GUIDELINES TO PROMOTE THE WELLBEING OF ANIMALS USED FOR SCIENTIFIC PURPOSES

THE ASSESSMENT AND ALLEVIATION OF PAIN AND DISTRESS IN RESEARCH ANIMALS:
GUIDELINES TO PROMOTE THE WELLBEING OF ANIMALS USED FOR SCIENTIFIC PURPOSES
THE ASSESSMENT AND ALLEVIATION OF PAIN AND DISTRESS IN RESEARCH ANIMALS

[This publication is a living document and will be updated from time to time. Please refer to the NHMRC website at http://www.nhmrc.gov.au/index.htm to download updates for incorporation into this folder.]
CONTENTS

HOW TO USE THIS DOCUMENT vii
DEFINITIONS ix
ABBREVIATIONS xi

PART I
ANIMAL WELLBEING AND SCIENTIFIC OUTCOMES
1 General principles for using animals in scientific activities 3
2 Wellbeing, stress, distress and pain 5
3 Effects of an animal’s wellbeing on scientific outcomes 13

PART II
PLANNING, CONDUCTING AND REVIEWING RESEARCH PROTOCOLS TO MAXIMISE WELLBEING AND MINIMISE PAIN AND DISTRESS IN ANIMALS
4 Planning new research protocols 17
5 Gaining approval for new research protocols 43
6 Implementing and reviewing strategies to promote wellbeing 45
7 Checklist for promoting animal wellbeing 49

PART III
FACTSHEETS
A Administration of substances A1
B Behaviour modification B1
C Biological sample collection C1
D Blood collection D1
E Environmental enrichment strategies E1
F Foetal and embryonic studies F1
G Food and water intake modification G1
H Humane killing and euthanasia H1
I Pain management: anaesthesia, analgesia and anxiolytics I1
J Polyclonal antibody production J1
K Surgical procedures K1
L Toxicology L1
M Tumour induction M1
N Wildlife research N1

APPENDIX: PROCESS REPORT

INDEX Index 1

GUIDELINES TO PROMOTE THE WELLBEING OF ANIMALS USED FOR SCIENTIFIC PURPOSES
TABLES

Table 4.1  Likelihood and consequence matrix for characterising risk  27
Table 4.2  Species-specific signs of pain and/or distress  37
Table A1  Common methods and routes of intravenous administration of substances  A3
Table A2  Recommended maximum injectable volumes for laboratory rabbits and rodents  A4
Table A3  Recommended needle gauge (G) and length for different species of animal  A4
Table A4  Procedures to minimise pain and distress when administering substances  A9
Table B1  Procedures for minimising pain and distress when modifying behaviour  B5
Table C1  Summary table for urine collection methods and their performance  C6
Table D1  Recommended site and volume of blood collection using calculated blood volume  D2
Table D2  Maximum volumes and recovery periods for blood collection  D4
Table D3  Signs and treatment of acute blood loss  D5
Table D4  Wellbeing issues to consider for blood collection in selected species  D7
Table E1  Environmental enrichment strategies for animal species used in scientific research  E2
Table G1  Procedures and points to consider when restricting the diet of animals used in scientific research  G6
Table H1  Methods of humane killing and euthanasia in rats and mice  H4
Table H2  Methods of humane killing and euthanasia in guinea pigs  H4
Table H3  Methods of humane killing and euthanasia in rabbits  H5
Table H4  Methods of humane killing and euthanasia in sheep and goats  H5
Table H5  Methods of humane killing and euthanasia in birds  H6
Table H6  Methods of humane killing and euthanasia in pigs  H6
Table H7  Methods of humane killing and euthanasia in fish  H7
Table I1  Inhalation anaesthesia: induction and maintenance concentrations of inhalation anaesthetics in laboratory animals  I15
Table I2  Injectable anaesthesia in rodents and rabbits  I15
Table I3  Injectable anaesthesia in other laboratory animals  I17
Table I4  Analgesia in rodents and rabbits: non-steroidal anti-inflammatory drugs (NSAIDs)  I18
Table I5  Analgesia in rodents and rabbits: opioids  I19
Table I6  Analgesia in other laboratory animals: non-steroidal anti-inflammatory drugs (NSAIDs)  I20
Table I7  Analgesia in other laboratory animals: opioids  I21
Table J1  Maximum volumes for injection of antigen with depot adjuvant for different animal species  J4
Table K1  Minimising surgical risks to animal wellbeing  K10
FIGURES

Figure 2.1  Schematic diagram of wellbeing  5
Figure 2.2  Model of stress and distress response  8
Figure 2.3  Nerve pathways involved in perception of, and response to, pain  10
Figure 4.1  Early planning for a project that might involve the use of animals  18
Figure I1  Example of pain management options classified according to the potential for the procedure to result in pain and distress ('invasiveness')  13
Figure I2  Site of action of analgesic agents in the pain pathway  113
HOW TO USE THIS DOCUMENT

These guidelines are to be read in conjunction with the Australian code of practice for the care and use of animals for scientific purposes (the Code).

The guidelines promote the wellbeing of animals used for scientific purposes, and aim to minimise their experience of pain and distress. To do this, the guidelines are divided into three parts.

- Part I provides background material to assist understanding and awareness of animal wellbeing and how it relates to scientific activities.
- Part II provides basic strategies for
  - planning research protocols to identify the risk of animal pain and distress
  - conducting research to manage the risk of animal pain and distress
  - reviewing research protocols to minimise animal pain and distress in future research.
- Part III provides factsheets on issues to consider for specific research protocols. They have been developed with the aim of providing guidance to investigators, rather than being prescriptive.

Please consult the Code for principles governing the use of animals for scientific purposes.

The online version of this document can be found at http://www.nhmrc.gov.au

The information in this document will be regularly reviewed, so please consult the above website for updates.

If you have any feedback or would like to notify us of updates, please contact us at: nhmrc.publications@nhmrc.gov.au
**DEFINITIONS**

These definitions have been taken from the Code and have been selected as the most relevant to these guidelines. See the Code for other definitions.

**Animal welfare**

An animal’s quality of life based on an assessment of an animal’s physical and psychological state as an indication of how the animal is coping with the ongoing situation as well as a judgement about how the animal feels (see also ‘Animal wellbeing’ and ‘Distress’).

**Animal wellbeing**

An animal’s present state with regard to its relationship with all aspects of its environment, both internal and external. It implies a positive mental state, successful biological function, positive experiences and freedom from adverse conditions.

**Distress**

The state of an animal that has been unable to adapt completely to stressors, and that manifests as abnormal physiological or behavioural responses. It can be acute or chronic and may result in pathological conditions. Distress is associated with negative emotional experiences and can be caused by either physical or psychological stressors, or both.

**Euthanasia**

The humane killing of an animal, in the interests of its own welfare, to alleviate pain and distress (see ‘Humane killing’).

**Humane killing**

The process of killing an animal with minimal pain and distress (see ‘Euthanasia’).

**Pain**

An unpleasant sensory and emotional experience associated with actual or potential tissue damage. It may elicit protective actions, result in learned avoidance and distress and may modify species-specific traits of behaviour, including social behaviour.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3Rs</td>
<td>the principles of Replacement, Reduction and Refinement</td>
</tr>
<tr>
<td>AEC</td>
<td>animal ethics committee</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiograph</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FCA</td>
<td>Freund’s complete adjuvant</td>
</tr>
<tr>
<td>HPA axis</td>
<td>hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>ID</td>
<td>intradermal</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>MAC</td>
<td>minimum alveolar concentration</td>
</tr>
<tr>
<td>NAD</td>
<td>no abnormalities detected</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NSAID</td>
<td>non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>PO</td>
<td>per os (administered by mouth)</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinyl chloride</td>
</tr>
<tr>
<td>SC</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operating procedure</td>
</tr>
<tr>
<td>VAP</td>
<td>subcutaneous vascular access port</td>
</tr>
</tbody>
</table>
PART I

ANIMAL WELLBEING AND SCIENTIFIC OUTCOMES
1 GENERAL PRINCIPLES FOR USING ANIMALS IN SCIENTIFIC ACTIVITIES

1.1 INTRODUCTION

These guidelines have been developed to assist investigators and members of animal ethics committees (AECs) to achieve the goals of the Australian code of practice for the care and use of animals for scientific purposes (the Code; NHMRC 2004), by promoting the wellbeing of animals used for scientific purposes.

To meet the requirements of the Code, scientists, animal carers and members of AECs must ensure that the use of animals in scientific activities is justified, and that there are no alternatives to using animals. For animals that are used, pain and distress must be alleviated or minimised.

Uncontrolled adverse effects on animal wellbeing directly affect the validity of research results and the number of animals used to achieve a scientific objective. Therefore, an animal’s potential to experience pain and distress while it is being used for scientific purposes has ethical, scientific and practical implications.

These guidelines focus on the practical issues involved in:

• promoting animal wellbeing
• minimising pain and distress (including recognising and assessing evidence that an animal is experiencing distress and determining whether this is associated with pain)
• developing strategies to effectively manage pain and distress and to promote animal wellbeing.

In addition to the potential effects of specific research procedures on the wellbeing of animals, animals can experience a range of stressors that are part of their daily living conditions and social environment. It is important that investigators consider the wellbeing of an animal over the whole of its life, whether the animal is bred specifically for research purposes or obtained from other sources.

1.2 LAWS AND REGULATIONS

The Code provides guidance for investigators, teachers, institutions, AECs and all people involved in the care and use of animals for scientific purposes. See Appendix 2 of the Code for state and territory legislation and codes of practice. For updated information on laws and regulations, see the NHMRC website1 and follow the links to information on animal wellbeing.

Concern about the wellbeing of animals used for scientific purposes, and the perception of the levels of pain and distress endured by such animals, have been translated into laws and regulations that seek to limit pain and distress in laboratory animals. The Code sets out the common framework for each Australian state and territory for ensuring the ethical and humane care and use of animals used in scientific activities; however, each Australian state and territory has its own legislation relating to the use of animals in research. The legislation can be downloaded from the AustLII database.2

1.3 KEY PRINCIPLES FOR PROMOTING ANIMAL WELLBEING

The Code outlines key principles for promoting the wellbeing of animals and the quality of scientific outcomes. The principles of Replacement, Reduction and Refinement (known as the 3Rs) aim to reduce the impact of scientific activities on animal wellbeing (Russell and Burch

1 http://www.nhmrc.gov.au
2 http://www.austlii.edu.au
and are pivotal to achieving the goals of the Code. Underlying these key principles is strong scientific evidence that animals experience pain and distress in a manner similar to humans; decisions regarding an animal’s wellbeing must be based on this premise. The 3Rs are defined as follows:

- Replacement—If a viable alternative method exists that would partly or wholly replace the use of animals in a project, the Code requires investigators to use that alternative. Examples of alternative methods include in vitro techniques and computer models.

- Reduction—A project must be designed to use no more than the minimum number of animals necessary to ensure scientific and statistical validity. However, the principle of reducing the number of animals used should not be implemented at the expense of greater pain and distress for individual animals.

- Refinement—Studies must be designed to avoid or minimise both pain and distress in animals, consistent with the scientific objective. Investigators must also be competent in the procedures they perform. Project design must take into account
  - the choice of animals, their housing, management and care and their acclimatisation
  - the choice of techniques and procedures
  - the appropriate use of sedatives, tranquillisers, analgesics and anaesthetics
  - the choice of appropriate measures for assessing pain and distress
  - the establishment of early intervention points and humane endpoints
  - adequate monitoring of the animals
  - appropriate use of pilot studies.

Other key principles in addition to the 3Rs include Justification and Responsibility:

- Justification—The Code requires projects using animals to be performed only after they are justified, weighing the predicted scientific or educational value of the project against the potential effects on the wellbeing of the animals. Thus, the justification must take into account all aspects of the project that may have an adverse impact on the animals.

- Responsibility—The Code states that investigators who use animals for scientific purposes have personal responsibility for all matters relating to the wellbeing of the animals. They have an obligation to treat the animals with respect and to consider their wellbeing as an essential factor when planning or conducting projects. To meet these responsibilities, it is essential that investigators are knowledgeable about all factors associated with the project that may affect the wellbeing of the animals they use, mechanisms to minimise these effects, the monitoring and assessment of adverse effects on animal wellbeing, and appropriate actions to take if adverse effects are observed.

Further reading—key principles underlying the Code


2 WELLBEING, STRESS, DISTRESS AND PAIN

This chapter discusses the concept of wellbeing and how it is affected by stress, distress and pain.

2.1 DEFINING THE CONCEPTS

The terms ‘animal wellbeing’ and ‘animal welfare’ are used interchangeably in many publications, but there is considerable debate as to the use and interpretation of these terms in various settings. The Code defines both and, in so doing, clarifies their meaning and provides a practical framework for their use.

As defined in the Code, the notion of animal welfare encompasses the different ways in which an animal may respond to its circumstances, ranging from a positive state of wellbeing to a negative state of distress. Criteria that define wellbeing and distress provide a basis for the critical evaluation of how an animal is coping in a given situation, and hence also provide evidence that informs our judgment about their welfare.

In this document, the terms ‘animal welfare’, ‘animal wellbeing’, ‘distress’ and ‘pain’ are used as defined in the Code.

2.2 WELLBEING

Animal wellbeing relates to evidence of how an animal is coping with a given situation and a judgment as to how the animal feels in these circumstances. Figure 2.1 outlines the way in which an animal perceives and experiences internal, external and environmental factors, and how this affects its wellbeing.

Wellbeing is an internal state involving quality of life that is affected by responses to internal and external factors. These factors may be good or bad, positive or negative. Individuals experience wellbeing differently, because of their different needs, goals, motivations and preferences. In addition, wellbeing in one individual can vary from time to time, and changes may or may not be orderly or predictable. As a protective mechanism, departures from optimal wellbeing generally cause normal adaptive coping responses designed to return the animal to its normal state of wellbeing. Ineffective responses may result in distress, disability, disease or death.

Figure 2.1 Schematic diagram of wellbeing

![Schematic diagram of wellbeing](image-url)
2.2.1 PHYSIOLOGICAL AND BEHAVIOURAL INDICATORS OF WELLBEING

Assessment of wellbeing involves using a combination of behavioural and physiological measures that indicate:

- the animal’s health status
- evidence of species-specific behaviours
- the status of the key indicators of the physiological and behavioural responses to a stressor (see Section 2.3.3 for details).

Animal behaviour is an important indicator of how an animal is interacting with its environment: changes in patterns of behaviour are often the first pointer as to how an animal is responding to and coping with change. Animal behaviour can be assessed by observation and during interactions with the researcher or animal carer. A number of factors can influence individual responses. Therefore, knowledge of species-specific behaviours as well as prior history is important. Documentation of the range and level of activities such as eating, drinking, play, grooming, sleeping, resting, interactions with conspecifics and exploration of the environment can be used to describe patterns of behaviour indicative of wellbeing. Species-specific differences will be seen in the types and levels of activities. Individual responses within this framework may be modulated by prior experiences.

Indicators of an animal’s state of health include general appearance, posture, coat condition, clinical signs (eg temperature, heart rate, respiratory rate), haematological and biochemical measurements, responses to handling, demeanour, temperament, maintenance of bodyweight or, in immature animals, rate of weight gain, and reproductive performance.

Although requiring sophisticated methods, the pattern of circadian rhythms in the physiological, immunological and neuroendocrine indicators of the stress response is a sensitive indicator of physiological adaptation.

Researchers and animal carers must be familiar with species-specific indicators of wellbeing; these are the basis for assessment of evidence of pain and distress. Absence of signs of disease or abnormal behaviours, together with positive evidence of health status and behaviour, indicate that an animal is probably coping with its current situation.

Further reading—assessing animal wellbeing


2.3 STRESS AND DISTRESS

2.3.1 STRESS

Stress is the response of the animal to a ‘stressor’ (external events or internal factors, including pain) and is a normal feature of life, serving important adaptive functions. The stress response consists of a combination of four general biological responses: behavioural, autonomic, neuroendocrine and immunological. The nature of this biological response varies between individuals and is influenced by factors such as previous experience, genetics, age and physiological state. Regardless of the combination of biological responses, the result is an alteration in the animal’s normal biological function as it attempts to adapt to or cope with the stressor, behaviourally and/or physiologically.

In most cases, this altered biological function has a minimal effect on the animal’s wellbeing; the stressor is either brief or it is eliminated, so biological function soon returns to normal. However, if the stress is not alleviated or if the stressor is large enough, the animal is forced into a prepathological state that makes it vulnerable to pathology such as disease, abnormal behaviour; reduced growth or some other type of undesirable shift in biological function. During this time, the animal experiences distress, and its wellbeing is threatened.

The degree and context of the stressor are crucial in determining whether distress occurs. The existence of subclinical stress may not affect normal biological function, but may make the animal vulnerable to the effect of a second subclinical stress; either stressor alone would have no effect on biological function, but their accumulated biological cost could result in distress.

2.3.2 DISTRESS

Distress occurs when, in magnitude or duration or both, the stress response is such that significant changes in biological function must occur for the animal to survive. For example, distress in animals results when a stressor (or a number of stressors) overwhelms the animal’s ability to cope with or manage a situation. Such a failure, from the animal’s point of view, arises directly from its capacity for sentience and the role of feelings and emotions in that experience.

Distress is not necessarily associated with pain, although pain will cause distress.

Various unpleasant experiences are often described and grouped together under the notion of suffering, including pain, distress, anxiety, fear, boredom and frustration. Suffering is the negative emotional state associated with distress; it can be due to adverse physical, physiological or psychological circumstances and is moderated by the cognitive capacity and experiences of the individual.

2.3.3 PHYSIOLOGICAL AND BEHAVIOURAL INDICATORS OF DISTRESS

The overall response of an animal to a stressor (which may or may not include a painful stressor) involves a variety of responses that are complex, closely integrated and complementary. The response may be modified by external or internal modifiers, such as experience, genetics, age, biological rhythms, the physiological or psychological state of the animal, the number of stressors (single or multiple) and their duration (acute or chronic), or the presence or absence of subclinical stress. The result is significant variation in responses among animals. Figure 2.2 shows a model of the stress and distress response.
Stress responses are broadly divided into behavioural, autonomic nervous system, neuroendocrine and immunological responses:

- Behavioural responses to a potential aversive stimulus are often the animal’s first line of defence. Some responses may be as simple as a reflex withdrawal or the ‘flight or fight’ response to protect the animal from injury; some convey the experience to others of the same or other species. Various species (e.g., prey species) may manifest tonic immobility (freezing), and avoidance behaviours can result from the animal learning from its experience. The nature of the behavioural response is determined by the species of animal, the location and intensity of any pain, and the environment. Acute, intermittent and chronic pain will produce different behavioural responses. Different individuals of the same species will behave differently in response to an identical pain stimulus. The absence of behavioural abnormalities does not necessarily imply that an animal’s psychological and physiological equilibrium is not disturbed.

- Autonomic nervous system responses (the ‘flight or fight’ response) can have marked, albeit short-term, effects on many biological systems. The results (increased metabolic rate, oxygen consumption, respiratory rate, heart rate, blood pressure etc) enable the animal to make quick physiological adjustments in response to sudden, short-term threats. Simultaneously, anabolic processes such as digestion, growth, reproduction and immune function are depressed. Learning and memory are also improved, enabling animals to react more adequately to similar stressors on subsequent exposures.

