



GUIDELINES FOR ANESTHESIA AND DRUG FORMULARIES

Anesthesia is the act of rendering the animal senseless to pain or discomfort and is required for surgical and other procedures.

Criteria for choosing an anesthetic agent: Many variables should be taken into account when a decision is made on the anesthetic protocol that will best suit the needs of the animal and the study. These include but are not limited to:

- Strain, breed, age, weight, and health of the animal
- Properties, mode of action, and duration of action of the anesthesia products
- Available equipment
- Recommendations from veterinary staff

The *Guide* states that FDA approved reagents must be used if available, unless compelling scientific justification is provided and approved by the IACUC. In addition, all anesthetic agents must be used before their expiration date with no exceptions (survival as well as non-survival procedures). In many cases, isoflurane is encouraged as the first choice anesthetic for surgery in rodents. Isoflurane has minimal cardiovascular adverse effects, and induction and recovery are typically rapid. It should be delivered as a known percentage (1-3% for maintenance; up to 5% for induction) in oxygen from a calibrated precision vaporizer. Scavenging of waste anesthetic gases is required when using this agent. An individual laboratory may have their own isoflurane vaporizers and several vaporizers are available for use in the Biological Resource Facility.

Handling and Restraint: Proper handling and restraint of the animal prevents injury and minimizes stress, both for the animals and the staff. In some cases, pre-medicating the animal with a tranquilizing drug may be useful in minimizing stress in the animals. This is particularly true for larger species.

Monitoring: All anesthetized animals must be constantly attended and monitored continually to assess whether the level of anesthesia is sufficient. Certain methods are fairly common between species. These include:

- Toe pinch: A gentle pinch, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal is too light. Any observed movement (withdrawing the paw, increase in heart or respiratory rate) indicates that the animal is not sufficiently anesthetized to do surgery.
- Jaw "tone": Generally a good indicator of muscle relaxation. The lower jaw is gently opened to its maximum extent. Any observed closing of the mouth or resistance to opening is an indicator that the animal is too light to do surgery.
- Respiratory rate: Good indicator of depth of anesthesia. Rapid, shallow respirations usually indicate the animal is too "light". Note that normal respiration rates vary among species.
- Heart rate: An increase in heart rate and/or blood pressure usually indicates a decrease in anesthetic depth. Normal heart rates vary greatly among species.
- Palpebral and corneal reflexes: These reflexes are lost in succession as anesthesia depth increases. In an ideal plane of anesthesia, the palpebral reflex should be abolished, but the corneal should be obtunded but not lost. To assess the palpebral reflex, gently touch the inner corner of the eye. There should be no response (no blink). To assess the corneal reflex, very gently touch the edge of the cornea with a gauge sponge or cotton q-tip. Movement of the eyelids in response to either test is an indication that the depth of anesthesia is not sufficient to do surgery. Care should be taken when assessing the corneal reflex, as damage to the cornea can result and thus this reflex should NOT be used routinely.

Body Temperature: Most anesthetic agents depress body temperature to a significant degree. Therefore, it is important that anesthetized animals be maintained on a surface that shields them from contact with cold surfaces and serves to minimize body heat loss. The use of a supplemental heat source is a good idea, but must be used with caution, since burns can occur from electric blankets, heat lamps, heat pads or water bottles that are too hot. Circulating hot water blankets or pads are ideal for use as a surgical or recovery table. The BRF has several available to use throughout the facility in procedure spaces.

Recovery: All animals recovering from anesthesia must be constantly attended until they have fully recovered. As a general statement, animals must be observed and the observation must be recorded at least every 15 minutes until the animal regains motor control. In the case of most animals, this is usually indicated by the animal starting to move around the cage and being able to stand and walk without falling. Observations may be recorded in a research notebook or a medical record notebook, but should be available for IACUC review.

Animals that have had any significant blood/fluid loss during surgery should be provided with fluid or blood replacement during surgery and/or the anesthesia recovery period. In small rodents, this is best accomplished via the intraperitoneal or subcutaneous route. In addition, special hydrating gel food tins can be provided during the first few days after surgery.

