

UNIVERSITY OF TEXAS AT ARLINGTON

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

ANESTHESIA IN LABORATORY ANIMALS SOP

I. Definitions

- A. Sedation is a mild degree of central depression in which the patient is awake but calm.
- B. Local anesthesia produces the loss of sensation in a limited area of the body.
- C. Regional anesthesia produces insensitivity in a larger but limited area of the body.
- D. Basal anesthesia is a light level of general anesthesia usually produced by pre-anesthetic agents. It serves as a basis for deeper anesthesia on administration of other agents.
- E. General anesthesia produces a completely unresponsive, disconnected, unconscious state.
- F. Surgical anesthesia produces unconsciousness accompanied by muscular relaxation to such a degree that surgery can be performed painlessly and without struggling on the part of the patient.
- G. Ambulatory is a state where the animal is able to stand upright and walk normally.

II. Background Information

- A. The selection of appropriate anesthetics should reflect professional veterinary judgment as to which best meets clinical and humane requirements as well as the needs of the research protocol. (*Guide*, [p. 121](#)).
- B. The selection of anesthetics depends on many factors, such as species, age, the type and degree of pain and the likely effects of particular agents on specific organ systems, the nature and length of the surgical procedure, and the safety of the agent (*Guide*, [p. 121](#)).

III. Responsibilities

- A. It is the responsibility of the Principal Investigator (PI) to consult, if needed, with the Attending Veterinarian to choose the appropriate anesthetic for a specific procedure.
- B. It is the responsibility of the PI to procure the anesthetics that have been approved in the protocol.
- C. It is the responsibility of the PI and other research personnel who will administer anesthetics to have completed the applicable training with the Animal Care Facility (ACF) Manager.
- D. It is the responsibility of the PI and lab staff to use the anesthetics that have been approved in the protocol and to use them in the manner approved in the protocol.
- E. It is the responsibility of the PI to use the appropriate safety equipment such as an anesthetic gas waste scavenging system.
- F. It is the responsibility of the ACF to coordinate yearly calibration of anesthesia machines.

IV. Anesthesia Requirements

A. Procedures

- 1. Fasting: Rodents and rabbits are generally not fasted before anesthesia. Water is not withheld.
- 2. Anesthetized animals of all species must be attended continuously from the onset of anesthesia until recovery.
- 3. Use of heat source: Small animals (rats, mice, hamsters) lose body heat rapidly while under anesthesia. It is recommended that a heat source (water circulating heat pad, heating pad, heat lamp, etc.) be used. Monitoring of body temperature with a thermometer may be necessary for long procedures.
- 4. Administering the anesthetic: Anesthesia agents can be administered by inhalation or injection. Supplemental doses can be administered as needed during the procedure.
- 5. Eye ointment – Eye ointment should be used in rodents to prevent drying of the eyes due to the loss of the blink reflex during anesthesia.
- 6. Monitoring anesthesia – Before an incision is made, ensure that the animal is in an adequate plane of anesthesia:

- a. For rodents – test the pedal withdrawal reflex by pinching a foot pad on each foot. If the animal pulls back, additional anesthetic may need to be administered. Monitor the animal's rate and depth of respiration (increase in depth and decrease in rate signify anesthesia) and re-check reflexes during the procedure. Monitor the color of the animal's ears, tail, mucous membranes, and foot pads. Anesthesia is too deep if mucous membrane turns gray and is no longer pink.
 - b. For non-rodent mammals – test responsiveness to painful stimuli and use monitoring equipment (EKG, Anal Probe for temperature, etc.) to check rate and depth of respiration (increase in depth and decrease in rate signify anesthesia). Listen to heart rate.
 - c. Vital records for USDA-covered species must be turned in to the ACF Manager.
7. Dehydration and volume depletion should be prevented during longer procedures by subcutaneous, intravenous, or intraperitoneal administration of warmed isotonic fluids (e.g., 0.9% saline for injection).
8. Post-procedure:
- a. Animals should be given a heat source. Be sure the animals do not get too hot or too cold. Animals should not have direct contact with the heat source and should be able to move away from it when ambulatory. It is suggested to place the heat source under half the holding enclosure so animals can move on/off heat at will.
 - b. At least one member of the research personnel must stay with the animals until they can maintain upright posture and walk normally about the cage.
 - c. Animals can be moved back into their home cage in animal room once they can ambulate on their own with appropriate balance.
 - d. Do not leave animals that have just undergone a procedure unattended with other unanesthetized animals.
 - e. Animals should be cleaned of any blood and skin preparation solution, such as betadine or chlorhexidine.
 - f. Depending on the type of surgery, fluids (usually saline or lactated ringers) may be administered to speed recovery or to replace fluids lost during the procedure. Fluids should be warmed prior to administration.
 - g. Once an animal is ambulatory and returned to its home cage and rack, it should be checked again within 24 hours. Animals should continue to be checked at least once every 24 hours until 72 hours post-operatively.
 - h. A record of surgery for each animal must be kept in a lab notebook for all surgeries and procedures to include but not limited to:
 - i. The animal's condition, including pain, appetite, excretions, body temperature, respiration, or heart rate with the date, time, and observer's initials.
 - j. Reversal agents – A reversal agent is any drug used to reverse the effects of anesthetics. These may be used to wake the animal up faster if it has been asleep for a long time or if the animal has a negative reaction to the anesthetic. Refer to formulary below and consult Attending Veterinarian as needed. The use of reversal agents must be described in the protocol.