- Hormones secreted from the hypothalamic-pituitary neuroendocrine system have a broad, long-lasting effect on the body. Virtually all the biological functions that are affected by
stress, including immune competence, reproduction, metabolism and behaviour, are regulated by these pituitary hormones. The neuroendocrine responses work to inhibit non-essential functions, such as growth and reproduction, in favour of maintenance and survival. These responses can also be graded, and vary according to the duration of exposure to the stressor (acute versus chronic).

- The immune system is modified by other stress-responsive systems, particularly the neuroendocrine system (the hypothalamic-pituitary-adrenal [HPA] axis). Recent research has identified a direct communication, mediated principally by cytokines, between the immune and nervous systems. The immune system in its own right is one of the major defence systems responding to a stressor.

It is important to realise that the responses to stress are closely integrated such that the overall effects on the animal are broad, affecting multiple body systems.

Further reading—response to stress and distress


2.4 PAIN

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. This definition is based on the definition from The International Association for the Study of Pain (Merskey and Bogduk 1994). Pain tolerance varies between individuals and is influenced greatly by environmental conditions and mental state. The inability to communicate verbally does not negate the possibility that a person or animal is experiencing pain and needs appropriate pain-relieving treatment.

2.4.1 CAUSES OF PAIN

Pain is a complex phenomenon involving the following components:

- transmission to the brain of a signal that identifies the site and intensity of a noxious stimulus
- interpretation by the brain that the noxious stimulus at that site is ‘pain’
- transmission of signals from the brain that will result in behaviours to withdraw from the noxious stimulus, promote recovery and enable social communication
- release of substances that will modify the response to, and experience of, pain
- experience of unpleasant feelings, including anxiety and fear.

3 http://www.iasp-pain.org/terms-p.html#Pain
Pain is caused by the detection of a noxious stimulus by the peripheral nerves, which send a signal along the sensory nerve fibres to the spinal cord, and up the spinal cord to the brain. This results in conscious awareness of pain. These pathways are illustrated in Figure 2.3. In some cases, the peripheral noxious stimulus results in a simple involuntary withdrawal response without the individual experiencing pain.

**Figure 2.3  Nerve pathways involved in perception of, and response to, pain**

Many factors moderate the experience of pain. In some instances, suppression of the signal from the noxious stimulus by the brain prevents the individual from being totally overwhelmed by a particularly intense pain and therefore allows some form of escape behaviour. Levels of anxiety also have a significant influence.

Various chemicals released during an inflammatory response to tissue damage may expand the area of pain and increase its intensity. In addition, the inflammatory response can lead to greater sensitivity to a light touch that would not normally be painful (peripheral sensitisation). Repeated pain impulses to the spinal cord (eg following surgery, injury, illness or disease) may result in hyperexcitability of the nerves within the cord and a persistent state of pain (central sensitisation). Once this happens, high doses of analgesics are required to relieve the pain.

All vertebrates possess the anatomical and neurophysiological components for the reception, transmission, central processing and memory of painful stimuli. Some of these features are also present in some higher-order invertebrates, such as octopus and squid. This, together with analyses of animal behaviour, supports the view that an animal may have subjective experiences of pain similar to those of humans.
2.4.2 PHYSIOLOGICAL AND BEHAVIOURAL INDICATORS OF PAIN

The physiological and behavioural changes associated with distress will also be evident when an animal experiences pain. However, the specific neurophysiological mechanisms that enable the experience of pain, and which underpin the sensory–motor and motivational–affective components of that experience, differentiate pain from other sensory inputs that cause distress. Consequently, animals will display a range of pain-related behaviours that are directed towards alleviating their experience of pain and promoting recovery. Pain-related behaviours vary with the circumstances and the level of injury and provide the basis for the differentiation of pain from other causes of distress and for the evaluation of the efficacy of pain management. Differences in behaviour patterns are seen between species, and an individual’s experience of and response to pain is variable. Further, the emerging evidence of the close relationship between nociceptive and immune pathways and the role of opioids in the regulation of pain and immunity highlights another aspect of the response to a stressor where there are specific pain-related effects.

Further reading—mechanisms for perception and transmission of pain, and discussion of the various types of pain

3 EFFECTS OF AN ANIMAL’S WELLBEING ON SCIENTIFIC OUTCOMES

Good experimental design is essential, but challenging, when complex biological systems are studied. The aim is to use animals that are in a stable and defined physiological state so that the response to the variable of interest is not confounded by unwanted influences. Studies in animals where there is not a stable baseline for reference can lead to incorrect interpretation of data due to the effects of a treatment being masked or confounded. Given the complexity and range of the physiological and behavioural responses associated with stress, distress and pain, there is a high risk of these effects confounding the collection and interpretation of data.

In addition to the potential effects of specific research procedures on their wellbeing, animals can experience a range of stressors that are part of their daily living conditions and social environment. Animals may experience physiological and behavioural perturbations associated with stress, distress or pain, which are induced as part of the experimental protocol, in which case the magnitude of the effect must be minimised commensurate with the aims of the study (humane endpoints). However, when these effects are incidental and not part of the experimental design, factors that cause such perturbations should be eliminated or controlled so as not to confound data collection and interpretation of results. Any response to stressors that results in fluctuations in physiological and behavioural measurements, however transient, may influence the reliability and interpretation of data.

If an animal’s wellbeing is compromised, the consequences can include:
- greater variability in the data
- a need for increased numbers of animals
- data that cannot be reproduced
- data points that are missing
- reduced credibility of data
- data that cannot be applied to other situations
- unpublishable data.

Clearly, in the design and execution of protocols, avoiding unintended effects on animal wellbeing involves much more than the selection of the appropriate anaesthetic or analgesic agent.

It is in the interests of good scientific practice to maintain the wellbeing of animals used in scientific activities and to identify, control and, if possible, eliminate factors likely to cause physiological or behavioural responses associated with stress, distress or pain. Reduced variability between animals should lead to reductions in the number of animals needed to achieve statistical significance. When stress, distress or pain are a predicted or unavoidable consequence of a research procedure, strategies to minimise or control these effects are an essential component of good experimental design. Part II of this document outlines strategies that investigators can use to maximise wellbeing and minimise pain and distress in animals, thereby reducing variability in scientific data.
Further reading—causes of stress, pain and distress and their impact on scientific data


PART II

PLANNING, CONDUCTING AND REVIEWING RESEARCH PROTOCOLS TO MAXIMISE WELLBEING AND MINIMISE PAIN AND DISTRESS IN ANIMALS
4 PLANNING NEW RESEARCH PROTOCOLS

This chapter provides information to help investigators decide whether animal experiments are needed to meet the aims of a specific research project. For projects that do require the use of animals, information is provided on all stages of the research process, such as choosing the right animal to use; sourcing, transporting and housing animals; designing the experiment; predicting and minimising pain and distress; training personnel; and publishing the data.

4.1 ARE ANIMALS NEEDED TO MEET RESEARCH AIMS?

Scientists using animals in scientific procedures have an ethical and legal obligation to ensure that the principles of Reduction, Refinement and Replacement are used wherever possible (see Chapter 1 of this document). Before developing a new research protocol using animals, the investigator should consider:

- whether the use of animals is justified
- if similar projects have been performed elsewhere
- whether the same results could be obtained using tissue culture or computer modelling or other alternatives to animals.

Investigators must weigh up whether the potential benefits of the scientific knowledge gained will outweigh harm to the animal. If animals are required for the research, the information in this section must be considered before submitting a proposal to the animal ethics committee (AEC).

Figure 4.1 is a flow diagram for making decisions (during the planning and design stages of a research project) about whether animals are needed. This decision-making process should be applied at the beginning of a project, and at regular intervals throughout.

See Appendix 5 of the Code for websites of organisations that promote the use of alternatives to animals in research.

If a research project does require the use of animals, investigators should follow the principles of Reduction and Refinement, where the minimum number of animals (or amount of animal tissue) is used to obtain the maximum amount of scientific information, and where methods of using animals are selected for their minimum impact.

Collaboration between investigators (both intra and inter-institutional) can reduce the number of animals or amount of animal tissue required for a particular research question. Investigators can also collaborate to develop methods of refinement, such as standard operating procedures, to promote animal wellbeing and maintain high ethical research standards.

See Section 2.2.17 of the Code for further information on including standard operating procedures in a research proposal.

For protocols that will be conducted at more than one institution, investigators must ensure that the AEC from each institution is aware of the proposal, and that appropriate approvals have been obtained for each component of the project.

See Sections 2.2.41–2.2.42 and 3.1.6 of the Code for further information about projects involving more than one AEC.
Figure 4.1 Early planning for a project that might involve the use of animals

- **Project that might involve use of living animals**
- **Define research objectives carefully**
- **Gather information**
  - Literature search of subject-specific databases (consult information professional)
    - To avoid duplication
    - To identify all possible approaches
  - Searches of specialised three Rs resources (consult information professional)
    - Websites
    - Internet discussion groups and mailing lists
    - Databases
    Specialised resources are listed in the Guide to Searching for Alternatives to the Use of Laboratory Animals (http://www.frame.org.uk).
- **Is the project still relevant and necessary?**
  - **Yes**
  - **No**
    - **Rethink**
- **Can it be done entirely without living animals?**
  - **Yes**
  - **No**
  - **Go ahead. Publish data highlighting three Rs aspects (eg in keywords, abstract and materials and methods)**
  - **Can a pilot study or other part of the study be done without living animals?**
    - **Yes**
    - **No**
    - **Implement reduction and refinement methods (eg strain, numbers, source, technique, husbandry)**
4.2 ANIMAL MODELS

All living things share common properties. The idea of 'studying common characteristics among species to shed light on the function of all species' goes back at least to Aristotle's Historia Animalium and underpins the value of comparative medicine (Dunlop and Williams 1996). Major discoveries about human and animal function and disease have come from comparative studies of animals. Here, animals are models or substitutes for the studies of humans or other animals.

Animal models can be used to study the function of cells, tissues and organs and how these are integrated into the function of whole individuals or, in a similar framework, to understand specific disease processes. Genetically modified animals are used to study the action of specific genes in the function of whole individuals, including the role of specific genes in disease processes. In fact, all animal use for scientific purposes relates to animals as models. Accordingly, every part of the present document on animal wellbeing and the alleviation of pain and distress applies to animal models.

Three main types of model used in biomedical studies have been described by Festing (2000):

- Exploratory models that are used to gain further understanding of biological or disease processes, or to describe such processes.
- Explanatory models that do not necessarily rely on the use of animals, and may instead use physical or mathematical model systems to unravel complex biological mechanisms. In keeping with the principle of Replacement, the use of explanatory models should be considered wherever possible.
- Predictive models, which are used in applied research to determine the likely impact of a treatment on humans, other animals or environments by extrapolating from animal models; for example, feeding rats with a chemical agent over a long period to test whether it causes cancer. Predictive models depend on the use of animals.

Animal models used in the study of human diseases most often involve the induction of a disease, either by the administration of a substance or exposure to an infection that will lead to pathological changes, or by a surgical intervention that may create a functional defect in an organ or alter the relationship between organs. In these induced models, although the endpoint of the disease process is usually similar to the situation in humans, often the pathological processes that have led to these changes differ. Recent advances with genetically modified animals overcome this problem and, as the progress of pathological changes is mimicked in the animal model, these models can be used to answer different and important questions. Use of genetically modified animals also facilitates targeting early endpoints to minimise the impact of the disease process.

There are also a number of animal models where the disease condition occurs spontaneously as a result of naturally occurring genetic variation. There is a significant body of literature that describes and defines a range of animal models of human diseases (eg Hau and Van Hoosier 2004). Investigators should consult such publications and electronic databases when proposing to use a specific model.

Since the concept of animals as models applies to all uses of animals for scientific purposes, the same general criteria apply to the selection and validation of a particular model. The investigator must first define the scientific questions being asked and determine which level of biological system is relevant to those questions. For example, is it a particular cell type, a tissue, an organ or the interaction between organs? Then it is possible to decide which species or strain provides the best analogue of the biological processes involved. Other factors such as availability, special housing needs or particular technical or ethical issues relevant to the use of a species or strain are taken into consideration in making the final choice. Through this process, the criteria that define the suitability of a specific animal model are defined and validated (van der Gulden et al 1993).

Researchers should use animals in research only after considering whether the predicted scientific or educational benefits of the project outweigh the potential adverse effects on the
wellbeing of the animals. The choice of animal model to answer a particular research question must have a sound scientific basis and not be unduly influenced by convenience or budget. To establish this essential balance, a number of issues need to be considered to determine how well the animal model meets the scientific goals and to validate its selection.

Models are chosen according to their perceived value at the time. This is an evolving process, and the ongoing process of definition and validation of models provides an excellent opportunity to optimise opportunities for Refinement. Decisions on a course of action should be made before the study is started, in order to ensure that it is correctly designed, executed and analysed to provide a valid result (Festing 2000).

Further reading—animal models


4.3 CHOOSING THE RIGHT ANIMAL

It is important to choose the right animal for a proposed research protocol. Biological variability can reduce the power of a research protocol to detect treatment effects, and increase the number of animals needed to maintain an adequate level of precision. On the other hand, biological variability itself may be important to the research. The most suitable animal to achieve the required outcomes must be used, and the reasons for choosing a particular species must be clear in the proposal.

See Section 1.14 of the Code for information on selecting suitable animals.

The following are issues to consider when deciding whether the animal is appropriate:

- Species—ensure that the species is the most appropriate for the proposed research protocol.

- Breed, strain and genetic variability—there can be wide variation between breeds of all species. Variability can be reduced by choosing the most appropriate animal model.
  - Outbred strains are mainly used in toxicology research; however, their phenotypic variability reduces precision and increases the number of animals required. In addition, the genetic definition of outbred strains is hard to control, and the equivalence of different colonies of animals is difficult to determine.
  - Inbred strains have a more uniform phenotype than outbred strains, allowing detection of smaller treatment responses and reducing the number of animals required.

- Health—ensure that the animal is free from disease, has a health status appropriate for the research purpose, and that, if it has been sourced from another facility, the source colony is of equivalent health status.

- Behaviour—ensure that the animal is behaviourally suited to the research environment. Investigators should select domesticated species and animals that have been habituated or accustomed to humans and the human environment.
Further reading—choosing the right animal and the effects of biological variability on scientific outcomes


4.4 SOURCING, TRANSPORTING, ACCLIMATISING AND HOUSING ANIMALS

4.4.1 SOURCING ANIMALS

Most animal species used in scientific activities are bred specifically for that purpose; farm animals are bred by suppliers. Wildlife species can be acquired from their natural habitat or from suppliers, either overseas or in Australia. Captive-bred animals, rather than wild-caught animals, should be used wherever possible.

Most native species are protected by state laws and guidelines, and by the Code. Investigators should check with the relevant state authorities about their use for scientific activities. Usually, a permit is needed, and there are specific requirements that must be met before native animals (or any wild-caught animals) can be collected, kept, killed, released or transported.

See Section 4.1 of the Code for further information on sourcing animals from Australian or overseas suppliers.

4.4.2 TRANSPORTING ANIMALS

Transporting animals for use in scientific activities can cause them distress, from excess noise, movement and unfamiliar environment and personnel. The extent of an animal’s distress depends on its species, sex, age, health and stage of pregnancy, number travelling together and social relationships. Distress is also affected by the duration and environmental conditions of the transport, and the level and quality of care administered throughout the journey.

The conditions and scheduling of transport must be planned to account for extremes of climate, species-specific requirements, and contingencies. The transport plan must be approved by the appropriate AEC.
To minimise pain and distress during transport, investigators should:

- use secure, comfortable and escape-proof containers
- provide appropriate food and water
- ensure all personnel responsible for handling and transportation are skilled and able to recognise signs of distress and pain
- ensure that appropriate holding accommodation is available on arrival and that there are no unnecessary delays when transferring animals to housing.

In addition, investigators should be aware of specific regulations for transporting animals by air⁴ and transferring genetically modified organisms. See Section 4.2 of the Code for further information on transporting animals for scientific activities.

### 4.4.3 ACCLIMATISATION

Introducing animals to a new facility, with associated changes in their living conditions and social groups, and new personnel, produces a stress response that, although often transient, can lead to distress. This stress, together with the effects of transport, necessitates the acclimatisation of animals before they are used in the research protocol. During the acclimatisation period, animals should be conditioned to handling and to the presence of the investigators. This period is also an important time for the investigators to familiarise themselves with the normal appearance and behaviour of their animals. Those animals that do not acclimatise should not be used in the research.

See Section 4.3 of the Code for further information on acclimatisation.

### Further reading—sourcing, transporting and acclimatisation


International Air Transport Association (IATA). [http://www.iata.org/index.htm](http://www.iata.org/index.htm)


---

⁴ [http://www.iata.org/ps/publications](http://www.iata.org/ps/publications)

Further reading—sourcing, transporting and acclimatisation


4.4.4 Housing and husbandry

Environmental conditions affect biological variation and the comfort of animals, and so contribute to Reduction and Refinement. To reduce variation from stress responses, animals should be kept in a safe and appropriate environment.

Animal accommodation should be designed and managed to meet the needs of each specific animal species. Species-specific behavioural requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy, contact with others of the same species, environmental enrichment and other requirements, should be taken into account. Investigators should take precautions to prevent the access of unauthorised people, and should have plans to cover emergencies, such as the breakdown of ventilation, lighting, heating or cooling (see Section 4.4.12 of the Code).

If an animal has a different health, genetic or disease status from the other animals, specific housing may be required. Requirements may also be dictated by the reproductive status of the animal, research needs, and previous housing experience.

Species-specific environmental requirements such as lighting, temperature, air quality, appropriate day and night cycles and protection from excessive noise and vibrations need to be considered. The need to provide ready access to food and water, and the need to provide clean accommodation free from pests and diseases, also need to be taken into account.

The number of animals in a cage, pen or container and the placement of these should allow social and environmental conditions suitable for the species to be maintained.

Environmental enrichment strategies can also improve the conditions for the animals (see the ‘Environmental enrichment strategies’ factsheet).

Adequate food and water must be provided. Animals have different nutrient requirements at different stages of their life. There is considerable variability in commercially prepared diets, which may have ingredients, contaminants or errors in composition that could affect research outcomes or the wellbeing of the animal. By using internationally recognised, balanced diets for their animals, investigators reduce the variation within and between studies, and hence avoid the need for duplication of experiments, reduce the number of animals required, and improve the quality of their research.

The quality of diet can be affected by food storage conditions and the frequency of feeding. Poor storage conditions may result in bacterial contamination or loss of bioactivity of supplements, which may not be recognised (see the ‘Food and water intake modification’ factsheet).

See Section 4.4 of the Code for further information on housing.
4.5 EXPERIMENTAL DESIGN

All research protocols should be well designed. However, given the ethical considerations associated with using animals in research, it is particularly important that studies using animals are well designed. The aim is to use as few animals as possible to get meaningful data, without using too few so that the study needs to be repeated or gives inconclusive results. This is the principle of Reduction, one of the 3Rs, along with Replacement and Refinement (see Chapter 1 of this document for more information on the 3Rs).

Studies must be designed to ensure that valid data can be obtained. Festing (2000) states that good experimental design means that the experiment should be:

- unbiased (for example, the treated and control groups have the same environment)
- precise (so that the chance of detecting treatment effects is as high as possible).

To achieve this, investigators must ensure their experimental design, objectives and hypotheses are thoroughly considered and completed before they start any research involving animals.

