

	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL	POLICY 34.05 Approval Date: 3.17.10 Revision: 2.1.11 Revision: 11.12.13 Revision: 3.16.17 Revision: 2.20.19
	Anesthesia	

I. Background and Purpose

Anesthesia is an integral part of many animal research and teaching protocols including but not limited to restraint, potentially painful and or distressing procedures, and surgery. The appropriate use of anesthetics in animals is required by federal regulations.

II. Who Should Read This Policy

All personnel engaged in or responsible for the administration of anesthetics to animals.

III. Definitions

Term	Definition
Expired drug	For drugs with an expiration date including month, day and year: the drug becomes expired one day after the expiration date. For drugs with expiration dates that include only month and year: the drug becomes expired on the first day of the next month.
Pharmaceutical grade compound	Any active or inactive drug, biologic, reagent, etc., manufactured under Good Manufacturing Practices (GMP) which is approved, conditionally approved, or indexed by the FDA or for which a chemical purity standard has been written or established by a recognized compendium (e.g., USP-NF, BP). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy.

IV. Policy

The following rules apply to the use of anesthetics in animal research and teaching unless a scientifically justified exception has been submitted to and approved by the IACUC.

1. The anesthetic regimen must alleviate pain and distress and be appropriate for the procedure and species.

2. The anesthetic regimen must be described in the applicable IACUC approved animal use protocol.
3. Anesthetic agents must not be used beyond their expiration date.
4. Anesthetic agents must be pharmaceutical grade products.
5. If anesthetics are not held within their original containers, they must be labeled with concentration (e.g., mg/ml) and expiration dates for each ingredient, including diluents.
6. Animals must be prevented from coming into physical contact with liquid form of inhalant anesthetics.
7. Animals must not be left unattended from the time of anesthetic induction until the animal is fully conscious, physiologically stable and able to maintain awake posture or positioning as appropriate for the species. Qualified individuals must closely monitor them during this time.
8. Storage and handling of anesthetic agents must be done in compliance with all applicable laws and regulations (e.g., controlled substances, EPA standards, etc.) and according to all applicable health and safety regulations (e.g., exposure to volatile anesthetic gases, etc.)
9. All animals must be monitored for basic physiologic function while under anesthesia. Monitoring requirements will vary according to species, breed, individual differences, temperature, type of anesthetic used, and the type of procedure performed. Monitoring of animals under anesthesia must be described in the protocol.
10. For all non-rodent mammals, peri-anesthetic records (pre, during and post) must be maintained, even when surgery is not performed. At a minimum, the record must indicate the time the animal was examined, the person who examined the animal, any treatments or medications administered, any abnormal findings, and (when applicable) the time of the recovery events, which are all listed on the Post-Anesthetic Recovery Record.
11. For rodents, birds, fish, amphibians and reptiles, anesthesia records are only required for surgeries and may be kept as part of the surgical record.
12. For anesthesia periods over 30 minutes, use of an external heat source to maintain body temperature is required and must be described in the Animal Use Protocol. Not all warming devices are acceptable:

- a. Acceptable warming equipment: Delta Phase pads, water recirculating heating pads, heating pads with a thermal feedback (such as rectal thermometer), Bair Hugger. These devices are always approved by the IACUC.
- b. Conditionally acceptable warming equipment: heating pads with temperature controls but without thermal feedback mechanisms. These devices may be used in rodents only, must be described on the IACUC protocol, and the PI must certify that animals are continuously monitored by trained laboratory personnel OR that the heating device will be calibrated routinely. If the investigator proposes to calibrate rather than continuously monitor, the method, frequency and record-keeping for the calibration must be included in the protocol.
- c. The use of heat lamps to warm animals is strongly discouraged. In the event that their use is proposed in an IACUC protocol, the IACUC will rarely approve such applications, and use of a heat lamp may be approved only for specific applications, only in rodents, and only with a detailed safety plan.
- d. The use of hand warmers that exceed 42C must be described in the protocol, including maximum temperature reached, safety precautions taken to prevent overheating and burns, and monitoring parameters.
- e. Not Acceptable: Heating pads with no or limited (e.g. high/low) temperature control, old or unmaintained warming equipment. Household electric blankets may not be used as they provide limited temperature control and do not disperse the heat evenly across the blanket which may expose the animal to excessive temperatures.

