WVU IACUC POLICY:
Anesthesia and Analgesia in Mice

Purpose

This document has been created by OLAR veterinary staff to serve 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. Rather, the following guidelines are 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, please contact the OLAR veterinary staff at 293-3737 or vetservices@hsc.wvu.edu.

More specific information on pre- and post-operative care for rodents undergoing survival surgical procedures may be found on the IACUC website at http://oric.research.wvu.edu/animal/guidlines

Definitions

Anesthesia: A pharmacologically induced and reversible lack of awareness and/or sensory perception
Analgesia: A relief or absence of pain sensation without loss of consciousness
Anesthetic: An agent that causes loss of sensation with or without loss of consciousness
Analgesic: An agent used to lessen or eliminate pain
Tranquilizer: A that reduces tension/anxiety, an anti-anxiety agent
Sedative: An agent or drug having a soothing, calming, or tranquilizing effect

Scope

These guidelines are intended for use by WVU research investigators and staff, veterinary staff, and IACUC members when writing, reviewing or designing animal use protocols involving painful and/or anesthetic procedures.

Special Considerations

Remember that body fat, or lack thereof, age, sex, and strain can all impact a mouse's response to anesthetic agents. Thus, all of these factors should be considered when contemplating an anesthetic regimen.

Preanesthetic fasting is not usually necessary in mice, however, if fasting is employed it should be limited to *no more than 2-3 hours* prior to the procedure, due to the high metabolic rate of small rodents. Water should *never* be restricted during the pre-anesthetic period.

Because mice have a greater body surface area to body mass ratio than larger animals, thermal support is critical to their survival and successful anesthetic recovery. Hypothermia during anesthesia results in prolonged recovery, additional stress to the animal, and increased potential for anesthetic death. Supplemental heat should be provided to mice undergoing anesthesia. Re-circulating warm water blankets and other isothermal heat sources such as Safe and Warm®, Snuggle Safe®, etc, are recommended for use with mice. Electric heating pads and heat lamps are generally discouraged because of uneven heating with the tendency to cause thermal injury, and an increased potential for ocular damage, respectively. Regardless of the source, the animal should never be placed directly in contact with the heat source, but should be separated from it by a towel or sterile drape.

Anesthesia Administration

Parenteral anesthesia may be administered to mice via **intraperitoneal** (IP), **subcutaneous** (SQ), or **intravenous** (IV) injection. Mice should *never* be given anesthetics by way of intramuscular (IM) injection!

Laboratory personnel who wish to obtain training or practice and increase proficiency with the approved parenteral administration techniques may contact OLAR veterinary services (3-3737) or the IACUC training associate (3-9368) to schedule a training or practice session at no charge.

Inhalation anesthesia may be delivered by chamber or facemask. **Flow-through anesthesia chambers** and **facemasks** require a gas anesthesia machine with an oxygen source and a precision vaporizer. A non-rebreathing system should always be used with mice. Anesthesia chambers and facemasks for mice are commercially available. Facemasks should be used for anesthetic events lasting more than 5 minutes. **"Drop jar" chambers** can be made of covered glass or plexiglass containers containing anesthetic-soaked absorbant material in the bottom (see table below for dose recommendations). It is important that chambers provide physical separation of the mouse from the anesthetic-soaked substrate. This can be done by utilizing a mesh grid or histopathology cassette. *Any time inhalant anesthesia is used, it is important to do so with an anesthetic system equipped with a gas scavenging system or in a fume hood to minimize occupational exposure to exiting gases.*

Monitoring and Recovery

Irrespective of the anesthetic used, the mouse must be monitored to avoid excess cardiac and respiratory depression, and insufficient anesthesia, characterized by poor muscle relaxation, movement in response to surgical stimulation or vocalization during an anesthetized procedure.