Before starting a research project, the experimental design must be approved by the relevant AEC (see Chapter 5 of this document for information on the AEC approval process). Festing and Altman (2002) recommend that, before starting an experiment, investigators should have protocols (preferably written) that include the following:

- clearly stated objectives and hypotheses of the research
- in the case of animal models, an explanation of why the model was chosen
- a good understanding of relevant scientific literature (including similar studies already done and reasons why more research using animals is required)
- precise details of the study design
- precise details of the statistical methods that will be used to analyse the data.

The following sections provide information on the areas that need to be considered when designing an experiment that is unbiased and precise.

4.5.1 STATISTICAL ANALYSIS

The design of experiments involving animals must ensure that statistically valid results are obtained using the minimum number of animals. Investigators may find it helpful to seek advice from their institution’s biostatistician before starting; experiments using animals are often collaborations between the investigator and the biostatistician. Other information can be found on websites and online statistics information packages, such as the FRAME website.6

Before starting, investigators should know exactly how the data will be analysed. The first step is clearly stating the purpose of the experiment: is it an exploratory study to determine

---

6 http://www.frame.org.uk/
a situation or effect; or is it a predictive study to examine the likely effect, size or impact of a test? Exploratory studies use graphs, diagrams, models and equations to describe results, while predictive studies use measures of fidelity (how closely the results resemble the object being modelled) and discriminating ability (how the results show the effect being modelled), often for extrapolation from animal models to humans (Festing 2000).

Another point that must be considered when designing the experiment is the sample size. Too small a sample will not allow the effect being studied to be detected with any degree of confidence; however, too large a sample is an unnecessary waste of animals. Biostatisticians can help the investigator to determine the correct sample size, taking into account factors such as the size of the effect of interest, the chosen significance level and the standard deviation.

4.5.2 METHODS USED

Before starting the research, it is also important to make sure that the methods used are designed to ensure the animals' wellbeing, and that random (uncontrolled) variables from biological variation, the species selected and housing conditions are taken into consideration. Unnecessary stress and discomfort can cause increased variation, affecting the accuracy of the results.

Other variables, such as circadian rhythms, measurement errors, and the age and quality of reagents, need to be considered. Contingency plans for unexpected animal deaths during the research are essential. For example, how will they affect the final results, taking into account the sample size; how can the maximum amount of information be salvaged (e.g., bodyweight, age, sex)?

4.5.3 AFTER GATHERING THE DATA

When designing the experiment, the final stages (e.g., writing up the results) should be considered. The methods, data and analyses must be accessible to other investigators. This information should be presented clearly, precisely, and in enough detail to allow it to be easily understood and replicated, including:

- the experiment's objectives and hypotheses
- the animals used (e.g., species, strain, source, type, health status)
- animal transport conditions and the length of the acclimatisation period before the experiment
- animal housing, dietary and water conditions
- the statistical methods used to analyse the data.

Overall, investigators must keep in mind that poorly designed studies using animals, or inappropriate statistical analysis of results, are a waste of animals, and this is unethical.

Further reading—experimental design


4.6 PREDICTING POTENTIAL PAIN AND DISTRESS

Aspects of a protocol that may impact on an animal are not confined to experimental procedures. Other potential sources of pain, stress and distress must be considered, such as capture, transport, handling, restraint, housing, social and physical environment, and phenotype. Prediction of potential pain and distress requires knowledge of the husbandry and handling of a particular animal, its normal behaviour and what can be expected if the particular procedures used cause adverse effects in the animal.

4.6.1 PILOT STUDIES

A pilot study is a small study that can be used to determine the gross effects of the research protocol on the wellbeing of the animals. Pilot studies are valuable in the management and planning of the research project, because they help to refine research protocols, therefore reducing the adverse impact on animals before large-scale studies are run. Pilot studies are also useful for helping to develop techniques and procedures.

Pilot studies should be regarded as integral to the overall project and should be assessed by the AEC according to the usual criteria applied to project approval. Including the results from a pilot study in the proposal to the AEC will help the committee assess the feasibility of the project.

See Sections 2.2.19 and 3.2.1 (xi) of the Code for information on pilot studies.

4.6.2 MANAGING RISKS

Risk management is a stepwise process for assessing and then implementing alternatives for mitigating risks. It has a potential application to pain and distress in animals where it could supplement the usual processes for diagnosis, prognosis and situational analysis.

Risk management depends heavily upon the distinction between hazards and risks. Hazards refer to the potential of agents or situations to cause adverse effects such as pain or distress. Risks are a function of the likelihood and severity of adverse effects produced by exposure to hazards. Risk management takes into account the context in which hazards occur and invokes one of the following three courses of action:

- accepting the risk (where the risk is insignificant and impossible to eliminate)
- avoiding the risk (where the risk is unacceptable and mitigation is not possible)
- reducing the likelihood of the risk or its consequences, or both, by the implementation of mitigation measures.

Risk assessment provides essential information for risk management. Classical risk assessment as it applies to biological issues such as disease or food safety consists of four steps, which could be modified for application to pain and distress in animals:

- hazard identification—identifying agents or situations that may lead to an adverse effect, such as pain or distress
- hazard characterisation—characterising the mode of action of the hazard and mechanisms for its effects
- exposure assessment—evaluating the likely actual levels and duration of exposure to the source of risk
- risk characterisation—integrating the likelihood and the severity of adverse consequences such as pain and distress.

Likelihood and consequence matrices may be useful adjuncts to the risk characterisation step and could be adapted to pain and distress in animals. Regardless of whether formal risk
management is applied, the crucial challenge is to devise effective measures for mitigating pain and distress. Table 4.1 shows a likelihood and consequence matrix of the sort that could be applied to pain and distress in animals.

Risk matrix

A matrix may be a useful tool for analysing the pain and distress impact of a model. The example below can be used to estimate probability, consequences and risk levels for animal models.

1. Assess the frequency of pain and distress associated with the procedure
   - frequent— if the chance of the event occurring repeatedly is highly likely (for example, animals experience pain and distress as a direct result of a procedure)
   - probable— if the chance of the event occurring again is likely as a result of the procedure, but on an irregular and unpredictable basis
   - occasional— if the chance of the event occurring again as a result of the procedure is unlikely
   - remote— if the chance of the event occurring again as a result of the procedure is highly unlikely
   - improbable— if the chance of the event occurring again cannot be distinguished from zero.

2. Assess the consequence for the animal with regard to the pain and distress caused by the procedure, and/or the consequence for the investigation (where death is not an endpoint)
   - catastrophic— the procedure causes severe pain or distress that cannot be alleviated (which is grounds for euthanasing animals without delay)
   - critical— permanent impairment and/or serious pain or distress occurs as a result of the procedure
   - marginal— temporary pain and/or distress occurs, but is alleviated in a short time
   - negligible— minor discomfort occurs for a short time.

3. Identify where the risk falls on a risk event status chart, such as in Table 4.1, below.

4. After identifying the consequences and their significance, make the necessary changes to the research protocol to reduce the likelihood of pain and distress for the animals.

<table>
<thead>
<tr>
<th>Table 4.1</th>
<th>Likelihood and consequence matrix for characterising risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severity of consequences</strong></td>
<td><strong>Likelihood</strong></td>
</tr>
<tr>
<td></td>
<td>Frequent</td>
</tr>
<tr>
<td>Catastrophic</td>
<td>High</td>
</tr>
<tr>
<td>Critical</td>
<td>High</td>
</tr>
<tr>
<td>Marginal</td>
<td>Medium</td>
</tr>
<tr>
<td>Negligible</td>
<td>Low</td>
</tr>
</tbody>
</table>
4.7 DEVELOPING STRATEGIES FOR ASSESSING, MINIMISING AND MONITORING PAIN AND DISTRESS

For each research protocol, the development of a strategy to assess, minimise and monitor pain and distress requires decisions to be made regarding:

- the clinical signs or observations that will be used to assess an animal’s wellbeing or clinical condition as the project progresses
- the clinical signs or combination of clinical signs that will indicate that intervention (including euthanasia) is necessary
- the actions that will be taken if a problem is detected
- the frequency of monitoring
- the people who will conduct the monitoring, and their training
- the system for the recording of observations.

The Code requires investigators to identify ‘all aspects of animal use and management, including handling and housing, that may adversely impact on the animals’ wellbeing and how this impact will be minimised’ (Clause 2.2.16 [ix]). When presenting an application to an AEC, it is essential that these matters are adequately addressed.

As outlined elsewhere in this document, aspects of a research protocol that may impact on an animal are not confined to experimental procedures. Other potential sources of pain, stress and distress, such as transport, handling, housing, social and physical environment, phenotype and health status, must also be considered.

The complexity of an animal’s response to stressors makes it difficult to rely on one simple measurement as an indicator of pain or distress. In addition, because animals cannot communicate their experiences directly to humans, their pain and distress can only be assessed by observing their behaviour and physiology. The challenge is to measure or evaluate pain, stress and distress in the animal, and to determine when a stress response develops to a stage at which the response has a deleterious effect on the animal’s wellbeing and leads to distress. In order to minimise pain and distress in animals, robust and practical systems must be developed for the prediction, monitoring and assessment of these states.

Important elements of such systems include:

- relevance of criteria to each animal species used in a research protocol
- relevance of criteria to the specific types of research protocol performed
- documentation of the criteria to be used for the monitoring of animal wellbeing
- documentation of the criteria that indicate when intervention (including euthanasia) will occur
- a flexible approach capable of dealing with the inevitable changes and unexpected events during the course of a project
- good communication, cooperation and respect between all parties, to ensure that problems are detected and managed quickly and effectively.
Once investigators have identified all potential sources of pain and distress associated with a particular project, they should determine:

- those signs that will indicate an animal’s wellbeing is compromised
- the most significant predictors of further deterioration in the animal’s condition
- the likely timing of the onset of these changes.

Based on these assessments, a monitoring strategy should be developed for the study, including documentation of the relevant signs, frequency of monitoring, intervention points and humane endpoints, and points for regular review.

4.7.1 ASSESSING THE IMPACT ON WELLBEING

So that adverse effects on the animal can be predicted and assessed, it is imperative that the observer be familiar with the normal and abnormal characteristics of each of the species used in a study.

The definition of ‘normal’ for a particular animal species may vary according to the housing or environmental conditions for the animal, the presence or absence of humans and other external stimuli, and whether the animal has been specifically bred as a research animal. It may also vary between strains or breeds within the same species, and even among individuals within a strain or breed.

During the acclimatisation period, researchers and animal carers should familiarise themselves with the ‘normal’ range of behaviours of a particular animal or group of animals. Measurements of physiological, biochemical and neuroendocrinological markers may also be made during this period to establish baseline levels. Establishment of normal circadian patterns is a sensitive indicator of physiological adaptation to a new environment and validates a stable baseline for physiological responses.

4.7.2 DEFINING APPROPRIATE SIGNS OR MONITORING CRITERIA

The clinical signs or observations that will be used to assess an animal’s condition must be defined. These include general signs of ill health or abnormality, and signs specific for the procedure.

So that appropriate clinical signs can be selected, it is imperative that investigators know the normal characteristics of the particular species, strain and individual animal that will be used. During the acclimatisation period, investigators should become familiar with the normal behaviour of the particular animal or group of animals in the research situation. Normal levels of physiological indices such as respiratory rate, heart rate, body temperature, and biochemical or hormonal markers may also be established during this period (see also Section 2.2 of these guidelines).

General signs of abnormality

The signs of abnormality in the animal should be identified. Signs of pain and distress vary not only with the species, but also between strains or breeds within the same species, and even among individuals within a strain or breed. Broad signs for a general preliminary screening might be:

- changes in physical appearance (eg injury, posture, coat texture, soiling of hair with urine or faeces)
- changes in bodyweight and related changes in food and water consumption
- changes in clinical signs of abnormal physiology (eg breathing frequency, heart rate, body temperature)
• changes in unprovoked behaviour (e.g. inactivity, self mutilation, compulsive behaviour)
• changes in responses to stimuli (e.g. aggression, excitability, righting reflex).

Behavioural indicators of acute pain could include vocalisations, abnormal appearance, posture, guarding or favouring, changes in gait and isolation.

Table 4.2 (at end of Chapter 4) summarises species-specific signs of compromised wellbeing, pain or distress, and can be used as a guide. These are intended to be a guide and are not listed in order of appearance or importance.

It is important to realise that, because many animals do not readily exhibit clinical signs of pain or distress, many criteria used to monitor animals are indicators of more substantial adverse effects rather than mild or moderate pain or distress. In addition, in many prey species such as the rat or mouse, signs of pain or distress may be transient and interspersed with normal behaviour (Roughan and Flecknell 2001). A ‘sick’ rat is often described as one that is hunched in the corner of a cage, with a rough coat. A rat behaving in this manner is no longer able to suppress pain-coping behaviour.

See Section 3.3 of the Code for more information on signs of pain or distress.

Specific signs of abnormality

Signs of abnormality relevant to a specific procedure need to be identified on a case-by-case basis; both the intended consequences of a given protocol and any potential, unintended complications need to be taken into account. In the case of a specific animal model, signs that will indicate the development of the intended effects should be identified. In relation to unintended complications, a simple approach is to consider the procedure that will be performed and to identify and list possible risks. In both circumstances, specific signs that will indicate the onset and progression of these adverse effects should be identified. As examples: in an animal model of chronic renal failure, biochemical markers of renal function would be used to identify the onset and progression of the disease, together with clinical markers of polydipsia, polyuria and weight loss; following abdominal surgery, peritonitis is a possible complication, signs of which will include fever, grunting or vocalisation on abdominal palpation and guarding of the abdomen.

When the risks of complications of a procedure are not known or the signs and time course of effects in a specific species are not well defined, a pilot study should be conducted; data will identify indicators of the intended effects and the risk of complications and inform strategies to achieve Refinement. Other sources of information in these circumstances include published results of similar experimental protocols and the experience of colleagues, veterinarians and animal technicians.

Extrapolation from human to animal also can be useful; that is, ask the question, ‘What would a human feel if they had to undergo this procedure (or when they experience this clinical condition)?’ This is an especially useful tool when teaching students about the concept of monitoring criteria.

Further reading—strategies to assess, minimise and monitor pain and distress


When animals are used for scientific purposes, in any circumstances where the experience of pain or distress is ethically justified as part of the study, actual or potential pain, distress or discomfort should be minimised or alleviated.

Effective monitoring strategies based on the criteria developed for a specific study should enable the detection of the predicted, intended effects in the animal model and the early detection and management of any complications, including unforeseen events.

In relation to the predicted effects on the animal model, researchers should determine which observations indicate the onset and stages of the effects as well as the predictors of a significant deterioration in the animal’s condition.

Intervention will be necessary to alleviate and manage complications, whether predicted or not. In the case of predicted complications, a plan to effectively manage such an event should be developed before the study commences. Throughout the course of a study, the frequency and kind of complications should be monitored and be subject to ongoing review and investigation to minimise or eliminate unwanted complications.

Intervention should also be considered and planned for managing the adverse effects of a specific experimental treatment. In many models, it may be possible to alleviate pain or distress without compromising scientific outcomes. Particular strategies will need to be customised to each project but could include an increase in the frequency of monitoring linked to onset or change in symptoms, provision of supportive therapy such as fluids, strategic use of analgesics or providing special housing conditions.

Humane endpoints

In contrast to other interventions, the setting of humane endpoints is solely dictated by predicted scientific outcomes. The notion of humane endpoints was first introduced as a Refinement strategy in toxicology so as to obtain verifiable and acceptable data in acute toxicity testing; the designated endpoint had to accurately predict that animals would die if the full course of the test proceeded.

However, this notion has now been widely developed as a key Refinement strategy in studies where there is potential for animals to experience pain or distress as part of the study. This is a rapidly emerging area and there are many examples of how humane endpoints have been developed and validated in a wide range of animal models (see examples in publications listed in Further reading below).
A number of definitions of humane endpoint have been developed, principally with the application to toxicology in mind. However, as described by Stokes (2000), humane endpoints can have a wider application as ‘criteria that can be used to end an animal study following the onset of pain and distress so as to avoid further pain or distress; or ideally, prior to the onset of potential pain and distress such that more than minimal pain and distress is completely avoided’. In many circumstances, data compatible with specific scientific goals can be obtained in the early stages of the development of an animal model.

Stokes (2002) has described the steps to be taken to develop and validate a humane endpoint, to identify the underlying pathophysiological mechanisms relevant to a particular animal model and, using these data, to identify biomarkers of the onset and sequence of events, to validate earlier endpoints. As noted by Richmond (1998), it is important that the development and validation of endpoints is evidence based.

In their Guideline (1998), the Canadian Council on Animal Care sets out a number of criteria against which a proposed endpoint should be checked by both researchers and members of AECs. These include:

- What is the scientific justification for the proposed endpoint?
- What is the expected time course from treatment until first signs of pain or distress?
- When will the effects on the animal be most severe?
- Is there a checklist of observations on which the endpoint is based?

Further reading—determining appropriate endpoints


4.7.4 DETERMINING ACTIONS

Actions that will be taken when a particular sign or combination of signs is observed in an animal should be defined. Such actions or interventions may include:

- promoting the animal’s comfort by providing supportive treatments (eg warmth, hygiene, fluids, nutrition and social needs)
- more frequent observation
- consultation with a veterinarian with appropriate experience
- administration of specific treatment (eg an analgesic agent)
- euthanasia of the animal
- removal of the animal from the protocol.

The Code requires investigators to act promptly to alleviate pain, which must take precedence over continuing or finishing the project (see Sections 3.3.5 and 3.3.10 of the Code). Alleviation of pain and distress may involve appropriate nursing as well as administration of specific drugs.

See also the ‘Pain management’ factsheet.

4.7.5 DETERMINING MONITORING FREQUENCY

The frequency of observations should be such that areas of concern and potential problems can be detected at an early stage, and therefore animal pain and distress can be alleviated as soon as possible, before they become too severe. If an animal is in a potentially critical period, the frequency of observations should increase. For example, in some experimental infections, hourly observations may be necessary to identify the point at which the selected endpoint has been reached and the animal’s pain or distress must be terminated.

4.7.6 TRAINING

All persons responsible for making observations of the animals from which an endpoint will be determined should be competent in evaluating the normal physiology, behaviour and body condition of the animals under observation, and the anticipated specific changes from normal. The research group, the AEC and the institution are responsible for providing appropriate training before the project begins. Training should be provided as needed, and should encompass not only techniques but also the responsibilities of the investigators for monitoring the animals. Training should incorporate workplace assessment, with further training as necessary.

Team approach

Monitoring strategies should be developed with input from all involved in the monitoring of the animals used for the research project, and from people with relevant experience with the species to be used and the procedures to be performed. This team approach should, where possible, include the investigators, research students, veterinarians and animal technicians. Going through the process of development of monitoring strategies can be used as a training tool for students.

