1.0 OBJECTIVE

1.1 The objective of the Standard Operating Procedure is to define the methods for animal anesthesia to be used in the MMRI Animal Facility. This procedure is meant to provide guidelines, for recommended anesthetic dosages. Dosages may be varied, within acceptable range to achieve a desired affect.

2.0 SCOPE

2.1 This procedure applies to all animals within the MMRI Animal Facility. This procedure includes general information regarding anesthesia, and how to properly use inhalant and injectable forms of anesthesia.

3.0 POLICY

3.1 It is the policy of MMRI that the appropriate sedatives, analgesics or anesthetics will be used when an animal may be subjected to procedures that involve more than momentary or slight pain or distress. Personnel involved in such procedures will be appropriately qualified and trained in these procedures. [3]

4.0 RESPONSIBILITIES

4.1 It is the responsibility of the Manager of the Animal Facility and the IACUC to insure that all personnel performing procedures that involve pain be trained in methods to alleviate pain as described in this standard operating procedure. The manager and the IACUC will be responsible for reviewing this procedure annually and revising it if necessary.

5.0 REFERENCES

[1] Guide for the Care and Use of Laboratory Animals, NIH Publication 86-23, 1985.
[2] Eisele, Pam, Overview of Anesthesia for Laboratory Rodents, Presentation at: NCB-AALAS Symposium, Dec. 1993.
[3] Code of Federal Regulations, Title 9, Subchapter A, Part 2, Subpart C, Section 31.

6.0 PROCEDURE

6.1 General Information

"The proper use of anesthetics, analgesics, and tranquilizers in laboratory animals is necessary for humane and scientific reasons. In accordance with the Animal Welfare Act, the choice and use of the most appropriate drugs are matters for the attending veterinarian's professional judgment. The veterinarian must provide research personnel with guidelines and advice concerning choice and use of these drugs."[1]

Anesthetic regimens used for rodents include induction with injectables or inhalants. Prior to anesthesia, evaluate the health and general condition of the rodent for any signs that could contribute to the risk of anesthesia. During the procedure, monitor the anesthetized rodent to insure an adequate surgical plane and that the animal is not experiencing pain or complications. Provisions should be made to support body temperature, oxygenation and fluid balance, during and after, a surgical procedure requiring anesthesia.[2]

6.2 Inhalant Anesthesia Procedures

These procedures can be used with either of the products listed above. Depending on the length of the procedure, it may be necessary to re-expose the animal to maintain the appropriate level of anesthesia.

6.2.1 Preparation of a chamber

6.2.1.1 Prepare an inhalation chamber under an active fume hood.

6.2.1.2 Saturate the gauze with inhalant. The gauze will be wet, but there will not be any liquid dripping off the gauze.

6.2.1.3 Place the soaked gauze at the bottom of the sealed chamber, underneath a plastic mesh rack.

6.2.1.4 Place the animal on the rack, and close the top of the chamber.

6.2.1.5 Begin procedure when the animal no longer responds to a ear or toe pinch.

6.2.1.6 When the procedure is in progress and reaches completion, follow the monitoring and recovery procedures listed in section 6.6 of this Standard Operating Procedure.

6.3 Injectable Anesthesia

6.3.1 Sodium Pentobarbital (64.8 mg/ml sodium pentobarbital in 10% ethanol)

Sodium pentobarbital is a barbiturate. Anesthetic effects from this class of drug are variable and are affected by such things as time of day, species, strain , and stress level. They are poor analgesics except at high levels and they are respiratory depressants. There is a narrow margin of safety and the recovery from the drug may be prolonged especially with the IP route of administration.[2]

6.3.1.1 Weigh the rodent.

6.3.1.2 Typical dose range of pentobarbital for rats and mice are:

Rats 30-40 mg/kg i.v.; Mice 40-90 mg/kg i.p.

6.3.1.3 Inject the desired dose intraperitoneally or intravenously.

6.3.1.4 Begin procedure when the animal no longer responds to a ear or toe pinch.

6.3.1.5 When the procedure is in progress and reaches completion, follow the monitoring and recovery procedures listed in section 6.4 of this Standard Operating Procedure.

