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Laboratory Animal Resources Guidelines

# Guidelines for Anesthesia and Analgesia in Rats

1. **Purpose**- This document has been designed by Indiana University Bloomington Laboratory Animal Resources veterinary staff as a guideline for tranquilization, anesthesia and analgesia of laboratory rats. This is not intended to be an inclusive tutorial on all possible drug combinations that can be used in rats. The following guidelines are also general recommendations and consequently do not include reference to specific research-associated concerns. If you have questions about the use of anesthetics or analgesics for your particular situation, or if you have questions or comments about this document, please contact LAR at x5-2356 or [lar@indiana.edu](mailto:lar@indiana.edu).
2. **Special Concerns in Rat Anesthesia**

There are several factors that should be considered prior to initiating an experimental procedure that requires anesthesia in the rat. Newly arrived animals should be **acclimated** to their new surroundings for at least **48 hours** before use. The age of the animals and their body weights should also be taken into account when selecting an anesthetic protocol. Preanesthetic fasting is usually not necessary; however, if fasting is employed is should be limited to no more than 2-3 hours due to the high metabolic rate of small rodents. **Water should never be restricted**.

There are 3 basic methods of anesthetic delivery for rodents: parenteral, inhalation, or a combination of both. **Parenteral anesthesia** may be administered via the:

* + **Intraperitoneal (IP) injection**, the animal is held in a head-down position and a 22-27 gauge needle is inserted into the lower left abdominal quadrant just off the midline.
  + **Subcutaneous (SC) injections** can be given by tenting the skin of the back and injecting into the space between the skin and underlying muscle.
  + **Intramuscular (IM) injections**, the drugs are usually delivered into the caudal thigh muscles or the muscles lateral to the backbone. IM injections should be reserved for non-recovery procedures because IM injection of irritating substances (such as ketamine) may lead to lameness, self-mutilation of the limb, or cutaneous ulceration.
  + **Intravenous (IV) injections** are most often given into the lateral tail veins. These vessels are to be dilated by placing the tail in warm (not hot) water for 1-2 minutes prior to injection or placing the animal on a warm **water** recirculating pad. Use of a 25 gauge needle should be adequate for IV injections in rats. Training in proper restraint and injection techniques is required for those persons performing these procedures.

**Inhalation anesthesia** may be delivered by a chamber, mask or endotracheal tube. Chambers can be made by using a large covered glass, plexiglass or plastic container with anesthetic soaked cotton balls or gauze squares in the bottom. See Table 1 for dose recommendations. The rat must be prevented from coming into direct contact with the liquid inhalant anesthetic by placing a mesh grid over the cotton/gauze. This method may be adequate for very short-term procedures such as retro-orbital bleeding or subcutaneous tumor implantation. Flow through anesthesia chambers and facemasks are preferred and require a gas anesthesia machine with an oxygen source and a precision vaporizer. Due to the small respiratory capacity in rats (2-4 ml), a nonrebreathing system should be used. Anesthesia chambers and facemasks are commercially available or can be made from a funnel or the end of a large syringe barrel. There are commercially available anesthesia chambers that have a fresh gas inlet as well as an outlet for exhaled gases. For anesthetic events lasting greater than 5 minutes and whenever facemasks are used, an **ophthalamic ointment** (e.g. Paralube® or Lacrilube®) must be applied to the eyes to prevent corneal drying and trauma. When using inhalant anesthesia, a fume hood or an anesthetic system equipped with a gas scavenging system should be used to minimize occupational exposure to exiting gases.

Endotracheal intubation is technically difficult in the rat. An intravenous over-the-needle catheter (size 14-20) with the stylet filed down may be suitable for intubation. If possible, the pharyngeal and laryngeal structures should be visualized with a laryngoscope before attempting intubation. Though rodent laryngoscopes are not commercially available at this time, laryngoscopes can be fabricated with a light source for use in rats. A Wisconsin pediatric laryngoscope size 0 blade may also be suitable. Some vendors have rat intubation kits with all the materials needed for intubation.

