

# Guidelines for the use of anaesthesia and analgesia in animals used for scientific purposes

The aim of these guidelines is to assist investigators to:

- Choose an anaesthetic/analgesic regimen that is appropriate for their projects
- Understand the correct process of administering anaesthesia to animals, from the arrival of the animal to full recovery
- Understand the general principles of how to monitor animals under and following anaesthesia
- Understand that there are regulatory requirements that determine how these drugs can be used, and to know how to access further information on how to obtain approvals and comply with these requirements

These guidelines related to anaesthesia being carried out by investigators without veterinary qualifications using laboratory animal species, wildlife and fish where there may be little veterinary oversight.

For domestic animal species and wildlife, a veterinarian needs to be involved in the choice of an anaesthetic or analgesic agent specific for the project and species.

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## Choosing an Anaesthetics and Analgesic Regimen

### **Anaesthetic Drugs**

When planning a project where pain or distress is a possibility, anaesthesia and/or analgesia must be used unless there is a specific reason for not using them, and this has been approved by the AEC. Investigators should consider their choice of anaesthesia and analgesia carefully. Different groups of drugs will have different uses, effects and side effects and so it is important to choose drugs that don't interfere with the systems being studied or if the research is intended to be used in human surgery, a drug regimen similar to those used in humans should be used.

A list of commonly used anaesthetics and analgesics and their common side effects, doses and routes of administration can be found in Appendix 1.

The following should be considered when choosing agents for a project:

#### **Route of Administration**

General anaesthetic drugs can be divided into three main groups:

- Inhalational agents (Isoflurane, halothane, methoxyflurane, sevoflurane) are administered via a vaporiser through a facemask or endotracheal tube into the lungs. They rapidly cross between the lungs and the blood and so are safe, with any overdose easily reversed. They provide good anaesthesia but no analgesia.
- Injected agents (ketamine, barbiturates, xylazine) are provided by injection and must be metabolised and excreted over time. This means the depth of anaesthesia cannot easily be altered once administered and they are less safe, but often provide degrees of analgesia and sedation.
- Absorbed agents (MS-222, Clove oil, Aqui-S) are mainly for amphibians, crustaceans and fish and are provided in a liquid and are absorbed through the skin or into the gills.

When anaesthetics are injected, the route of administration will affect the way the anaesthetic progresses:

#### **Intravenous (IV)**

Administration of anaesthesia into the vein and so the systemic circulation. Administration via the IV route results in an immediate effect and depending on the dose and drug, a rapid loss of consciousness. When administered IV, drugs can be given to effect giving better control over depth so that overdoses can be more easily prevented. They are reliable as long as the drug is administered correctly, so that the exact dose given will be known. IV administration requires more skill and training than other routes of administration, especially in smaller species.

#### **Intramuscular (IM)**

Administration into the body of a muscle or muscle group. Muscles are highly vascular and so the effect will be fairly rapid but not as rapid as via IV. IM injections can be painful especially where large volumes are needed so large volumes should be avoided or more than one injection given in more than one site. Some agents can cause muscle necrosis so be sure to check that the drug can be given by this route.

#### **Sub-cutaneous (SC)**

An injection under the skin. This is an easy method of delivery but absorption can be slow especially in animals with a lot of sub-cutaneous fat, so it can result in an unreliable and variable effect. Some drugs are irritant and cause the overlying skin to become necrotic and slough off, so care should be taken choosing a drug for this method and also being careful when injecting irritant drugs IM, IP or IV to ensure that none of the drug escapes or is injected SC.

#### **Intraperitoneal (IP)**

The injection is made in the abdomen with the aim of the drug being injected into the peritoneal cavity. The final injection site can be variable especially if the operator is unskilled or the animal moves, and the injection given into an intestine, bladder, liver or other viscera delaying or minimising the effect. If no effect is observed in the expected time, care should be taken administering a second as it may lead to overdose once the initial dose is finally absorbed. Care should be taken to keep the animal still during the injection to prevent tearing of viscera or blood vessels which can result in peritonitis or bleeding.

#### **Combination Drug Regimens**

Since no single drug will have all the properties required for a successful and pain-free anaesthetic, two or more drugs with differing effects may be combined into a regimen. Combining drugs also has the benefit of reducing the individual doses of each which can reduce the chance of an overdose and the development of side effects and improves recovery.

#### **Pre-emptive Analgesia**

It has been shown that the provision of an analgesic before the painful procedure begins (pre-emptive analgesia) reduces the degree and duration of pain. Pre-emptive analgesia must be included in the anaesthetic drug regimen for painful/invasive procedures.

## Local Anaesthetics

If local or regional anaesthesia is going to be used, a sedative or other agent should be provided to reduce the stress on the animal and prevent the animal from moving during the procedure. Pre-emptive analgesia must also be given.

Topical local anaesthetics may be used for minor procedures such as injections but they don't penetrate deep enough to be used in procedures that go deeper than the dermis

Local anaesthetics cannot take the place of an analgesic regimen as they're length of action is not sufficient

## Other Considerations

If the drug regimen required is different to those used in standard practice, then the investigator must provide the AEC with appropriate references in the literature that support its safety and efficacy for use in that circumstance.

Analgesics are generally provided as injections, but oral formulations and preparations are becoming available and some transdermal preparations can be used on larger animals.