6.3.2 Ketamine-Xylazine Cocktail

Ketamine (100 mg/ml) Vetalar TM

Xylazin (100 mg/ml) OR (20 mg/ml) Rompun TM

These two drugs are typically mixed together, and used as a cocktail. Xylazine should never be used without other anesthetic drugs. Ketamine alone is also unsatisfactory for surgical anesthesia due to inadequate muscle relaxation.

All injectables should be 10-25% of original dose and wait 2-3 minutes for effect.

When preparing a large batch of cocktail for a number of rodents, prepare the batch 24 hours in advance of use. Completely label the batch vial with concentration, species, date prepared, and expiration date. Expiration date is two weeks past the date prepared. Expired solution (maximum 3.5 weeks) can be used, **IF** the animals will be anesthetized for a terminal procedure and a notation is made on the study data record sheet.

6.3.2.1 Weigh the rodent.

6.3.2.2 Typical dose range of Ketamine/Xylazine cocktail for rats and mice are:

Rats	70.5 mg Ketamine	3.4 mg Xylazine per Kg i.p./ i.m.
Mice	7.05 mg Ketamine	0.34 mg Xylazine per 100 g i.p.

6.3.2.3 Aseptically prepare the rat cocktail with 0.9% saline such that the volume per rat is 0.1 ml per 100 grams of body weight. (See example in figure one.) Prepare cocktail for mice that would be 0.01 ml per gram of body weight.

6.3.2.4 Inject the desired dose.

6.3.2.5 Begin procedure when the animal no longer responds to a ear or toe pinch.

6.3.2.6 When the procedure is in progress and when it reaches completion, follow the monitoring and recovery procedures listed in section 6.6 of this Standard Operating Procedure.

6.4 Monitoring and Recovery

6.4.1 Monitor the animal closely, overexposure to the anesthesia can be fatal. Monitor the animal's heart and respiration rate during the experimental procedure.

6.4.2 Throughout the procedure, help the animal maintain it's body temperature by providing an external heat source. (i.e. heat lamp, thermal mattress, hot water bottle or warm towel) Apply gel lubricant to the eyes, to protect the corneas from drying out.

6.4.3 When procedure is complete, continue to monitor and support normal body temperature until animal is completely conscious.

6.4.4 When the animal is semi-conscious and able to maintain a sternal position, place animal back into a clean cage.

6.4.5 Continue to monitor animal until it has fully regained consciousness.

6.4.6 Fast the animal until it has been fully conscious for 30 minutes.

6.4.7 If the procedure has been long, it may be necessary to provide fluids to the animal. Give one of the following; Lactated Ringers, Dextrose, or sterile Physiological Saline, in a sub-cutaneous injection at a rate of 3-12 mL depending on size and species.

Figure One

Ketamine-Xylazine Cocktail

Dose: Ketamine: 70.5 mg/kg xylazine: 3.4 mg/kg
Concentration out of the bottle: Ketamine 100 mg/ml Xylazine: 100 mg/ml
Each rat to receive **0.1 ml** of cocktail per **100 grams** of body weight.

Example Calculation:

To determine how much cocktail will be needed for 25 rats weighing approx. 200g :

$$25 \text{ rats} \times \frac{200 \text{ g}}{\text{rat}} \times \frac{0.1 \text{ ml cktl}}{100\text{g}} = 5.0 \text{ ml cktl}$$

Add a bit extra to cover the weight variability: 5.0 mls x 33% = 1.65 mls
Total amount of cocktail needed: 5.0 + 1.65 = 6.65 mls.

Use the tables below to prepare the cocktail from the concentrated bottle solution.

Ketamine-Xylazine Cocktail

Dose	Approx # of rats	final volume mls	Ketamine 100 mg/ml	Xylazine 100 mg/ml	0.9% Saline
70.5k/3.4x		10	7.1 ml	0.34 ml	2.64 ml
70.5k/3.4x		5.1	3.6 ml	0.17 ml	1.32 ml
70.5k/3.4x	8-10	2.5	1.8 ml	0.09 ml	0.66 ml
70.5k/3.4x	4-5	1.25	0.9 ml	0.05 ml	0.33 ml

*(Lisa: *change volumes to be 2,3,4,5,6,7,8,9, 10)*