* 1. **Prevention of Hypothermia**

In order to prevent hypothermia during anesthesia, which slows recovery and further stresses the animal, supplemental heat should be provided in the form of a recirculating warm water blanket or isothermal heat source (e.g. Gaymar Stryker Heating/Cooling T/Pump, Snuggle®Safe). Electric heating pads are discouraged because of uneven heating and tendency to cause thermal injury. Regardless of the heat source, the animal must never be placed directly on the heat but should be separated from it by a towel or sterile drape. Covering the animal with a sterile drape also helps to conserve body temperature. When covering an anesthetized animal, be careful not to place excessive pressure over the thorax.

* 1. **Monitoring and Recovery**

Parameters that should be monitored in an anesthetized rat include anesthetic depth,

* + - respiratory rate (normal undisturbed = 70-110/min., a fall of 50% is acceptable during anesthesia),
    - mucous membrane color (should be pink not blue),
    - pulse (normal= 260-500/min.), and
    - rectal temperature (normal= 35.9-37.5oC or 96.6-99.5oF).

Anesthetic depth may be assessed by recumbancy and loss of movement, loss of blink reflex, muscle relaxation, and loss of response to stimulation (such as a toe pinch). Movement of the chest wall may be used to determine respiratory rate, and palpation of the chest wall may be used to assess pulse. A pulse oximeter probe may be placed on a foot or at the base of the tail to measure oxygen saturation. An ophthalmic ointment (Paralube®) must be applied to the eyes of any animals receiving injectable anesthetics or to those animals anesthetized with gas anesthetics for greater than 5 minutes. Depth of anesthesia may be assessed by an inability to remain upright, loss of movement, loss of blink reflex, muscle relaxation and loss of response to reflex stimulation (such as a toe pinch). **Because the rat has a greater body surface area to body mass ratio than larger animals, thermal support is critical to their recovery.** Heat loss may be minimized by placing a warm water blanket drape between the animal and the table or by administering warmed subcutaneous or intraperitoneal fluids during or after surgery (5-10 ml/kg/hr). Fluids such as warmed saline or lactated Ringer’s solution are also important for correcting volume deficits. Nutritional support is also critical following recovery and food can be provided on the cage floor or in the form of nutrient gels available from LAR.

It is highly preferable to recover all animals in separate clean cages without bedding, within the room used for surgical preparation so that they can be continually monitored. If a large number of surgeries are being conducted at one time, animals may be housed together following anesthesia but prior to recovery only if they are continually observed (at least once every 2-3 minutes) by a member of the research lab to assure that more alert rodents are not cannibalizing nonresponsive cage mates. Animals should be monitored every 5-10 minutes by laboratory staff until they are fully recovered and ambulatory. A standard rodent diet should be provided as soon as the animal has recovered sufficiently to move and eat. Moist chow can be used to encourage eating.

1. **Anesthesia** 
   1. **Preanesthetic Agents and Anesthetic Agents**

Atropine (0.05 mg/kg-0.1 mg/kg SC) may be administered subcutaneously as a preanesthetic agent approximately 15 minutes before anesthesia. Atropine prevents the drop in heart rate and excessive salivation that may be caused by agents such as inhalant anesthetics and ketamine. Glycopyrrolate (0.01-0.02 mg/kg SC) is an effective alternative to atropine when given 15 minutes prior to surgery. These agents are not frequently used for rodent anesthesia.

* 1. **Preparation of Drugs to be administered in vivo**

Dilution of injected drugs allows more precise dosing, but may shorten the shelf life of the compound. Aseptic technique must be observed as mixtures (cocktails) are prepared; this includes using sterile vials, syringes and needles, wiping the cover of each vial or bottle with 70% ethanol or isopropanol, diluting with Sterile Water for Injection or sterile PBS (phosphate buffered saline) and not reusing needles used for dilution or administration. As with undiluted drugs, only new, sterile needles must be used for withdrawing aliquots from the cocktail and for administering injections. Diluted drugs must be labeled and dated, then discarded after 6 months, or at the expiration date of any of the components, whichever comes first.