For rodents, some analgesics can be given in the water or in pre-prepared gel drops to avoid having to handle the animals frequently, which can add to the distress being experienced.

Whenever possible, inhalational anaesthesia should be the first choice because it is easy to control the depth of anaesthesia and results in a fast recovery.

Where the anaesthesia is for a minor, painless procedure, inhalational anaesthesia should be used.

For painful procedures, inhalational anaesthesia requires pre-emptive analgesia.

## Preparation and Pre-anaesthesia

### Pre-anaesthetic Fasting

Some species should be fasted before the administration of anaesthetic drugs in order to empty the upper gastrointestinal system and prevent regurgitation and aspiration of gastric contents. Twelve hours is sufficient for most mammals

**Rodents and lagomorphs** do not vomit/regurgitate, and so there is no need to fast these species to prevent regurgitation and aspiration of gastric contents.

**Dogs, pigs, cats, horses and non-human primates** must be fasted before anaesthesia.

**Ruminants** (cattle, sheep, goats and deer) fasting may not allow the upper gastrointestinal tract to clear sufficiently, so fasting in these species is often not performed, however, as a precaution ruminant should be positioned with their head raised higher than their abdomen if anaesthetised or heavily sedated to prevent regurgitation.

### Animals must be in good health and wellbeing before undergoing an anaesthetic

Performing an anaesthetic on an animal can be a risky procedure, therefore it's important to ensure the animal is in good health and wellbeing before the anaesthesia and procedures. If for some reason it isn't in good health then it's important to know what challenges may arise so an anaesthetic regimen can be chosen and appropriate precautions put in place. This will in turn result in less adverse events and produce superior outcomes for the research. In order to achieve this, the following points should be considered:

- Animals should be provided optimal transport and housing before being used
- Animals must be acclimatised to the new surrounds, handling and other procedures according to the requirements of the AEC, before any painful procedure is performed or anaesthesia is administered

- The animal's monitoring records should be inspected before an animal is anaesthetised and any abnormalities investigated to determine whether the animal is suitable for use
- Animals must receive a brief physical examination immediately before undergoing anaesthesia
- SPF rodent supply and housing minimises disease and ensures the animals are in optimal health. Animals from non-SPF suppliers or that are housed in other circumstances may require a more thorough health examination and/or a longer acclimation/quarantine period before undergoing anaesthesia

### **Handling and restraint**

Anaesthesia and sedation work better if the animals are calm. If animals are stressed, it may increase the amount of anaesthesia or sedation required to achieve the same results. This in turn can result in extended recovery periods, or anaesthetic emergencies.

- Animals should be handled in a calm and quiet manner, with the aim to minimise any stress, struggling, fear or anxiety
- Animals should be acclimated to the handling and restraint procedure
- Handling, restraint and induction should be done in a quiet environment

## **Anaesthesia**

### **Premedication**

In larger animals, particularly those not accustomed to handling or human interaction, premedication with a sedative is advisable. Other drugs can also be administered at this stage to ensure they are in the system by the time the anaesthesia and procedure begins.

- Sedatives, analgesics, anticholinergics (atropine) and antibiotics may be used prior to anaesthesia
- Sedatives calm the animal, facilitate handling and induction and reduce the dose of anaesthetic agent needed
- Anticholinergics reduce salivary and respiratory secretions in some species, but are not used in ruminants
- Analgesics should be given as a premedicant to any animal undergoing a procedure with the propensity to cause pain or where the anaesthetic agent being used does not provide sufficient analgesia itself (for example inhalation anaesthetics)
- If pre-medication is given, it's important to allow sufficient time for the drugs to take effect before anaesthetising the animal, this will depend on the species, drug and route by which it is given

### **Induction of Anaesthesia**

The induction of the anaesthesia is extremely important as it can affect how the anaesthesia progresses, for example, in rodents receiving an IP injection of anaesthesia struggle, the dose can be mis-administered resulting in reduced effect. The following should be considered:

- The induction of anaesthesia should be smooth, with minimal distress to the animal
- Induction should take place in a quiet environment, and preferably in an area away from other animals
- Animals undergoing a general anaesthetic or heavy sedation must have a sterile eye ointment or moisturising agent administered to their eyes to prevent dehydration of the cornea

## Maintenance

The main aim during the maintenance stage of an anaesthetic is to keep the animal at a level of anaesthesia at a depth that is appropriate for the procedure, while monitoring the animal's physiological vital signs to detect and respond to any abnormalities.

The animal must be assessed to be in an adequate depth of anaesthesia before any procedure can commence and be maintained in a sufficient depth of anaesthesia so that it does not show any perception of pain.

- Some or all of the following may be used to assess depth of anaesthesia, depending on the species (see Appendix 3):
  - Righting reflex
  - Withdrawal reflex
  - Palpebral reflex, pupillary reflex and eye position (some species)
  - Ear flick, tail pinch reflex
  - Muscle tone of the jaw
  - Anal sphincter tone
  - Physiological data such as respiratory and heart rates but also blood pressure, PO<sub>2</sub> (pulse oximetry)
- Physiological data can be measured, depending on the species of animal, the procedure being undertaken and the equipment available to assess the animal's physiological wellbeing while under anaesthetic.
- The minimum physiological data to be collected includes:
  - Anaesthetic depth
  - Respiration rate
  - Heart rate
  - Mucous membrane colour
- If neuromuscular blocking agents for paralysis are to be used, then the animal must be ventilated and assessed to be in deep anaesthesia before they are administered and the following physiological parameters must be monitored at least every 5 minutes and more frequent monitoring around the times of noxious stimulation.
- Always ensure the animal is in a stable surgical depth of anaesthesia before beginning any procedure

For details on anaesthetic emergencies and resuscitation for rodents, see Appendix 4.