4.7.7 DOCUMENTING THE MONITORING STRATEGY

Accurate documentation of the monitoring strategy ensures that all persons involved with the care of the animals are aware of the basis for determining the presence and severity of pain and distress. This facilitates:

- the assessment of an animal as its clinical condition changes
- the determination of whether an intervention point has been reached
- review of the effectiveness of the monitoring strategy as a project progresses.
There are many different methods for the documentation of the monitoring strategy. Morton and Griffiths (1985) described a scoring system as a strategy for animal monitoring. This system has since been refined into the binary score-sheet system or monitoring checklist (Canadian Council on Animal Care 1998, Morton 2000). A recent survey of current practices in the United Kingdom for the monitoring of animals demonstrated that, in the absence of practical techniques that could feasibly be used to assess animals objectively, binary score systems or monitoring checklists appeared to be the most effective way of assessing animals and recording observations (Hawkins 2002). The survey also revealed that reluctance to use scoring systems was generally due to lack of time to implement them and a lack of awareness that checklists can be continually adapted and tailored to protocols, and be binary rather than numerical.

**Monitoring checklist**

A monitoring checklist should include the following elements:

- general signs of abnormality for the species, strain or individual
- specific signs of problems that may arise from the procedure performed
- documentation of points at which some sort of intervention is required
- documentation of endpoints at which euthanasia is necessary
- provision for details of any treatments given, so that their effectiveness can be assessed.

Other factors that can be included are details of any special husbandry requirements, and identification of any samples that should be taken from an animal should its euthanasia be necessary in the absence of the research team.

The descriptions of the monitoring criteria should be phrased so that a ‘negative’ sign is used to indicate ‘no problems’, and a ‘positive’ sign is used to indicate that there may be a potential or actual problem indicated by the clinical sign or behaviour. For example, the term ‘isolation’ should be used rather than ‘social interaction’, and ‘laboured respiration’ rather than ‘respiratory pattern’.

The inclusion of a NAD (no abnormalities detected) box in the checklist should be considered. This box could be used by an experienced person who has little difficulty assessing whether or not an animal, or group of animals, is unwell. If an animal is unwell, the detailed checklist should then be used to make judgments about actions to be taken. The chief investigator for the project must ensure that there is no misuse of the NAD box by inexperienced people.

**Specificity of a monitoring checklist**

Ideally, a monitoring checklist should be specifically designed for each species and for each procedure. Monitoring criteria will differ according to the type of research protocol, as well as between species and individuals. For some projects, several different monitoring checklists may be necessary in order to cover different phases of the work. A monitoring checklist must also be relevant for the procedure. For example, a generic checklist for mice could be used as a starting point, but should not necessarily be used for all projects involving mice.

Simple checklists can be developed for use during periods in the project when the wellbeing of the animals is of less concern; for example, during the acclimatisation period or when an animal has recovered from a particular procedure. A simple checklist could incorporate a NAD box, with the more detailed monitoring checklist used if any abnormality is detected (see Section 6.1 of these guidelines for more information).

**4.7.8 INVOLVING THE ANIMAL ETHICS COMMITTEE**

Agreement on the monitoring strategy should form part of the application process to the AEC as required by the Code (Section 2.2.16 [x]). The AEC can be involved in the finetuning of the monitoring criteria and intervention points in consultation with the research team. In this way,
all criteria for monitoring and subsequent actions are agreed to and documented before the project begins. The AEC must also ensure that the investigators have the appropriate experience and/or training to effectively implement the monitoring strategy.

Further reading—monitoring strategies


4.8 TRAINING PERSONNEL

Scientific activities involving animals are best seen as a collaborative effort between investigators, animal care experts (including veterinarians), technical staff, teachers and students. To this end, everyone who works with animals for scientific purposes must have adequate training and support so that they can care for and use animals in compliance with the Code. This will ensure that:

- there is minimal pain and distress for the animals
- all personnel involved have appropriate knowledge and skills relevant to the use of animals
- personal safety of the experimenter is maintained during animal handling
- best scientific outcomes are achieved.

Provision of appropriate training (specific to a particular procedure and specific species) before the beginning of a project should be addressed by the research group, the AEC and the institution. Training should be provided on a needs-assessment basis, and should encompass not only techniques but also the responsibilities of the investigators with respect to monitoring of animals.

Research institutions must ensure that there are enough people with the appropriate skills to care for animals. In addition, the research institution must ensure that all personnel involved receive appropriate information relevant to their responsibilities under the Code (including translations where necessary).

4.8.1 SUPERVISING STUDENTS

Students working with animals must have:

- close, competent supervision
- been instructed in the appropriate methods of handling and caring for animals
- demonstrated that they are capable of performing the necessary tasks with care and competence.

Supervisors must ensure that, before using animals, students receive instruction in the ethical and legal responsibilities as well as in the appropriate methods for animal care and use.

The Australian Code for the Responsible Conduct of Research (the Research Code, 2007), which replaced the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), specifies that institutions need to ensure that research trainees are aware of, and comply with, government and institutional guidelines for ethical requirements for research using animals (see Section 3.2 of the Research Code).
## Table 4.2 Species-specific signs of pain and/or distress

Note: These are listed alphabetically, and not in order of importance. In order to detect signs of pain and distress, the researcher must know the behaviour of individual animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Signs of pain and/or distress</th>
</tr>
</thead>
</table>
| Amphibian   | • Ataxia  
             • Blood spots on skin  
             • Dry or dull skin  
             • Dry, dull appearance to the tongue  
             • Lethargic with poor responses to simple stimuli  
             • Muscle wastage, especially in the thigh region, as a result of decreased feed intake or inactivity  
             • Staring, dry eyes |
| Bird        | • Ataxia  
             • Change in wing posture/position  
             • Decreased use of an affected limb  
             • Escape reactions with vocalisation and excessive movement  
             • Eyes partly closed  
             • Fluffed appearance  
             • Inactivity  
             • Increased respiratory effort  
             • Loss of appetite or reduced faecal component in dropping  
             • Neck retracted  
             • Not perching  
             • Prominent sternum  
             • Reduced escape reactions  
             • Self mutilation and other stereotypic behaviour (especially psittacines and ratites) |
| Cat         | • Abnormal posture  
             • Apprehensive facial expression  
             • Ataxia  
             • Crying or yowling  
             • Desperate attempts to escape  
             • Isolation  
             • Loss of appetite  
             • Marked panic or aggression  
             • Overgrooming  
             • Panting  
             • Pupillary dilatation  
             • Quiet  
             • Self mutilation  
             • Tendency to hide  
             • Ungroomed appearance  
             • It should be noted that cats in pain or distress will still purr when petted. |
<table>
<thead>
<tr>
<th>Species</th>
<th>Signs of pain and/or distress</th>
</tr>
</thead>
</table>
| Cattle  | • Abdominal pain—characteristic stance with one foot placed directly in front of the other  
          • Adoption of rigid posture  
          • Ataxia  
          • Bellowing  
          • Dull  
          • Grunting  
          • Head held low  
          • Kicking of painful area  
          • Lack of rumination  
          • Little interest in surroundings  
          • Loss of appetite  
          • Sudden drop in milk yield (milking cows)  
          • Teeth grinding  
          • Violent reaction to handling  
          • Weight loss |
| Dog     | • Abnormally apprehensive or aggressive  
          • Adopting an abnormal posture  
          • Ataxia  
          • Biting or scratching at painful regions  
          • Change in breathing pattern  
          • Decreased alertness  
          • Growling without apparent provocation  
          • Loss of appetite  
          • Quiet  
          • Restlessness  
          • Shivering  
          • Stiff body movements  
          • Unwillingness to move  
          • W himpering or howling  
          It should be noted that dogs in pain or distress may still wag their tails when petted. |
| Ferret  | • Anorexia  
          • Ataxia  
          • Change in breathing pattern  
          • Crying or grunting  
          • Decreased use of one or more limbs  
          • Dull, dry or sparse hair coat  
          • Increased aggression  
          • Lack of ‘play’ activity  
          • Persistent piloerection of tail hair (bottlebrush tail)  
          • Screaming  
          • Sunken eyes  
          • Weight loss |
| Fish    | • Abnormal opercular movements  
          • Abnormal swimming behaviour  
          • Change in skin and scale colour  
          • Change of eye colour  
          • Colouring of skin or eye darkened  
          • Fin or tail damage  
          • Gulping  
          • Skin lesions |
| Guinea pig | • Abnormal vocalisation (urgent, repetitive squealing)  
              • Agitated appearance  
              • Ataxia  
              • Dragging of the back legs  
              • Lack of spillage of food or water in the cage  
              • Unusually quiet behaviour |
<table>
<thead>
<tr>
<th>Species</th>
<th>Signs of pain and/or distress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>• Ataxia &lt;br&gt; • Changes in postural expression &lt;br&gt; • Elevated heart rate &lt;br&gt; • Frequent lying down and rising &lt;br&gt; • Kicking at the abdomen &lt;br&gt; • Lameness &lt;br&gt; • Loss of appetite &lt;br&gt; • Pawing the ground &lt;br&gt; • Rolling &lt;br&gt; • Shallow breathing with increased rate &lt;br&gt; • Sweating &lt;br&gt; • Unwillingness to move</td>
</tr>
<tr>
<td>Koala</td>
<td>• Aggression &lt;br&gt; • Ataxia &lt;br&gt; • Conjunctivitis &lt;br&gt; • Drinking of free water &lt;br&gt; • Ear flicking &lt;br&gt; • Eating a few tips of fresh leaves and then ‘falling asleep’ &lt;br&gt; • Head shaking &lt;br&gt; • Perianal moist dermatitis &lt;br&gt; • Reduction in the number of faecal pellets passed &lt;br&gt; • Shaking of the whole body in juveniles &lt;br&gt; • Teeth grinding &lt;br&gt; • Vocalisation</td>
</tr>
<tr>
<td>Macropod</td>
<td>• Ataxia &lt;br&gt; • Attempts to escape &lt;br&gt; • Body trembling &lt;br&gt; • Decreased food intake &lt;br&gt; • Ear flicking &lt;br&gt; • Flinching &lt;br&gt; • Head shaking &lt;br&gt; • Licking excessively at forearms and flanks, resulting in wetting of these areas with saliva &lt;br&gt; • Recumbent &lt;br&gt; • Reduced flight distance &lt;br&gt; • Symmetrical alopecia &lt;br&gt; • Teeth grinding &lt;br&gt; • Thinning of the hair &lt;br&gt; • Thumping of the ground &lt;br&gt; • Unable to move or lift head &lt;br&gt; • Vocalisation</td>
</tr>
<tr>
<td>Mouse</td>
<td>• Ataxia &lt;br&gt; • Change in the normal group behaviour &lt;br&gt; • Decreased activity &lt;br&gt; • Eating of bedding or neonates &lt;br&gt; • Excessive licking and scratching &lt;br&gt; • Hunched posture &lt;br&gt; • Loss of appetite &lt;br&gt; • Piloerection &lt;br&gt; • Ungroomed appearance &lt;br&gt; • Unusually docile or aggressive when handled &lt;br&gt; • Vocalisation</td>
</tr>
<tr>
<td>Species</td>
<td>Signs of pain and/or distress</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Non-human primate</td>
<td>- Aggression</td>
</tr>
<tr>
<td></td>
<td>- Apprehensive facial expression</td>
</tr>
<tr>
<td></td>
<td>- Ataxia</td>
</tr>
<tr>
<td></td>
<td>- Clenching of teeth</td>
</tr>
<tr>
<td></td>
<td>- Crouched position with arms across chest</td>
</tr>
<tr>
<td></td>
<td>- Decreased activity</td>
</tr>
<tr>
<td></td>
<td>- Decreased vocalisation</td>
</tr>
<tr>
<td></td>
<td>- Loss of appetite</td>
</tr>
<tr>
<td></td>
<td>- Rocking</td>
</tr>
<tr>
<td></td>
<td>- Self biting or self mutilation</td>
</tr>
<tr>
<td></td>
<td>- Vocalisation (grunts and moaning)</td>
</tr>
<tr>
<td>Pig</td>
<td>- Ataxia</td>
</tr>
<tr>
<td></td>
<td>- Changes in gait and posture</td>
</tr>
<tr>
<td></td>
<td>- Changes in vocalisation</td>
</tr>
<tr>
<td></td>
<td>- Decreased activity</td>
</tr>
<tr>
<td></td>
<td>- Hiding in bedding</td>
</tr>
<tr>
<td></td>
<td>- Lack of normal social behaviour</td>
</tr>
<tr>
<td></td>
<td>- Tooth grinding</td>
</tr>
<tr>
<td></td>
<td>- Unwillingness to move</td>
</tr>
<tr>
<td>Platypus</td>
<td>- Abrasions on the bill</td>
</tr>
<tr>
<td></td>
<td>- Ataxia</td>
</tr>
<tr>
<td></td>
<td>- Flinching</td>
</tr>
<tr>
<td></td>
<td>- Males growl and attempt to stab with their spurs</td>
</tr>
<tr>
<td></td>
<td>- Reduction of the amount of tail fat with chronic stress</td>
</tr>
<tr>
<td>Possum</td>
<td>- Will depend upon the particular species of possum and background (eg wild caught, captive bredd, or hand reared)</td>
</tr>
<tr>
<td></td>
<td>- Change in skin and fur appearance</td>
</tr>
<tr>
<td></td>
<td>- Difficulty in climbing or walking</td>
</tr>
<tr>
<td></td>
<td>- Failure to produce normal faecal pellets</td>
</tr>
<tr>
<td></td>
<td>- ‘Hunched’ appearance</td>
</tr>
<tr>
<td></td>
<td>- Lack of appetite</td>
</tr>
<tr>
<td></td>
<td>- Unusual activity</td>
</tr>
<tr>
<td>Rabbit</td>
<td>- Aggressive behaviour</td>
</tr>
<tr>
<td></td>
<td>- Apprehensive</td>
</tr>
<tr>
<td></td>
<td>- Ataxia</td>
</tr>
<tr>
<td></td>
<td>- Change in activity</td>
</tr>
<tr>
<td></td>
<td>- Changes in breathing pattern</td>
</tr>
<tr>
<td></td>
<td>- Dull demeanour</td>
</tr>
<tr>
<td></td>
<td>- Excessive scratching and licking</td>
</tr>
<tr>
<td></td>
<td>- Face towards back of the cage</td>
</tr>
<tr>
<td></td>
<td>- ‘Hunched’ appearance</td>
</tr>
<tr>
<td></td>
<td>- Loss of appetite</td>
</tr>
<tr>
<td></td>
<td>- Reactions to handling exaggerated</td>
</tr>
<tr>
<td></td>
<td>- Teeth grinding</td>
</tr>
<tr>
<td></td>
<td>- Ungroomed appearance</td>
</tr>
<tr>
<td></td>
<td>- Vocalisation</td>
</tr>
<tr>
<td>Species</td>
<td>Signs of pain and/or distress</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Rat        | - Ataxia  
|            | - Change in the normal group behaviour  
|            | - Decreased activity  
|            | - Eating of bedding or neonates  
|            | - Excessive licking and scratching  
|            | - Hunched posture  
|            | - Loss of appetite  
|            | - Piloerection  
|            | - Red staining around the eyes and nose (porphyrin)  
|            | - Ungroomed appearance  
|            | - Unusually docile or aggressive when handled  
|            | - Vocalisation  |
| Reptile    | - Abnormal body shape (lumpy)  
|            | - Ataxia  
|            | - Aversive movements away from the unpleasant stimulus  
|            | - Flinching  
|            | - Lethargy  
|            | - Muscle contractions  
|            | - Partially closed eyelids (lizards)  
|            | - Skin colour changes  
|            | - Weight loss  |
| Sheep or goat | - Abnormal grinding of the teeth  
|            | - Ataxia  
|            | - Change in activity  
|            | - Change in rumination  
|            | - Changes in posture and facial expression  
|            | - Dull demeanour  
|            | - Foot stamping  
|            | - General reluctance to move  
|            | - Grunting  
|            | - Little interest in surroundings  
|            | - Loss of appetite  
|            | - Rapid, shallow breathing  
|            | - Vocalisation  
|            | - Weight loss  
|            | The view that sheep tolerate pain and distress without overt signs is incorrect.  |
| Turtle     | - Abnormal appearance of the eye  
|            | - Abnormal appearance of the shell  
|            | - Abnormal breathing  
|            | - Ataxia  
|            | - Discharges around mouth or nose  
|            | - Inactivity  
|            | - Lameness  
|            | - Spots on the skin  
|            | - Swelling or gaping of the mouth  |
5 GAINING APPROVAL FOR NEW RESEARCH PROTOCOLS

This chapter outlines the purpose and responsibilities of animal ethics committees (AECs), and what must be considered when submitting a research proposal to an AEC.

5.1 ANIMAL ETHICS COMMITTEES

All studies using animals must be approved and monitored by an AEC. AECs are responsible for ensuring, on behalf of institutions, that all care and use of animals complies with the Code, the use of animals is justified and the principles of Reduction, Replacement and Refinement are followed.

Institutions are responsible for ensuring that any use of animals for scientific purposes is approved and monitored by an AEC. Before a project using animals can begin, any new protocols must be approved by the research institution’s AEC.

See Section 2.1 of the Code for the responsibilities of institutions using animals in research.

An institution’s AEC must have at least four people, including at least one person in each of the following categories:
- someone with qualifications in veterinary science and experience relevant to the activities of the institution (Category A)
- someone with recent experience in using animals in scientific activities or teaching (Category B)
- someone committed to, and experienced in, improving animal wellbeing (Category C)
- someone independent of the institution who has never used animals in scientific activities or teaching (Category D).

Institutions, AECs, investigators and teachers are responsible for complying with the Code.

See Section 2.2 of the Code for more information about the roles and responsibilities of AECs.

5.2 SUBMITTING A PROPOSAL TO THE ANIMAL ETHICS COMMITTEE

Before submitting a proposal to the AEC, the investigator must have considered the issues discussed in Chapter 4 of this document. These are summarised below:
- Is it necessary to use animals?
- Has the study been designed to produce valid results?
- Is a pilot study required?
- Have appropriate species/animals been selected?
- Are suitable facilities, equipment and environmental conditions available?
- Are all personnel involved suitably trained?
- Are there strategies for minimising and monitoring pain and distress?

After approval has been received from the AEC, investigators must not deviate from the approved protocols without seeking AEC approval. Investigators must also notify the AEC if any adverse or unforeseen events occur; and suspend research until AEC advice has been obtained.

The AEC and investigator must ensure that there are contingency plans for identifying and responding to emergencies (see Sections 2.2.35 and 2.2.36 of the Code).
A proposal to an AEC must have enough information for the committee to be satisfied that the proposed use of the animal in the research project is justified. It should include:

- the project title
- the expected project timeframe
- the names, roles and experience of all personnel
- the source of animals and required permits
- details of housing and procedures to be used
- the potential benefits of the project
- an overview of the project
- how the principles of Reduction, Replacement and Refinement are being applied
- how animals will be monitored
- justification for the use of animals in the project
- any practical considerations, such as special risks to other animals or humans
- a declaration that the project complies with the relevant legislation and the Code.

See Section 3.2 of the Code for a detailed list of questions to consider before submitting a proposal to the AEC.

See Section 2.2.16 of the Code for a detailed description of the information required for a proposal to the AEC.
6 IMPLEMENTING AND REVIEWING STRATEGIES TO PROMOTE WELLBEING

This chapter outlines the main steps in minimising and managing pain and distress in animals used in research protocols during the conduct of a protocol.

6.1 USING THE MONITORING STRATEGY

Once the project has started, the first few animals undergoing a novel procedure must be observed very carefully to determine whether the predictions of the likely adverse effects were accurate. The initial study should ideally be timed so that the critical period for the animals occurs during normal working hours. This will help to ensure that appropriate observations are made and that suitable advice is readily available (for example, from veterinarians or senior investigators).