* 1. **Preferred Anesthetics**
     1. Isoflurane has become the anesthetic agent of choice for both short and lengthy procedures due to its rapid and reliable recovery. If using a precision vaporizer to deliver anesthetic, the machine must be compatible with the specific inhalant anesthetic. Sevoflurane is another common inhalation anesthetic with a quick recovery time. Best administered using a precision vaporizer but may also be administered via nose cone containing small amount of anesthetic. Without a vaporizer the dose of isoflurane is very high, and cannot be titrated. Diluting the isoflurane in mineral oil is recommended to lessen the dose of isoflurane the animal will receive when a vaporizer is not used. Refer to Guidelines for the use of Isoflurane anesthesia without a vaporizer for rodents. Survival surgery requires concurrent pre-emptive analgesia.

The injectable anesthetic combination of choice is Ketamine 80-120 mg/kg IP + xylazine (Rompun®) 5-10 mg/kg IP to produce 30-45 minutes of anesthesia. If one needs more anesthetic, only use 1/3 the original calculated dose of ketamine. Xylazine should not be redosed due to its hypotensive effects.

**Table 1. Inhalant Anesthetics Used in Rats**

|  |  |  |
| --- | --- | --- |
| **Drug** | **Dosage** | **Comments** |
| Isoflurane (Forane®, Aerane®) Recommended | 3-4% for induction  1-2% for maintenance | 300 μl in a 500 ml container- chamber induction for brief anesthesia.  Maintenance requires use of a calibrated vaporizer. |
| Sevoflurane | 4-6% induction  0.5-3% maintenance | Requires use of a calibrated vaporizer. |

**Table 2. Injectable Anesthetics and Tranquilizers Used in Rats**

|  |  |  |  |
| --- | --- | --- | --- |
| **Drug** | **Dosage & Route** | **Duration of Anesthesia** | **Comments** |
| Sedatives/Tranquilizers | | | |
| Diazepam (Valium®) | 3-5 mg/kg SC (sedation) |  | Sedation only |
| Midazolam (Versed®) | 1-2 mg/kg SC (sedation) |  | Sedation only |
| Barbiturates | | | |
| Pentobarbital (Nembutal®) | 40-50 mg/kg IP sedation  70-85 mg/kg IP anesthesia | 20-60 min.  80-95 min. | Poor analgesic in rats. Dose sufficient to produce surgical anesthesia may cause severe respiratory depression and death. Give diluted in saline (<10 mg/ml). |
| Dissociatives | | | |
| Ketamine (Ketoset®)\* | 50-100 mg/kg IM | Unproven | Poor muscle relaxation and insufficient analgesia for major surgery. Lower doses appropriate for sedation only. |
| Ketamine + Acepromazine (Promace®) | Ket 75-80 mg/kg IM, IP +  Ace 2.5 mg/kg IM, IP | 20-30 min. | Light anesthesia |
| Ketamine + Diazepam (Valium®) | Ket 40-80 mg/kg IP =  Diaz 5-10 mg/kg IP | 45-60 min. |  |
| Ketamine + Medetomidine# | Ket 60-75 mg/kg IP +  Med 0.25-0.5 mg/kg SC, IP | Surgical anesthesia 20-30 min.;  Sleep time 60-120 min. | Light anesthesia.  Females more sensitive than males. |
| Ketamine + xylazine# (Rompun®)  **Recommended** | Ket 40-90 mg/kg IP +  Xyl 5-10 mg/kg IP | 45-90 min. | Thermal support is crucial. To prolong anesthesia, supplement with 1/3 dose of ketamine only. Xylazine can be reversed with 1-2 mg/kg Yohimbine IP. |
| Ketamine + xylazine1 + acepromazine  (Triple sedative) | Ket 31.25 mg/kg IP, IM +  Xyl 6.25 mg/kg IP, IM +  Ace 1.25 mg/kg IP, IM  (0.04-0.05 ml/100g) | ~20-30 min. | Sedative- not appropriate for anesthesia alone; 60-120 min. sleep time, 30-40 min. anesthesia |
| Other | | | |
| Telazol®= Tiletamine + zolazepam | 20-40 mg/kg IP or  20 mg/kg IM | 30-60 min. | Anesthesia variable. Corneal, pedal and swallowing reflexes remain intact. |
| Telazol® + Butorphanol | Tel 20-40 mg/kg IP +  But 1.25-5 mg/kg IP | 59-140 min. | Good analgesia but transient hypotension, bradycardia, and dose-dependent respiratory depression. |
| Telazol® + Xylazine | Tel 20-40 mg/kg IP +  Xyl 5-10 mg/kg IP | 130-200 min. | Good analgesia but marked cardiovascular depression. |
| Propofol (Diprivan®) | 7.5-10 mg/kg IV for induction, then 44-55 mg/kg/hr continuous IV infusion | 8-11 min.  3 hr. | Titrate as needed |
| Urethane | 1000 mg/kg IP |  | Caution! Prolonged anesthesia; terminal procedures only; carcinogenic and mutagenic |

