UNT DIVISION OF RESEARCH & INNOVATION Research Integrity & Compliance	**Institutional Animal Care and Use Committee** **Standard Operating Procedures**

Title: Standards for Anesthesia

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Approval/Date:

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REVISION HISTORY

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PURPOSE

Appropriate anesthetics must be used in a manner that meets clinical and humane requirements with consideration of the needs of the research and must be justified in a UNT approved animal use protocol. Selection of anesthetic agents and use protocols should reflect professional veterinary judgment and depends on many factors, such as: species, age, the type and degree of pain to the test system involved, the likely effects of particular agents on specific organ systems, the nature and length of the surgical procedure, and the safety of the agents use.

SCOPE

It is the responsibility of the Principal Investigator to ensure use of appropriate anesthetics and administration protocols through consult with the UNT AV (Attending Veterinarian) for information if the PI is unsure. All methods and agents should be described and approved through the IACUC in an Animal Use Protocol prior to their use.

Some anesthetic agents are controlled substances and will require a DEA license. It is the responsibility of the PI to have this license or include an approved collaborator with such.

It is the responsibility of the PI to ensure staff administering anesthetic agents and monitoring anesthetized animals are adequately trained to do so.

DEFINITIONS AND ABBREVIATIONS

UNT- University of North Texas, Denton

IACUC- Institutional Animal Care and Use Committee

SOP/SOP's- Standard Operating Procedure(s)
AUP- Animal Use Protocol
PI- Principal Investigator
USDA- United States Department of Agriculture
LAF- Laboratory Animal Facility
AV- Attending Veterinarian
DEA- Drug Enforcement Administration which monitors use and issues licenses for use of Controlled Substances to researchers.
CRI- Constant Rate Infusion
NSAID- Non-Steroidal Anti-Inflamatory Drugs

PROCEDURES

I. Use of Anesthetic Agents

- **A.** Only use the anesthetic agents and dose ranges that are listed in your approved protocol.
- **B.** Calculate dosages by recent body weight when using an injectable.
- **C.** An inventory list of all anesthetics should be kept.
- **D.** Controlled Agenets
  1. Drugs under the control of the Drug Enforcement Agency (DEA) must be stored in a locked cabinet in a secure area.
  2. A written record is required when controlled drugs under the control of the DEA are used and must include:
     a) how much of the drug you have
     b) when and how much of the drug is used, and for what purpose.

II. Anesthesia Guidelines

- **A.** Fasting may be necessary prior to anesthetic procedures for most mammals to ensure any possible regurgitation during induction does not enter the airway and/or lungs and to prevent bloating in ruminant species.
  1. Rodents and rabbits do not require fasting.
  2. Ruminants are fasted from food for 24- 48hrs and from water for 12-24hrs
  3. Other mammals should be fasted from food for 12 hours prior to anesthetic induction.
- **B.** Body temperature maintenance is important as the body is unable to thermoregulate as normal while under anesthesia.
  1. Loss of core body temperature can lead to hypothermia and decreased blood pressure and cardiac function, and in turn may take longer for animals to recover from anesthetic agent usage as well as cause permanent organ system damage if prolonged.
  2. Blankets and surgical drapes may be sufficient for very short procedures. During longer procedures the use of an external/controlled heat source such as a water circulating heat pad, electric heating pad, warm air circulating blankets, warm IV fluids, etc. may be needed as the body will lose heat rapidly while under anesthesia.
- **C.** Use of anesthetic agents cause a loss of blink reflexes that can cause permanent eye damage and blindness from lack of ocular moisturization. Appropriate eye lubricants and ointments should be used from time of induction through the recovery period.
- **D.** Anesthetic agent administration
  1. Anesthetic agents can be administered via inhalation or injection depending on the agent.
  2. Inhalents may be administered through a calibrated vaporizer with an appropriate

scavenging system.

a) Due to the occupational health and safety risks of using a drop method for isoflurane delivery, the IACUC does not recommend this method and if preferred must be described in an approved Animal Use Protocol with extensive justification.