## Heat Loss

Hypothermia is a particular risk in rodents, neonatal and juvenile animals and animals smaller than about 20kg and so supplementary heating needs to be provided to these animals in the form of a heat pad or other system. Heat lamps should not be used as they can result in hyperthermia, burning or dehydration

Hypothermia may also be a problem with larger animals if surgery is carried out in cool facilities and if surgery goes for longer periods of time

If the animal is hypothermic their recovery time will be longer. If animals are taking too long to recover then hypothermia should be considered as a cause

## Fluid Loss

Animals can experience fluid loss while under anaesthesia through blood loss and evaporation from exposed viscera or through respiration and being unable to drink during and after a procedure

Fluid loss can be reduced by minimising and controlling blood loss and irrigating viscera with warm sterile saline

Animals undergoing anaesthesia should be given supportive fluid therapy at 3-5% of their body weight using warmed sterile isotonic fluids such as 0.9% saline for injection, lactated ringers or PBS given subcutaneously (rodents) or intravenously (larger animals) depending on the procedure involved and any veterinary advice provided

### **Recovery from anaesthesia**

Once the procedure is complete and the anaesthesia ended, the animal must be allowed to recover in a quiet, dark, warm area that is away from other animals and the animal must be supervised closely until it has fully recovered.

The animal should not be returned to its regular housing until it has fully recovered from the anaesthesia, can ambulate normally to source feed, water or escape other animals that may be aggressive and the analgesia provided has been shown to be effective at managing any pain.

## **Post-anaesthetic Care and Analgesia**

**It's the policy of the JCU AEC that all animals undergoing painful and/or invasive procedures must receive analgesia for as long as they are assessed to be experience pain or discomfort afterwards.** They must be monitored more closely for several days post-anaesthesia to ensure they have fully recovered from the procedure, for the signs of post-procedure adverse events and to ensure that any analgesia regimen is working.

- Unrelieved pain has a significant negative consequence on the physiology of an animal and will confound research results.
- Where appropriate analgesia is not provided, animals in pain and discomfort are more likely to self-traumatize surgical wounds, show decreased appetite and become quiet and depressed in mentation. This poses an increased risk of post-operative infection and wound dehiscence, as well as dehydration, weight loss and general malaise. This is primarily a welfare concern and may also compromise research outcomes by creating altered physiological and hormonal states in the animal under investigation, leading to invalid results
- Animals must be monitored in accordance with the approved monitoring plan for the protocol and any pain or distress addressed as outlined in that protocol.
- Signs of pain or distress that indicate that an analgesic may be required or the current analgesic regimen must be reviewed include:
- Behavioural responses:
  - Abnormal posture
  - Social isolation
  - Aggression (people or other animals)
  - Decreased appetite
  - Self-mutilation
  - Reluctance to move
  - Lethargic
  - Abnormal vocalisation
  - Decreased groom/unkempt coat
  - Pica (eating abnormal materials eg bedding)
  - Grinding of teeth

- Physiological responses:
  - Increased heart rate and blood pressure
  - Increased respiratory rate and/or effort
  - Weight loss
  - Pupillary dilation
  - Changes in body temperature.

## Record Keeping

Investigators are required to keep accurate records for anaesthesia and their monitoring of the animals during and after anaesthesia. These records must be provided to the AEC with the protocol application and then be used as outlined in that protocol.

These records must contain enough information to show the following:

- Details of the animal's pre-anaesthetic health assessment and its body weight
- Details of any pre-medication and other drugs given to facilitate the anaesthesia or a procedure including the dose, route and time of administration and any adverse events that occur as a result
- Details of any anaesthetic agents including the dose, route and time of their administration and any adverse events that occur as a result
- Enough monitoring criteria observed at sufficient frequency to ensure the animal's wellbeing and accurately assess wellbeing and depth of anaesthesia (see Appendix 2).
- Details of the animal's depth of anaesthesia and physiological data while from pre-induction examination to recovery and return to the animal's normal housing.
- Details of any adverse events that may occur
- The time the anaesthesia ended and the animal is allowed to recover
- Details of the monitoring received while recovering including any support provided such as heat mats or fluid therapy or analgesia
- The time the animal was returned to its regular housing

The anaesthetic records must be made available to the AEC, AWO, JCU WH&S or Queensland Health Medicines and Poisons inspectors if requested.

Monitoring records must be in line with the appropriate legislation and the University's data retention requirements. Paper documents can be destroyed after being converted to digital versions.

## Regulatory requirements

In Queensland, anaesthetics and analgesics are controlled (Schedule 4 or 8 – S4, S8) drugs and in order for investigators to possess or use therapeutic substances, or for veterinarians to use the drugs in a research setting, they or their direct supervisor must hold the appropriate approval from Queensland Health and comply with JCU policies and procedures related to their use. These can be found at:

<https://www.jcu.edu.au/work-health-and-safety/drugs-and-poisons>

All investigators are required to obtain the appropriate approvals, understand the legislation and follow the regulatory requirements closely.