Subcutaneous (SC), Intraperitoneal (IP), Intravenous (IV)

\*Ketamine alone is not adequate for deep anesthesia or procedures that are painful. It is only to be used for immobilization.

#Reversal of α2agonists such as xylazine and medetomidine can be accomplished by giving atipamazole (Antisedan®) 1-2.5 mg/kg IM, IP, SC or IV

* 1. **Analgesia**

Unrelieved pain can have profound negative physiologic consequences, which may alter research results. Mice show a variety of responses to pain, some of which may be fairly subtle and easily missed on casual examination. Pain evaluation in mice consists of evaluating behavioral and physiologic parameters.

|  |  |
| --- | --- |
| Behavioral Signs of Pain | Physiologic Indicators of Pain |
| Reluctance to move | Elevated blood pressure |
| Abnormal posturing | Elevated heart rate |
| Social isolation | Elevated respiratory rate |
| Decreased appetite | Changes in body temperature |
| Vocalization | Dilated pupils |
| Decreased grooming |  |
| Aggression |  |
| Self-mutilation |  |
| Piloerection |  |
| Squinted eyes/pale eyes |  |

For short-term management (less than 7 days) of moderate to severe pain, the LAR staff recommends SC injections of buprenorphine (0.05-0.1 mg/kg) 2-3 times per day. A single injection of buprenorphine will typically last 8 hours, but there is considerable variation in duration. The animals should be observed carefully so the optimum dose and frequency can be determined.

Table 3. Analgesics Used in Rats

|  |  |  |
| --- | --- | --- |
| Drug | Dose | Duration |
| Buprenorphine (Buprenex®)a | 0.01-0.05 mg/kg SC, IP | 6-12 hrs. |
| Buprenorphine SR | 1-1.2 mg/kg SC | Lasts for 3 days |
| Carprofen (Rimadyl®) | 5 mg/kg SC or IP, 10 mg/kg PO | 24 hrs. |
| Flunixin (Banamine®) | 2.5 mg/kg SC | 12-24 hrs. |
| Ibuprofen | 15 mg/kg PO | 4 hrs. |
| Ketoprofen | 5 mg/kg SC, IP | 24 h |
| Meloxicam (Metacam®) | 2 mg/kg SC, 5 mg/kg PO | 12-24 hrs |
| Morphine a,b | 2.5 mg/kg SC, IP | 2-4 hrs. |
| Tramadol | 5 mg/kg SC, IP |  |
| Lidocaine 1% | 4 mg/kg (0.4 ml/kg) | 1.5-2 hours |
| Bupivacaine 0.25% | 1-2 mg/kg (0.4-0.8 ml/kg) | 4-12 hours |

Subcutaneous (SC), Intraperitoneal (IP), Intravenous (IV), oral (PO)

a In addition to being an analgesic, this drug also acts as a sedative. If this drug is administered as an animal is recovering from anesthesia, the animal must be observed carefully for cumulative sedative effects of the anesthetics and the analgesics.

b This drug has a broad range of recommended doses. It is recommended that the animal be given the lowest dose in the range and be observed for signs of pain or discomfort. Additional analgesic may be administered if necessary at the next scheduled dosing time.

* 1. **Local Anesthetics**

Lidocaine and bupivacaine are the two most commonly used local anesthetics. Lidocaine has a rapid onset (1-2 minutes) and short duration (1.5-2.0 hours) of action; bupivacaine has a slower onset (5-10 minutes), but a much longer duration of action (4-12 hours, site dependent). Maximum safe doses for most species are:

**Lidocaine: 4 mg/kg (0.4 ml/kg of a 1% solution)**

**Bupivacaine: 1-2 mg/kg (0.4-0.8 ml/kg of a 0.25% solution)**

These doses can be diluted in sterile saline to provide a larger injection volume. IV administration of lidocaine or bupivacaine can cause cardiovascular effects (e.g., hypotension, dysrhythmias) and central nervous system depression followed by seizures. To avoid these adverse consequences, each animal should be weighed individually and the maximum safe dose calculated for that individual. Aspiration should always be performed prior to injection to ensure that IV injection is avoided.

