

**THE UNIVERSITY OF MELBOURNE
ANIMAL WELFARE COMMITTEE**

**GUIDELINES FOR GENERAL ANAESTHESIA AND ANALGESIA
OF COMMON LABORATORY ANIMALS**

Introduction

Anaesthesia means without ('an') feeling ('aesthesia') and general anaesthetic agents are drugs which typically produce a progressive central nervous system depression with loss of sensation when introduced into the circulation.

Policy

In line with the requirements of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004) the use of local or general anaesthetics, analgesics or tranquillisers must be appropriate to the species and should at least parallel their use in current medical or veterinary practice. Anaesthesia and surgery must be performed by competent staff with appropriate training and experience. There must also be adequate monitoring of the depth of anaesthesia and respiratory depression. Adequate monitoring, treatment and care, including analgesia of post-operative animals, must be provided, and investigators must ensure that they are fully informed of the condition of the animals at all times. Alleviation of pain or distress must always take precedence over continuing or finishing a procedure that is under way.

Guidelines

General Anaesthesia

General anaesthetic agents are either:

(i) **injectable** - administered using a needle and syringe by the intraperitoneal (ip), subcutaneous (sc), intramuscular (im), or intravenous (iv) routes. Agents are classified as barbiturates, narcotics, sedatives - hypnotics (sleep inducing agents), steroids, or dissociative agents

OR

(ii) **inhalants** - administered by the open/semi-open or closed/semi-closed methods. Examples of inhalant anaesthetics are methoxyflurane, halothane and isoflurane.

Depending on the agent, anaesthetics are given individually or in combination.

Choice is always based on:

- species/strain, weight, sex and age of the animal
- anticipated duration of the procedure and whether it is to be recovery or not
- appropriateness for the procedure, including possible interaction with other substances used;
- availability of the equipment required to administer agent(s)
- safety for the worker; and
- reliability of the anaesthetic agent to produce a steady-state between anaesthesia/analgesia, muscle relaxation and optimum physiological function.

Inhalants can be used for induction and maintenance of anaesthesia. Alternatively, anaesthesia may be induced by an injectable agent and maintained by a gaseous agent. Inhalants have the advantage of being rapidly eliminated from the system, mostly via the lung, which allows rapid recovery. They are particularly useful for long procedures and avoid the need for repeated doses of injectable agents which can be cumulative. When used with an appropriate vaporiser and oxygen delivery system, a means of assisted respiration is always available in an emergency. (Refer to Appendix I for some recommended anaesthetic choices in the common laboratory animals ie rabbits and rodents.)

Pre and Post-Operative Care of Animals Undergoing Procedures

In addition to the requirement for choosing the right anaesthetic for the procedure, proper consideration must be given to pre- and post-operative care, including by non-pharmacological means.

1. Pre-operative Care

- Before any procedure is undertaken, animals must be checked for health. Note especially any signs of respiratory disease or discharge. It is important to use healthy animals which have been obtained from a reliable source.
- While it is generally recommended that food intake be reduced or withdrawn before a procedure (to allow for emptying of stomach contents distally and reduction in the risk of asphyxiation by regurgitation) this is not considered necessary/advisable in the case of rabbits and rodents.
- Animal(s) should be transferred (in their cage/s) to a comfortable corner of the research laboratory for a 2-4 hour conditioning period, and observed.
- Immediately before administration of any premedication drug(s) eg atropine, animals should be weighed, and the premedication and anaesthetic drug doses calculated.

- The next step is to administer the pre-medication agent(s) and a tranquilliser. Rarely used in rats and mice they can be useful in larger laboratory animals such as the guinea pig or rabbit to reduce bronchial secretions and reduce the risk of cardiac depression. It should be remembered, however, that because of the presence of serum atropinase in a high proportion of rabbits, response to this drug may be variable. To overcome this problem, administer atropine sc every 30 minutes. In general, premedication agents help to calm the animal, reduce the calculated anaesthetic dose required, and provide a smooth induction.
- Administer the anaesthetic 20–30 minutes after administration of the premedication agent(s). Where a volatile anaesthetic is to be given, use an anaesthetic mask to deliver the agent. In the case of rabbits or larger animals, intubation may be used, but only by people who are expert in the technique.
- At this point, consideration should be given to the administration of pre-emptive analgesia ie the administration of an analgesic agent before a surgical intervention. Pre-emptive analgesia is thought to block pain pathways during surgical procedures and reduce pain experienced in the post operative period. Long acting non-steroidal anti-inflammatory agents and/or opiates may be used.