Animals should be checked against all criteria listed in the monitoring checklist developed during the planning stages of the project. A ‘negative’ sign should be recorded if the sign is absent, or a ‘positive’ sign if the abnormality is present. If unsure, record a ‘positive slash negative’ (+/-) sign. Gradations of the ‘positive’ sign can be used to indicate severity of the abnormality. Other signs that were not predicted at the planning stage may be important, so animals should be examined for other abnormal clinical signs and these should be recorded. Therapeutic medications should be recorded, so that their effectiveness can be assessed (for example, for pain relief or the reversal of abnormal clinical signs).

A single checklist could be used for an individual animal or for a group of animals, depending on the nature of the study and the species involved.

Monitoring checklists invariably take time to complete. Nevertheless, suitable recognition must be given to their importance in the overall monitoring strategy, particularly during the review stages. Rather than abandoning the use of checklists, or using them reluctantly or carelessly, investigators should develop systems to address this issue. For example, use of the NAD (no abnormality detected) box can reduce the time taken for an experienced person to complete the checklist. Simple checklists can be used during the acclimatisation period or when an animal has recovered from a particular procedure.

To ensure that all involved in the care of an animal can make informed decisions about the animal’s wellbeing, monitoring checklists should be kept with the animal. This practice can easily be overlooked, with monitoring records being kept with the investigator’s other experimental records.

6.2 ASSESSING ANIMALS AND TAKING SUBSEQUENT ACTIONS

Use of monitoring records such as checklists allows comparisons between time points and reduces the variability in interpretation of signs. This makes it easier to assess an animal as its clinical condition changes and to determine whether an intervention point has been reached.

If the criteria in the checklist have been framed correctly, an abnormality observed in an animal will be recorded as a ‘+’ sign, making it easily and quickly detectable within the checklist. Subsequent actions are determined by the nature of the abnormalities and the actions or endpoints that have been agreed to and documented in the checklist. If a treatment is administered (eg analgesia), the response of the animal to the treatment should be evaluated to determine if further treatment is required.

Problems must always be dealt with immediately upon detection. At the very least, the detection of one or more abnormalities will result in more frequent observations. The aim is to detect problems before they result in pain or distress to the animal, or in the animal’s death.
The Code requires investigators to act promptly to alleviate pain, and this must take precedence over continuing or finishing the project. In addition, anaesthetic, analgesic and tranquillising agents must be used that are appropriate to the species and the scientific or educational aims of the protocol, and in line with current medical or veterinary practice (see Sections 3.3.5–3.3.10 of the Code, and the ‘Pain management’ factsheet of these guidelines).

If an animal dies unexpectedly, an autopsy should be performed so that the cause of death can be determined and the monitoring strategy altered if necessary.

6.3 REVIEWING THE MONITORING STRATEGY

The value of written monitoring records is particularly important during the review process.

6.3.1 WHEN TO REVIEW THE STRATEGY

The monitoring strategy should be reviewed as the project progresses. This is particularly important for new procedures or when the effects of a procedure on an animal may not be clear. There should be a review in the event of any unpredicted or unexpected problem. Reviews should also be scheduled for strategic points or at regular intervals throughout the project; for example, after the use of the first group of animals, at the conclusion of a particular section of the study, or even on a weekly basis.

6.3.2 WHAT TO REVIEW IN THE STRATEGY

A monitoring checklist should be treated as a ‘living document’, and the suitability and relevance of the monitoring criteria should be constantly reviewed. Signs that are found to be relevant to the procedure can be added, and any that are found to be irrelevant can be deleted.

A review should include the effectiveness of the criteria used to determine that an action must be taken, including euthanasia, especially if animals are found dead during the procedure. Could the deterioration in the condition of the animal have been detected earlier using less severe signs?

A review should also include the effectiveness of any therapy that was administered. Was the animal’s pain alleviated? Did the abnormal clinical sign resolve?

The research protocol should be amended or refined in the light of any adverse events.

6.4 INVOLVING THE ANIMAL ETHICS COMMITTEE

The animal ethics committee (AEC) must be advised promptly of any problems or adverse events. These requirements are included in the Code, which states that ‘when an animal dies unexpectedly, or is euthanased due to unforeseen complications, an autopsy should be performed’ (Section 3.3.24) and that ‘investigators, teachers and animal facility managers should promptly notify the AEC of any unexpected adverse events that may impact on the wellbeing of an animal in their care’ (Section 2.2.28). While the Code currently uses the word ‘should’ rather than ‘must’, most AECs would view with concern any adverse event that is not reported or any failure to conduct an autopsy when an animal dies unexpectedly. Reporting to the AEC enables the committee to assist with any investigation of the incident, to prevent its recurrence and to prevent any compromise of animal wellbeing and the experimental model. Reporting also educates the AEC by informing it of any problem with a procedure that has been approved.

The AEC should be involved in the review process. At a minimum, AEC approval should be required for any changes to intervention points or humane endpoints. AEC approval must be obtained before the implementation of any amendment to the approved protocol. AEC representatives may be involved in the ‘day-to-day’ evaluation of the effectiveness of the
monitoring criteria or endpoints, particularly if a project is associated with specific animal wellbeing concerns or involves a pilot study.

The AEC should also be involved with the review of the monitoring strategy following an adverse event or unexpected death.

The use and effectiveness of monitoring checklists should be actively reviewed by the committee during its routine inspections of research in progress, when project records are examined.

As part of annual and final reporting to the AEC, the investigator must report whether the wellbeing of the animal was consistent with the predictions in the protocol (see Section 2.2.37 of the Code).

6.5 STANDARD OPERATING PROCEDURES

A standard operating procedure (SOP) is a detailed written description of a standardised procedure, which should be available to all researchers likely to use the procedure. SOPs are useful because they:

- reduce inconsistencies in methods used within one laboratory
- help to ensure that the interpretation of observed treatment differences is not confounded by inconsistencies in procedural methodology
- provide a record of how a particular procedure was developed over time
- can be used to train new personnel.

SOPs should at least include:

- the title, and date on which they were approved or reviewed by the AEC
- the environment where the task should be undertaken
- clear step-by-step instructions for undertaking the task in a prescribed manner
- the name of the investigator or teacher and the skills necessary to implement the SOP
- personal protective equipment or clothing to be worn while undertaking the task
- clean-up and waste disposal guidelines.

The AEC should review SOPs at least every three years, or when there are changes in, or modifications to, procedures, equipment or dose rates.

See Section 2.2.17 of the Code for further information on SOPs.
7 CHECKLIST FOR PROMOTING ANIMAL WELLBEING

The checklist below can be used to prompt investigators to think of the issues to be considered when planning and conducting research protocols using animals, to promote wellbeing and to minimise pain and distress.

### Planning the study

- Determine whether alternative, non-animal techniques could be used
- Anticipate the extent of pain and distress and work out the ways in which it can be controlled
- Choose the most humane methods possible
- Balance the anticipated pain and distress to individual animals against the possibility of lesser pain to a greater number
- Design the research protocol to last for the shortest possible time (eg choosing the earliest practicable endpoint)
- Learn the normal behaviour of the species and the signs of pain and distress
- Consider whether the proposed techniques are the best possible ones that could be used

### Conducting the study

- Monitor animals for changes in behaviour and signs of pain and distress throughout the study
- Provide animals with adequate pain management, including anaesthesia or analgesia
- Provide palliative treatment for pain and distress (eg postoperative nursing, comfortable bedding, optimal environmental temperature and humidity, minimal noise etc)
- Kill humanely and without delay any animal that appears to be suffering unforeseen pain and distress that cannot be promptly alleviated
- Evaluate unforeseen complications and determine adequacy of criteria for intervention and humane endpoint

### Reviewing techniques and promoting strategy

- Continue to review techniques and refine them whenever possible
- Review SOPs
- Review husbandry SOPs
- Continue to review procedures for the care and management of animals in holding facilities
- Continue to review procedures to ensure good practice

### Reporting to the AEC

- Report to the AEC as required

AEC = animal ethics committee; SOP = standard operating procedure
PART III

FACT SHEETS
A ADMINISTRATION OF SUBSTANCES

WHY ADMINISTER SUBSTANCES?

Substances are administered to achieve an experimental outcome, or for a therapeutic purpose. Many different kinds of substances may be used, and the scientific justification, techniques and routes of administration are numerous. It is beyond the scope of this document to list all these aspects; therefore, the emphasis has been to detail the more common procedures, and their refinement.

There is potential for both the procedure and the substance(s) to cause pain or distress, and to impact upon the wellbeing of the animal and the scientific validity of data. The experience and skill of the person administering the substance, and the prior acclimatisation and training of the animal, contribute to this impact. Therefore, refinement of all aspects should be investigated during the planning and preparatory stages of a scientific project.

HOW ARE SUBSTANCES ADMINISTERED?

The following routes are those most commonly used to administer substances. The route chosen will place physical limits on the volume that can be given and will influence bioavailability of test materials (Svendsen and Hansen 1998). The requirements of the study and the potential risk to the animals will be key factors in the route chosen.

**Oral (by mouth, PO)**

- In drinking water: The substance must be water soluble and palatable (no aversive taste or odour). Check that the substance is not readily degraded by heat or light before choosing this method. In some cases, light-sensitive materials can be protected by wrapping the bottle in aluminium foil.

- In food: The substance is incorporated into the pelleted diet or a customised ration, which must be palatable (no aversive taste or odour). Check that the substance is not degraded by heat that is generated during the manufacturing process. (Examples: high and low salt diets; diets with additional fat, cholesterol, antibiotics.) Another method involves adding the substance to blocks of jelly, agar or gelatine. Animals require time to become accustomed to the different food item or diet. The new food is best introduced in combination with the existing diet, with a gradual increase in the amount or proportion over several days.

- Into the mouth: Palatable, non-corrosive liquids and pastes can be placed in the mouth using a pipette, a gavage needle (bird-feeding needle used for small species, see below), drenching gun (livestock species), feeding bottle +/- teat, plastic tubing, or a syringe. The animal will swallow the liquid; therefore, take care to administer in small aliquots to avoid spillage. The volume that the animal will accept voluntarily will vary with the amount of material present in the stomach and the palatability of the material. Palatability may be improved by adding sugar or flavouring (eg raspberry) or another ingredient (eg peanut butter). Substances in either a liquid or a powder can also be administered in a capsule in some species such as dogs, cats and primates.

- By gavage: The substance, dissolved or suspended in a suitable vehicle, is administered using either a modified blunt-ended stainless steel needle with a bulb on the end, or by using plastic tubing, or a combination of both. In the gavage procedure, the needle or tubing is passed down the oesophagus into the stomach and the solution is dispensed by an attached syringe or a funnel (livestock species). The gavage needle size and length are dependent on the species and the animal’s age. Length is determined by the distance from the nose to the stomach, and this should be estimated by measuring the needle or tube against anatomical landmarks. Hold the animal with its head tilted backwards so that the neck is extended and measure from the nose to the xiphisternum (bottom of the ribcage). This will give a rough
Part III  Administration of substances

idea of the length of the needle or tubing to be used, and the depth to which it is inserted. This can be marked on the needle or tube with a non-toxic marker pen. If irritant materials are administered, saline or water must be used to rinse the oesophagus and mouth as the needle is withdrawn.

Gavage is the most reliable method of oral administration as the animal receives the designated dose without spillage, but it also poses the highest risk to the animal’s wellbeing. It is also the appropriate method for administering substances that are unpalatable or may irritate the mouth or oesophagus, but not the stomach. The other methods for oral administration depend upon the animal’s health and predilection for food and water.

The volume that can be safely administered by gavage depends on the size (volume) of the stomach, and the amount of food and water already present in the stomach. To maximise the gavage volume, food can be removed for a few hours before the procedure. As a guide, the maximum volume given by gavage is 10 mL/kg (BVAAWF et al 2001).

Care must be taken to ensure that the gavage needle/tube does not cause damage from being placed incorrectly: this can occur if the needle/tube is placed in the trachea rather than the oesophagus, or if there is perforation of the oesophagus or the stomach. If the substance is injected into the trachea by accident, then the animal will cough and immediately display breathing difficulties. If this occurs, it should be immediately euthanased. If perforation occurs, tissue irritation and infection will result, and the animal will eventually show signs of pain or distress, or die. Animals showing signs of pain or distress or other unexpected adverse effects following gavage should be humanely killed and an autopsy performed to determine the cause of the problem.

By injection

Intravenous (IV, injection into a vein)

This is the most effective method of administration and is the route chosen when the biological availability of the test substance needs to be accurately monitored or when rapid distribution is required. However, it is not always feasible in species that do not have large, readily accessible superficial veins or when it is not practicable to establish an alternative access route. In those species that do have veins that can be injected through the skin (percutaneously), the use of local anaesthetic (injected subcutaneously at the proposed site, or applied to the skin as a cream) could be considered as a refinement. Substances can be administered as either a single bolus, a slow injection, usually over 5–10 minutes, or a chronic infusion (see below).

The methods and routes of IV administration of substances for commonly used laboratory animals are listed in Table A1.
### Table A1  Common methods and routes of intravenous administration of substances

<table>
<thead>
<tr>
<th>Species</th>
<th>Method and route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits</td>
<td>The marginal ear vein is relatively simple to use, and up to 10 mL can be given as a bolus in a conscious rabbit. Continuous infusion into this vein is also possible provided the rabbit is restrained. For long-term infusion, a catheter in the jugular is preferable.</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>Injection into the saphenous, femoral, jugular and auricular (ear) veins is possible. Only saphenous and auricular veins can be injected percutaneously (through the skin). Other veins need to be exposed via a skin incision, and therefore require the animal to be anaesthetised.</td>
</tr>
<tr>
<td>Mice and rats</td>
<td>The lateral and ventral tail veins can be used; warming the tail helps. The femoral and jugular veins can be used, but to access them usually requires skin incision and general anaesthesia. Catheterisation of the jugular or femoral vein is essential for the continuous infusion of substances. For short-term infusion in the anaesthetised rat or mouse, either vein is satisfactory. The jugular vein is more suitable for longer infusion with an indwelling catheter.</td>
</tr>
</tbody>
</table>
| Other species   | - Jugular vein: This vessel is readily accessible and can be injected percutaneously in many larger species, such as sheep, cattle, horses, dogs, cats and geese.  
- Cephalic vein: This vein is located on the forearm near the flexure of the elbow joint. It is used for injections in the dog and cat.  
- Brachial vein (wing vein in birds): This vein is located on the ventral surface of the wing, and can be used for injections in larger birds such as the goose and domestic fowl.  
- Femoral vein: This vein is located on the inner thigh, in the groin area. It can be injected percutaneously in many species, including sheep, dogs, marmosets and macaques.  
- Saphenous vein: This vein is located on the inner and outer surfaces of the hindleg, in the hock or 'ankle' area. It can be injected percutaneously in many species, including dogs and macaques. |

**Intraperitoneal (IP, injection into the peritoneal cavity of the abdomen)**

Intraperitoneal is more commonly used in rodents but can be used in rabbits and guinea pigs. No anaesthesia is needed, and the injection is made in the lower abdomen. A guard can be placed around the needle to prevent too deep an injection into the peritoneum and accidental injection into the bowel.

**Subcutaneous (SC, injection under the skin)**

Subcutaneous is commonly used in all species. The solutions should be pH 7.4 and isotonic. Injections are given in the scruff or flank. Larger volumes can be administered for dehydrated animals by injection at several sites. The animal need not be anaesthetised. Absorption from this route is slow, particularly for oily solutions. Rotate site of administration if injections are given more than once.

**Intramuscular (IM, injection into the muscle)**

Intramuscular injections can also be given to the conscious animal. The usual site is the hind limb thigh muscles; the needle is inserted laterally to prevent damage to the sciatic nerve. The needle should not be inserted too deeply, but must be inserted into the muscle and not the skin. The volumes injected are small, to prevent muscle damage. Absorption from IM injection is slow, but faster than SC sites as muscle is more richly supplied with blood vessels.

**Other sites**

Other sites can also be injected (eg intra-articular, transdermal, intradermal, intrathecal, intra-ocular). Refer to the 2001 Joint Working Group Report on Refinement and Diehl et al (2001) in the Reference section for further information.
Volume and needle size

Needle size will depend on size of animal, route of injection, skin thickness and the viscosity and volume of the substance to be injected (see Tables A2 and A3).

The typical syringe sizes would include 1, 2, 5, 10 and 20 mL. Choice will depend on the volume to be injected.

For gavage needles (oral dosing), the needle gauge chosen will be determined by the viscosity of the substance to be administered and the size of the animal. For example, an adult rat is gavaged with a modified 16-gauge needle; weanlings and mice require modified 19-gauge or 21-gauge needles.

<table>
<thead>
<tr>
<th>Route</th>
<th>Volume</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>2–5 mL (aqueous solutions)</td>
<td>Volume depends on the looseness of skin. Distension of the skin is painful, so minimise this by using multiple sites (up to 4 per session).</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>0.05 mL/site (mouse)</td>
<td>Volumes refer to aqueous solutions that are rapidly absorbed. Halve volume/site for oily solutions.</td>
</tr>
<tr>
<td></td>
<td>0.2 mL/site (rat, guinea pig)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mL/site (rabbit)</td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Maximum bolus volume is 1% of animal’s bodyweight</td>
<td>Volumes refer to aqueous solutions that are rapidly absorbed. Reduce volume for oily solutions. Distension of the abdomen is painful.</td>
</tr>
<tr>
<td>Intravenous</td>
<td>Maximum bolus volume is 1% of animal’s bodyweight</td>
<td>Volume refers to aqueous solutions. Bolus injection should be given slowly, over 1 minute. Greater volumes can be administered if much slower infusion rates are used.</td>
</tr>
<tr>
<td>Oral (gavage)</td>
<td>0.05–0.1 mL/site</td>
<td>The volume depends on the thickness of the skin. Maximum number of sites is 6.</td>
</tr>
<tr>
<td>Rabbits and rodents</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Based on BVAAWF/FRAME/RSPCA/UFAW

<table>
<thead>
<tr>
<th>Route</th>
<th>Mice</th>
<th>Rats</th>
<th>Guinea pigs</th>
<th>Rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>25–26 G x 13–25 mm</td>
<td>23–26 G x 13–25 mm</td>
<td>23–26 G x 13 mm</td>
<td>21–25 G x 25–38 mm</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>26–30 G x 13–25 mm</td>
<td>25–30 G x 13 mm</td>
<td>25–30 G x 13 mm</td>
<td>25–30 G x 13–25 mm</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>25–27 G x 13–25 mm</td>
<td>23–26 G x 13–25 mm</td>
<td>23–26 G x 25 mm</td>
<td>21–26 G x 25–28 mm</td>
</tr>
<tr>
<td>Intravenous</td>
<td>25–30 G x 13 mm</td>
<td>23–26 G x 13 mm</td>
<td>25–30 G x 13–25 mm</td>
<td>21–26 G x 13–25 mm</td>
</tr>
<tr>
<td>Intradermal</td>
<td>27–30 G x 13 mm</td>
<td>27–30 G x 13 mm</td>
<td>25–30 G x 13 mm</td>
<td>25–30 G x 13 mm</td>
</tr>
</tbody>
</table>

Source: Based on BVAAWF/FRAME/RSPCA/UFAW
Continuous administration of substances

Substances may need to be administered continuously for a period of time. There are a number of ways of achieving this requirement.

