0	
Total volume	10		10	

**Rat Cocktail Dosage: Use 0.125 to 0.150 ml per 100 g BW injected IP or SQ**  
**Mouse Cocktail Dosage: Use 0.1 to 0.2 ml per 25 g BW injected IP or SQ**

**Intraperitoneal (IP) injections** - Restrain the animal using the appropriate techniques. Tilt the animal's head downward and inject into its lower right quadrant of the abdomen. The lower right quadrant is used because there is less chance of injecting into a major organ. In rats, it is helpful to have one person restrain and another to do the injection.

- Mice - use a 26 ga needle and inject no more than 0.3 ml in any one spot. If the volume of the solution injected is larger, divide the injection equally into both the right and left lower quadrants.
- Rats - use a 21 ga needle and injection no more than 3.0 ml in any one spot. If the volume of the solution injected is larger, divide the injection equally into both the right and left lower quadrants.

**Subcutaneous (SQ) injections** - These can be performed by restraining the rat under a cloth and accessing the skin above the shoulders. Pull up the skin to form a tent and inject using a 21 or 26 ga needle into the space created.

#### **Inhalation:**

Inhalation anesthesia is very useful for many surgical procedures. An advantage is there is a rapid induction and recovery phase and less cardiopulmonary depression. An isoflurane anesthesia system is located in room N110E. It is supplied with an induction chamber, separate non-rebreathing nosecones for mice and rats, a stereotaxic non-rebreathing nosecone, and a heated surgical bed. Directions for using the system are found on the cart holding the anesthesia equipment.

**Monitoring during surgery** - The anesthetized animal should be draped in such a way to allow access to the mouth and nose to monitor oxygenation status. The animal should be maintained at an adequate anesthetic level where the breathing is regular (not shallow - too deep or rapid - too light) and the mucous membrane of the nose and mouth remain pink (not blue - not enough oxygen). The animal should **not** have a blink reflex when the inner corner of the eye is touched and should **not** withdraw its feet in response to a toe pinch (loss of pedal withdrawal reflex). The animal should be assessed every 5 minutes during surgery. Adjust level of anesthesia as necessary. Use of the surgical heating bed is suggested to maintain the animal's core temperature. Rodents are prone to hypothermia due to their large surface area to mass ratio therefore must be kept warm during surgery and while recovering from anesthesia. The first dose of analgesia should be given at the start of surgery as pre-emptive analgesia. By doing this, there may be less need for extended use of analgesics after surgery. Be cautious when using an opioid analgesic as these can depress respiration. Generally they are safe to use at analgesic levels however the dosage of anesthetic may need to be reduced. Analgesics may also be administered immediately post-operatively.

**Monitoring during recovery** - Following surgery, the animal should be placed in a clean cage on a paper towel or absorbent lab diaper to prevent aspiration of bedding. The cage can be placed partially on a heating pad set to "low" to allow the animal to adjust the level of heat by moving away from the source. A

heat lamp may also be used. The animal must be monitored at least every 5 minutes until it is fully conscious and then returned to its regular room. Place some food on the floor of the cage and make sure water is accessible. Food pellets can be placed in glass dishes and moistened with water along with baby style fruit sauces to encourage eating. Cereal treats may be added to the cage of rats to encourage them to eat and drink.

### Analgesics:

Monitor the animal each day following surgery for at least 4 days. Record body weight and assess amount of eating and drinking. Be aware that rodents may mask signs of pain. Painful procedures are defined by the USDA as “any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied, that is, pain in excess of that caused by injections or other minor procedures”. Signs of pain or discomfort are decrease in food and/or water consumption, decrease in weight, abnormal posture, aggressiveness towards handling, reluctance to move, grinding of teeth, and ruffled or unkempt fur.

- **Local anesthetics** - These can be used topically at the surgical site to provide effective analgesia immediately post-op. They do not significantly delay wound healing. Bupivacaine (Marcaine™ 0.25%) at 1 drop per centimeter of incision length can be placed onto the surgical incision at the time of closure and will take effect in 5-10 minutes with a duration of effect for 4 to 12 hours.
- **Non-steroidal anti-inflammatory drugs (NSAID)** - NSAIDs such as aspirin, ibuprofen (Advil™, Motrin™), carprofen (Rimadyl™), and meloxicam (Metacam™) are useful for mild to moderate pain or discomfort. These drugs can be used for follow-up dosing after an initial dose of an opioid pain reliever. They can be dosed orally (through drinking water) or SQ injection and should be used for no longer than 3 to 5 days post-operatively. They are metabolized rapidly and have very few adverse side effects. Dosages of NSAIDs are in the following table:

NSAID drug	Injection dosage (SQ)	Oral dosage Mice	Oral Dosage Rats
Carprofen	5 mg/kg SID	27 µg/ml* (5 mg/kg/day)	50 µg/ml* (5 mg/kg/ml)
Ibuprofen	N/A	30 mg/kg 4.7 ml Children's Motrin in 500 ml water	15 mg/kg 2.35 ml Children's Motrin in 500 ml water
Ketoprofen	5 mg/kg SID	N/A	N/A
Meloxicam	5.0 mg/kg SID Mice 2.0 mg/kg SID Rats	1.7 µg/ml (0.3 mg/kg/day)	10.89 µg/ml (1.0 mg/kg/day)

\*light sensitive in drinking water

In addition to dosing by injection (most recommended route) or in drinking water, some NSAIDs may be administered in gelatin.

#### Carprofen in Jell-O™ recipe for rats (raspberry or strawberry flavor)

Prepare according to Jigglers recipe, use one 100 mg tablet of Carprofen crushed in 500 ml water. Pour into ice cube tray and store in refrigerator.

Yields 0.2 mg/ml. A 5 mg dose equals 7.5 ml cube (1.5 tsp). Up to 10 mg can be given per day.

Note: Because of the rat's tendency to not consume new food items (neophobia), the gelatin must be fed for several days unmedicated.

- **Opioid analgesics** - These controlled substances are used for moderate to severe pain. Opioid analgesics can sometimes cause adverse side effects, such as nausea, constipation or pica (eating of non-nutritive substances such as bedding). An effective way to treat moderate pain is to use an initial SQ injection of a opioid analgesic immediately post-op and then switch to NSAID. Opioid analgesics are controlled substances and accurate records must be kept of their use. Some facilities administer Buprenorphine in Jell-o™. Buprenorphine is less effective orally and some studies show that it does not provide adequate analgesia when dosed orally. Buprenorphine has been shown to increase activity levels and food intake in rats. This effect may be mistaken for an analgesic effect in post-operative rats.
  - Dilute one vial of Buprenex™ (buprenorphine 0.3 mg/1 ml) with 19 ml sterile saline into a glass vial. Final concentration is 0.015 mg/ml. Dosage is 0.05 mg/kg every 12 hrs (BID) for up to 3 days post-op. For example, a 200 gm rat would receive 0.6 ml SQ. A 400 gm rat would receive 1.3 ml SQ. A 20 gm mouse would receive 0.06 ml SQ.