V. Related Documents

UCSD Documents	Policy 7. Surgery in Non-Rodent Mammals Policy 35. Surgery in Rodents, Birds, Reptiles, Amphibians and Fish Policy 31. Non-pharmaceutical Grade Compounds
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	<p>Safe Use of Anesthetic Gas</p> <p>Rodent Anesthesia Equipment</p> <p>Vendors for Isoflurane Calibration and Maintenance</p> <p>Guideline for the Use of Tranquilizers, Analgesics, and Anesthetics in Rodents and Rabbits</p> <p>Guidelines for the Use of Tricaine Methanesulfonate (MS-222)</p> <p>Guidelines for the Use of Avertin</p> <p>Guidelines for the Use of Urethane</p>
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VI. Additional Information

The following information is provided by the Animal Care Program (online at [ACP Beacon Homepage](#) under the tab “Animal Health”) to help you conform to this policy, the Guide for the Care and Use of Laboratory Animals, and federal laws and regulations:

Stabilization/Acclimation:

Stabilization/acclimation periods allow the animal to rehydrate, resume normal eating patterns and recover from the stress of transport, all of which are beneficial prior to being anesthetized. Minimum acclimation periods:

- Rodents - 48 hours
- Other animals (rabbits & larger) - 72 hours

Stabilization/acclimation periods may need to be longer depending on the study. Keep in mind that the longer that an animal is allowed to stabilize/acclimate the more physiologically stable they are.

Withholding Food Prior to Anesthesia:

- Rodents and rabbits do not need to be fasted; exception is GI surgery
- Cats, Dogs, Primates, Pigs - 8 to 12 hours
- Small ruminants (sheep, goats, calves) - 24 hours
- Neonates - not usually fasted

Withholding Water Prior to Anesthesia:

- Small ruminants (sheep, goats, calves) - 8 to 12 hours
- Other common laboratory animals - not generally necessary

Common Anesthetics (Injectable and Inhalant) For Use in Rodents:

The most common injectable anesthetic combination used in mice and rats is ketamine/xylazine given either IP (Intraperitoneal) or SC (subcutaneous). The most common inhalant anesthetic used in both rodents and larger animals is isoflurane. For dosages and duration of effect see [Guideline for the Use of Tranquilizers, Analgesics, and Anesthetics in Rodents and Rabbits](#).

Common Anesthetics (Injectable and Inhalant) For Use in Birds:

The most common injectable anesthetic combination used in birds is Ketamine combined with an alpha 2 agonist (either xylazine or dexmedetomidine) given IM. Ketamine can also be combined with other various tranquilizers depending on the degree of sedation or depth of anesthesia desired. Exact dosages are dependent on species; thus it is recommended to contact an ACP veterinarian to prepare the proper anesthetic regime for your protocol.

The inhalant anesthetic agent of choice for use is isoflurane. Anesthesia can be induced via a face mask or in an induction box. Anesthesia can then be maintained with the use of a face mask or an endotracheal tube. The most optimal method is to intubate and maintain on isoflurane. The general guidelines recommend a dosage of 3-5% isoflurane for induction and 1.5-2.5% isoflurane for maintenance anesthesia.

Tricaine Methanesulfonate (MS 222) Use in Fish and Amphibians:

Tricaine Methanesulfonate (Finquel, MS 222, Tricaine-S) is used for anesthesia and euthanasia of fish and amphibians. Tricaine Methanesulfonate comes as a powder which is hazardous and **must** be handled and prepared according to the [UCSD Guidelines for the Preparation, Storage and Use of Tricaine Methanesulfonate](#).

Dose Ranges for immersion anesthesia:

- Fish - 25 to 200mg/L;
- Amphibians - 0.5 to 3 gm/L

Final solution must be buffered to pH 7-7.5. The exact dose will be determined by variables such as species, size, desired depth of anesthesia, etc.

Tribromoethanol (Avertin) Use in Mice:

Tribromoethanol is an injectable anesthetic that has been used in mice. It was once manufactured under the trade name Avertin but this pharmaceutical grade product is no longer commercially available. Tribromoethanol is only available as a non-pharmaceutical grade product and its use must be scientifically justified over the use of other pharmaceutical grade anesthetic agents and must receive IACUC approval.

The disadvantages of this anesthetic agent include:

- When given IP (Intraperitoneal) it is an irritant to the peritoneal cavity, especially at high doses or concentrations and/or with repeated use. It can cause peritoneal adhesions and/or intestinal ileus. Intestinal ileus and death can occur several weeks after use.
- If not properly stored, it will degrade in the presence of heat and/or light to produce toxic byproducts that can be both nephro and hepatotoxic. Death can occur up to 24 hours after use of degraded product.
- Repeat administration of this drug for anesthesia can be associated with an increased incidence of morbidity and mortality.

ACP veterinarians **do not** recommend the use of this anesthetic.

Delivery Techniques for Inhalant Anesthetics:

Delivery of isoflurane is commonly performed in all animals with the use of a precision vaporizer. However, in rodents, isoflurane can be delivered using either a precision vaporizer or by using the "open drop technique". Both of these techniques are described below:

Precision Vaporizer Use:

- Provides optimal delivery of inhalant anesthetic in all animals and commonly uses 100% oxygen as the carrier gas.
- A mixture of nitrous oxide and oxygen may be used instead of oxygen alone but nitrous oxide in animals is less potent than it is in people and does not produce the same effect.
- Allows the level of anesthesia to be precisely titrated for procedures lasting minutes or several hours.
- Always used for the inhalant anesthesia of large animals.
- Waste anesthetic gases can be scavenged either passively (e.g. charcoal canister) or actively (e.g. house vacuum, downdraft table, snorkel, hard ducted chemical fume hood). Charcoal canisters are ineffective for the scavenging of nitrous oxide.
- Always adhere to [EH&S scavenging requirements](#).