Subcutaneous implant

This is commonly used for hormone administration. The substance is pelletised, or is sealed in a short length of silastic tubing, and placed subcutaneously in a fold of loose skin in a location such as at the back of the neck, or on the flank. The substance gradually diffuses out of the pellet/tubing and is absorbed.

Osmotic minipump

The osmotic minipump resembles a drug capsule in shape, and comes in a range of sizes that make it suitable for different species and administration (infusion) periods. The principle of operation is one of osmotic pressure gradients; fluid from the surrounding tissues enters the capsule, and the solution of the substance being administered passes out. The osmotic minipump is filled with a solution of the substance, and then implanted either subcutaneously at the back of the neck (between the shoulder blades) or intraperitoneally. A small catheter can be attached to the minipump and placed into a vein, which enables the substance to be delivered intravenously. Great care must be taken when filling the minipump to prevent air bubbles forming in the tube. The filled pump should be primed in normal saline at 37°C until implantation, or it will malfunction and cause dehydration of the tissues. The manufacturer’s instructions for use should be consulted for further details. A minipump can administer substances for many days but may need to be replaced during longer administrative protocols.

Permanent indwelling venous catheter

When used in laboratory rats and mice, the catheter is often placed in the jugular vein in the neck, exteriorised (passed out of the body) through the skin at the back of the neck, then attached to an infusion system for the constant infusion of substances. The animal and catheter tubing are tethered to a mobile swivel arm to restrict movement of the animal within the cage and prevent entanglement of the plastic tubing. Modern equipment has been refined to permit greater animal mobility and comfort. The swivel contains sensors that detect movement of the tubing caused by the animal’s movements, and prevents tangling by causing a compensatory movement of the cage base (which sits on a computer-controlled, motorised turntable).

With larger animals, if it is necessary for the catheter to be continuously infused, the animal needs to be confined in a crate, stall or cage. In many situations, it may be possible to disconnect the catheter from the infusion system for periods of time, permitting the animal more mobility. In these situations, the tubing is filled with an anticoagulant solution such as heparinised saline, plugged closed and placed in a pouch or bandage in an area where it is difficult for the animal to dislodge it (such as on the back).

(See the ‘Surgical procedures’ factsheet for details on the management of implanted catheters and devices).

WHAT ARE THE ESSENTIAL ANIMAL WELFARE ISSUES TO CONSIDER?

From the perspective of the animal’s wellbeing, it is important to know for the species involved and using the formulation and route proposed:

- Has the test substance(s) previously been used in that species?
- If so, what effects have been reported?
- Are there any known side effects and, if so, how can these be ameliorated?
- Does the vehicle to be used present a risk of an adverse reaction?
- Are there any specific risks associated with the route and volume proposed?
The substance(s) being administered

The substance and its liquid solvent (vehicle, diluent) must be appropriate for the route of administration, the species and the scientific purpose. The chemical properties of the substance and solvent need to be known from prior in vitro studies, and these should include: the physical properties of the solution— in particular, pH, solubility, viscosity, purity, sterility; the physiological properties such as irritancy, toxicity, and other biological effects; and the stability and biocompatibility of the formulation both before and after administration.

Where possible, physiologically compatible solvents are used. Physiological solutions include sterile, pyrogen-free solutions designated for IV medical/veterinary use (e.g., normal [isotonic] saline or phosphate-buffered saline). Water for injection is sterile, free of contaminants, and pyrogen free. It is used for medical and veterinary purposes such as dissolving powdered drugs or vaccines.

Where sterility is unknown, solutions can be filtered to exclude microbes, or tested for the presence of viruses, bacteria and pyrogens. Alternatively, some substances may be sufficiently stable to permit use of sterilisation methods such as autoclaving.

Substances that do not dissolve in physiological solutions are more problematic as they present a greater risk of irritancy and lack of biocompatibility. A range of possible solvents, suspensions and emulsions may need to be investigated and the potential risks identified (see, for example, the International Conference on Harmonisation guidance document on risk assessment of solvents7).

Adjuvants may be needed when substances are injected for the purposes of immunisation. Refer to the factsheet on ‘Polyclonal antibody production’ for more information on issues associated with the use of adjuvants.

Solutions for injection should be close to pH 7.0 in order to reduce the risk of tissue damage. The order of tolerance for a substance with a pH in the range 4.5–8.0 is: oral > IV > IM > SC (Waynforth and Flecknell 1992). However, as noted in the 2001 Working Party Report (BVAAWF et al. 2001), irritancy will also depend on the concentration and pK (ionisation point) of the components.

Refrigerated solutions should be warmed to room temperature before administration, or ideally to body temperature (37°C) where this is compatible with the stability of the substance being administered.

The animal

Acclimatisation to the new surroundings and training to the administration procedure can minimise animal distress, particularly when animals are unaccustomed to handling and substances are to be administered on more than one occasion. Where possible, rewards (positive reinforcement) should be used when training animals to cooperate with the procedure.

The species, sex, age, bodyweight, temperament and health status of the animal must be appropriate for the proposed administration protocol. These should be checked before animal use commences. Animals that are not suited for the scientific purpose should be excluded from the study.

If the effects of the administration of a substance are unknown, or if the optimal animal factors, dose or route of administration are not certain, a pilot study with a small group of animals should be considered.

After dosing, animals must be monitored for adverse effects, pain and distress. There must be adequate monitoring to address the possibility that animals may develop pain or distress unexpectedly, or in the period between observations.

---

7 http://www.fda.gov/cder/guidance/index.htm
Neonate rodents undergoing injection procedures may subsequently be rejected or killed by their mother (mismothering). It is important to be aware of the maternal behaviour of the species, and the differences in behaviour of breeds, strains or individuals. Avoid introducing foreign smells to the skin and hair of young animals by wearing gloves, and by avoiding spillage of chemicals or blood on the animal’s skin. Remove the entire litter of animals and any bedding, and handle all the young in a litter in the same way. Ensure that the animals are mixed with their original soiled bedding so that they reacquire the ‘right smell’ before returning them to the mother en masse. Avoid unnecessary disturbance when observing the animals post-administration.

**Personnel**

Personnel performing the administration procedures must be experienced and skilled in the procedure. Appropriate training and supervision must be provided before less experienced personnel undertake procedures. Sympathetic handling of the animals is very important in order to reduce animal fear; conditioning of animals to handling can have a significant effect on the biodynamics of test substances (see, for example Shyu et al 1987). Personnel should be encouraged to develop standard operating procedures (SOPs) as the basis of quality management.

**Aseptic technique**

Contamination and infection may result from administration of contaminated substances, use of non-sterile needles and syringes, transfer of infection between animals by equipment, or introduction of microbes when puncturing the skin. The risk of contamination or infection entering the site is high for contaminated substances or equipment; therefore, these deserve close attention. Infection may be transferred between animals, so a different needle should be used for each animal wherever practical. However, the risk of contamination with skin microbes is comparatively low, so the requirement to prepare the skin varies.

In situations where SC or intradermal injections require close monitoring (e.g. immunisation solutions containing adjuvants), this can be facilitated by clipping the hair over the site to be injected. If the skin or hair overlying the intended injection site is grossly contaminated and requires cleaning, the hair at the site can be clipped and the skin cleaned with antiseptic skin solution. Note that use of antiseptic or alcohol skin preparations may cause irritation of the skin in some sensitive areas (e.g. scrotum), and residues may interfere with some substances being administered (e.g. live virus vaccines). The need for skin preparation should be evaluated in each specific case.

**Injection technique**

In addition to the topics discussed above (needle size, single use of needles, injection site and volume injected), it is important that injection technique and positioning be correct. Correct positioning requires knowledge of the anatomy of the species, and selection of a suitable needle. Needle guards may be used to limit the depth the needle can be placed into a tissue. Anatomical landmarks can be located by palpation of the site.

Once the correct location is identified, the needle is inserted quickly and firmly to the required depth. The syringe plunger is withdrawn a short distance to ensure that the needle has not accidentally entered an organ or structure such as the thorax, bowel or a blood vessel. If the placement appears correct, the substance is injected steadily until complete, and the needle is withdrawn. If hazardous or infectious substances have been injected, this technique may need to be modified to prevent the injected material leaking from the needle and contaminating the body surface. This would involve withdrawing the syringe plunger a short distance while the needle is still under the skin.
Just as the needle leaves the skin, apply gentle but firm pressure to the surface with a gauze swab, and massage the injection site to disperse the injected substance. Maintain pressure if there are signs of bleeding, especially following IV injections. Once bleeding stops, carefully wipe away traces of blood using a swab dampened with water. This will reduce the tendency for the animal to lick or chew the site.

**Restrainment of animals**

Animals may require restraint when substances are being administered, particularly when injection methods are used. An appropriate method of restraint for the species and procedure should be identified, and where possible the animal should be trained to the restraint procedure. Prolonged periods of restraint cause distress; therefore, the period should be minimised. In some cases, use of anaesthesia or anxiolytic drugs may be more effective and less distressing for the animal than use of physical restraint alone. Animals require close monitoring when under restraint.

**WHAT ARE THE SCIENTIFIC ISSUES?**

From a scientific perspective, as highlighted in a review by Claassen (1994), the influence of the route of administration on the bioavailability and biopharmaceutics of the test substance is an important consideration. Other factors that need to be taken into account when assessing the scientific outcomes are the stability of the formulation, the biological activity of the test substance in the species involved and whether the availability of the active ingredient(s) is limited by the volume that can be administered by the selected route in that species. Also, as demonstrated in a number of pharmacological studies, the confounding effects of the stress response need to be considered.

**HOW ARE PAIN AND DISTRESS MONITORED?**

A monitoring sheet should be prepared that is tailored to the particular study and substance and that addresses the anticipated effects on animal welfare of the administered substance and the associated technique. The earlier section listing species-specific signs of pain and distress should be referred to when developing the monitoring sheet. Humane endpoints should be incorporated into the monitoring plan and included in the monitoring sheet and other documentation.

**HOW CAN PAIN AND DISTRESS BE MINIMISED?**

Table A4 summarises general procedures and other considerations for minimising pain and distress associated with administration of substances.
Table A4  Procedures to minimise pain and distress when administering substances

<table>
<thead>
<tr>
<th>Planning the administration of a novel substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Investigate a number of alternative methods of administration in order to identify the most suitable route.</td>
</tr>
<tr>
<td>• Investigate the physicochemical properties of the substance such as solubility, stability, pH, irritancy, toxicity.</td>
</tr>
<tr>
<td>• Perform a risk assessment for the preparation and use of the substance—identify risks to animal welfare and incorporate refinement strategies to minimise the risk of adverse effects.</td>
</tr>
<tr>
<td>• For substances where there is a lack of knowledge, consider in vitro evaluation of its biocompatibility prior to in vivo study.</td>
</tr>
<tr>
<td>• Perform a pilot study to establish the correct animal, technique, dose rate, route, frequency of administration, and other aspects relevant to its biological properties such as its metabolism and route of excretion.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minimise the volume of the substance and the frequency of its administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Investigate use of a solvent/vehicle that is physiologically compatible and suitable for the route of administration.</td>
</tr>
<tr>
<td>• Prepare a suitable monitoring strategy for the post-administration period. Ensure that monitoring frequency is sufficient to detect both expected and unexpected effects, and that there is a plan in place for managing animal pain and distress.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Use a route that is suitable for administering the substance, while minimising the impact on the animal.</td>
</tr>
<tr>
<td>• For substances requiring frequent administration, investigate oral dosing via food or water.</td>
</tr>
<tr>
<td>• For substances requiring frequent IV administration, consider use of an indwelling venous catheter.</td>
</tr>
<tr>
<td>• For substances requiring frequent SC or IP administration, consider use of an osmotic minipump or an implant.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Identify the most suitable species, strain, sex, age, bodyweight and health status.</td>
</tr>
<tr>
<td>• Acclimatise the animal to the facility and the personnel.</td>
</tr>
<tr>
<td>• Train the animal to the handling and restraint procedure before commencing studies with repeated administration of substances.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Perform a risk assessment for the use of the technique and any associated restraint—identify risks to animal welfare and incorporate refinement strategies to minimise the risk of adverse effects.</td>
</tr>
<tr>
<td>• Identify and address gaps in training, knowledge or equipment needed to perform the technique.</td>
</tr>
<tr>
<td>• Monitor the animal for the known and the unanticipated effects, including the impact on animal welfare.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Identify experienced and skilled personnel, and personnel with training gaps.</td>
</tr>
<tr>
<td>• Address gaps in knowledge and skill with training and supervision.</td>
</tr>
<tr>
<td>• Identify personnel with responsibility for animal monitoring, and develop a roster that includes monitoring for after normal working hours and weekends; identify personnel to contact in the case of emergencies.</td>
</tr>
</tbody>
</table>

IP = intraperitoneal; IV = intravenous; SC = subcutaneous
REFERENCES


B BEHAVIOUR MODIFICATION

WHAT IS BEHAVIOUR MODIFICATION?

In some research projects, animal behaviour is modified or manipulated to achieve a research outcome. This may involve treatments such as use of rewards, or biological stressors, including physical restraint, social deprivation, administration of drugs, restriction of food or water, or exposure to stimuli that cause pain or distress. Section 3.3.44 of the Australian code of practice for the care and use of animals for scientific purposes (the Code) specifies the animal welfare considerations that must be observed.

The behaviour of animals is modified or manipulated in many fields of research, including psychology, animal behaviour science (ethology), pharmacobehavioural and pain research, genetic research, and neuroscience.

HOW IS BEHAVIOUR MODIFIED?

The following strategies are commonly used to modify behaviour:

- **Choice**—the animal is given a number of options and can make a decision about its behaviour based on preference.

- **Manipulating social variables**—including population density, early social experience, introduction of new animals ('intruders') into an established group, social separation, isolation or loss (NIMH 2002).

- **Rewards**—a reward (positive reinforcer) can be offered when an animal successfully completes a task it is trained to do. In some cases, the reward is a ‘treat’ such as highly palatable food or fluid. In other cases, the animal’s access to food or water is limited before a training session, and the animal receives additional food or water as the reward. (See the ‘Food and water intake modification’ factsheet for more information.)

- **Punishment**—stimuli that are disliked, stressful or painful to the animal are used (aversive situations, negative reinforcers). Animals will respond by escaping or avoiding the stimuli if possible. The stimuli that are considered aversive can vary between species, but may include novel or unfamiliar cages; unpleasant tastes; the presence of a predator; an unfamiliar animal or social group; extremes of light, temperature or sound; a puff of air in the face; and electric shocks or other painful stimuli.

- **Stressors**—causes of distress (a perceived lack of control, such as close physical restraint) are stressors for most species. Other procedures or situations perceived to be stressors vary between species.

- **Modifying the environment of free-living animals**—including the manipulation of habitat, nests or food resources; artificial situations and staged encounters between animals of the same or different species; capture for study; human–animal interactions; taming or acclimatising the animal to humans (NIMH 2002).

- **Neurological deficits**—creation of abnormalities in the structure or function of the nervous system; for example, by genetic changes, by the use of chemicals toxic to the nervous system, or by physically damaging specific areas of the brain, spinal cord or nerves. These abnormalities resemble naturally occurring human neurological diseases that involve behavioural or sensory dysfunction. Examples include chemically induced models of Parkinson’s disease; physical damage to blood vessels to cause a stroke; and strains of mice with a high genetic predisposition to epilepsy or seizures.

- **Drugs**—changes to the function of the nervous system by the use of chemicals. For example, analgesic drugs alter the animal’s perception of pain, and will delay or eliminate the behavioural response to a painful stimulus.

- **Experimental apparatus**—equipment that contains the animal, presents stimuli or records behavioural responses. Experimental apparatus includes
- restraint devices (metabolic cage, head restraint, tether, restraint chair)
- recording chambers (including activity monitor, telemetry platform)
- special apparatus (eg open field area, maze, running wheel, rotating drum, raised beam, operant chamber/‘Skinner box’)
- spatial learning and memory apparatus used with rodents (eg mazes such as radial arm, Barnes circular platform, Morris water maze).

Awake-behaving neuroscience studies—includes studies of the ‘higher’ functions of the brain, such as perception, memory and motor control, which involve the active participation of the animal. These studies require an extensive preliminary stage, during which the animal is trained to perform a task and then surgically implanted with data collection or monitoring devices. Data are then collected daily over a period that may extend for years (particularly in the case of non-human primates).

W H A T  A R E  T H E  E S S E N T I A L  A N I M A L  W E L L B E I N G  I S S U E S T O  C O N S I D E R?

Using food and water as rewards after restriction

Controlling the delivery of food or fluids can influence the behaviour of a wide variety of animals. Food or fluid can be used as a reward, even in well-fed (satiated) animals (ASAB 2003). Food or water restriction is usually achieved by limiting the daily quantity available to the animal, or limiting the animal’s daily period of access (Toth and Gardiner 2000, NIMH 2002). This will cause the animal to experience hunger and thirst. If the restriction is prolonged, the animal may become dehydrated or lose bodyweight. The impact of food or water restriction is described further in the ‘Food and water intake modification’ factsheet. Severe deprivation of water or food must not be used (NHMRC 2004).

Using aversive stimuli or punishment to motivate behaviour

Aversive stimuli or punishment cause animals to experience fear, distress, anxiety or pain. The basic behavioural and physiological (fear and stress) responses are minimised if the animal is able to control the aversive stimulus. Situations in which animals cannot influence or control the aversive experience are particularly distressing to them. Fear and stress behavioural responses include:
- escape (eg stopping contact with an aversive stimulus, such as an electrified cage floor)
- avoidance (a learnt behaviour that prevents an aversive encounter, such as not stepping on an electrified floor, or pressing a lever that turns off the electric shock)
- decreases in grooming, food intake, level of activity, exploration, sexual activity, mothering behaviour and loss of bodyweight
- increases in ‘freezing’ behaviour.

These response behaviours are species typical or ‘hard wired’, but the aversive stimuli that evoke them are learned (NIMH 2002). Severe negative or aversive sensory stimuli must not be used, and painful or noxious stimuli should be avoided. If their use is necessary, the level and duration of the stimulus must be minimised, and escape from the stimulus must be available (NHMRC 2004; see also section 3.3.44 of the Code).

Social behaviour

Most laboratory species have a preference for social housing, so animals are usually housed in social groups rather than in isolation. Social grouping has both potential beneficial and adverse behavioural and physiological effects:
- The positive effects of social grouping include grooming and parenting, social attachment (bonding), and promotion of infant development. Stimulating the brain with sensory inputs
affects the growth and interconnectedness of the brain, thereby affecting function. Enrichment of environmental stimuli has permanent effects on the brains of rats, and this effect can even occur in aged animals. Social deprivation can result in stunted growth (NIMH 2002).

- Negative effects include aggression, fighting, and immunological and cardiovascular changes and depression induced by social stress. In many cases, the negative effects on subordinate animals in social-dominance hierarchies subside over time. However, if food or water is restricted, subordinate animals may continue to be adversely affected (NIMH 2002).

Social isolation can be distressing to social animal species. The distress is due to lack of visual, physical, auditory and olfactory contact with other individuals with which the animal is familiar. Physically isolated animals no longer have cage-mates to assist with grooming. In some situations, animal wellbeing or research protocol requirements justify individual housing, such as when:

- individual animal food and water intake is to be recorded (eg in pharmacological and metabolic studies)
- pharmacological effects on an individual’s behaviour may reduce its ability to feed, or predispose it to attack by cage-mates
- behaviour modifying and recording devices are attached to the cage, and individual animal responses are required (NIMH 2002).