B. Classifications of Anesthetics

1. Injectable - Effects of these agents cannot be reversed quickly. The drug must be metabolized, excreted, or counteracted by another drug to terminate anesthetic action. Examples include sodium pentobarbital and ketamine/xylazine cocktail.
2. Inhalants - Effects of these agents can be reversed quickly. The agent is eliminated when the administration is discontinued as the animal exhales. The most common inhalant is isoflurane.

C. Requirements for Use of Anesthesia

1. Always use the anesthetic that is listed in protocol.
2. Calculate the dose by body weight if using an injectable.
3. Drugs under the control of the Drug Enforcement Agency (DEA) must be stored in a locked cabinet in a secure area. (<https://www.ecfr.gov/current/title-21/chapter-II/part-1301/subject-group->

4. A written record is required when drugs under the control of the DEA are used (how much of the drug was used, for what purpose, and how much is remaining).
 5. An inventory list of anesthetics should be kept.
- D. The following listings of anesthetics (and some reversing agents) and the corresponding doses for each species are approved by the IACUC. If another drug not on this list is to be used, it must be described in the animal use protocol for approval by the IACUC.

Drug Formulary by Species

Mouse

Drug	Dose and Route	Frequency	Notes
Inhalation anesthetics			
Isoflurane	1-3% inhalant to effect (up to 5% for induction)	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer. Drop method not acceptable.
Ketamine combinations			
Ketamine-Xylazine-Acepromazine	70-100 mg/kg (K) + 10-20 mg/kg (X) + 2-3 mg/kg (A) SC or IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole or Yohimbine
Ketamine-Medetomidine	50-75 mg/kg (K) + 0.5-1 mg/kg (M) IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole

Ketamine-Xylazine	80-100 mg/kg (K) +5-10 mg/kg (X) IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole or Yohimbine
Ketamine-Midazolam	80-100 mg/kg (K) + 4-5 mg/kg (M) IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures, but may be useful for restraint. If re-dosing, use ¼ to ½ dose of ketamine alone.
Ketamine alone	100-200 mg/kg IP	As needed	Deep sedation, but not surgical anesthesia. Rarely should be used alone. Examples are sedation prior to euthanasia via cervical dislocation or a minor non-invasive procedure.
Reversal agents			
Atipamezole	0.1-1.0 mg/kg subcutaneous or IP	Any time medetomidine or xylazine had been used	More specific for medetomidine than for xylazine
Yohimbine	1.0-2.0 mg/kg SC or IP	For reversal of xylazine effects	
Other injectable anesthetics			
Sodium pentobarbital (Nembutal)	40-50 mg/kg IP	Recommended for acute/ terminal procedures only, with booster doses as needed	Consider supplemental analgesia (opioid or NSAID) for invasive procedures
Tribromoethanol (avertin)	250-500 mg/kg IP	May be used once for survival procedure (re-dosed as needed during procedure, but no separate events) and once for terminal/acute procedure	Diluted Avertin Solution must be used within 30 days of initial preparation and be properly stored. Lower concentration (1.25%) less likely to cause peritonitis.
Propofol	12-26 mg/kg IV	As needed	IV administration only, short acting and requires constant infusion, so therefore limited usefulness in mice. Respiratory depression upon induction is possible.

Rat

Drug	Dose and Route	Frequency	Notes
Inhalation anesthetics			

Isoflurane	1-3% inhalant to effect (up to 5% for induction)	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer. Drop method not acceptable.
Injectable anesthetics			
Ketamine-Xylazine	75-100 mg/kg (K) +5-10 mg/kg (X) IP or IM (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole or Yohimbine
Ketamine-Dexmedetomidine	75-100 mg/kg (K) + 0.15 mg/kg (D) IP or IM (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole.
Sodium Pentobarbital (Nembutal)	40-50 mg/kg IP	As needed	Recommended for acute/terminal procedures. If used for survival surgery, should use supplemental analgesia.
Reversal agents			
Atipamezole	0.1-1.0 mg/kg IM or IP	Any time medetomidine or xylazine had been used	More specific for medetomidine than for xylazine

USDA-Covered Species (Rabbits, Hamsters, Guinea Pigs, etc.)