FORMULARIES FOR ANESTHESIA BY SPECIES

Abbreviations used: *IH:* Inhaled agent; *IP:* Intraperitoneal injection; *SC:* subcutaneous injection; *IV:* intravenous injection; *IM:* intramuscular injection; *CS:* **controlled substance** regulated by the Drug Enforcement Agency. *Special licensing required to procure; appropriate record keeping logs required.*

MICE

Inhalant Agents - IH

Drug Name	DOSE (%) for IH route	FREQUENCY	NOTES
Isoflurane	1-3% for maintenance 5% for induction	Whenever general anesthesia is required	Must use precision vaporizer. Survival surgery requires concurrent preemptive analgesia.
Nitrous oxide (N ₂ O)	Up to 60% with oxygen	For deep sedation	<i>Not acceptable as sole agent for surgery</i> – used with an inhalant anesthetic to potentiate effect and to lower required anesthetic dose

Injectable Agents

Drug Name	DOSE (mg/kg) and Route	FREQUENCY	NOTES
Ketamine (CS) + Xylazine + Acepromazine	(100)+ (2.5) +(2.5) (in same syringe) IP	Duration ~20-40 min	May not produce sufficient surgical-plane anesthesia for major procedures. If redosing, use ketamine alone.
Ketamine (CS) + Xylazine	(90-150) + (5-10) (in same syringe) IP <i>SC also effective</i>	Duration ~20-40 min	May not produce sufficient surgical-plane anesthesia for major procedures. If redosing, use ketamine alone.

Ketamine (CS) + Medetomidine	(50-75) + (0.5) (in same syringe) IP	Duration ~30-40 min	May not produce sufficient surgical-plane anesthesia for major procedures. For redosing, use ketamine alone.
Sodium pentobarbital (CS) (Nembutal)	60-90 IP	For general anesthesia. Use booster doses as needed. ~20-60 min duration	Recommended for terminal/acute procedures and most survival procedures. Has no analgesic properties.

RAT

Inhalant Agents - IH

Drug Name	DOSE (%) for IH Route	FREQUENCY	NOTES
Isoflurane	1-3% for maintenance 5% for induction	Whenever general anesthesia is required	Must use precision vaporizer. Survival surgery requires concurrent preemptive analgesia
Nitrous oxide (N ₂ O)	Up to 60% with oxygen	Whenever deep sedation is needed	<i>Not acceptable as sole agent for surgery</i> – used with an inhalant anesthetic to potentiate effect and to lower required anesthetic dose

Injectable Agents

Drug Name	DOSE (mg/kg) and Route	FREQUENCY	NOTES
Ketamine (CS) +Xylazine	(40-80) + (5-10) (in same syringe) IM	~20-30 min duration	May not produce sufficient surgical-plane anesthesia for major procedures. If redosing, use ketamine alone.
Ketamine (CS) + Xylazine + Acepromazine	(40) + (8) + (4) (in same syringe) IM	~20-40 min	May not produce sufficient surgical-plane anesthesia for major procedures. If redosing, use ketamine alone.
Ketamine (CS) +Xylazine	(80-100) +(10-20) (in same syringe) IP	~20-40 min duration	Sufficient for major surgeries. If redosing, use ketamine alone.
Sodium pentobarbital (Nembutal) (CS)	50-80 IP	For general anesthesia. ~20-60 min duration Use booster doses as needed.	Recommended for terminal/acute and most survival procedures. No analgesic properties.

SWINE

Inhalant Agents - IH

Drug Name	DOSE (%) and Route - IH	FREQUENCY	NOTES
Isoflurane or Sevoflurane	1-3% inhalant to effect (up to 5% for induction). Up to 8% for Sevoflurane	Whenever general anesthesia is required	Must use precision vaporizer. Mask induction possible with neonatal pigs. Survival surgery requires preemptive analgesia
Nitrous oxide (N ₂ O)	Up to 60% with oxygen	Whenever deep sedation is required	<i>Not acceptable for surgery as sole agent</i> – used with inhalant anesthetic to potentiate effect and lower required dose of anesthetic

Sedation and Pre-anesthesia

Ketamine (CS) + Xylazine	(15-20) + (1.1-2.2) (in same syringe) IM	For sedation or pre-anesthesia (~15 min) Useful for endotracheal intubation	May require large volumes – consider using Telazol® or Telazol® combination as alternative
Telazol® (CS) (tiletamine with zolazepam). When reconstituted with 5 ml sterile water, vial contains 50 mg/ml of each agent.	6 – 8 mg/kg IM (When reconstituted, dosing is .06 - .08 ml/kg based on 100 mg/ml of combined active ingredients)	For sedation or pre-anesthesia (~15 min)	Note that Telazol® must be refrigerated once reconstituted.
Xylazine + Telazol® (CS)	(2.2) + (4.4) IM	For sedation or pre-anesthesia (20-30 min)	Must be stored refrigerated once reconstituted
Ketamine (CS) + acepromazine	(33) + (1.1) IM	For short procedures or preanesthesia	
Xylazine + Telazol (CS) + butorphenol (CS)	2.2 + 4.4 mg/kg + 0.22 mg/kg IM		
Propofol	4-6 mg/kg IV bolus	As preanesthetic to facilitate intubation; requires sedation beforehand	Causes respiratory apnea if injected too rapidly; rapid recovery