Local anesthetics are available in a variety of concentrations with or without adrenaline. Adrenaline causes vasoconstriction and prolongs the action of the local anesthetic. Adrenaline should not be used in animals that have suspect cardiac compromise.

* 1. **Neonatal Rodent Anesthesia**

A rodent neonate is defined as a rat <6 days of age. There are several anesthetic methods currently presented in the literature for use in neonatal rodents. These include injectable, inhalant, and physical methods. Hypothermia is the primary physical method utilized in neonatal rodent anesthesia and it is believed to provide anesthesia/analgesia by decreasing neural conduction and synaptic transmission. However, the cooling process itself may be painful and for this reason direct contact with the cooling agent should be avoided. Neonatal rodents demonstrate an increased sensitivity to most injectable anesthetic agents and these have been associated with a high anesthetic mortality in neonates. Inhalant anesthetics are considered safe in neonatal rodents but may have a longer induction time than adult rodents because of their tolerance to hypoxia.

Parental cannibalism is a common problem with neonatal rodent anesthesia. This problem can be reduced by ensuring that the neonate is fully recovered before returning to the dam. Additional steps can also be implemented to reduce cannibalism including smearing pups with soiled bedding from the mother’s cage, placing the pup back in the middle of the litter, and masking scent cues.

* + 1. Anesthesia methods
       1. Physical Methods- Hypothermia- can only be performed in Neonatal rodents < 6 days old and should not be used for procedures lasting longer than 30 minutes.
          1. Place neonates either on a latex covered bed of crushed ice, in a cut off finger of a latex glove and place in ice water (animal’s head must be held above water to prevent water aspiration and death) or a paper lined test tube and placing in crushed ice/ice water.
          2. Animals have reached proper plane of anesthesia when pedal reflex is lost (animal does not respond to toe pinch).
          3. Once proper plane is reached animals are removed from ice bath and placed on a chilled cold pack or bed of ice.
          4. Use fiber optic light during procedure because incandescent bulbs can warm surgical field.
          5. Following anesthesia animal should be re-warmed slowly. Rapid warming can cause tissue damage. Patient can be re-warmed on a circulating water heating pad (40oC) or in an incubator (33oC).
          6. Pups can be returned to dam once they are able to crawl.
       2. Inhalant anesthetics

Table 1. Inhalant Anesthetics

|  |  |  |  |
| --- | --- | --- | --- |
| Stage of Anesthesia | Route | Oxygen (L/min.) | Isoflurane (%) |
| Induction | Mask or Chamber | 0.5-1 | 4-5 |
| Maintenance | Mask | 0.5-1 | 1-2 |

* + - 1. Injectable Anesthetics  
         Ketamine/Xylazine- Mice >7 days, 50-150 mg/kg Ket + 5-10 mg/kg Xylazine  
         Intraperitoneal (IP) 27 g needle, 1 ml syringe; maximum volume 0.5 ml  
         Subcutaneous (SC) 27 g needle, 1 ml syringe; maximum volume 1 ml
  1. **Emergency Resuscitation**

Attempts at resuscitating mice that have received an excessive dose of anesthetic or are experiencing cardiac or respiratory arrest for any reason, are typically unrewarding. Chest compressions often do not restore circulation, and artificial ventilation is difficult in the mouse. A rubber bulb with attached tubing large enough to fit over the nose may be used to periodically inflate the lungs. Respiratory depression can be treated by the administration of doxapram (Dopram®) 5-10 mg/kg IV or IP. If respiratory depression reoccurs, the doxapram should be administered repeatedly at approximately 10-15 minute intervals. Supportive care for animals which reach too deep a level of anesthesia includes raising the body temperature to normal, providing supplemental oxygen through a facemask or nosecone, and administering reversal agents if available (e.g. Yohimbine at 2.1 mg/kg IP or atipamazole 1-2.5 mg/kg IP or SC as needed to reverse xylazine or medetomidine).

