### PURPOSE

This Standard Operating Procedure is intended to provide information on correctly monitoring and recovering animals from anesthesia. You must have prior approval from the IACUC before doing any procedure involving vertebrate animals at CU Boulder.

### BACKGROUND

The following is based on principles described in the *Guide for the Care and Use of Laboratory Animals* and the NIH Guidelines for anesthetic depth assessment, correctly monitoring anesthetic depth and recovering animals for anesthesia.

### PROCEDURES

- Administer anesthetic agent using appropriate techniques and equipment.
- Administration of anesthetics can be via injectable or inhalation.
- Only pharmaceutical grade anesthetics should be used unless there is a scientific justification for using another agent that includes why pharmaceutical grade agents cannot be used in that particular case.
- With inhalant anesthesia, use a properly maintained, annually calibrated vaporizer for anesthetic agent delivery.
- Administer analgesics at induction of anesthesia so that they are at appropriate blood levels when the procedure is completed. If the procedure is survival and the animal will be sedated longer than 5 minutes, apply sterile pharmaceutical or vet grade ophthalmic ointment to the eyes to prevent ulceration.

#### Assessment of depth of anesthesia

- Pedal response – most common method to assess for depth of anesthesia in rodents.
  - Give a firm pinch to both a fore limb paw and hind limb paw to make sure the anesthesia is effective from head to toe of the animal.
- Other forms of monitoring for anesthetic depth that may be used (as appropriate) include
  - Heart rate
  - Respiratory rate
  - Blood pressure
  - Electroencephalography (EEG)
  - Capnography

#### Intraoperative monitoring

- Do not leave an animal unattended while under anesthesia for any length of time.
- Animals must be monitored at least every 5 minutes while under anesthesia.
- Monitoring includes the routine evaluation of anesthetic depth and physiologic functions and conditions.
- What is monitored, and documented, will vary based on the species and procedure being performed.
- In rodents, the most common methods are toe pinch (for anesthetic depth) and respiration (both pattern and depth).
- Other monitoring parameters may include body temperature, cardiac rates, EEG, capnography, and blood pressure.
- A general guideline is to monitor the animals approximately every 5 minutes.
- Parameters that will be monitored should be outlined in the IACUC protocol.
- Documentation of the anesthetic event (induction time, dose of anesthesia, general summary of procedure, recovery, and any unusual occurrences) should be performed in an appropriate location (i.e. lab notebook, anesthesia form, cage cards). Please consult with the veterinarian during the development of your protocol on what should be documented.
- Anesthesia should be appropriate for the duration and type of surgery being performed.
  - When using a gas anesthetic, it should be noted that these agents do not provide residual analgesia once the animal recovers and therefore proper pain relief must be considered.
When using injectable agents, in most cases a cocktail is used in order to achieve anesthesia. Some of these cocktails provide pain relief.

- Please consult with the veterinarian when developing your protocol to determine the most appropriate anesthetic and analgesic for your specific surgical technique.

- Maintenance of normal body temperature minimizes cardiovascular and respiratory disturbances caused by anesthetic agents and is of particular importance in small animals where the high ratio of surface area to body weight may easily lead to hypothermia.
- A direct temperature controlled heating pad should be used intraoperatively for all procedures greater than 5 minutes.

- Fluid (warmed) replacement is generally a necessary component of intraoperative therapy depending on the duration and nature of the procedure.
  - It is highly recommended that for surgeries that last for more than 20 minutes or that might have a significant amount of blood loss that subcutaneous fluids be provided to assist with recovery. The amount of fluids will vary based on the species involved and procedure being performed, please consult with the veterinary staff during the preparation of your protocol.
  - As a general guideline, most animals will receive a one-time subcutaneous injection of approximately 20-40 ml/kg of saline or lactated Ringers solution (generally 0.5-1 ml for a 25 gram mouse and 5-10 ml for a 250 gram rat).
  - If you are performing surgeries where the animal is under anesthesia longer than one hour, repeated fluid dosing may be necessary.
  - Please consult with the veterinarian in the development of the surgical protocol to determine the optimal volume to provide for your specific procedure.

- For aquatic species (including amphibians), care should be taken to keep the skin surfaces moist and minimize drying during surgical procedures.

### Postoperative care

- Animals MUST be monitored at least every 15 minutes until they are able to maintain sternal recumbency.
- The intensity of monitoring will vary with the species and the procedure and may be greater during the immediate anesthetic recovery period.
- Animals should be in a clean, dry, warm (i.e. in a cage halfway on a direct temperature controlled warming blanket so they have a way to escape the heat if needed) and comfortable area where they can be observed frequently by trained personnel.
- Particular attention should be given to thermoregulation, cardiovascular and respiratory function, electrolyte and fluid balance, and management of postoperative pain or discomfort.
- Additional care may be warranted, including long-term administration of parenteral fluids, analgesics, and other drugs, as well as care of surgical incisions.
- Appropriate documentation of procedures must be performed to include the type of procedure, anesthetics analgesics used and the time that they were provided, including the dose, and also the postoperative observations.
- Animals should not be left alone or returned to their housing rooms until fully recovered. An animal is considered recovered when the righting reflex is restored.
- After recovery from anesthesia, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and to behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision site for dehiscence, bandaging as appropriate, and timely removal of skin sutures, clips, or staples.

### REFERENCES


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i From the transcript of an OLAW webinar “Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals”

Agents for sedation, analgesia, or anesthesia should be veterinary or human pharmaceutical-grade compounds, when available, unless the use of a non-pharmaceutical chemical or formulation is scientifically necessary, appropriately justified and approved by the IACUC. The use of a non-pharmaceutical-grade euthanasia agent must meet the same standards. If no equivalent veterinary or human drug is available for experimental use, then the highest-grade equivalent chemical reagent should be used and formulated aseptically and with a non-toxic vehicle as appropriate for the route of administration. Recent exorbitant cost increases of pentobarbital have placed it logistically into the unavailable category. Pentobarbital from a reagent or analytical-grade powder, properly prepared by a pharmacist or other knowledgeable individual (e.g., chemist, veterinarian, researcher), with assurance of appropriate storage and handling, and approval by the IACUC is acceptable. IACUC approval can be institution-wide for the drug prepared in this fashion for all approved users.

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