Severe social deprivation or negative social interactions must not be used. When social species are physically isolated, visual, auditory and olfactory social contact should be maintained (NHMRC 2004).

Ethological research

The potential adverse effects of ethological research are as follows:

- The human observer may attract the attention of predators, or disturb animal parenting (see Section 5.1.2 of the Code).
- Providing food or other materials to attract animals and facilitate observations may result in an unsustainable increase in population density.
- Trapping and removing an animal from its territory might disturb social hierarchies, and risk stress or injury for the individual animal (NIMH 2002, APA 2004).

Induced neurological deficits

After neurological deficits have been induced, and depending on the site and severity of the pathology, animals may have only a limited ability to care for themselves. Their place in the social hierarchy is likely to be low once the deficit is created, and this may result in conflict and reduced ability to get enough food when housed with the group. In some situations, they may need to be housed in physical isolation (see ‘Social behaviour’ above). Pair-housed non-human primates may be cared for in part by their cage-mate (NIH 1991).

Restraint of animals

Prolonged periods of close restraint may be aversive and stressful to an animal. This is likely to occur if the animal has no control over the experience (ie cannot avoid or minimise the restraint), if the animal is temperamentally unsuited, or if acclimatisation or restraint training has been inadequate.

Most behavioural studies with awake animals require the animal to be restrained or confined to a certain workspace. This enables the investigator to bring the animal close to the behavioural task, to provide sensory stimulation in a quantifiable way, and to monitor physiological variables, including the electrical activity of brain cells.

Many studies on the brain require that the animal be restricted to a constrained working space and that a head holder be implanted onto its skull to immobilise its head during sessions. This
kind of constraint can only be done without causing undue distress if the animal is properly conditioned to the restraint, the behavioural task and the reward protocol (NIH 1991).

In postsurgical recovery, it is appropriate for the animal to be restrained to prevent self injury, to minimise the risk of wound contamination, to protect the wound and limit access to it by the animal, and to protect any implants or cannulae during the acute phase of healing. This restraint may be by protective bandaging, a jacket, or a confining cage (NIH 1991, NIMH 2002). The animal’s tolerance of the restraint must be monitored carefully.

**HOW ARE PAIN AND DISTRESS RECOGNISED OR MEASURED?**

General signs of pain or distress may be monitored in the following ways.

- Monitoring for dehydration: Acute fluid deficiency may result in rapid loss of more than 15% of bodyweight, thirst, dryness of mucous membranes, reduced urine output, reduced food consumption, loss of skin elasticity (‘tenting’ in response to skin pinch test), sunken eyes, tremor, lethargy, and shock or cardiovascular collapse (NIH 1991). (For further information, see the section on fluid restriction in the ‘Food and water intake modification’ factsheet.) Dehydration is a common complication that may not be readily recognised at an early stage without close and careful monitoring.

- Monitoring for failure to grow and loss of bodyweight: Chronic (long-term) food or fluid deficiency results in reduced food intake and loss of bodyweight by more than 15% (NIH 1991, Toth and Gardiner 2000). (See the section on fluid restriction in the ‘Food and water intake modification’ factsheet for further information.) In young, growing animals, reduced growth rate rather than loss of bodyweight may be observed. This can be determined by comparison with control animals that have free access to food. Loss of bodyweight is a particular concern when it occurs rapidly (over hours or days), or when there is loss of skeletal muscle mass. In all cases where the research is likely to impact upon an animal’s food or water intake, substantial scientific justification must be provided to the AEC when seeking approval.

- Monitoring for loss of mobility or body function: Unkempt or soiled appearance, reduced or absent urinary or faecal output, abnormal posture, generalised or localised (eg in a limb) weakness or loss of function, or general decreased movement should be monitored carefully.

- Monitoring for pain or distress associated with use of aversive stimuli or restraint: Increases in biting, avoidance, escape and ‘freezing’ behaviours.

- Monitoring for general signs of physiological and psychological distress, including
  - unkempt appearance; abnormal posture; tear production, including red tears or porphyria; teeth grinding; increased vocalisation; increased or decreased movement; self isolation; increased or decreased aggression; rapid, open-mouthed or exaggerated breathing
  - disturbance to social hierarchies (see ‘Social behaviour’ above)
  - reduced food intake, bodyweight, level of activity, exploration, sexual activity, mothering behaviour (see ‘Using aversive stimuli or punishment to motivate behaviour’ above).

**HOW CAN PAIN AND DISTRESS BE MINIMISED?**

Positive reinforcement is the preferred method for motivating an animal to modify its behaviour or perform specific tasks (NHMRC 2004). Table B1 summarises general procedures and other considerations for minimising the adverse effects of behaviour modification.
### General procedures

- In all cases, limit the duration and severity of the event.
- In the case of restraint, see the United States National Research Council Guidelines (NRC 1996).
- In the case of distress and stress, permit coping and control.
- In the case of fear or fighting, permit avoidance or escape.
- In the case of pain, see the ‘Pain management’ factsheet and the American Psychological Association Guidelines (APA 2004).

### Restraint

- The method of restraint used must be appropriate to the species and allow the animal to rest in a natural position. For example, a rodent can be placed in a loosely fitting bag, which is then placed in a box or tube, while a non-human primate could sit in a restraint chair.
- Animals should be properly accustomed (‘behaviourally conditioned’) to the restraint device. The ideal indicator of acceptance is the animal's voluntary movement into the device.

### Individual and group housing

- When new social groups or pairs are to be formed, it may be possible to house the animals in close proximity to enable familiarisation and the establishment of dominance hierarchies before they come into physical contact.
- In some species (e.g., mice), postpubertal males fight and injure other males. In such cases, the animals should not be housed together.
- Where individual housing is unavoidable, animals should still be in visual, auditory and olfactory contact. Human caregivers may be a source of social enrichment (NIMH 2002, NHMRC 2004).

### Induced neurological deficits

- The scientific need to induce debilitating deficits in animals must be rigorously justified, and investigators must demonstrate that they are capable of providing the special care that these animals require.
- The number of animals with induced neurological deficits should be minimised.
- Mammals should be replaced with less sentient, non-mammalian species whenever possible.
- The research protocol must be refined to reduce or eliminate pain, discomfort and mortality (NIH 1991).

### Humane endpoints

- Animals that fail to adapt to the experimental conditions should be excluded.
- The presence of lesions, illness or severe behavioural change during restraint necessitates temporary or permanent removal of the animal from the apparatus or experiment.
- Incompatible social groups or pairs should be separated and more appropriate companions found.
- Animals that lose more than 10% of bodyweight (acutely) or 15% of bodyweight (longer term) on restricted food or fluid access protocols are to be removed from the research protocol. The endpoint criteria for specific cases must be scientifically justified and approved by the animal ethics committee.
- Animals showing signs of dehydration, which include thirst, dryness of mucous membranes, reduced urine output, reduced food consumption, loss of skin elasticity (‘tenting’ in response to skin pinch test), lethargy or shock/cardiovascular collapse, are to be removed from the experimental protocol.
CONCLUSION

To ensure that animal wellbeing is maintained during scientific experiments, the Australian Code of Practice (NHMRC 2004) expects investigators to meet animals' behavioural needs as well as their biological needs. In some studies, animal behaviour is modified or manipulated to achieve an experimental outcome, and pain or distress may result. A number of behavioural modifications do cause an animal pain, distress and suffering, and all steps must be taken to ensure that this is minimised. Even those modifications that do not necessarily result in pain, distress and suffering may have that result if inappropriately administered. All steps must be taken to ensure that pain and distress are minimised. Therefore, research involving behaviour modifications must be scientifically justified, approved by the relevant animal ethics committee and conducted in a way that minimises any adverse effects for the animals.

REFERENCES


C BIOLOGICAL SAMPLE COLLECTION

See the factsheet ‘Blood collection’ for more information on that topic.

WHY COLLECT BIOLOGICAL SAMPLES?

Biological samples (body fluids, secretions and excretions) are collected from a living animal for analysis of biochemical, metabolic, toxicological, immunological and physiological changes. Samples collected may include urine, tears, nasal secretions, saliva, genital tract secretions, semen, milk, cerebrospinal fluid and faeces. The sample type, volume and frequency of collection must be humane and appropriate for the scientific purpose.

Whatever the sample to be collected, care should be taken to minimise stress and injury to the animal. The sample should also be collected as aseptically as possible and care should be taken to avoid cross-contamination of samples.

Urine

Urine analysis enables monitoring of:

- the health of the animal
- presence, absence and concentration of drugs excreted in the urine
- glucose, protein, and other substance levels
- biochemical changes.

Urine analysis may be quantitative or qualitative. Quantitative urine analysis enables monitoring of urinary pH, protein, glucose, bilirubin, haemoglobin, ketone, urobilinogen, creatinine and the concentration of excreted drugs, metabolites or other substances. Qualitative urine analysis is generally used to monitor such things as renal function, renal disease, evaluation of nutritional and/or endocrine abnormalities and the excretion of drugs and/or metabolites.

Nasal secretions

Nasal secretions and samples from the conjunctiva are generally collected for analysis of bacterial or other infectious agents.

Saliva

Salivary samples can be used in studies of the secretory immune system and the digestive system, to measure cortisol in a relatively non-invasive way and to detect signs of infectious disease; for example, avian influenza in birds. Scrapings of the buccal mucosa are used as a source of DNA (deoxyribonucleic acid) and in virological studies.

Milk

Milk can be used for serological testing, bacterial examination and the excretion of drugs or metabolites.

Faeces

Faeces are collected to look for the presence of internal parasites, evaluation of nutritional or endocrine abnormalities, the excretion of drugs or metabolites and the presence of blood associated with gastrointestinal disease.
Genital tract secretions

Vaginal samples are collected for the establishment of the stage of oestrus or to look for bacterial or other infections or other cellular abnormalities.

Semen

Semen samples are collected to look at the quality of the ejaculate and for artificial insemination. Bacterial or other infection and/or other cellular abnormalities can also be detected.

Cerebrospinal fluid

Cerebrospinal fluid (CSF) analysis is used to detect the presence of inflammatory disease, infection and the presence of tumours. CSF is analysed for cellularity, cell morphology, protein content, bacteria and to determine if medicines or other compounds of interest cross the blood-brain barrier.

HOW ARE BIOLOGICAL SAMPLES COLLECTED?

NOTE: It is important that animals are handled and conditioned correctly to facilitate the collection of biological samples.

Urine

Methods used for urine collection will differ according to whether qualitative or quantitative assessment is required. Small volumes are required for qualitative studies, and quantitative studies require volumes to be collected over a set time period (usually 24 hours).

Urine can be collected in several different ways:

- by voiding in the conscious animal
- via urinary catheter under general anaesthesia
- by cystocentesis under general anaesthesia.

These methods can be categorised according to criteria such as ease of collection, quality of sample and levels of pain and distress caused to the animal. Specific methods of urine collection are described below; further details are provided in Table C1.

- Urine collection without intervention—the plastic cling wrap is placed on top of a sheet of white paper and the mouse is put on the cling wrap. As soon as the mouse urinates, it is placed back in its box and the urine aspirated with an adjustable air displacement pipette. Volumes of 10–250 µL can be obtained in a short timeframe with no pain or distress to the mouse (Kurien et al 2004).
- Urine collection with mild intervention—the mouse or rat is held over a Petri dish and gentle transabdominal pressure is applied over the bladder. Volumes of 30–100 µL have been collected in this way, with minimal distress to the animal (Kurien et al 2004).
- Capillary tube method of urine collection—this method is useful for collecting urine from male rats. The rat is held with one hand and gentle transabdominal pressure is applied over the bladder. Excreted urine is collected immediately using the capillary tube (Kurien et al 2004).
- Metabolic cages—this method is used for 24-hour urine collection, and many different types are commercially available. However, this method of urine collection is potentially stressful to the animal, and they may need to be acclimatised before stable measurements can be obtained (Damon et al 1986, Vadiei et al 1990, Gomez-Sanchez and Gomez-Sanchez 1991). Stress is likely to be increased where the metabolic cage is substantially smaller than the home cage and where wire mesh has been used for the floor. Studies have shown that a
more complex housing environment, compared with a barren cage, buffers anxiety responses to potential stressors (Kurien et al 2004). Therefore, providing a specially designed bottomless nest box that provides shelter of opaque or semi-opaque material inside the metabolic cage is recommended.

- Cystocentesis—the animal is anaesthetised and urine collected using cystocentesis. This method allows sterile urine to be collected directly from the urinary bladder, eliminating contamination that can occur as the urine exits the urethra. The puncture site must be prepared aseptically and sterility maintained throughout the procedure. It is possible to perform this procedure in the conscious, restrained animal; however, because this is more stressful and painful for the animal and the potential for complications such as puncturing internal organs or introducing infection is greater, this method would need to be justified before being used (Kurien et al 2004). Investigators must ensure that pain and distress are prevented or minimised as much as possible during this procedure.

**Tears**

Depending on the species, light anaesthesia may be required during tear collection to minimise discomfort to the animal and to obtain an uncontaminated sample.

Conjunctival samples should be taken with a sterile cotton, gauze or dacron swab, moistened with saline before applying to the conjunctiva for up to one minute. The swab should be handled in a sterile manner at all times, placed in transport medium, refrigerated and sent to the laboratory without delay (OIE 2004).

**Nasal secretions**

Depending on the species, light anaesthesia may be required when taking nasal secretions to minimise discomfort to the animal and to obtain an uncontaminated sample.

Samples should be taken with a sterile swab moistened with transport medium. The swab should be allowed to stay in contact with the nasal secretions for up to one minute. The swab should be handled in a sterile manner at all times, placed in transport medium, refrigerated and sent to the laboratory without delay (OIE 2004).

**Saliva**

Depending on the species, collection of mixed saliva from the oral cavity can be simple and non-invasive. Light anaesthesia may be required in some instances. Ruminants secrete saliva continuously, and saliva can be collected without inducing animals to chew. Chewing may need to be stimulated in non-ruminant animals to create a flow of saliva. Unmixed mucoid or serous saliva can only be collected by catheterising the ducts of appropriate salivary glands. In small animals such as mice, saliva production can be stimulated via the administration of pilocarpine hydrochloride with subsequent collection using a glass pipette in the anaesthetised animal.

Samples should be taken with a sterile cotton or polyester swab moistened with appropriate transport medium. Samples should be treated according to their purpose. For example, cortisol declines significantly in samples at room temperature.

Swabs of cotton or other material can be used to collect buccal cells from all species.

**Milk**

Milk samples are taken after cleaning and drying the teat(s). The use of antiseptics should be avoided. The initial few drops of milk should be discarded before the sample is collected. Milk for serological testing should not be frozen. Samples should be refrigerated and sent to the laboratory without delay.
Rodgers (1995) describes a technique for collecting multiple milk samples from rats that causes minimal stress because of the speed of the milking and the fact that it can be performed without the use of continuous restraint or anaesthesia. It involves the use of oxytocin to facilitate milk let-down. Delongaes et al (1997) describe another method for milking rats that requires anaesthesia. This method may be preferable where the use of oxytocin could interfere with the research results.

Vaginal secretions

Samples of vaginal secretions should be taken with a sterile cotton gauze or dacron swab. The perivaginal area should be cleaned before samples for bacterial culture are taken (avoid skin preparation with antiseptic solutions). The swab should be handled in a sterile manner and applied to the vaginal area gently to minimise discomfort to the animal. The swab is then placed in transport medium, refrigerated and sent to the laboratory without delay. Samples for oestrus detection are examined under the microscope immediately (Waynforth and Flecknell 1987, OIE 2004).

Semen

Semen collection methods include massage, natural mating and collection from the female, electroejaculation, artificial vagina and collection after euthanasia. Methods are species specific and aversive to many species. Anaesthesia should be considered whenever possible.

Cerebrospinal fluid

This technique requires a high degree of skill, and practice should first be gained using cadavers. Samples are collected under anaesthesia with the animal in ventral recumbency with the head elevated and at an approximate 50° angle to the horizontal (Waynforth and Flecknell 1987). The site of the puncture in small animals is the cerebromedullary cistern (cisterna magna), which lies between the occipital bone and the atlas. In larger animals, the lumbosacral interarcuate space is used; however, this method is more difficult. Before taking the sample, the hair should be shaved and the skin prepared in an aseptic manner. The whole process must be conducted in a sterile environment, because any contamination of the CSF can lead to bacterial meningitis. For rats, a 24-gauge needle attached to polyethylene tubing can be used (Waynforth and Flecknell 1987). Resistance may be felt as the needle passes through the supraspinous and interspinous ligaments. When the needle enters the cisterna magna, a sudden decrease in resistance is felt, and the CSF should flow immediately. CSF samples should be clear, although blood contamination can occur: the degree of contamination will depend on the interval between repeated cisternal punctures. To minimise contamination, 3–7 days should be allowed to lapse between punctures (Waynforth and Flecknell 1987).

Faeces

Faecal examination can be both qualitative and quantitative. Small volumes are required for qualitative studies and are collected either from the cage floor or directly from the rectum in a restrained animal. In the latter case, the object used for collection must be lubricated and of a diameter that causes the least discomfort to the animal. Quantitative studies require all faeces to be collected over a set time period (usually 24 hours). A metabolism cage is the usual method; however, although separation of urine and faeces does occur, cross-contamination may be a problem. If uncontaminated samples are required, they can be collected using an ‘anal cap’ (Waynforth and Flecknell 1987).
WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

If pain and distress to the animals are to be minimised, the least invasive method that gives a sample that is compatible with the aims of the investigation should be used.

Rodgers (1995) states that, with a gentle touch and patience, most people can become adept at milking rats. This recommendation of a very gentle touch and a good deal of patience should be applied to any sampling technique in relation to any species if the best samples are to be obtained.

HOW ARE PAIN AND DISTRESS MONITO RED?

Observation of physical and behavioural changes, anorexia, weight loss and dehydration, using a standardised assessment system for that species, is mandatory if signs of pain and distress are to be identified and managed.

HOW CAN PAIN AND DISTRESS BE MINIMISED?

The following general considerations for minimising the adverse effects of biological sample collection should be used to guide the selection of methods:

- Pain and distress during biological sample collection can be minimised by using the method that has the potential to cause the least tissue damage.
- When samples will be taken from a conscious animal, and the sampling procedure will be repeated regularly during an investigation, the animal should first be acclimatised to the restraining device (eg through mock runs).
- Suitably trained staff using methods that inflict the least pain must perform the biological sample collection.
- The faster the procedure can be performed in the conscious animal, the better the samples, because the physiological changes induced by stress are minimised.
- Use a reward system when taking samples from a conscious animal and, where the sampling procedure will be repeated regularly during an investigation, to encourage a positive association with the sampling procedure.