2. Monitoring throughout the period of anaesthesia

The following items should be monitored throughout an anaesthetic procedure:

- palpebral reflex
- pedal reflex (NOTE that this reflex is not particularly reliable in rodents), tail pinch reflex (rats and mice) or ear pinch reflex (rabbits, guinea pigs), as well as general muscle tone
- body temperature (animals must be kept warm throughout the procedure eg use a thermostatically-controlled heating pad or cover small rodents with bubble wrap)
- respiratory rate
- heart/pulse rate, tissue colour, capillary bed refill time
- (if possible) also EEG (or EKG) activity, pO₂ and blood pressure.

The use of neuromuscular blocking agents must have specific approval from the AEC. These agents, which induce immobilisation through muscle paralysis, must always be used in conjunction with a general anaesthetic. The use of a neuromuscular blocking agent alone is not acceptable. The neuromuscular blocking agent should not be given until an adequate depth of general anaesthesia has been achieved. Extreme care must be taken to ensure that anaesthesia and loss of consciousness is

maintained for as long as the procedure takes and until the neuromuscular blocking agent wears off. The animal will need to be artificially ventilated and the following monitored closely:

- sudden changes in heart rate or arterial blood pressure with the application of noxious stimuli
- pupil size (this can be confounded by premedication with atropine which dilates the pupil); and
- end tidal pCO₂ (which should be no greater than 4.7%) and direct arterial pCO₂.

3. Post-operative Care

- Place the animal on its side, head extended, in a clean, dry, warm (30 °C) box on shredded paper free of any dangerous objects which could lead to asphyxiation/injury (eg sawdust, water containers or other material which might act as a noose) in a quiet warm room away from strong light and in a position where recovery can be monitored. Ensure that animals do not become hypothermic.
- Where an endotracheal tube has been used, it should be removed very gently as soon as the swallowing reflex returns. Watch for any sign of regurgitation and if necessary reposition the head to a level below that of the rest of the body so that any accumulated fluid drains away.
- Restrict access to food and water until full recovery has taken place. When recovery is several hours, animals should be turned carefully every 20 minutes or so to avoid fluid congestion in the lungs.
- Provide warm fluid replacement by injection, ie. Na lactate, Hartmans solution or 0.9% (w/v) ie normal saline sc or ip. Give approximately 3–5% of the animals' pre-anaesthetic body weight in ml, eg 1–1.5 ml for a 30 g mouse.
- Provide post-operative nursing, antibiotics and analgesia as necessary (see Appendices II and III). Once animals have fully recovered, continue to monitor progress for a further 24–48 hours. Observation of surgical wounds is essential as a check on progress of healing.
- Clinical records must be kept and made available to all staff involved. Duties of staff must be clearly defined and ways of dealing with emergencies established. Animals found to be in a severe state of pain or distress which cannot be alleviated promptly must be euthanased immediately.

Further Information can be found in 'Careful How You Hold Me - an insight into caring for laboratory animals' CD ROM University of Melbourne, 1999.

References

Flecknell, P. A. *Laboratory Animal Anaesthesia*. 2nd edition Academic Press, London (1996).

Hau, J & Van Hoosier, G.L *Handbook of Laboratory Animal Science Vol 1 Essential Principles and Practices* (2003)

Hillyer, E.V and Quesenberry, K.E (eds) *Ferrets, Rabbits and Rodents: Clinical Medicine and Surgery*. W.B. Saunders Company, Philadelphia (1997)

APPENDIX I

ANAESTHETIC AGENTS

As a general rule, the smaller the species the more rapid the metabolic rate and therefore a requirement for a slightly higher anaesthetic dose rate than larger species. When calculating anaesthetic doses, make allowance for the weight of the bowel contents in animals with a large caecum. In the case of the guinea pig or rabbit, the weight of the bowel contents can be up to 20% of the animal's real body weight. To be quite safe, lower dosages should be administered initially with incremental (ie one third of the original calculated dose) top-up doses given every half hour or so to maintain anaesthesia. Alternatively, animals should be maintained on gaseous anaesthesia.

Monitor body temperature and maintain at 37-38 °C for rabbits and rodents. Use a thermostatically-controlled heating pad, or cover animals in bubble wrap.

RATS AND MICE		
Premedication Agent		
Injectable Agents	Dose	Comments
atropine (Atropine injection, <i>Apex</i> 0.6 mg/ml)	Rat: 0.05 mg/kg sc or ip Mouse: 0.04 mg/kg sc or ip	Rarely used in rats and mice. Reduces salivary and bronchial secretions. Protects heart from vagal stimulation, bradycardia, and possible cardiac arrest induced by surgery or drugs such as xylazine.