Due to the nature of their actions, anaesthetics, sedatives, tranquilisers and analgesic drugs are particularly prone to misuse and so failure to meet regulatory requirements can result in severe penalties for individuals, their supervisors and supervising institutions.

## Glossary

<b>Anaesthetic (General)</b>	a drug that causes a controlled and reversible loss of consciousness
<b>Anaesthetic (Local)</b>	an agent that can be administered to a local area or a nerve that causes a loss of sensation in that area or the area a nerve supplies
<b>Analgesic</b>	a drug that provides relief from pain
<b>Anti-cholinergic</b>	a drug which inhibits the cholinergic nervous system with no anaesthetic or analgesic properties. The cholinergic nervous system controls involuntary smooth muscle movement in the gut as well as at other visceral sites but also salivation and mucus secretion in the mouth, throat and nose (see Appendix 1)
<b>Anti-inflammatory</b>	a drug that acts to reduce the inflammatory response to injury including swelling, redness and pain
<b>Anxiety</b>	an intense feeling of worry, fear or apprehension
<b>Anxiolytic</b>	a drug that reduces anxiety
<b>Distress</b>	a negative mental state that indicates when an animal is unable to cope with a degree of underlying pain, anxiety, fear, stress. In animals, it is defined by changes in behaviour and physiology
<b>Neuromuscular blocker</b>	a drug that blocks neuromuscular transmission at the neuromuscular junction with the muscle cells and so causes paralysis. These drugs have no anaesthetic or analgesic properties.
<b>Pain</b>	a highly unpleasant physical sensation provoked through nervous stimulation
<b>Scheduled drugs</b>	the classification applied to restricted drugs as a part of their regulation. Examples are Schedule 8 (drugs of addiction), Schedule 4 (prescription only medication)
<b>Sedative</b>	a pharmacological agent that causes drowsiness and reduced excitement and movement
<b>Tranquiliser</b>	a drug that whose principal actions is to reduce anxiety
<b>Wellbeing</b>	an animal is in a positive mental state and is able to achieve successful biological function, to have positive experiences, to express innate behaviours, and to respond to and cope with potentially adverse conditions. Animal wellbeing may be assessed by physiological and behavioural measures of an animal's physical and psychological health, of the animal's capacity to cope with stressors, and species-specific behaviours in response to social and environmental conditions

## Administration

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## Appendix 1

### DRUGS GROUPS COMMONLY USED IN ANIMAL ANAESTHESIA, ANALGESIA AND SEDATION

#### Sedatives/tranquilisers

**Benzodiazepines:** diazepam, midazolam, zolazepam (only available in combination with tiletamine, marketed as Zoletil in Australia)

**Description:** These drugs provide sedation, muscle relaxation and are anxiolytics. In anaesthesia they are often used in combination with other drugs such as ketamine or tiletamine.

**Advantages:** They have a large safety margin and little effect on the cardiovascular system. They potentiate the effects of anaesthetic agents meaning that when they are given as a premedicant, less anaesthetic is required making the procedure safer and recovery faster.

**Disadvantages:** These are controlled drugs and so their use requires special storage, handling and record keeping.

**Alpha2-adrenergic agonists:** xylazine, medetomidine, detomidine

**Description:** CNS depressants and sedatives with a mild analgesic effect that can be reversed by the administration of an alpha2-adrenergic antagonist such as yohimbine

**Advantages:** These drugs provide good CNS depression and muscle relaxation and can be used alone for immobilisation for minor procedures (blood collection, eye examination) to facilitate handling or prevent movement, although a local anaesthetic and analgesia should be provided for any surgical procedure. As a premedication they can potentiate the effects of anaesthetics reducing the dose required.

**Disadvantages:** Side effects such as cardiovascular depression, hypotension and bradyarrhythmias can occur with resulting reduced cardiac output. They can cause abortion in pregnant animals. They can cause respiratory depression and arrest when administered with some analgesic agents, so care must be taken to monitor respiration rates when used with opiates.

**Local anaesthetics:** lignocaine, bupivacaine, cocaine

**Description:** These agents block the transmission of nerve impulses by affecting electrolyte transmission across the cell membrane. They are usually administered as an injection either around the area of an incision (local block) or a nerve (regional block).

**Advantages:** They can play a part in the analgesia regimen and also allow for minor procedures to take place in sedated animals, so preventing the requirement for a general anaesthesia.

**Disadvantages:** Some local anaesthetics can cause muscle necrosis in high doses, with bupivacaine being the most myotoxic so care should be taken when using them in muscle layers. Even though they exert most of their effects at the site of injection that are absorbed systemically and so at high doses can result in cardiac and respiratory depression and arrhythmias, convulsions and seizures.

## Anaesthetics

**Inhalant agents:** halothane, isoflurane, sevoflurane, methoxyflurane

**Description:** Inhalant anaesthetics are a group of volatile liquid anaesthetics that are administered as vapours and are absorbed through the lungs into the blood stream. They should be provided using an appropriately designed and calibrated anaesthetic vaporiser and breathing system, however in the past bell jars and induction chambers with the liquid provided on gauze has been used. Older agents include ether and chloroform however these agents are flammable and are toxic/carcinogenic so are no longer acceptable.