Parameters that should be monitored for mice during anesthesia include:

1. **Anesthetic depth**: assessed by an inability to remain upright, loss of purposeful voluntary movement, loss of blink reflex, muscle relaxation, and loss of response to reflex stimulation (toe or tail pinch with firm pressure)
2. **Respiratory rate and pattern**: assessed by observing chest wall and abdominal movements (normal respiratory rate for an awake mouse at rest is 180/min, a slow rate drop of 50% is acceptable during anesthesia with a regular even pattern)
3. **Mucus membrane color**: mucous membranes should be pink (not grey, blue, or white)

An ophthalmic ointment, such as Paralube® or Lacrilube®, must be applied to the eyes of any animal receiving injectable anesthetics or anesthetized with gas anesthetics for >5 minutes.

Placing a warm water re-circulating blanket and drape between the mouse and the table, or administering warmed fluids (SQ or IP) during or after the surgery (not to exceed 5-20 ml/kg/hr) can minimize heat loss. Warm fluids such as normal saline or lactated Ringer's solution are also important for correcting volume deficits following surgical procedures.

Nutritional support is critical during post-procedure recovery periods. Placing moistened rodent chow on the cage floor is recommended to encourage animals to eat following anesthetic events.

Mice should be recovered from anesthetic events in a clean cage with only a clean paper towel or no bedding with constant observation until such time as they are deemed to be stable and recovered. If a large number of procedures are being conducted in the same time period, mice may be housed together following anesthesia and prior to recovery, but only if they can be continually observed (at least every 2-3 minutes) by a member of the research staff to assure that alert mice do not harm more sedated cagemates.

Animals may not be returned to animal housing rooms following an anesthetized procedure until they are alert and fully ambulatory.

General Anesthetics

Isoflurane is generally the anesthetic agent of choice (when study parameters allow) for both short and lengthy procedures due to its rapid and reliable recovery. When using a precision vaporizer to deliver inhalent anesthetic, the machine must be compatible with the specific agent being used (isoflurane vs sevoflurane).

The injectable anesthetic combination of choice is ketamine (20-120 mg/kg IP) with xylazine (5-10 mg/kg IP) to produce 30-45 minutes of anesthesia. **NOTE**: When using this combination, if additional anesthetic is needed, only re-dose 1/3 the original calculated dose of ketamine. Xylazine should *not* be re-dosed due to its profound cardiovascular effects.

Table 1: Inhalant Anesthetics Used in Mice

Drug	Dosage	Comments
Isoflurane (Forane®, Aerane®) Recommended	4-5% for induction 1-2% for maintenance	300µL in a 500mL container for chamber induction: Brief anesthesia only Maintenance requires use of a calibrated vaporizer
Sevoflurane	To effect	Requires use of a calibrated vaporizer

Table 2: Injectable Anesthetics and Tranquilizers for Mice

Drug	Dosage & Route	Duration of Anesthesia	Comments
Barbiturates			
Methohexitone	8-16 mg/kg IV	5 minutes	Respiratory depression / poor analgesia
Pentobarbital (Nembutal®)	30-40 mg/kg IP for sedation 40-50 mg/kg IP for anesthesia	10-60 minutes	Respiratory depression / poor analgesia Immobilization / anesthesia
Thiopental (Pentothal®) (Soon to be permanently off the market)	25-40 mg/kg IV 50 mg/kg IP	10 minutes Unproven	Dose dependent respiratory depression and hypothermia Surgical anesthesia
Dissociatives			
Ketamine (Ketoset®) Not recommended	100-200 mg/kg IP	Unproven	Poor muscle relaxation / mild analgesia
Ketamine + acepromazine	80-120 mg/kg + 5-10 mg/kg IP	20-30 minutes Unproven	For sedation/ immobilization only. Not considered sufficient for surgical anesthesia
Ketamine + dexmedetomidine (Dexdomitor®)	60-75 mg/kg + 1.0 mg/kg IP	20-30 minutes	For surgical anesthesia. Anesthetic depth varies – dexmedetomidine should *not* be re-dosed
Ketamine + diazepam (Valium®)	100 mg/kg + 5 mg/kg IP	20-30 minutes	Sedation/immobilization

