Laboratory Animal Resources Guidelines

**Guidelines for Anesthesia and Analgesia in Mice**

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 mice. This is not intended to be an inclusive tutorial on all possible drug combinations that can be used in mice. 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 Mouse Anesthesia**

There are several factors that should be considered prior to initiating an experimental procedure that requires anesthesia in the mouse. Newly arrived animals should be **acclimated** to their new surroundings for at least **48-72 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 right or 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. **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 or 27 gauge needle should be adequate for IV injections in mice. Intramuscular (IM) injections are not recommended in mice. Dosing inaccuracies and complications such as tissue irritation, lameness and self-mutilation can result. Training in proper restraint and injection techniques is required for those persons performing these procedures.

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.

Inhalation agents may be delivered by a chamber or facemask. 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 mouse must be prevented from coming into direct contact with the liquid inhalant anesthetic by placing a mesh grid over the cotton/gauze. Flow through anesthesia chambers and facemasks require a gas anesthesia machine with an oxygen source and a precision vaporizer. Due to the small respiratory capacity in mice, a nonrebreathing system should be used. Anesthesia chambers and facemasks are commercially available. 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.

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. See also *Guidelines for Surgery in Rodents*.

1. **Monitoring and Recovery**

Regardless of the anesthetic administered, the mouse must be monitored to avoid excessive depression of cardiac and respiratory functions, or insufficient anesthesia. This is characterized by poor muscle relaxation, movement in response to surgical stimulation or vocalization. Parameters that should be monitored in an anesthetized mouse include anesthetic depth, respiratory rate and pattern (normal undisturbed RR = 180/min., a slow rate drop of 50% is acceptable during anesthesia), mucous membrane color (should be pink not blue or grey). An ophthalamic 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). Movement of the chest wall and observation of abdominal movements may be used to assess respiratory rate. **Because the mouse 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 the 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. 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. To prevent injury or cannibalization, anesthetized mice should not be placed back in a cage with other mice until they are fully ambulatory.

1. **Anesthesia**
2. **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. **Preferred Anesthetics**

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.

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 Mice**

|  |  |  |
| --- | --- | --- |
| **Inhalation anesthetics** – 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. For further information, please refer to the [Guidelines for the Use of Isoflurane Anesthesia without a Vaporizer](https://lar.indiana.edu/doc/Guidelines_for_the_use_of_Isoflurane_anesthesia_without_a_vaporizer.pdf) for rodents. Survival surgery requires concurrent pre-emptive analgesia. | | |
| **Drug** | **Dosage** | **Comments** |
| Isoflurane (Forane®, Aerane®) Recommended | 4-5% 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 | 5-7% induction  3-4% maintenance | Requires use of a calibrated vaporizer. |

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Drug** | **Dosage & Route** | **Duration of Anesthesia** | **Comments** |
| Barbiturates | | | |
| Thiopental (Pentothal®) | 25-50 mg/kg IV  50 mg/kg IP | 10 min.  Unproven | Dose dependent respiratory depression and hypothermia |
| Pentobarbital (Nembutal®) | 40-50 mg/kg IP sedation  70-85 mg/kg IP anesthesia | 10-300 min. | Respiratory depression/poor analgesia. Consider supplemental analgesia (opioid or NSAID) for invasive procedures, especially when used on a survival basis. |
| Dissociatives | | | |
| **Ketamine combinations** – These dose combinations vary depending upon the type of procedure and the age/strain of the animal. A higher ketamine dose with lower xylazine dose is often used for very young/very old or critical patients. | | | |
| Ketamine (Ketoset®)\* | 100-200 mg/kg IP | Unproven | Poor muscle relaxation/mild analgesia |
| Ketamine + Medetomidine\* | 75 mg/kg K + 1 mg/kg M IP |  | Surgical anesthesia 20-30 min.;  Sleep time 60-120 min. |
| Ketamine + Dexmedetomidine | 50-75 mg/kg + 500 ug/kg IP | 20-30 minutes | May not produce surgical plane anesthesia for major procedures. If re-dosing, administer ketamine only at ¼ to ½ the original dose. |
| **Ketamine + xylazine (Rompun®)**  **Recommended** | 80-120 mg/kg ketamine IP + 5-10 mg/kg xylazine IP | 30-45 min. | Anesthetic depth varies from sedation to anesthesia |
| Ketamine + xylazine + acepromazine (Triple sedative) | 13 mg/kg ketamine + 0.66 mg/kg xylazine + 0.33 mg/kg ace IP (0.01-0.03 ml/100g) | Unproven | Sedative- not appropriate for anesthesia alone |
| Ketamine + acepromazine | 44 mg/kg ketamine I +0.75 mg/kg ace IP | 20-30 min. | Sedation only |
| Ketamine + diazepam (Valium®) | 100-200 mg/kg ketamine IP + 5 mg/kg dia IP | 20-30 min. | Sedation/immobilization |
| Ketamine + midazolam (Versed®) | 100 mg/kg ket IP +  5 mg/kg mid IP | 20-30 min. | Immobilization |
| Other | | | |
| Urethane | 1000 – 1500 mg/kg IP |  | Caution! Prolonged anesthesia; terminal procedures only; carcinogenic and mutagenic |
| Tribromoethanol (Avertin) | 200 – 240 mg/kg IP | Use fresh solution (<1 week of age) See recipe below. | May be used for only a single survival procedure. Expect 15-20 minutes anesthesia time. (Booster as necessary during procedure). |
| Propofol (Diprivan®) | 12-26 mg/kg IV | 5-7 min. | Titrate as needed |