**Advantages:** These agents are quickly absorbed into the blood stream from the lungs and so act rapidly. Since they are excreted from the blood into the lungs rather than requiring urinary excretion or metabolism) anaesthesia is rapidly reversed once the precision of anaesthesia ends. This makes them safe and the anaesthesia more controlled.

**Disadvantages:** These drugs have no analgesic effects and so a pre-emptive analgesic must be provided when being used for painful procedures. Operator exposure is a risk and so these agents must only be used with the appropriate equipment including vaporisers, anaesthetic circuits and exhaust and scavenger systems. To operate this equipment, users must be appropriately trained.

**Dissociative anaesthetics:** ketamine, tiletamine (only available in combination with the benzodiazepine zolazepam, marketed as Zoletil in Australia)

**Description:** These drugs are types of hallucinogens that work by inducing a dream-like state or euphoric anaesthesia and amnesia where the animal becomes mentally detached from the procedures they are undergoing. They are usually provided in combination with other drugs, particularly benzodiazepines, analgesics or alpha2-adrenergic agonists for use in general anaesthesia in animals.

**Advantages:** These drugs have a wide range of safety and can be used in pregnant animals. Animals maintain pharyngeal reflexes and tone so will not regurgitate under ketamine anaesthesia.

**Disadvantages:** Ketamine is a controlled drug (Schedule 4 appendix D) meaning that it can only be used with the appropriate authority and under strict conditions as it is prone to misuse. They can cause an increase in cerebral blood flow and intracranial pressure and can induce seizures in susceptible animals. In recovery animals may show delirium and react violently to stimuli, so animals should be recovered in a dark, quiet and safe environment. They provide poor muscle relaxation and animals cannot be intubated with ketamine alone.

**Barbiturates:** thiopentone, pentobarbitone

**Description:** Barbiturates are fast-acting central nervous system depressants. They are an early class of anaesthetics and so are rarely used in modern anaesthesia except in research or euthanasia). When used, they should only be used with an analgesic agent and generally only for terminal procedures. They are usually given intravenously or intraperitoneal as they can cause tissue irritation or and necrosis if given by other routes.

**Advantages:** These drugs can provide a relatively stable and long anaesthesia when used by experienced operators. They are excellent drugs for use in terminal studies or for euthanasia.

**Disadvantages:** Barbiturates must only be used if justified as superior drugs are now available. They have many side-effects and low margins of safety and their actions can vary due to their complex metabolism and excretion pathways. Due to the long excretion times (especially

pentobarbitone) recovery is protracted and unpleasant with nervous stimulation, tremors and vocalisation.

**Others:**

### **Tribromoethanol (Avertin)**

**Description:** Avertin is an injectable anaesthetic causing generalised CNS depression. It is no longer available commercially, but is often made from scratch in laboratories for use in animals. It has a number of side effects and should not be used in animals unless considerable justification is provided and there is no other viable alternative and then only in mice and rats for terminal procedures.

**Advantages:** Avertin causes varying degrees of anaesthesia and muscle relaxation but there are no advantages over other newer superior agents.

**Disadvantages:** Anaesthesia depth and duration is inconsistent; the drug is extremely irritant and can cause peritonitis and typhilitis. It degrades quickly producing toxic by-products and its metabolites are nephrotoxic and hepatotoxic.

## **Analgesics and anti-inflammatories**

**Opiate and opioid drugs:** fentanyl, buprenorphine, morphine, codeine

**Description:** These are natural and synthetic drugs that provide potent analgesia, however the potency, duration of action and specific effects will vary depending on the drug used. They are mostly given by injection, but can be given orally although the dose needs to be higher for oral administration.

**Advantages:** They are potent analgesics and also cause a degree of euphoria and sedation so they allow for a slight reduction of anaesthesia. Their main site of action is the CNS. While there are reports that they can have anti-inflammatory actions, these are usually the less-commonly used drugs and at doses way over the normal therapeutic doses.

**Disadvantages:** These drugs can be addictive and there can be a withdrawal in animals receiving drugs regularly over several days when administration stops. They produce respiratory depression and resulting acidosis which can lead to cerebral oedema and swelling, which can be important in neurological studies. Due to their propensity for misuse, their use is highly regulated with strict and often confusing requirements for authorisation, use and storage.

**Non-steroidal anti-inflammatories (NSAIDs):** ibuprofen, aspirin, flunixin, carprofen, ketoprofen, paracetamol

**Description:** This group of drugs act on the prostaglandin chemokine pathways involved in inflammation, pain and swelling. They are less potent analgesics than opiates but do not have the same strict controls placed on their use. They act peripherally at the sites of inflammation. NSAIDs are best started a day before the painful procedure in order to give maximum analgesic effect.

**Advantages:** NSAIDs do not cause sedation and can be combined with opiates to provide a combination of peripheral anti-inflammatory and central pain relief. They aren't restricted drugs and so their use is easier to facilitate.

**Disadvantages:** NSAIDs have an effect on the gastrointestinal system causing ulceration and irritation and can also affect renal function particularly in dehydrated animals. Because NSAIDs affect the inflammatory process, which is an important part of healing and often the subject of the

research project, their use may be contraindicated in some research projects. Paracetamol has been reported to lead to weight loss and reduced water consumption (when given in drinking water) and increased liver and renal indices when given at a high (300mg/kg) dose for 7 days. If prolonged treatment is required, lower doses or an alternative agent should be used. It can also lead to changes in responses during behavioural testing.