Ketamine + midazolam (Versed®)	100 mg/kg + 5 mg/kg IP	20-30 minutes	Sedation/Immobilization
Ketamine + xylazine (Rompun®) Recommended	80-120 mg/kg + 5-10 mg/kg IP	25-35 minutes	Anesthetic depth varies from sedation to anesthesia Xylazine should *not* be re-dosed
Ketamine + xylazine + acepromazine	80-100 mg/kg + 5-10 mg/kg + 3 mg/kg IP	30-40 minutes	Surgical anesthesia
Tiletamine/zolezepam (Telazol®)	60-80 mg/kg	Unproven	Sedation/immobilization
Other			
Alphachloralose	100-120 mg/kg	300-400 minutes Unproven	Light anesthesia **Non-survival** procedures only
Alphaxalone/alphadolone	10-15 mg/kg IV	5 minutes	Surgical anesthesia
Chloral hudrate	400 mg/kg IP	30 minutes	Light anesthesia
Methohexital (Brevital®)	10-20 mg/kg IV	5 minutes	Surgical anesthesia
Metomidate + fentanyl	50-60 mg/kg + 0.06 mg/kg SC	40-60 minutes	Surgical anesthesia
Propofol (Diprivan®)	12-26 mg/kg IV	5-10 minutes	Surgical anesthesia
Tribromoethanol (Avertin®)* Not recommended	125-250 mg/kg IP	15-45 minutes	Surgical anesthesia Avoid re-dosing

SC = subcutaneous IP = Intraperitoneal IV = Intravenous

*__Tribromoethanol (Avertin®)__ should not be used routinely without scientific justification and IACUC approval. Tribromoethanol is an irritant, especially at high doses and/or concentrations, or with repeated use. It can result in peritonitis, and the risk of fatal peritonitis increases with each subsequent use in the same animal. It degrades in the presence of heat or light to produce toxic byproducts, resulting in nephrotoxicity and hepatotoxicity. The effects of tribromoethanol are unpredictable in mice <16 days and in animals with altered carbohydrate metabolism (e.g. obesity and diabetes models).

Cautions for use: Because Avertin® is not commercially available as a pharmaceutical grade drug, it must be made in the laboratory from the reagents tribromoethanol and tertiary amyl alcohol. It must be carefully prepared under aseptic conditions with a working dilution of 1.25% prepared fresh. Sterile filter the prepared solution with a 0.2 micron filter. Store in a brown glass or foil-covered container at 4°C for no longer than 4 months. Do not use if the solution becomes discolored or has a precipitate. Check the pH before each use and use only when pH >5. It is not to be used twice in one animal on a survival basis. If used a second time in the same animal, that use should be for a terminal/acute procedure only.

Table 3: Antagonists to Anesthetics used in Rodents

Drug	Dosage & Route	Anesthetic	Comments
Atimpamezole (Antisedan®)	0.1–1 mg/kg IP or SC	Any regimen using xylazine or dex-medetomidine	Highly specific alpha-2 adrenergic receptor antagonist. Reverses the effects of xylazine or dexmedetomidine
Yohimbine (Yobine®)	2.0 mg/kg IP or SC	Any regimen using xylazine	Alpha-2 adrenergic receptor antagonist.

SC = subcutaneous IP = Intraperitoneal IV = Intravenous

Local Anesthetics

The two most commonly used local anesthetics in veterinary patients are lidocaine and bupivacaine. Lidocaine has a rapid onset (1–2 min) and a short duration of action (1½ –2 hr). Bupivacaine has a slower onset (5–10 min) and a much longer duration of action (4–12 hr, site dependent).

Maximum safe doses for most veterinary species are:

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

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

These doses can be diluted in sterile saline to provide a larger injection volume. IV administration of lidocaine or bupivacaine can result in cardiovascular effects including hypotension and dysrhythmias, as well as central nervous system depression and seizures. To avoid these adverse consequences, the maximum safe dose should be calculated for **each individual animal** based on **body weight**. Always aspirate prior to injection to insure that IV injection is avoided.

PLEASE NOTE: local anesthetics are available in a variety of concentrations with our without epinephrine. Epinephrine causes vasoconstriction and prolongs the action of the local anesthetic. Adrenaline should *not* be used in animals with suspected cardiac compromise.