RATS AND MICE		
Anaesthetic Choices		
Injectable Agent/s	Dose/Method	Comments
pentobarbitone 60 mg/ml (Nembutal, <i>Boehringer Ingelheim</i>)	Rat: 40–50 mg/kg ip Mouse: 40–60 mg/kg ip Young: 10–20 mg/kg ip	Medium to long acting barbiturate, lasts 30–40 minutes. Considerable variation between individuals.
*alphaxalone 9.0 mg/ml /alphadolone 3.0 mg/ml (Saffan/Alfaxan, <i>Jurox</i>)	Rat: 9–12 mg/kg iv Mouse: 10–15 mg/kg iv	*Note: Must not be used with non steroidal anti-inflammatory drugs eg carprofen
methohexitone Na (Brietal, <i>Eli Lilly</i>)	Rat: 50–65 mg/kg ip 10–15 mg/kg iv Mouse: 10 mg/kg iv	5–15 mins of anaesthesia

RATS AND MICE		
Anaesthetic Choices		
Inhalants	Dose/Method	Comments
methoxyflurane (Penthrane, <i>Abbott</i>)	Pour several ml onto a wad of cotton wool in the bottom of a large jar. Place over this a perforated metal platform. Lower the animal carefully onto the platform, loosely replace jar lid and wait about 40–60 seconds or until animal is soundly asleep. Remove the animal and replace the lid on jar to prevent further escape of gas into the working environment.	Because of its nephrotoxic properties this is no longer used in Australia for medical or veterinary purposes. It can be obtained but is very expensive. Once the animal has been induced, continue to maintain anaesthesia using a mask. A mask can be prepared by pouring 0.5–1.0 ml of Penthrane onto a little cotton wool placed in the bottom of a wide-mouthed test tube or a very small jar. Invert the test tube or jar onto the bench top when not in use.

<p>halothane (Halothane, <i>Rhone Meieux</i>; Halothane, <i>VCA</i>)</p> <p>or</p> <p>isoflurane (Forthrane, <i>Abbott</i>; Aerrane, <i>Zeneca Pharmaceuticals</i>)</p>	<p>Must be delivered in O₂ via a specialised vaporiser. Require scavenging for human safety. Can be used with a mask, endotracheal tube or in a clear container.</p>	<p>Allows rapid induction and recovery. Also O₂ source readily at hand if needed for resuscitation.</p> <p>In most cases, gaseous anaesthesia is the method of choice.</p>
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GUINEA PIGS		
Premedication		
Injectable Agent	Dose/Route	Comments
<p>atropine (Atropine injection, <i>Apex</i>) 0.6 mg/ml</p>	<p>0.05 mg/kg sc</p>	<p>Used to decrease salivary and bronchial secretions (guinea pigs have relatively narrow airways) and protect heart from vagal stimulation ie bradycardia and possible cardiac arrest</p> <p>Give diazepam 10–15 mins after atropine to sedate the animal.</p>
<p>diazepam (Pamlin injection, <i>Parnell Laboratories</i>; Valium, <i>Roche</i>)</p>	<p>5 mg/kg ip or im</p>	<p>Give diazepam in a separate syringe from the atropine. This produces heavy sedation but no analgesia. Good for sedation for minor non-invasive procedures. If used as a premedication to injectable anaesthesia, dose should be reduced.</p> <p>After a further 10–15 mins, administer one of the following anaesthetic choices.</p>

GUINEA PIGS		
Anaesthetic Choices		
Injectable Agent	Dose/Route	Comments
urethane (<i>Merck</i>)	1500 mg/kg ip or iv	Long duration (about 3 hours), non-recovery only. Solution needs to be freshly made up on the day and may be carcinogenic to the handler.

GUINEA PIGS		
Anaesthetic Choices		
Inhalant Gaseous Agent	Dose/Route	Comments
methoxyflurane (Penthane, <i>Abbott</i>)	In container	Wide safety margin, non-irritant
halothane (Halothane, <i>Rhone Meieux</i> ; Halothane, <i>VCA</i>)	In O ₂ using a vaporiser as recommended by the manufacturer	Can induce hypotension
isoflurane (Forthrane, <i>Abbott</i> ; Aerrane, <i>Zeneca Pharmaceuticals</i>)	In O ₂ using a vaporiser as recommended by the manufacturer	Can induce hypotension Guinea pigs are difficult to intubate but can be successfully maintained on a face mask

RABBITS		
Anaesthetic Choices		
Injectable Agent	Dose Rate	Comments
alphaxalone 9 mg/ml /alphadolone 3 mg/ml (Saffan/Alfaxan, <i>Jurox</i>)	6-9 mg/kg iv (ear vein).	Very effective and safe
propofol (Diprivan, <i>Zeneca</i> ; Rapinovel, <i>Schering-Plough</i>)	10 mg/kg iv	Produces light anaesthesia for 5–10 minutes or can be used for induction followed by maintenance on gas as for guinea pigs. Intubation is difficult in rabbits and requires practice. A close-fitting face mask can be used to maintain anaesthesia. Because of the risk of laryngeal spasm, induction using gaseous anaesthesia in rabbits is generally not recommended.
medetomidine* (Domitor, <i>Ciba-Geigy</i>)	Give im and follow with isoflurane gas.	Anaesthesia for 30–40 minutes. Domitor can be reversed with Atipamezole (<i>Antisedan, Ciba Geigy</i>) 1mg/kg ip or sc.