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 or Yohimbine 0.2 mg/kg IV or 1- 2.1mg/kg IP. Side effects of yohimbine include CNS excitement, muscle tremors, salivation, and increased respiratory rate.

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 Mice**

|  |  |  |
| --- | --- | --- |
| Drug | Dose | Duration |
| Buprenorphine (Buprenex®)a,c | 0.05-0.1 (2 mg/kg) mg/kg SC or IV | 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 | 7.5-40 mg/kg PO (0.2 mg/ml water) |  |
| Ketoprofen | 5 mg/kg SC | 24 h |
| Meloxicam (Metacam®) | 2 mg/kg SC, 5 mg/kg PO | 24 hrs |
| Morphine a,b | 2-5 mg/kg SC | 1-4 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.

c Naloxone 0.01-0.1 mg/kg IV, IP can be given as opiod reversal agent once as needed to reverse respiratory depression. Note that reversal will also remove the analgesic effect of the opioid.

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 mouse <10 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
2. 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.
3. 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.
4. Animals have reached proper plane of anesthesia when pedal reflex is lost (animal does not respond to toe pinch).
5. Once proper plane is reached animals are removed from ice bath and placed on a chilled cold pack or bed of ice.
6. Use fiber optic light during procedure because incandescent bulbs can warm surgical field.
7. 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).
8. Pups can be returned to dam once they are able to crawl.
9. 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 |
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**Buprenorphine Dilution and Dosage Chart**

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

**Dilution for Mice**: 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 mice according to the following chart. Buprenorphine is **light sensitive** so prepare dilution in an **amber bottle** or cover bottle with **foil**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mouse** | **Dosage** | | |
| **Weight** | **0.05 mg/kg** | **0.075 mg/kg** | **0.1 mg/kg** |
| 15 g | 0.025 ml | 0.04 ml | 0.05 ml |
| 20 g | 0.03 ml | 0.05 ml | 0.07 ml |
| 25 g | 0.04 ml | 0.06 ml | 0.08 ml |
| 30 g | 0.05 ml | 0.08 ml | 0.1 ml |
| 35 g | 0.06 ml | 0.09 ml | 0.12 ml |
| 40 g | 0.07 ml | 0.1 ml | 0.13 ml |
| 45 g | 0.08 ml | 0.11 ml | 0.15 ml |
| 50 g | 0.08 ml | 0.12 ml | 0.17 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 Mice:** 1.0 ml carprofen (50 mg/ml) + 49.0 ml D5W (5% dextrose) to make a final concentration of 1 mg/ml. Using this dilution, dose mice according to the following chart.

|  |  |
| --- | --- |
| **Mouse** | **Dosage** |
| Weight | 5 mg/kg |
| 15 g | 0.08 ml |
| 20 g | 0.1 ml |
| 25 g | 0.12 ml |
| 30 g | 0.15 ml |
| 35 g | 0.18 ml |
| 40 g | 0.2 ml |
| 45 g | 0.22 ml |
| 50 g | 0.25 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).

