GUIDELINES FOR ANESTHESIA AND ANALGESIA IN LABORATORY ANIMALS

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1. Background

Federal regulations and guidelines mandate that animals undergoing potentially painful procedures be provided with adequate anesthesia and analgesia. In addition, for these procedures, a veterinarian must be consulted regarding the appropriate anesthetic and analgesic agent and dose for the species being used and the procedure being performed. The standard of care is to prevent animal pain whenever possible and to treat animal pain whenever diagnosed. Exceptions to these principles are permitted only if scientific justification is provided in the Animal Use Protocol (AUP) and approved by the Animal Care and Use Committee (ACUC).

2. Training

All personnel who perform anesthesia must be appropriately trained. The Principal Investigator (PI) is responsible for assuring that research personnel receive appropriate training and certification prior to performing any procedure. New anesthetists are trained and supervised by the PI, or appropriate designated personnel, until they are competent to perform the procedure independently (please refer to ACUC Policy on Training and Education and ACUC Post-Approval Monitoring Guidelines). All new anesthetists, including PIs, must be observed by the Office of Laboratory Animal Care (OLAC) Veterinary staff or the OLAC Trainer and the individual's competency certified to the ACUC prior to working independently. The OLAC Veterinary staff or OLAC Trainer is available to provide assistance with, or training in, aseptic technique and the proper administration of anesthesia, analgesia, and euthanasia.

3. Anesthetics

- Inhalant anesthetics (e.g., isoflurane) – Delivery of inhaled anesthetics by mask or endotracheal tube via a precision vaporizer is recommended for all non-aquatic species. Adjusting the inhaled percentage of anesthetic gas to deepen anesthesia is far safer than repeated re-dosing of injected drugs. Volatile anesthetics are easier to decrease as well, even compared to drugs for which there is an injectable antagonist or reversal agent. A disadvantage of the inhalant anesthetic agents is the lack of residual analgesia once the vaporizer has been turned off; pre-emptive analgesia is necessary. Visit the OLAC website at www.olac.berkeley.edu for information regarding vaporizer and scavenger availability and training.
    - Isoflurane via the open drop jar method
      The open drop jar method is imprecise and can only be used for short-term procedures (30-60 seconds) in small rodents and birds. This method contains no provisions for scavenging of anesthetic waste and therefore must be performed under a fume hood to protect personnel. Please reference the SOP below (Appendix A) for additional guidance on method and materials needed to perform this technique.
- Injectable anesthetics (e.g., ketamine combinations, dexmedetomidine) – Injectable anesthetics are appropriate for many procedures. There is, however, a great deal of variation in depth and duration of anesthesia among rodent strains and individual animals.
- Immersion anesthetics (i.e., buffered MS-222) – Immersion anesthetics are appropriate for aquatic species, such as amphibians and fish. Different solution strengths may be appropriate for induction and maintenance of anesthesia.
- Local anesthetics (i.e., lidocaine, bupivacaine) – Local anesthetics are are considered adjuncts to either inhalant or injectable anesthetics, provide additional analgesic coverage, and aid in preventing “windup phenomenon”.
- Cryoanesthesia - Only for mice up to 7 days of age. Mouse pup must not be placed in direct contact with ice-water slurry.
- Please visit the OLAC website for appropriate dosages and routes of administration by drug for some common laboratory animals.
- Dilutions and mixtures of drugs may change the chemical composition of a drug and could alter its shelf life. For this reason, even if it is earlier than the manufacturer’s drug expiration date, all dilutions or mixtures made from a drug must be discarded after one month.

4. Analgesics

For the use of any analgesic agent, OLAC veterinarians should be consulted regarding the appropriate agent and dose for the species being used and the procedure being performed.

- Opioids (i.e., buprenorphine, morphine) – Opioids are very effective analgesics for surgical pain but may have effects on cardiovascular and respiratory function, intestinal motility, and can be sedating.
- Non-steroidal anti-inflammatory agents (i.e., meloxicam, carprofen, ketoprofen) – Newer, longer-lasting non-steroidal anti-inflammatory analgesics (NSAIDs) may have longer durations of action than available opioids. These drugs are frequently co-administered with an opioid to combine potency of effect with duration of action.
- Please visit the OLAC website for appropriate dosages and routes and frequency of administration by drug for some common laboratory animals.
- Dilutions and mixtures of drugs may change the chemical composition of a drug and could alter its shelf life. For this reason, all dilutions or mixtures made from a drug must be discarded after one month from the date of preparation unless a different shelf-life is specified by the manufacturer.

5. Best Practices

The following issues must be considered in developing a protocol for anesthesia and analgesia.