Neonatal Rodent Anesthesia

A neonatal mouse is defined as a mouse pup < 10 days of age. There are a variety of anesthetic techniques currently described in the literature for use in neonatal rodents including injectable, inhalant, and physical methods.

Neonatal rodents demonstrate an increased sensitivity to most **injectable** anesthetic agents and these have been associated with high anesthetic mortality in neonates. **Inhalant** anesthetics are considered to be safe and effective in neonatal rodents, but they may have a longer induction time than adult rodents, and this should be anticipated when using these anesthetics. **Hypothermia** is the primary physical method utilized in neonatal anesthesia and is believed to provide anesthesia 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.

NOTE: it is important to make sure the neonate is fully recovered from anesthesia prior to placing back in the mother's cage in order to reduce the likelihood of parental cannibalism.

Table 4: Neonatal anesthesia methods

Inhalant Anesthetics (Neonatal)			
Stage of Anesthesia	**Route**	**Oxygen (L/min)**	**Isoflurane (%)**
Induction	Mask or Chamber	0.5–1.0	4–5
Maintenance	Mask	0.5–1.0	1–2
Injectable Anesthetics (Neonatal)			
Mouse Age	**Drug**	**Dose & Route**	**Comments**
> 7 days	Ketamine + Xylazine	50–120 mg/kg + 5–10 mg/kg **IP**	27 g needle 1 mL syringe max. vol. 0.5 mL
> 7 days	Ketamine + Xylazine	50–150 mg/kg + 5–10 mg/kg **SC**	27 g needle 1 mL syringe max. vol. 1 mL
Physical Anesthesia Method (Neonatal)			
Mouse Age	**Technique**	**Procedure**	
< 6 days	Hypothermia	Place neonate(s) either 1) on a latex covered bed of crushed ice; 2) on a paper towel-lined test tube in crushed ice/ice water Animals have reached their proper plane of anesthesia when they no longer respond to a firm toe pinch Once the proper anesthetic plane is reached, remove the neonates from the ice bath and place them on a chilled cold pack or bed of ice Only fiber optic light sources should be used during neonatal hypothermia-anesthetic procedures, as incandescent bulbs will warm the surgical field. Following anesthesia, the animals should be warmed *slowly* (rapid warming can cause tissue damage) on a circulating warm water blanket (40°C) or in an incubator (33°C) Animals can be returned to the dam once they are fully awake and able to crawl	

Analgesia

Unrelieved pain can have profound negative physiologic consequences and, subsequently, alter research results. Mice show a variety of responses to pain, some of which may be fairly subtle and easily overlooked on cage side exam. Physiologic and behavioral parameters should be evaluated when assessing pain in mice. Indicators of pain in mice include:

BEHAVIORAL:
Reluctance to move
Abnormal posturing
Social isolation
Decreased appetite
Vocalization
Decreased grooming
Aggression
Self-mutilation

PHYSIOLOGIC:
Elevated blood pressure
Elevated heart rate
Elevated respiratory rate
Changes in body temperature
Pupil dilation

Table 5: Analgesics Used in Mice

Drug	Dose & Route	Duration
Buprenorphine (Buprenex®)*	0.05–0.1 mg/kg SC or IV	6–12 hrs
Butorphanol (Torbutrol®)*	1.0–5.0 mg/kg SC	4 hrs
Morphine*§	2–5 mg/kg SC	1–4 hrs
Carprofen (Rimadyl®)	5 mg/kg SC	24 hrs
Meloxicam (Metacam®)	1–2 mg/kg PO or SC	12–24 hrs
Flunixin (Banamine®)	2.5 mg/kg SC	12–24 hrs

SC = Subcutaneous IP = Intraperitoneal IV = Intravenous PO = By mouth

* In addition to being an analgesic, this drug also has sedative properties. 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 analgesic.

§ 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.

References

This document was adapted from "ULAM Mouse Anesthesia and Analgesia Guidelines" referenced below.

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