## Additional Contacts

|  |  |  |  |
| --- | --- | --- | --- |
| ***Subject*** | ***Contact*** | ***Phone*** | ***Email*** |
| Veterinary Concerns | LAR Veterinarians | 855-2356 | [lar@indiana.edu](mailto:lar@indiana.edu) |
| Policy | IACUC Administrator | 855-5138 | [biacuc@indiana.edu](mailto:biacuc@indiana.edu) |

## References

ACLAM July 2006 Guidelines for the Assessment and Management of Pain in Rodents and Rabbits.

Arras M, Autenried P, Rettick A, Spaeni D, Rulicke T. 2001. Optimization of intraperitoneal injection anesthesia in mice: drugs, dosages, adverse effects, and anesthesia depth. Comp Med 51: 443-456.

Bonaparte (Convenor) D, Cinelli P, Douni E, Herault Y, Maas A, Pakarinen P, Poutanen M, Lafuente MS, Scavizzi F. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents: A report of the Federation of European Laboratory Animal Science Associations Working Group. *Lab Anim.* 2013; 47(3): 134-145.

Braden GC, Brice AK, Hankenson FC. Adverse Effects of Vapocoolant and Topical Anesthesia for Tail Biopsy of Preweanling Mice. *JAALAS*, May 2015; 54(3): 291-298.

Castelhano-Carlos MJ, et. al. 2010. Identification methods in newborn C57BL/6 mice: a developmental and behavioral evaluation. *Lab Anim.* 1-16.

Dahlborn K, Bugnon P, Nevalanen R, Raspa M, Verbost P, Spangenberg E. Report of the Federation of European Laboratory Animal Science Associations Working Grou on animal identification. *Lab Anim.* 2013; 47: 2-11.

Diesch TJ, Mellor DJ, Johnson CB, Lentle. 2009. Electoencephalographic responses to tail clamping in anesthetized rat pups. Lab Anim 43(3): 224-31.

Flecknell P. 2009. Laboratory Animal Anesthesia. 3rd Edition. Elsevier, Burlington, MA.

Garrels W, Cleve N, Niemann H, Kues WA. Rapid non-invasive genotyping of reporter transgenic mammals. *Biotechniques.* May 2012, p 1-4.

*Guide for the Care and Use of Laboratory Animals*, National Research Council, 8th Edition, pp 120-123.

Hankenson FC, et al. 2008. Evaluation of tail biopsy collection in laboratory mice (*Mus musculus*): vertebral ossification, DNA quantity, and acute behavioral responses. *JAALAS*; 47(6): 10-18.

Hankenson FC, Braden-Weiss GC, Blendy JA. Behavioral and Activity Assessment of Laboratory Mice (*Mus musculus*) After Tail Biopsy Under Isoflurane Anesthesia. *JAALAS,* Sept. 2011; 50(5): 686-694.

Jones CP, Carver S, Kendall LV. Evaluation of Common Anesthetic and Analgesic Techniques for Tail Biopsy in Mice. *JAALAS*; Nov. 2012; 51(6): 808-814.

NIH “Guidelines for Toe Clipping of Rodents” [Internet]. May 2010. Available at <http://oacu.od.nih.gov/ARAC/>

NIH “Guidelines of the Genotyping of Mice and Rats”. May 2010; <http://oacu.od.nih.gov/ARAC/documents/Rodent_Genotyping.pdf>

Norecopa “Supplementary Statement on Toe Clipping in Rodents”. March 19, 2010. <http://www.norecopa.no/norecopoa/vedlegg/Supplementary-statement-190310.pdf>.

Paluch LR, Lieggie CC, Dumont M, Monette S, Riedel ER, Lipman NS. Developmental and Behavioral Effects of Toe clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. *JAALAS*. 2014 Mar; 53(2): 132-140.

Philbert RA, Zadorozhnyaya I, Beach SRH, Brody GH. A Comparison of the Genotyping Results Using DNA Obtained from Blood and Saliva. *Psychiatr Genet*. 2008 December; 18(6): 275-281.

Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002. Health Research Extension Act of 1985, Public Law 99-158, Animals in Research, Principle V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

Schaefer DC, et al. 2010. Analysis of physiological and behavioral parameters in mice after toe clipping as newborns. *Lab Anim.* 44:7-13.