- Multi-modal drug administration – Using a combination of agents of analgesic agents that have different mechanisms of action is recommended. This allows for lower doses of each drug while minimizing the side effects that may occur when using a single agent.
- Pre-emptive analgesia – Pre-emptive analgesia or administration of pain relief *before* the painful stimulus is recommended and is thought to prevent the "wind-up phenomenon[1]":
    - To ensure that pain is being treated as the general anesthetic is wearing off;
    - To lower the overall amount of general anesthetic required;
    - To allow for improved cardiovascular stability
- Frequency of analgesic administration – Scheduling of analgesic doses and frequencies should be carefully planned with consideration of the duration of action required to prevent the need for late night or early morning dosing. For example, many analgesics administered at 5 pm will be ineffective before 8 am the next morning. Multimodal analgesia is recommended to combine potency of effect with duration of action. Animals should be checked when the last dose of analgesic is expected to fall below therapeutic levels for signs of pain or distress. If animals

[1] Pain wind-up is a phenomenon of increased central pain sensitization in which repeated painful stimulation of peripheral nerves increases the strength of pain signal reaching the brain. This process leads to increased pain in which less stimulation is needed to increase pain response to otherwise nonpainful stimuli and spontaneous pain.

exhibit signs of pain or distress, the OLAC veterinary staff must be consulted and a protocol amendment submitted if necessary.

- Supportive care – Non-pharmaceutical methods to enhance the administration of anesthetic and analgesic agents should be used and include:
    - Keeping the animal warm during and after anesthetic procedures (acceptable sources of ancillary heat include: circulating warm water or warm air blanket, infrared warming pad, chemical warming pads, thermal gel packs)
    - Fluid administration
    - Keeping recovering animals isolated in a quiet area
    - Providing supplemental foods

  Contact the veterinary staff for additional information on supportive care.

6. Monitoring

- Animals must never be left unattended during anesthesia or while recovering from anesthesia.
- The depth of anesthesia should be monitored continuously and parameters recorded at least every 15 minutes during all procedures.
- Plans for intra- and post-operative monitoring must be included in the AUP. Monitoring of respiratory rate and character is facilitated by the use of transparent drapes. Monitoring anesthesia includes: assessing the animal's responsiveness to painful stimuli, character of respiration, and color of the ears, tail, mucous membranes, or foot pads, and adjusting anesthetic depth as needed. Withdrawal reflex (toe, tail or fin pinch) is recommended for assuring adequate depth of anesthesia prior to first incision and as a repeated check throughout the procedure.
- Depending on the procedure and animal species, other monitoring may be indicated such as heart rate, blood pressure, body temperature, and tissue oxygenation. Monitoring should be recorded through the post-operative period to complete recovery.
- Dose ranges and titration – All drugs, dose ranges, and routes of administration must be listed in the AUP. Dose ranges are starting points which must be titrated up or down for the individual animal, or for the particular application (procedures conducted, animal age and strain differences). When experience indicates that the recommended dose range is consistently too high or too low for the particular application, the veterinarian should be informed, and a protocol amendment submitted to the ACUC. Anesthetics are always titrated to effect. It is not acceptable to conduct surgical procedures unless the animal is fully anesthetized.

7. Recordkeeping

- Administration of anesthesia and analgesia and peri-operative monitoring must be recorded. Depending on the species, records may be kept in the animal's individual medical record, or in laboratory records and on blue post-operative cage cards provided by OLAC.
- Please refer to ACUC Guidelines on Recordkeeping for Surgical Procedures on Laboratory Animals for additional information.

8. Controlled Substances

- Several commonly used anesthetics and analgesics (i.e., opioids, ketamine) are controlled substances and require special authorization and procedures to be completed prior to use in animal research.
- More information can be found at the Office of Environment, Health & Safety (EH&S) website (https://www.ehs.berkeley.edu/controlled-substances)
- Once they are obtained, controlled substances, including any dilutions made, carry special storage and record keeping requirements.

9. References

- Caro CC et al (2013). J Am Assoc Lab Anim Sci. Sep; 52(5): 577–583.
- Carpenter, J.W. (2005). *Exotic Animal Formulary*. (3rd Ed.). Philadelphia, PA: Elsevier Saunders.
- Hawk, C.T., Leary, S., & Morris, T. (2005). *Formulary for Laboratory Animals*. (3rd Ed.). Ames, Iowa: Blackwell Publishing (https://www.upstate.edu/iacuc/pdf/Formulary_for_Lab_Animals_3rd_ed.pdf)
- Principles and Practice of Veterinary Technology, Fourth Edition: Margi Sirois (2017)

Call 3-VETS if there is an animal emergency (510-643-8387)

APPENDIX A
Isoflurane Bell Jar/Open Drop Jar Method SOP

Justification for the use of bell jar/open drop jar anesthesia:

Please note the bell jar/ open drop jar method for administering isoflurane is imprecise and can only be used for short-term procedures (30-60 seconds) in small rodents and birds. A vaporizer must be used for procedures of longer duration.
The method contains no provisions for scavenging of anesthetic waste and therefore must be performed under a fume hood to protect personnel.