Open Drop Technique:

- Not an optimal technique for anesthetizing rodents as they are exposed to approximately 33% isoflurane. In comparison, 5% isoflurane delivered from a precision vaporizer results in a deep plane of anesthesia. If an animal is not closely monitored using the open drop technique an overdose and subsequent death are possible.
- Generally used in rodents for procedures lasting 30-45 seconds (e.g. blood collection, tail clipping) For longer periods of anesthesia a precision vaporizer is recommended.
- A clear glass or plastic induction chamber/container is used to facilitate observation of the animal as it becomes anesthetized.
- The chamber/container should be of sufficient size that the animal can move freely and will not become hypoxic before it becomes anesthetized. A source of oxygen may be delivered into the chamber/container.
- A grid or other barrier must be used to prevent the animal from coming into direct physical contact with the liquid inhalant as it can be irritating to the skin.
- Waste anesthetic gases are scavenged by working in a hard-ducted chemical fume hood, on a downdraft table, or under an EH&S approved snorkel. Do **not** work bench top with this delivery technique.
- Always adhere to EH&S scavenging requirements.

Monitoring Animals During Anesthesia:

Following administration of an anesthetic it is essential to assess that the required depth of anesthesia has been achieved. It is also important to monitor the vital signs of the animal and the function of any anesthetic equipment that is in use. The extent of monitoring necessary to achieve these goals will depend upon the anesthetic regime used, the animal's clinical condition, and the invasiveness of the procedure. The frequency of monitoring is stated in the protocol and documented during the procedure.

Common parameters monitored in all animals:

- Heart rate
- Respiratory rate
- Body temperature
- Movement in response to a noxious stimulus (e.g. skin incision, toe or tail pinch)

Additional parameters frequently monitored in large animals:

- Jaw tone, palpebral reflexes
- Blood pressure
- Pulse quality
- Urine output
- SPO₂
- ETCO₂
- EEG
- Blood gases/pH and/or other blood work

ACP recommendations for anesthetized animals:

- Body temperature supplementation in all animals whenever the procedure and recovery time exceed 30 minutes
- An Anesthetic Record for large animals is required and must include observations from induction of anesthesia to extubation and return to cage/kennel and should include documentation of any therapeutic drugs and/or fluids administered
- Notations of monitored parameters should be made on an anesthetic record every 5-10 minutes (even where monitoring is continuous)
- Anesthetic record is recommended for rodents and other small animals

Monitoring Animals During Recovery from Anesthesia:

Animals recovering from anesthesia must be closely monitored by qualified individuals until they have regained consciousness, are physiologically stable and are no longer

recumbent. Documentation must be kept in large animals (rabbits and larger) during the recovery period.

If a separate record is kept for recovery from anesthesia it must at a minimum include the following:

- Time of all animal evaluations
- Initials of person making observation
- Any treatments or medications administered
- Any abnormal findings
- When applicable, a timeline of recovery events

General parameters that should be monitored during the animal's anesthetic recovery include the following:

- Body temperature
- Heart rate and character of peripheral pulse
- Respiratory rate and pattern of respirations
- Capillary refill time (CRT)
- Jaw tone
- Response to toe/tail/medial claw pinch
- Palpebral reflex secondary to gentle stroking of the eye lashes
- Mucous membrane color

Guidelines for anesthetic recovery:

- Animals (if intubated) are extubated when their swallowing reflex has returned.
- When recovery is prolonged animals should be periodically turned from side to side to prevent pulmonary hypostatic congestion (accumulation of blood in the down-side lung).
- Keep animals warm as hypothermia prolongs recovery from anesthesia. This can be accomplished by
 - Increasing room temperature
 - Use of water circulating heating blanket
 - Use of other IACUC approved heating device (e.g. Bair Hugger, Deltaphase pads)
- Animals must be kept dry and clean (any excreta removed immediately)
- Hydration is assessed by noting perfusion of the mucous membranes, skin turgor and urine production. Fluids should be provided as necessary and may be given IV, SC or IP depending on the species of animal.
- If indicated in the protocol the animal should be given an appropriate analgesic pre-emptively prior to awakening from anesthesia if possible. The most common opioid analgesic used in animals is buprenorphine.

Calibration of Vaporizers and Maintenance of Anesthesia Machines:

The calibration of vaporizers and routine maintenance of anesthesia machines should be done no less than once every three years per [EH&S policy](#).