Injectable Agents

Sodium pentobarbital (Nembutal) (CS)	20 - 40 single or intermittent IV bolus (subsequent continuous infusion at 5-40 mg/kg/hr)	For general anesthesia as needed; requires preanesthetic or sedative beforehand to facilitate IV catheterization	Initial bolus will induce apnea and prompt intubation should follow. No analgesic properties; consider supplemental analgesia (opioid or NSAID) for invasive procedures.
Propofol	0.83-1.66 IV bolus (followed by continuous infusion of 14-20 mg/kg/hr)	Requires prior sedation to facilitate IV injection	Initial bolus can cause apnea if injected too rapidly; Ultrashort acting, rapid recovery, monitoring critical.

RABBITS

Inhalant Agents - IH

Drug Name	DOSE (%) and Route - IH	FREQUENCY	NOTES
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.

Sedation and Pre-anesthesia

Ketamine (CS)	50 IM, SC	Sedation alone	Lasts about 30 minutes, no analgesia
Ketamine (CS) + Acepromazine	(25-30) + (1) IM, SC	Mild sedation,	Facilitates intubation
Xylazine	3 IM, SC	Heavy sedation	Some analgesic properties
Medetomidine	0.25 SC	Sedation	Can be reversed with atipamezole (0.50).
Acepromazine	1 IM, SC	Moderate sedation	Hypotensive
Diazepam or midazolam (CS)	1-2 IV, IM	Good sedation	

Injectable Agents

Ketamine (CS) + Acepromazine	(35- 40) + (5) IM, SC	Surgical anesthesia	Acepromazine vasodilates, facilitating IV injections; lasts about 30 minutes
Ketamine (CS) + medetomidine	(35) + (0.5) IM, SC	Surgical anesthesia	
Propofol	10 IV	Surgical anesthesia	Short duration, causes apnea on rapid injection, IV injection requires prior sedation, useful prior to gas anesthesia

XENOPUS (FROG)

Drug Name	DOSE	ROUTE	NOTES
Finquel® (Tricaine methanesulfonate; MS-222) (buffer with NaHCO ₃ ; pH 7-7.5)	500-2000 mg/L Bath	Immersion in shallow liquid (avoid drowning) Induction in 10-30 minutes; maintained by skin contact with moist cloth soaked with anesthetic solution.	Amphibians must be kept moist over their entire bodies during anesthesia and recovery. Do not fully immerse as this may result in drowning. Recovery may take 3-6 hrs. Chemical Hazard – use appropriate PPE and disposal.

OTHER:

- This formulary is not an exhaustive list of useful anesthetic agents. Other agents may be available and investigators are encouraged to contact the veterinarians for consultation.
- For *Non-Survival* surgical procedures, anesthetic agents besides those listed above may be useful. For example, some investigators find that chloral hydrate and urethane provide long-term anesthesia for rodents in non-survival neurophysiological recording studies. When such agents are nonpharmaceutical grade, scientific justification and appropriate considerations for obtaining sterility as well as safe handling must be provided in the protocol application to the IACUC. The investigator should consult IACUC guidelines on using non-pharmaceutical grade agents as well as the veterinarian for appropriate dosing and other considerations. In addition, the investigator must be cognizant of expiration dates for such materials and not use for anesthesia after expiration.

References

- CITIprogram.org: Working with Swine in Research Settings (*requires registration*).
- Hawk, CT et. al. 2005. *Formulary for Laboratory Animals, 3rd Ed.* Blackwell Publishing.
- Levin-Arama, M. et al. 2016. Subcutaneous compared with intraperitoneal ketamine-xylazine for anesthesia of mice. *JAALAS* 55(6): 794-800.
- Wolfman, S. 1994. *Handbook of Laboratory Animal Management and Welfare*, Oxford Press.