Materials needed:

Fume hood
Bell jar or other glass container of known volume with tightly fitting lid
Mesh platform (plastic or woven wire)
Cotton balls or gauze squares
Conical tube(s)
Isoflurane
Propylene glycol (1,2-Propanediol USP grade): For use in recovery procedures only
Recovery cage with supplemental heat source

Method:

Induction-bell jar:
Working inside a fume hood, soak a cotton ball or gauze with the appropriate amount of isoflurane (*see chart below for concentration guidelines*).

Cover the isoflurane/propylene glycol soaked cotton ball (or gauze) with a mesh platform (*Figure 1*). This is to prevent the rodent from coming in direct contact with the anesthetic agent. Place one animal at a time in the jar (*Figure 2*) and close the lid tightly (*Figure 3*).

Monitor the animal closely while in the jar with a focus on respiration rate. Once the animal has lost the righting reflex and breathing has slowed, remove the animal from the bell jar. Check the color of the mucous membranes, rate of respiration, and withdrawal reflexes. The procedure may begin if there are no reflexes but the mucus membranes and respiration appear normal.

Induction-conical tube:
Place the cotton ball or gauze in the conical tube at the end as to avoid direct contact with the animal (*Figure 4*). Then, in the fume hood, wet a cotton ball or gauze with the isoflurane/propylene glycol mixture.

After anesthesia induction of animal in the bell jar, place the animal's nose in the conical tube. Make sure the nose of the animal remains close to the terminal portion of the conical tube. Do not put the entire face in the nose cone; there should be space for air to move around the animal's face.

When the procedure is complete, place the animal in the recovery cage or euthanize as outlined in the approved AUP.

Clean the bell jar before the next use.

Figures 1-4

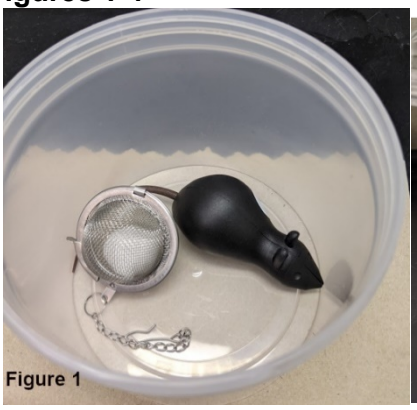
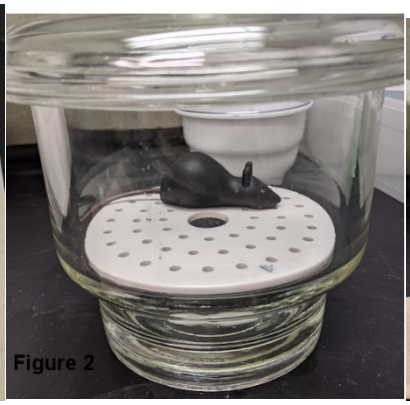


Figure 1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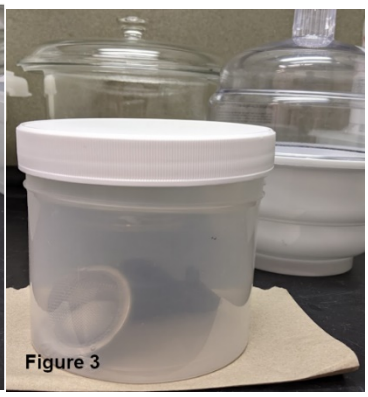
Figure 3


Figure 4

Anesthetic Chamber Guidelines:

Concentration of Isoflurane (%)	Internal Volume of Anesthetic Chamber				
	1L	2L	3L	4L	5L
1	0.05	0.10	0.15	0.20	0.26
2	0.10	0.20	0.31	0.41	0.51
3	0.15	0.31	0.46	0.61	0.77
4	0.20	0.41	0.61	0.82	1.02

5	0.26	0.51	0.77	1.02	1.28

Volumes in the shaded area are in mL and indicate the volume of isoflurane to be applied to a cotton swab or gauze in the bell jar.
From: *"Anesthesia and Analgesia in Laboratory Animals" 2nd edition pg. 86*

REFERENCES:

- https://iacuc.wsu.edu/documents/2016/06/wsu_sop_3.pdf/
- Anesthesia and Analgesia in Laboratory Animals 2nd edition pg. 86