3. Injectables may be administered through a calculated Constant Rate Infusion (CRI) or single injection that may be repeated as needed during the procedure to maintain an adequate and consistent plane of anesthesia.

E. Monitoring Anesthesia

1. Before any physical manipulations or incisions are made, ensure that the animal is at an adequate plane of anesthesia.
   a) Causing pain or excitement prior to the animal being at a surgical plane can be hazardous and may result in the need for more anesthetic agent to reach that plane (which can adversely affect cardiac function).
2. Failure to maintain a sufficient and consistent plane/depth of anesthesia can cause adverse effects, therefore the animals vitals and reflexes must be monitored throughout procedures.
3. Depth of anesthesia may be evaluated through a combination of observing some of the following (this is not an exhaustive list of means to evaluate surgical plane, but includes the most common methods):
   a) Tail and toe pinch reflexes- acute pressure applied to these sensitive areas should not elicit a reflex at a surgical plane of anesthesia.
   b) Muscular jaw tone- jaw muscles should be loose and easily manipulated at a surgical plane of anesthesia.
   c) Pupillary dilation- blinking and or fixed pupils should not be noted at a surgical plane of anesthesia, pupils should be dilated
   d) Heart rate- should be steady and lower than at time of induction, sudden increases in rate may indicate an indadequate plane of anesthesia depending on procedural manipulations.
   e) Respriations- should be steady and appropriate to maintain SPO2 and CO2. Increased or decreased rate of voluntary respirations that result in changes to SPO2 and/or CO2 may indicate an inadequate plane of anesthesia.
   f) Temperature- should remain steady and appropriate, sudden increases and decreases in body temperatures may indicate an inadequate plane of anesthesia.
   g) Blood pressure (Venous and/or Arterial)- should remain steady and appropriate to maintain organ functions. Increases and decreases in average pressures may indicate an inadequate plane of anesthesia.
4. Anesthetic and Surgery Records
   a) Records for all anesthetic and surgical procedures should be kept to document anesthesia monitoring and should include test system primary vitals.
   b) It is recommended that vitals be evaluated and documented in 5 minute intervals to appropriately identify trends and changes to assess plane of anesthesia.
   c) A Surgery/Post-op Report must be completed for each procedure and each animal. (See IACUC Procedure 02.28 Required Record Keeping)
5. Post Procedure Monitoring- following any anesthetic procedures, methods of monitoring and assisting recovery are required.
   a) Lab staff must stay with the animals until they have recovered from anesthesia:

should be breathing appropriately to maintain oxygen levels without assistance and regained ambulatory control.

b) Body temperature should continue to be maintained. Animals should be provided with an indirect heat source and monitored to ensure they do not get too hot or too cold.

c) Warm fluids may continue to be administered to speed recovery and to replace fluids lost during the procedure.

III. Classifications of Anesthetic Agents

A. Injectables

1. Effects of these agents cannot often be reversed quickly. The drug must be metabolized, excreted or counteracted by reversal drug to terminate anesthetic action.
2. Examples include sodium pentobarbital, Ketamine/Xylazine cocktail.

B. Inhalants

1. Effects of these agents can be reversed quickly when needed. The agent is eliminated when the administration is discontinued and the animal inhales Oxygen/ room air.
2. The most common inhalants are isoflurane and sevoflurane.

C. Dissociative Agents

1. These agents depress the central nervous system and produce a state of catalepsy (ie. Ketamine).
2. Most effective when combined with tranquilizers and sedatives (e.g., Xylazine, diazepam).

IV. Approved Agents

A. The following list of anesthetics (and reversal agents) and the corresponding doses for each species must be considered for use by the Principal Investigator. If another drug or dosage that is not on this list is to be used, the Attending Veterinarian must be consulted prior to inclusion in an Animal Use Protocol.