Symonds EL, Fenech M. A method for non-invasive genotyping of APCmin/+ mice using fecal samples. *Biol Proced Online.* 2012. 14: 1, Jan. 30, 2012.

University of Tennessee Mouse and Rat Toe Clipping. <https://www.uthsc.edu/research/compliance/iacuc/documents/mouse-and-rat-toe-clipping.pdf>

University of Pennsylvania IACUC Guideline Rodent Identification. <http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/iacucguideline-rodentidentification.pdf>

## Buprenorphine Dilution and Dosage Chart

**Buprenorphine (Buprenex®)** 0.3 mg/ml in boxes of 5 1 ml vials

**Dilution for Rats**: 1.0 ml Buprenorphine (0.3 mg buprenorphine/ml) + 9.0 D5W (5% dextrose in water) for injection to make a final concentration of 0.03 mg/ml. Using this dilution, dose rats according to the following chart. Buprenorphine is **light sensitive** so prepare dilution in an **amber bottle** or cover bottle with **foil**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Rat** | **Dosage** | | |
| **Weight** | **0.01 mg/kg** | **0.03 mg/kg** | **0.05 mg/kg** |
| 100 g | 0.03 ml | 0.1 ml | 0.17 ml |
| 125 g | 0.04 ml | 0.12 ml | 0.21 ml |
| 150 g | 0.05 ml | 0.15 ml | 0.25 ml |
| 175 g | 0.06 ml | 0.18 ml | 0.29 ml |
| 200 g | 0.07 ml | 0.20 ml | 0.33 ml |
| 225 g | 0.08 ml | 0.22 ml | 0.38 ml |
| 250 g | 0.08 ml | 0.25 ml | 0.42 ml |
| 275 g | 0.09 ml | 0.28 ml | 0.46 ml |
| 300 g | 0.1 ml | 0.30 ml | 0.50 ml |
| 325 g | 0.11 ml | 0.32 ml | 0.54 ml |
| 350 g | 0.12 ml | 0.35 ml | 0.58 ml |
| 375 g | 0.12 ml | 0.38 ml | 0.62 ml |
| 400 g | 0.13 ml | 0.40 ml | 0.67 ml |
| 450 g | 0.15 ml | 0.45 ml | 0.75 ml |
| 500 g | 0.17 ml | 0.50 ml | 0.83 ml |

Stable for up to 30 d at 21oC or 4oC- Jappinen A, Kokki H, Naaranlahti TJ, Rasi AS. Stability of buprenorphine, haloperidol and glycopyrrolate mixture in 0.9% sodium chloride solution. Pharm World Sci. 1999: 21(6): 272-4.

## Dilution for Carprofen

**Carprofen (Rimadyl®)** 50 mg/ml 10 ml bottle

**Diluent:** 5% Dextrose (D5W)

**Stability:** stable up to 7 days stored at 4oC, protected from light (amber vials).

**Dilution for Rats:** 1.0 ml carprofen (50 mg/ml) + 9.0 ml D5W (5% dextrose) to make a final concentration of 5 mg/ml. Using this dilution, dose rats according to the following chart.

|  |  |
| --- | --- |
| **RATS** | **Dosage** |
| Weight | 5 mg/kg |
| 100 g | 0.10 ml |
| 125 g | 0.12 ml |
| 150 g | 0.15 ml |
| 175 g | 0.18 ml |
| 200 g | 0.20 ml |
| 225 g | 0.22 ml |
| 250 g | 0.25 ml |
| 275 g | 0.28 ml |
| 300 g | 0.30 ml |
| 325 g | 0.32 ml |
| 350 g | 0.35 ml |
| 375 g | 0.38 ml |
| 400 g | 0.40 ml |
| 450 g | 0.45 ml |
| 500 g | 0.50 ml |

Solutions stable for **1 week** refrigerated at 4oC.