The COX inhibitors (ibuprofen, meloxicam etc) can cause gastrointestinal ulceration and renal damage if given for long periods or in dehydrated animals.

## Other agents

### **Anticholinergics:** Atropine

**Description:** Anticholinergic drugs, such as atropine, block the neurotransmitter acetylcholine and so the conduction of nerves in the parasympathetic nervous system. The parasympathetic nervous system regulates much of the body's unconscious actions and supplies the gastrointestinal system, glands such as salivary and sexual glands and the heart through the vagus where it acts to slow the heart beat and cardiac output.

**Advantages:** Anticholinergics therefore act to reduce salivation and prevent bradycardia (slowing of the heart rate), which can often occur during anaesthesia.

**Disadvantages:** Because the parasympathetic nervous system has slightly different effects in different species, anticholinergics are not recommended in ruminants as they increase salivation.

### **Neuromuscular blockers:** succinylcholine, vecuronium, pancuronium

**Description:** These drugs block acetylcholine at the neuromuscular junction and so cause complete muscular paralysis, but have no anaesthetic or analgesic possible meaning the animal will be conscious and aware of its surroundings. Because they cause paralysis, animals who have received these drugs must be ventilated, given sufficient pain relief and be maintained under a good level of anaesthesia and have physiological measures monitored extremely carefully as normal signs of anaesthetic depth will not be used eg reflexes, eye position etc. They can only be used by experienced anaesthetists and their use must be clearly justified in the AEC application.

**Advantages:** These drugs cause muscle relaxation and so are beneficial when carrying out surgery such as bone, or muscle surgery or to help facilitate intubation.

**Disadvantages:** The use of neuromuscular blockers will mean that reflexes, commonly used to assess anaesthesia depth, will no longer be present. This can result in animals undergoing painful procedures while in insufficient depths of anaesthesia. For this reason, these drugs carry restrictions to their use as outlined in the guidelines.

## Appendix 2

### RECOMMENDED DRUGS AND DOSAGES FOR LABORATORY RODENTS

For other acceptable anaesthetic and analgesic regimens and doses for rodents and other species, see [NHMRC Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes](#)

#### Anaesthetic Agents

Drugs and Combinations	Mouse		Rat	
	Dose	Comment	Dose	Comment
Ketamine Xylazine	75-100mg/kg IP 8-10mg/kg IP	20-30 min	80-100mg/kg IP 5-10mg/kg IP	40-60 min
Ketamine Xylazine Isoflurane	40-100mg/kg IP 5-10mg/kg IP 1.2-2%	10-30 min (ket/xyl), iso will last as long as being admin	40-100mg/kg IP 5-10mg/kg IP 1.2-2%	15-60 min, iso will last as long as being admin
Zolazepam/Tiletamine (Zoletil)	40mg/kg IP		80mg/kg IP	Restraint only, does not provide surgical anaesthesia
Alfaxalone	Females 40-80mg/kg IP Males 80-120mg/kg IP	60-80 minutes	Females 25mg/kg IP Males 40mg/kg IP	60-80 minutes
Xylazine	8-10mg/kg IP		5-10mg/kg IP	

#### Analgesic Agents

Drugs and Combinations	Mouse		Rat	
	Dose	Comment	Dose	Comment
Buprenorphine Injectable	0.1-0.2mg/kg SC q8 hours	8 hours duration	0.01-0.05mg/kg SC q8 hours	8 hours duration
Buprenorphine in Drinking Water <sup>#</sup>	0.5mg/kg or 2.5mL/160mL water	Continuous	0.5mg/mL or 2.5mL/160mL water	Continuous
Buprenorphine in Jelly <sup>#</sup>	0.5mg/kg PO SC q8 hours	8 hours duration	0.5mg/kg PO SC q8 hours	8 hours duration
Ibuprofen	40mg/kg /day PO	Gavage	40mg/kg/day PO	Gavage
Ibuprofen* (Nurofen for Children 40mg/mL)	0.4mg/mL or 1mL/100mL drinking water	Continuous	0.4mg/mL or 1mL/100mL drinking water	Continuous

Paracetamol	300mg/kg PO	Gavage	300mg/kg PO	Gavage
Paracetamol* (Panadol Elixir 48mg/mL)	1-2mg/mL or 2-4mL/100mL drinking water	Continuous	2-4mg/mL or 4-8mL/100mL drinking water	Continuous
Meloxicam (Metacam)	1mg/kg/day PO, SC	24 hours	2mg/kg/day PO, SC	24 hours
Carprofen (Rimadyl)	5mg/kg/day SC, IP, PO	24 hours	5mg/kg/day SC, IP, PO	24 hours

#NB because buprenorphine is partially metabolised in the liver, it must be given at a higher dose when given orally to achieve the same blood levels and analgesic effect

\*Assumes a 25g mouse drinks 5mL/day and a 300g rat drinks 30mL/day

A NSAID analgesic can be combined with an opioid analgesic to increase the pain relief, however, two NSAIDs cannot be given concurrently as this can increase the chances of toxic reactions such as gastric ulceration.