Mouse

Drug	Dose/ Route	Frequency	Notes
Inhalation anesthetics			
Recommended: Isoflurane or Sevoflurane	1-3% inhalant to effect (up to 5% for induction). Up to 8% for Sevoflurane	Whenever general anesthesia is required	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer
Nitrous oxide (N20)	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			
Recommended: Ketamine-Xylazine-Acepromazine	70-100 mg/kg (K) +10-20 mg/kg (X) + 2-3 mg/kg (A) (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ketamine alone. May be partially reversed with Atipamezole or Yohimbine
Ketamine-Medetomidine	50-75 mg/kg + 0.5-1 mg/kg IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ketamine alone. May be partially reversed with Atipamezole
Ketamine-Xylazine	80-100 mg/kg +5-10 mg/kg IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ketamine alone. May be partially reversed with Atipamezole or Yohimbine

Ketamine-Midazolam	80-100 mg/kg + 4-5 mg/kg IP (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures, but may be useful for restraint.
Ketamine alone	100-200 mg/kg IP	As needed	Deep sedation, but not surgical anesthesia. Should not often use alone.
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 (as a general rule, Atipamezole is dosed at the same volume as Medetomidine, though they are manufactured at different concentrations.)
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 (boosted as necessary during procedure) 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. As this is a non-pharmaceutical substance, scientific justification is needed for use in survival surgeries. *see preparation protocol below
Propofol	12-26 mg/kg IV	As needed	Only useful IV, so therefore limited usefulness in mice. Respiratory depression upon induction is possible.

***Avertin Preparation**

Using 100% stock avertin:

Mix by adding Tribromoethanol to Tertiary Amyl Alcohol and dissolve by heating and stirring. Add distilled water and continue until the solution is well mixed.

Store out of light, either wrapped in foil in an amber glass bottle at 4° C.

Solution may have to be warmed to dissolve. Mixture should be clear before use.

- Warning! Decomposition can result from improper storage.
- 2.5% Diluted Avertin Solution should have ph= 7.4 and must be used within 30 days of initial preparation and be properly stored. Be sure to label the container with the date of preparation.
- For use in mice, dilute the 100% to 2.5% (1:40) using diluent, water or isotonic saline.
- Dosages for mice may vary with different preparations of Avertin. Dosage should be redetermined each time a 100% stock is made up. Test for best effect in a few mice before choosing dose. Allow 5-10 min to take effect.

Rat

Drug	Dose/ 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

Injectable anesthetics			
Recommended: Ketamine-Xylazine	75-100 mg/kg +5-10 mg/kg IP or IM (in same syringe)	As needed	
Ketamine-Dexmedetomidine	75-100 mg/kg + 0.15 mg/kg IP or IM (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If re-dosing, use ketamine alone. May be partially reversed with Atipamezole
Sodium Pentobarbital (Nembutal)	40-50 mg/kg IP		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 (as a general rule, Atipamezole is dosed at the same volume as Medetomidine, though they are manufactured at different concentrations.)

Rabbit

Drug	Dose/ Route	Frequency	Notes
Inhalation anesthetics			
Recommended: Isoflurane or Sevoflurane	1-3% inhalant to effect (up to 5% for induction). Up to 8% for Sevoflurane	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.
Nitrous oxide (N20)	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			
Recommended: Ketamine-Xylazine	35-50 mg/kg + 5-10 mg/kg 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 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 not be often used alone. Note that IM Ketamine combinations often sting upon injection.
Ketamine-Medetomidine	35-50 mg/kg + 0.5 mg/kg 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 ketamine alone. May be partially reversed with Atipamezole . Note that IM Ketamine combinations often sting upon injection.
Ketamine-Xylazine-Acepromazine	35-40 mg/kg + 3-5mg/kg + 0.75-1.0 mg/kg IM or SC (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures. If redosing, use 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 + ˜2 mg/kg IM or SC (in same syringe)	As needed	May not produce surgical-plane anesthesia for major procedures, but may be useful for restraint. 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 (as a general rule, Atipamezole is dosed at the same volume as Medetomidine, though they are manufactured at different concentrations).
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, with booster doses 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.

REFERENCES

1. The Guide for the Care and Use of Laboratory Animals.
2. Animal Welfare Act
3. UNT IACUC Procedure 02.28 Required Record Keeping

APPENDICES

IACUC Standard Operating Procedures