Anesthesia for USDA-covered species varies widely. Consult Attending Veterinarian before protocol submission.

Drug	Dose and Route	Frequency	Notes
Inhalation anesthetics			
Isoflurane	1-3% inhalant to effect (up to 5% for induction)	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer. Drop method not acceptable.

Sevoflurane	1-3% inhalant to effect (up to 8% for induction)	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer
Ketamine combinations			
Ketamine-Diazepam	6-11 mg/kg (K) + 0.05-0.2 mg/kg (D) IV (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures.
Ketamine-Medetomidine	5-10 mg/kg (K) + 0.6 -1 mg/kg (M) IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole.
Ketamine-Xylazine	50-100 mg/kg (K) +5 mg/kg (X) IM 200 mg/kg (K) + 10 mg/kg (X) IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole. Note that IM Ketamine combinations often sting upon injection.
Ketamine-Midazolam	5-10 mg/kg (K) + 0.1-0.5 mg/kg (M) IM or SC (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures, but may be useful for restraint. If re-dosing, use ¼ to ½ dose of ketamine alone. Note that IM Ketamine combinations often sting upon injection.
Ketamine alone	100 mg/kg IM 200 mg/kg IP	As needed	Deep sedation, but not surgical anesthesia. Should rarely be used alone. Examples are sedation prior to euthanasia via cervical dislocation or a minor non-invasive procedure.
Reversal agents			
Atipamezole	0.1-1.0 mg/kg SC or IP	Any time medetomidine or xylazine had been used	More specific for medetomidine than for xylazine
Other injectable anesthetics			
Sodium pentobarbital (Nembutal)	20-60 mg/kg IP	Recommended for acute/ terminal procedures only, with booster doses as needed	Consider supplemental analgesia (opioid or NSAID) for invasive procedures

Drug	Dose and Route	Frequency	Notes
Inhalation anesthetics			
Isoflurane	1-3% inhalant to effect (up to 5% for induction)	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer. Mask or chamber induction without injected pre-medication may result in breath-holding and injury.
Sevoflurane	1-3% inhalant to effect (up to 8% for induction)	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia.
Nitrous oxide (N2O)	Up to 60% with oxygen	Whenever deep sedation or general anesthesia is required	Not acceptable for surgery as sole agent – must be used with inhalant anesthetic to potentiate effect and lower required dose.
Ketamine combinations			
Ketamine-Xylazine	35-50 mg/kg (K) + 5-10 mg/kg (X) IM or SC (in same syringe or with xylazine administered 10-20 minutes in advance)	As needed	May not produce surgical-plane anesthesia for major procedures. If redosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole or Yohimbine. Note that IM Ketamine combinations often sting upon injection.
Ketamine alone	20-60 mg/kg IM or SC	As needed	Deep sedation, but not surgical anesthesia. Should rarely be used alone. Examples are sedation prior to euthanasia via cervical dislocation or a minor non-invasive procedure like catheter placement. Note that IM Ketamine combinations often sting upon injection.
Ketamine-Medetomidine	35-50 mg/kg (K) + 0.5 mg/kg (M) IM or SC (in same syringe, or with medetomidine administered 10-20 minutes in advance)	As needed	May not produce surgical-plane anesthesia for major procedures. If redosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole. Note that IM Ketamine combinations often sting upon injection.
Ketamine-Xylazine-Acepromazine	35-40 mg/kg (K) + 3-5mg/kg (X) + 0.75-1.0 mg/kg (A) IM or SC (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If redosing, use ¼ to ½ dose of ketamine alone. May be partially reversed with Atipamezole or Yohimbine. Note that IM Ketamine combinations often sting upon injection.

Ketamine-Midazolam	35-50 mg/kg (K) + ~2 mg/kg (M) IM or SC (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures, but may be useful for restraint. If redosing, use ¼ to ½ dose of ketamine alone. Note that IM Ketamine combinations often sting upon injection.
Reversal agents			
Atipamezole	0.1-1.0 mg/kg subcutaneous or IP	Any time medetomidine or xylazine has been used	More specific for medetomidine than for xylazine
Yohimbine	~ 0.2 mg/kg IV or SC	For reversal of xylazine effects	
Other injectable anesthetics			
Sodium pentobarbital (Nembutal)	20-60 mg/kg IV	Recommended for terminal/acute procedures only, re-dose as needed	Consider supplemental analgesia (opioid or NSAID) for invasive procedures. Apnea is common at anesthetic doses.
Propofol	12-26 mg/kg IV	As needed	Only useful IV, so therefore limited usefulness. Respiratory depression upon induction is possible.