## Appendix 3

### MONITORING OF ANAESTHESIA DEPTH AND ANIMAL WELLBEING

Signs / Criteria	Species where it should be used in monitoring anaesthesia	Procedure	Stages of anaesthesia				Comments
			Conscious (at rest or pre-anaesthesia)	Light anaesthesia or sedation	Surgical-depth anaesthesia	Too Deep (at risk of anaesthetic crisis)	
<b>Voluntary muscle movement</b>	All species	Stimulate the animal with touch, restraint or sound.	Present and normal, animal perceives the stimulus centrally and reacts normally to it. Response may be turn head to site of stimulus, struggle, kick, vocalise or move away from stimulus	No response to stimulus.	No response to stimulus	No response to stimulus	
<b>Withdrawal Reflex</b>	All species	Pinch skin firmly with fingers or forceps or a pin or needle prick, this can be done between the digits or in rodents, to the side of the digits, the ear or any other loose skin.	Animal will react immediately with only light pinch, will turn head towards the area in preparation to bite while at the same time pulling the pinched area away.	A harder pinch is required to illicit a response which will be milder, the animal may lift its head from the table but not in a fully coordinated action. There will be some withdrawal of the limb but both responses will be reduced.	No response to pain either a withdrawal or movement of the head.	No response to pain either a withdrawal or movement of the head.	



Stages of anaesthesia							
Signs / Criteria	Species where it should be used in monitoring anaesthesia	Procedure	Conscious (at rest or pre-anaesthesia)	Light anaesthesia or sedation	Surgical-depth anaesthesia	Too Deep (at risk of anaesthetic emergency)	Comments
<b>Palpebral reflex</b>	Cats, dogs, rodents, (larger animals have unreliable ocular reflexes)	Gently touch the lateral corner (canthus) of the eye finger.	Animal will blink	Animal will blink.	No response.	No response.	
<b>Jaw tone</b>	Larger rodents, cats, dogs, larger animals	Attempt to open the animals mouth	Animal will resist attempts to open the mouth and/or move its head away.	There may be some resistance to opening of the mouth requiring increased force (can prevent intubation) but no movement of the head.	Jaw opens easily.	Jaw opens easily.	
<b>Swallowing reflex</b>	Rabbits, cats, dogs, larger mammals	Attempt to pull the tongue forward with clean gauze.	Animal will swallow and move its head away.	Animal will swallow.	No response	No response	

			Stages of anaesthesia				
Signs / Criteria	Species where it should be used in monitoring anaesthesia	Procedure	Conscious (at rest or pre-anaesthesia)	Light anaesthesia or sedation	Surgical-depth anaesthesia	Too Deep (at risk of anaesthetic crisis)	Comments
<b>Respiration (rate and depth)</b>	All species	Observation of chest, stethoscope, respiratory monitors, movement of re-breathing bag (anaesthetic machines and intubated animals)	Respiratory rate will be fast/normal and responsive to stress such as stress from handling or restraint or excitement.	Respiratory rate will decrease to a baseline normal, but will increase in response to stimulation especially painful stimuli.  NB Some animals may 'breath hold' when in light anaesthesia in response to being exposed to inhalation anaesthetics. They will show all other signs of light anaesthesia in terms of reflexes.	No response to stimuli from pain.	No response to stimuli from pain but at increasing depth respiratory rate will fall and eventually the animal will go into respiratory arrest.	Respiratory rate needs to be assessed in terms of changes in rate from the baseline rather against species normal values. The rate should be measured after stable anaesthesia is provided but before surgical stimulation and this used as the baseline against which changes are measured.
<b>Heart rate</b>	All species	Pulse rate at various sites, stethoscope, heart monitors and pulse oximeter, feel the rate through the chest.  It may be difficult to monitor heart rate in small rodents however the heart	Heart rate will remain fairly constant in any animal but is different between animals depending on species, age, breed, size and health status.	There may be a slight decrease in heart rate due to relaxation (an effect of the heart rate before anaesthesia being artificially high due to handling and preparation for anaesthesia).	If painful stimulus is applied, there should be no change in rate.	If painful stimulus is applied, there should be no change in rate.  The heart rate may go below baseline if the animal is in deep anaesthesia or in cases of overdose.	Heart rate needs to be assessed in terms of changes in rate from the baseline rather against species normal values. The rate should be measured after stable anaesthesia is provided but before surgical stimulation and this used as the baseline against

		beats can be seen through the chest.  Depth of anaesthesia can be assessed by monitoring rate after painful stimulus applied.		If painful stimulus is applied the heart rate will increase.			which changes are measured.
<b>Mucous membrane/skin colour</b>	All species (but may be hard to see the animal's colour in heavily pigmented animals)	Observation, pressing the gums, looking in to the eyes, any exposed skin can be used but the eyes, mouth and nose are best, the ears can be used in some hairless animals. Pulse oximetry can give a more accurate reading of blood and circulatory wellbeing.	Pink, when pressure is applied to the gums of larger animals the colour should return within 3 seconds	No effect.	No effect.	The animal's colour may become pale or blue if the respiratory rate is decreased to an extent to which the blood is not being oxygenated.	This is an assessment of the degree of oxygenation of the blood and also the state of the peripheral circulatory system. It should be monitored but is not very helpful for assessing depth

These monitoring criteria should be used to monitor animals while under anaesthetic, and be included in the anaesthesia monitoring records. Animals should be confirmed to be in a surgical depth of anaesthesia before any painful procedure begins. The more criteria, and higher the frequency of monitoring, the better will be the assessment of the animal's wellbeing and anaesthetic depth. Note: The above table is to be used as a guide only as there are many factors other than anaesthetic depth that can affect reflexes and especially physiological factors. The choice of anaesthetic agent, analgesia, health status, species, age and procedure all can affect the above. For example, heart and respiratory rates increase can be in response to light anaesthesia with the animal feeling pain, but could also be due to blood loss causing decreased amounts of oxygenated blood. Therefore, to give more anaesthetic could result in an overdose and death of the animal, so careful assessment is required before assuming it is the depth of anaesthesia causing changes to monitoring criteria. It is best to use many criteria when monitoring animals in order to prevent mistakes being made.