**Mouse Anesthetic Dose**

Ketamine (100 mg/kg) + Xylazine (10 mg/kg)

* 1. **ml Ketamine (100 mg/ml) + 0.5 ml xylazine (20 mg/ml) + 8.5 ml D5W or normal saline for injection OR + 0.1 ml xylazine (100 mg/ml) + 8.9 ml for injection**

**Mice receive 0.1 ml/10 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 of 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

**0.01-0.03 ml/100g mouse**

**Ketamine/Xylazine/Acepromazine** (alternate recipe) for IP administration:

Ketamine 65 mg/kg

Xylazine 13 mg/kg

Acepromazine 2.0 mg/kg

To prepare cocktail:

Ketamine (100 mg/ml) 1.0 ml

Xylazine (20 mg/ml) 1.0 ml

Acepromazine (10 mg/ml) 0.3 ml

Sterile water or saline 7.7 ml

Cocktail total volume: 10.0 ml

Dose anesthetic cocktail based upon individual mouse body weight:

|  |  |
| --- | --- |
| Mouse Body Weight (g) | Volume of cocktail (ml) |
| 20 g | 0.13 ml |
| 25 g | 0.16 ml |
| 30 g | 0.20 ml |
| 35 g | 0.23 ml |

This cocktail is useful for longer more invasive surgical procedures in mice. It provide anesthesia for 45-60 minutes. If the acepromazine is eliminated, the anesthesia will be shorter and the recovery faster.

**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 Mice**

**0.2 ml atipamezole (5 mg/ml) + 4.8 ml sterile saline** to make final concentration of **0.2 mg/ml** solution. This makes a 5 ml dilution of atipamezole which is enough to reverse medetomidine in approximately 25-35 mice weighing between 25-40 g.Using this dilution,dose mice at **0.05 ml solution/10 g body weight SC** according to the following chart.Dose is administered as **1 mg/kg** atipamezole SC.

|  |  |
| --- | --- |
| **Mouse** | **Dosage** |
| **Weight** | **1 mg/kg** |
| 10 g | 0.05 ml |
| 15 g | 0.08 ml |
| 20 g | 0.10 ml |
| 25 g | 0.13 ml |
| 30 g | 0.15 ml |
| 35 g | 0.18 ml |
| 40 g | 0.2 ml |
| 45g | 0.22 ml |

**Bupivicaine Dilution for Rodents**

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

**Dilution for Mice**

* 1. ml bupivacaine (50 mg/ml) + 19.9 ml 0.9% saline to make a final concentration of 0.25 mg/ml solution. Using this dilution, dose mice at 0.1 ml solution/mouse (25 g). Dose is administered as **1-2 mg/kg** bupivacaine.

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| **Mouse** | **Dosage** | |
| **Weight** | **1 mg/kg** | **2 mg/kg** |
| 10 g | 0.04 ml | 0.08 ml |
| 15 g | 0.06 ml | 0.12 ml |
| 20 g | 0.08 ml | 0.16 ml |
| 25 g | 0.1 ml | 0.2 ml |
| 30 g | 0.12 ml | 0.24 ml |
| 35 g | 0.14 ml | 0.28 ml |
| 40 g | 0.16 ml | 0.32 ml |
| 45 g | 0.18 ml | 0.36 ml |

**Tribromoethanol (Avertin) recipe:**

**100% stock solution:**

Dissolve 10 g 2, 2, 2-tribromoethanol in 10ml amylene hydrate (tertiary amyl alcohol, 2 methyl-2-butanol). Make sure fully dissolved, heat up to 50o C. Solution s should be clear. Store wrapped in foil (light sensitive solution, ok to use brown glass bottle), at -20oC. Date and label bottle. Stock solution can be kept for up to one year.

**1.25% working solution (12.5 mg/ml):**

Mix 0.5 ml of stock solution with 39.5 ml sterile isotonic saline. Recommended to be used the same day it is prepared, but can be stored at 4oC or frozen at -20oC for up to a week, in a foil wrapped container or brown bottle. Use the frozen aliquots the same day after thawed to 37o C and shaken; discard frozen aliquots after 1 week. Date and label all bottles. Recheck pH prior to use. If pH <5, the solution becomes discolored or if precipitate is present after shaking, these are indicators that the solution has decomposed. If any of these are noted in the solution, do not use and discard.

In keeping with IACUC policy, tribromoethanol must be prepared, sterilized with a 0.2-micron

filter, stored and used with aseptic technique.

Dosing from working solution: 200mg/kg-240mg/kg

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| Mouse Body Weight (g) | Volume (ml) working solution |
| 20 g | 0.3-0.4 ml |
| 25 g | 0.4-0.5 ml |
| 30 g | 0.5-0.6 ml |
| 35 g | 0.6-0.7 ml |