## Ketamine/Xylazine Dilution for Rodents

**Ketamine (Ketaset®)** 100 mg/ml in 10 ml vial

**Xylazine (Rompun®, Anased®)** 20 mg/ml or 100 mg/ml 20 ml vial

**Diluent:** 5% Dextrose (D5W) or normal saline (0.9% NaCl)

**Stability:** stable for 28 days stored under ambient conditions and at 4oC, protected from light (amber bottle).

### Rat Anesthetic Dose

Ketamine (40-90 mg/kg or 75 mg/kg) + Xylazine (10 mg/kg)

**3.75 ml Ketamine (100 mg/ml) + 2.5 ml xylazine (20 mg/ml) + 3.75 ml D5W or normal saline for injection OR + 0.5 ml xylazine (100 mg/ml) + 5.75 ml water for injection**

**Rats receive 0.2 ml/100 g body weight**

Ketamine and xylazine diluted as above with D5W (5% dextrose) or normal saline are chemically and physically stable after storage for 28 days under ambient conditions or 4oC protected from light.

### Triple Sedative (Ketamine + Xylazine + Acepromazine)

4 ml Ketamine 100 mg/ml + 1 ml Xylazine (20 mg/ml) + 1 ml Acepromazine (10 mg/ml)=  
66.66 mg/ml Ketamine + 3.33 mg/ml Xylazine + 1.66 mg/ml Acepromazine   
**0.04-0.05 ml/100g rat**

## Atipamazole (Antisedan®) Dilution and Dosage Chart

To Reverse Medetomidine (Dormitor®) or Xylazine (Rompun®)

**Atipamazole (Antisedan®)** 5 mg/ml 10 ml vial

**Diluent:** normal saline (0.9% NaCl)

**Stability:** stable for 28 days under ambient conditions and at 4oC, protected from light (amber bottles).

### Dilution for Rats

**2 ml atipamezole (5 mg/ml) + 8 ml sterile saline** to make final concentration of **1 mg/ml** solution. This makes a 10 ml dilution of atipamezole which is enough to reverse medetomidine or xylazine in approximately 25-30 rats weighing between 300-400 g.Using this dilution,dose rats at **0.1 ml solution/100 g body weight SC** according to the following chart.Dose is administered as **1 mg/kg** atipamezole SC.

|  |  |
| --- | --- |
| **Rat** | **Dosage** |
| **Weight** | **1 mg/kg** |
| 100 g | 0.10 ml |
| 125 g | 0.12 ml |
| 150 g | 0.15 ml |
| 175 g | 0.18 ml |
| 200 g | 0.20 ml |
| 225 g | 0.22 ml |
| 250 g | 0.25 ml |
| 275 g | 0.28 ml |
| 300 g | 0.30 ml |
| 325 g | 0.32 ml |
| 350 g | 0.35 ml |
| 375 g | 0.38 ml |
| 400 g | 0.40 ml |
| 450 g | 0.45 ml |
| 500 g | 0.50 ml |

## Bupivicaine Dilution for Rodents

**Bupivicaine (Sensorcaine®, Marcaine®)** 0.5% (50 mg/ml) 20 ml bottle?= $3.32

### Dilution for Rats

0.2ml bupivacaine (50 mg/ml) + 9.8 ml 0.9% saline to make a final concentration of 1 mg/ml solution. Using this dilution, dose rats according to the following chart at 0.1 ml. Dose is administered as **1-2 mg/kg** bupivacaine SC.

|  |  |  |
| --- | --- | --- |
| **Rat** | **Dosage** | |
| **Weight** | **1 mg/kg** | **2 mg/kg** |
| 100 g | 0.1 ml | 0.20 ml |
| 125 g | 0.12 ml | 0.25 ml |
| 150 g | 0.15 ml | 0.30 ml |
| 175 g | 0.18 ml | 0.35 ml |
| 200 g | 0.20 ml | 0.40 ml |
| 225 g | 0.22 ml | 0.45 ml |
| 250 g | 0.25 ml | 0.50 ml |
| 275 g | 0.28 ml | 0.55 ml |
| 300 g | 0.30 ml | 0.60 ml |
| 325 g | 0.32 ml | 0.65 ml |
| 350 g | 0.35 ml | 0.70 ml |
| 375 g | 0.38 ml | 0.76 ml |
| 400 g | 0.40 ml | 0.80 ml |
| 450 g | 0.45 ml | 0.9 ml |
| 500 g | 0.50 ml | 1.0 ml |