RABBITS		
Anaesthetic Choices		
Inhalant Gaseous Agent	Dose/Route	Comments
<p>halothane (Halothane, <i>Rhone Meieux</i>; Halothane, <i>VCA</i>)</p> <p>or</p> <p>isoflurane (Forthrane, <i>Abbott</i>; Aerrane, <i>Zeneca Pharmaceuticals</i>)</p>	<p>In O₂ using a vaporiser as recommended by the manufacturer</p>	<p>Can induce hypotension</p> <p>Because of the risk of laryngeal spasm, induction using gaseous anaesthesia in rabbits is generally not recommended. Anaesthesia should be induced using an injectable anaesthetic agent and maintained using a gaseous agent. . Intubation is difficult in rabbits and requires practice. A close-fitting face mask can be used to maintain anaesthesia.</p>

APPENDIX II

ANALGESIA

For any surgical procedure, a pain management plan appropriate for the procedures and the species must be developed, implemented and reviewed if necessary (Australian Code of Practice, 3.3.25) Analgesia is best given at the time of, or just after, induction of anaesthesia and before any procedure commences. Pre-emptive analgesia greatly reduces recovery time and the need for post-operative analgesic administration. There are two main categories of analgesics: non-steroidal anti-inflammatory agents and opiates. They can be used alone or in combination. There are several drugs in each category with different durations of action. Choice of analgesic should be made carefully, taking into account the aim of the experiment and the advice of the AEC. The following table provides a number of species-dependent suggestions.

Analgesic Drug Alternatives	RATS	MICE	GUINEA PIGS	RABBITS
pethidine (Pethidine injection, <i>Parnell</i>)	10–20 mg/kg im or sc 2–3-hourly	10–20 mg/kg im. or sc 2–3-hourly	10–20 mg/kg sc or im 2–3-hourly	10 mg/kg sc or im 2–3-hourly
buprenorphine (Temgesic, <i>Reckitt & Colman</i>)	0.01–0.05 mg/kg sc 8–12-hourly	0.05–0.1 mg/kg sc 12-hourly	0.05 mg/kg sc 8–12-hourly	0.01–0.05 mg/kg sc 8–12-hourly
carprofen (Rimadyl, <i>Pfizer</i>)	4 mg/kg sc 24-hourly	5 mg/kg sc 24- hourly	4 mg/kg sc 24-hourly	4 mg/kg sc 24-hourly

APPENDIX III

ANTIBIOTICS

Always use aseptic technique for all surgical procedures to minimise the risk of infection.

Antibiotic Alternatives	RATS	MICE	GUINEA PIGS	RABBITS
Ampicillin (Ampicyn, <i>Rhone-Poulenc Rorer</i> ; Austrapen, <i>CSL</i>)	10–30 mg/kg sc 8-hourly	10–30 mg/kg sc 8-hourly	Toxic	Toxic
Oxytetracycline (Terramycin 100, <i>Pfizer</i> ; Tetravet 100, <i>Hoechst Roussel</i>)	6–10 mg/kg sc or im 12-hourly	6–10 mg/kg sc or im 12-hourly	Toxic	15 mg/kg sc or im 24-hourly
enrofloxacin (Baytril, <i>Bayer</i>)	5 mg/kg sc 12-hourly	5 mg/kg sc 12-hourly	5 mg/kg sc or im 12-hourly	10 mg/kg sc or im 12-hourly
trimethoprim - sulphadoxine (Tribactral or Tribrissen, <i>Jurox</i>)	30 mg/kg sc 12–24- hourly	30 mg/kg sc 12–24- hourly	30 mg/kg sc 12–24-hourly	30 mg/kg sc 12–24-hourly
doxycycline (Vibravet, <i>Pfizer</i>)	2.5 mg/kg po ^a 12-hourly	2.5 mg/kg po 12-hourly	2.5 mg/kg po 12-hourly	2.5 mg/kg po 12-hourly

^a po, *per os*

OTHER SPECIES

For other species or for information about other anaesthetics/analgesics, refer to resources listed under References and contact your department's nominated veterinarian.