## Appendix 4

### ANAESTHETIC EMERGENCIES AND RESUSCITATION IN RODENTS

While there are a large number of possible causes of anaesthetic emergencies, the most common in healthy research animals are:

#### Insufficient depth of anaesthesia

- If the animal starts to react to stimuli, move, vocalise or struggle then it means the depth of anaesthesia is insufficient for the procedure and the animal may be experiencing pain and distress
- The most likely explanation is that the animal didn't receive (injectable) or isn't receiving (inhalational) enough anaesthetic agent and there are many possible causes
- The first action that should be taken is to immediately stop the procedure and act to ensure the animal doesn't contaminate the surgical field before troubleshooting the cause
- Injection anaesthesia:
  - Always be prepared with an extra, smaller, safe dose of injectable anaesthetic or have an anaesthetic machine nearby and ready in case the animal is too light, especially if the injection was IP, however, be careful not to overdose the animal which can be a consequence of giving an extra dose
- Inhalational anaesthesia:
  - Check the vaporiser to see what concentration of gas the animal is receiving and turn it up by 2% while troubleshooting, then check the oxygen to ensure that it's flowing and flowing at the correct flow rate and that there is anaesthetic in the machine
  - If on a mask, check to make sure it's fitted correctly, then check the tubing between the animal and the anaesthetic machine to ensure all tubing is connected in the right way
  - If an induction chamber is attached to the same machine by way of a 3-way tap, check to ensure the tap is in the correct position to allow flow to the mask
  - Check for leaks in the system by blocking the outlet of the tubing and pressing the oxygen flush. This should cause pressure to build in the system and if there is a leak, there may be a sound from that site and the pressure in the system will dissipate without having to remove the blockage
- To prevent this from occurring, before starting a procedure ensure:
  - Any injectable agents are correctly administered at the correct dose rate
  - The anaesthetic machine and its tubing are correctly connected and the oxygen is on (with a spare bottle nearby if possible) – test the machine before using it for the first time
  - The vaporiser is full when you start and there is enough oxygen to last the procedure
  - Masks/nose cones fit correctly

- The animal is in a stable surgical depth of anaesthesia before beginning any procedure

### **Animal is too deep**

- If the animal has been given or is being given too much anaesthesia, respiratory arrest will be the first sign seen and this will be followed fairly quickly by cardiac arrest.
- If respiratory arrest, and even early cardiac arrest, are detected early enough the animal may respond well to resuscitation and supportive treatment
- Before respiratory arrest occurs, there will be a gradual decrease in respiratory rate. This is why it is extremely important to constantly monitor the animal during the procedure so that respiratory changes can be detected early on and action taken
- To prevent this from occurring ensure:
  - Any injectable agents are correctly administered at the correct dose rate
  - Supportive fluids are administered and a heating mat is used
  - The animal is in a stable surgical depth of anaesthesia before beginning any procedure
  - The minimum concentration of anaesthesia is used to achieve the required depth of anaesthesia
  - The animal's respiration is monitored constantly so that any decrease in respiratory rate will be noticed, and action taken before arrest occurs
  - That any 'top up' doses of injectable anaesthesia are no more than 30% of the original dose

(NB opiate analgesics such as buprenorphine can cause respiratory depression which, when combined with other anaesthetic agents, may predispose animals to respiratory arrest if it is given as pre-emptive analgesia. If you are administering an opiate analgesic pre-emptively and have had unexpected deaths, then reconsider your anaesthesia/analgesia regimen or doses and monitor the animals closely especially during recovery)

### **Resuscitation**

As said above, there are many possible causes of respiratory arrest and emergencies, however, depending on what is being monitored it may not be possible to detect all of them. If respiratory arrest occurs then basic resuscitation can be commenced, that should be adequate for the causes outlined above if commenced early enough. If an animal stops breathing the following steps should be taken:

- Turn off the vaporiser (if inhalational anaesthesia), remove the animal from the breathing circuit
- Stop the procedure, being sure to protect the surgical field from contamination
- Check for a heartbeat and mucous membrane colour
- Commence chest compressions by gently pressing on the ribs, which will compress both the heart and lungs so that ventilation can take place
- flush the system with oxygen for 3-4 seconds then return the animal to the breathing system
- Administer supportive fluids and ensure the animal is on a heating pad
- Continue chest compressions until the animal recovers or until no improvements in the condition (eg recommencement of heart beat) have been seen for at least 10 minutes

- If the animal is on inhalational anaesthesia, the ventilation from resuscitation will allow the anaesthetic to dissipate fairly quickly and the animal may become too light so be ready to administer the anaesthetic again
- If the animal has received an injectable anaesthetic, then this could take much longer before the agents are metabolised and the animal will recover so support may need to be provided for longer and the chance of success is less