Methods of urine collection are listed in Table C1. The methods are rated in terms of pain and discomfort for the animal, quality of sample, and ease of collection.
### Table C1  Summary table for urine collection methods and their performance

<table>
<thead>
<tr>
<th>Method of urine collection</th>
<th>Species</th>
<th>Quality of sample</th>
<th>Pain and discomfort</th>
<th>Ease of urine collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary voiding/micturition/free-catch collection, eg cling-wrap catch, petri dish</td>
<td>mouse</td>
<td>good</td>
<td>low</td>
<td>easy</td>
</tr>
<tr>
<td>Voluntary voiding/micturition/free-catch collection, eg cling-wrap catch, petri dish</td>
<td>rat</td>
<td>good</td>
<td>low</td>
<td>easy</td>
</tr>
<tr>
<td>Voluntary voiding with massage and collection via capillary tube</td>
<td>rat</td>
<td>good</td>
<td>medium</td>
<td>moderately hard</td>
</tr>
<tr>
<td>Metabolic cage with specially designed bottomless nest boxes that provide shelter for small rodents</td>
<td>mouse</td>
<td>fair</td>
<td>medium</td>
<td>easy</td>
</tr>
<tr>
<td>Metabolic cage with specially designed bottomless nest boxes that provide shelter for small rodents</td>
<td>rat</td>
<td>fair</td>
<td>medium</td>
<td>easy</td>
</tr>
<tr>
<td>Metabolic cage</td>
<td>rabbit</td>
<td>fair</td>
<td>medium</td>
<td>easy</td>
</tr>
<tr>
<td>Voluntary voiding with massage</td>
<td>rabbit</td>
<td>good</td>
<td>medium</td>
<td>moderately hard</td>
</tr>
<tr>
<td>Restraint and cystocentesis</td>
<td>mouse</td>
<td>excellent</td>
<td>high</td>
<td>hard</td>
</tr>
<tr>
<td>Restraint and cystocentesis</td>
<td>rat</td>
<td>excellent</td>
<td>high</td>
<td>hard</td>
</tr>
<tr>
<td>Restraint and cystocentesis</td>
<td>rabbit</td>
<td>excellent</td>
<td>high</td>
<td>hard</td>
</tr>
<tr>
<td>Anaesthesia and cystocentesis</td>
<td>rabbit</td>
<td>excellent</td>
<td>low</td>
<td>hard</td>
</tr>
<tr>
<td>Anaesthesia and cystocentesis</td>
<td>rat</td>
<td>excellent</td>
<td>low</td>
<td>hard</td>
</tr>
<tr>
<td>Anaesthesia and cystocentesis</td>
<td>mouse</td>
<td>excellent</td>
<td>low</td>
<td>hard</td>
</tr>
</tbody>
</table>

Source: Adapted from Kurien et al (2004), Tables 1 and 2.

### REFERENCES


D BLOOD COLLECTION

Although blood is a biological sample, there is enough information on blood collection to warrant a separate factsheet. See the ‘Biological sample collection’ factsheet for information on other types of samples.

WHY COLLECT BLOOD?

Blood is collected from a living animal for analysis of biochemical, metabolic, toxicological, immunological and physiological changes. The volume, frequency, method, site and duration of collection must be humane and appropriate for the scientific purpose.

Blood collection is a valuable scientific tool. It enables the investigator to monitor, in a dynamic setting, many factors, including:

- the presence, absence and concentration of drugs in the circulation
- haematological and biochemical changes (eg in a stress response)
- exogenous or endogenous toxins
- development of immunity
- blood parameters (eg packed cell volume, blood urea nitrogen) as a measure of the health of the animal
- the presence or absence of bacteria, viruses or protozoa determined by culture or direct examination.

HOW IS BLOOD COLLECTED?

Blood can be collected:

- by puncturing a blood vessel (venipuncture—from jugular, cephalic, lateral tail, marginal ear, saphenous, brachial [in birds], mammary, femoral, sublingual, cranial vena cava, and anterior facial veins)
- from the heart, under general anaesthesia and as a terminal procedure
- from the retrobulbar sinus under anaesthesia—this method is controversial, and there are reports that this procedure causes histological damage to structures around the eye even in anaesthetised rats, including haemorrhages and inflammatory reactions in the puncture track, retro-orbital periosteum, eye muscles and Hardaerian gland (Hem et al 1998); therefore, this procedure must only be performed by skilled personnel
- from catheters—chronic catheters enable blood to be sampled without the added stress of many handlings (see the ‘Surgical procedures’ factsheet for the management of catheters).
Table D1 shows the recommended site and volume of blood collection for various animal species.

### Table D1  Recommended site and volume of blood collection using calculated blood volume

<table>
<thead>
<tr>
<th>Species</th>
<th>Total blood volume (using blood vol of 7% body weight [mL])</th>
<th>Recommended site for blood collection (see Table D4 for alternatives)</th>
<th>≤7.5% Minor bleed (mL)</th>
<th>7.5–10% Moderate bleed (mL)</th>
<th>10–15% Major bleed (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (26 g)</td>
<td>1.82</td>
<td>Anterior facial vein or saphenous vein</td>
<td>&lt;0.14</td>
<td>0.14–0.18</td>
<td>0.18–0.27</td>
</tr>
<tr>
<td>Rat (250 g)</td>
<td>17.5</td>
<td>Saphenous vein</td>
<td>&lt;1.31</td>
<td>1.31–1.75</td>
<td>1.75–2.63</td>
</tr>
<tr>
<td>Rabbit (4 kg)*</td>
<td>280</td>
<td>Lateral ear vein</td>
<td>&lt;21.0</td>
<td>21.0–28.0</td>
<td>28.0–42.0</td>
</tr>
<tr>
<td>Dog (10 kg)*</td>
<td>497</td>
<td>Jugular or cephalic vein</td>
<td>&lt;37.2</td>
<td>37.2–49.7</td>
<td>49.7–74.5</td>
</tr>
<tr>
<td>Macaque (5 kg)*</td>
<td>350</td>
<td>Saphenous vein</td>
<td>&lt;26.3</td>
<td>26.3–35.0</td>
<td>35.0–52.5</td>
</tr>
<tr>
<td>Minipig*</td>
<td>2100</td>
<td>Cranial vena cava</td>
<td>&lt;157</td>
<td>157–210</td>
<td>210–315</td>
</tr>
</tbody>
</table>

*Blood volume taken should always be the minimum possible for the tests required. For example, minor bleeding to monitor haematology and biochemical parameters requires a volume of no greater than 10 mL, and in many cases much less than this.

Source: Adapted from Diehl (2001)

All forms of blood collection via mutilation, including the transection of blood vessels and the amputation of toes, are inhumane and therefore unacceptable.

### Planning blood collection

When planning the research project, investigators should identify the most appropriate type of blood collection tube, whether anticoagulants will be required, how blood samples will be stored, and how blood and sera will be separated. The site of the blood collection will determine whether the animal needs to be anaesthetised. These decisions should not be left until the animal has been restrained.

In addition, investigators should include in the research proposal regular handling of animals to acclimatise them before blood samples are taken (see 'How can pain and distress be minimised?' below).

### Preparing animals for blood collection

It is important to maintain asepsis throughout sampling. Hair and superficial skin debris over the vein should first be removed. The method for hair removal will depend on the site of the vein and the species of animal, with plucking, clipping or shaving commonly used. The clipped or plucked area should be cleaned with warm water with the addition of a disinfectant such as chlorhexidine (Hibitane). These agents should be subsequently removed with plain water to avoid contaminating the sample. Alternatively, the cleaned skin can be swabbed with 70% alcohol and allowed to dry.

Small quantities of blood can be taken by pricking with a solid, triangularly pointed cutting needle (OIE 2004).

Topical application of anaesthetic creams, where appropriate, will significantly alleviate any discomfort associated with venipuncture. Flecknell et al (1990) found that application of a
local anaesthetic cream 30–60 minutes before blood collection was beneficial in dogs, cats and rabbits, but did not influence the response of rats to a tail vein injection.

The size of the needle to be used for blood collection must be carefully considered. There is evidence, based on the use of a disturbance index (Barclay et al 1988), that a large-bore needle (relative to the size of the vein) may result in no more stress to the animal than a small-bore needle. This is because the larger bore provides for faster blood removal and therefore shorter handling time (Hem et al 1998).

The blood may be collected in a haematocrit tube, eppendorf tube, syringe or even a vacuum tube if the size of the vein allows it. The receptacle and the use of anticoagulants will be dictated by the volume of blood required and the type of testing to be performed, and should be decided at the design phase of the project.

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

Animal wellbeing considerations must dictate not only the method of blood collection (ie the least painful to the animal), but also the volume of blood that may be withdrawn at any one time and the number of times that an animal may be bled in a given timeframe. In addition, the investigator must establish the minimum volume required and the frequency of collection before the site is chosen. The four main areas of impact on animal wellbeing associated with blood collection are excessive blood loss, thrombosis, bruising and inflammation of the vein (Morton et al 1993).

Blood sampling and haemorrhage may have negative effects on the health and wellbeing of laboratory animals. Minor blood loss causes the sympathetic nervous system to react, increasing the heart rate, constricting the vessels in the skin and muscles to increase circulating blood volume, and releasing hormones to decrease urine output to help replace blood volume. With moderate blood loss, the animal’s blood pressure will drop despite compensatory mechanisms. Cholinergic release directs blood away from the skin, muscles and gut, but metabolic changes occur with ensuing tissue anoxia and acidosis (Morton et al 1993, Diehl 2001).

With severe blood loss, tissue anoxia, hypercapnia and acidosis can result in irreversible tissue damage and death.

The investigator must establish:

- the minimum volume of blood required for the experimental protocol
- the frequency of blood collection
- the time period over which blood is collected
- the site of blood collection that will deliver the volume required with minimal effects on the animal’s wellbeing
- availability of suitably skilled staff to conduct the sampling
- the likely effect of removal of this volume of blood from the animal (clinically, organ perfusion and oxygenation)
- whether the effects of the blood collection will interfere with the aim of the research project
- protocols for the monitoring of the animal for the effect of blood sampling, both acute and chronic, and action to be taken to minimise suffering.

Animal wellbeing considerations for blood collection are summarised in Table D4, at the end of this factsheet.
Volume of blood collection

Blood collection limits can be expressed as a percentage of blood volume. Blood volume can be approximated by assuming it is 5.5–7% of the animal’s bodyweight. This means blood volume can be estimated at 70 mL/kg (Morton et al. 1993, Diehl 2001).

The recommended maximum volume of blood collected as a single sample blood collection is 10–15% of the circulating blood volume (Morton et al. 1993). If larger volumes are to be collected, the animal should receive fluid replacement (injection of sterile isotonic saline) equal to the volume collected, and be monitored for signs of haemorrhagic shock (rapid heart rate, pale mucous membranes, cold skin and extremities, increased respiration rate).

Removal of 30% or greater of the circulating blood volume is hazardous to the animal’s health, and there is a significant risk of shock or death.

A single sample blood collection can be performed every two weeks; however, in this situation, haematocrit (packed cell volume) and other haematological assessments must be made regularly, as there is an increased risk of anaemia developing over time.

If blood is required to be sampled on a repeated and more frequent basis than that indicated above, the recommended maximum volume of blood for repeat bleeds at intervals of less than two weeks is 7% of the circulating blood volume per week. This amount may be withdrawn once a week without compromising a healthy animal. Larger amounts can be withdrawn if the recovery time is extended. Table D2 shows the maximum volumes and recovery periods for blood collection. Haematological assessments must be made regularly, because there is an increased risk of anaemia developing if samples are collected frequently over long periods of time. Consider placement of an indwelling venous cannula if many samples of blood are to be collected over a short timespan (hours or a few days). This will reduce the number of venipunctures.

<table>
<thead>
<tr>
<th>Period of collection</th>
<th>% of blood volume collected</th>
<th>Approximate recovery period in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single bleed</td>
<td>Up to 7% (minor bleed)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10% (moderate bleed)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>15% (severe bleed)</td>
<td>3</td>
</tr>
<tr>
<td>Over a 24-hour period</td>
<td>Up to 7%</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>2-3</td>
</tr>
<tr>
<td></td>
<td>15%</td>
<td>4-6</td>
</tr>
</tbody>
</table>

Note: These recommendations make no allowance for pregnancy, lactation, illness, or the effects of genetic manipulation.

Source: Diehl (2001)

Blood collection limits can also be expressed as a percentage of bodyweight (Hem et al. 1998). As a general rule, a blood volume equivalent to about 0.5–1% of the animal’s bodyweight can be withdrawn as a single sample (Wolfensen and Lloyd 1994, Diehl 2001), and this can usually be repeated once every one to two weeks without damaging the animal’s health. Alternatively, daily samples not exceeding 0.05% of bodyweight may be taken.
HOW ARE PAIN AND DISTRESS MONITORED?

The investigator must be aware of the signs of adverse effects of blood collection, and must monitor for the signs of acute or chronic blood loss.

Acute blood loss

If the volume of blood removed exceeds the guidelines provided, then hypovolaemic shock may result. Signs and treatment of acute blood loss are described in Table D3.

<table>
<thead>
<tr>
<th>Signs of acute blood loss</th>
<th>General principles of treatment of acute blood loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased respiration, cold extremities, restlessness, pale mucous membranes</td>
<td>Fluid replacement: warmed normal saline or dextrose saline at a volume of less than or equal to 5% of bodyweight</td>
</tr>
</tbody>
</table>

**Table D3  Signs and treatment of acute blood loss**

<table>
<thead>
<tr>
<th>Species examples</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-g mouse</td>
<td>Max 1.5 mL SC or IP. Support: warmth, soaked cotton wool balls, jelly</td>
</tr>
<tr>
<td>250-g rat</td>
<td>Max 12.5 mL SC or IP. Support: warmth, soaked cotton wool balls, jelly</td>
</tr>
<tr>
<td>3-kg rabbit</td>
<td>150 mL slow IV drip or SC, IP in multiple sites</td>
</tr>
<tr>
<td>15-kg dog</td>
<td>750 mL slow IV drip</td>
</tr>
</tbody>
</table>

IP = intraperitoneal; IV = intravenous; SC = subcutaneous

Chronic blood loss

The effects of chronic blood loss are more subtle than those of acute blood loss. Packed cell volume, haemoglobin levels, red cell count and reticulocyte count should be monitored regularly. Signs of chronic anaemia include pale mucous membranes, loss of muscle mass, reduced activity (and exercise intolerance) and increased respiratory rate.

Bleeds should be suspended until blood parameters are back in the normal range, and then the frequency and volume of blood taken should be reduced.

EFFECTS ON RESEARCH DATA

Some methods of blood collection can affect research data. For example:

- some anaesthetic agents affect blood values
- indwelling catheters can affect blood values
- poor aseptic technique can result in bacterial contamination of the sample
- handling and housing conditions can affect the blood sample
- poor venipuncture technique can affect blood values
- choice of sampling site can affect the speed of blood collection, and therefore the quality of the sample (Loeb and Quimby 1999).
HOW CAN PAIN AND DISTRESS BE MINIMISED?

The following sections and Table D4 summarise general procedures and other considerations for minimising the adverse effects of blood or body fluid collection.

For all procedures, the animal restraint time should be reduced to an absolute minimum, because this will reduce the risk of excessive bleeding caused by stress-induced hypertension.

General procedures

- Pain and distress during blood collection can be minimised by using the method that has the potential to cause the least tissue damage.
- Blood collection must be performed by suitably trained staff using the least painful method.
- Animals should be handled regularly so they are less stressed when they are restrained for blood sampling.

Blood collection

- Staff skilled in restraint and in the sampling technique are essential.
- Gentle warming of the animal with a heat lamp before blood collection from the tail vein will reduce sampling time and thus reduce stress.
- Topical application of anaesthetic creams 30–60 minutes before the procedure has been shown to significantly reduce the discomfort of venipuncture in dogs, cats and rabbits.
- Inadequate restraint will prolong sampling time, increase the risks to the animal and reduce the quality of the sample.
- When multiple sampling is required, alternating the site of collection should be considered.
Table D4  Wellbeing issues to consider for blood collection in selected species

<table>
<thead>
<tr>
<th>Species</th>
<th>Routes of blood collection</th>
<th>Preferred route</th>
<th>Suitable for repeat bleeds</th>
<th>Volume of blood able to be collected</th>
<th>Potential for tissue damage</th>
<th>Anaesthesia required</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Anterior facial vein or submandibular puncture</td>
<td>Yes</td>
<td>Yes (consider alternating sides)</td>
<td>+</td>
<td>Low</td>
<td>No</td>
<td>Needle used to puncture vein or a lancet may be used. Vessel is superficial but not visible without removing hair.</td>
<td>Gole et al (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral tail vein</td>
<td>Yes</td>
<td>+ or +</td>
<td></td>
<td></td>
<td>Low</td>
<td>No</td>
<td>Vasodilation may be necessary to promote bleeding by applying warmth to the tail. Animals must be monitored for heat stress.</td>
<td>Diehl (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac puncture</td>
<td>No</td>
<td>+++</td>
<td></td>
<td></td>
<td>Moderate</td>
<td>Yes, general</td>
<td>Can lead to pericardial bleeding and cardiac tamponade and should only be used for terminal bleeds.</td>
<td>Diehl (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral tail vein</td>
<td>Yes</td>
<td>+ (+)</td>
<td></td>
<td></td>
<td>Low</td>
<td>No</td>
<td>Vasodilation may be necessary to promote bleeding by applying warmth to the tail. Animals must be monitored for heat stress.</td>
<td>Diehl (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac puncture</td>
<td>No</td>
<td>+++</td>
<td></td>
<td></td>
<td>Moderate</td>
<td>Yes, general</td>
<td>Can lead to pericardial bleeding and cardiac tamponade and should only be used for terminal bleeds.</td>
<td>Diehl (2001)</td>
</tr>
<tr>
<td>Species</td>
<td>Routes of blood collection</td>
<td>Preferred route</td>
<td>Suitable for repeat bleeds</td>
<td>Volume of blood able to be collected</td>
<td>Potential for tissue damage</td>
<td>Anaesthesia required</td>
<td>Comment</td>
<td>Reference</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------</td>
<td>-----------------</td>
<td>-----------------------------</td>
<td>-------------------------------------</td>
<td>----------------------------</td>
<td>----------------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>Dog and cat</td>
<td>Cephalic vein</td>
<td>Big dog, cat</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>No, although topical local anaesthesia 30–60 mins before the procedure will reduce discomfort</td>
<td>Monitor the site for tissue reaction. The site should be changed if it becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.</td>
<td>Diehl (2001) Flecknell et al (1990)</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>Small dog, cat</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>No, although topical local anaesthesia 30–60 mins before the procedure will reduce discomfort</td>
<td>Monitor the site for tissue reaction. The site should be changed if it becomes red, swollen or inflamed.</td>
<td>Diehl (2001) Flecknell et al (1990)</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Marginal ear vein</td>
<td>Yes</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>Yes, local infiltration and topical local anaesthesia 30–60 mins before the procedure will reduce discomfort</td>
<td>Monitor the site for tissue reaction. The site should be changed if it becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.</td>
<td>Diehl (2001) Flecknell et al (1990)</td>
</tr>
<tr>
<td>Central ear artery</td>
<td>Yes</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>Yes, local infiltration or topical local anaesthesia 30–60 mins before the procedure will reduce discomfort</td>
<td>Monitor the site for tissue reaction. The site should be changed if it becomes red, swollen or inflamed. Local anaesthetic infiltration can lead to prolonged constriction of blood vessels and tissue necrosis.</td>
<td>Diehl (2001) Flecknell et al (1990)</td>
<td></td>
</tr>
<tr>
<td>Cardiac puncture</td>
<td>No</td>
<td>+++</td>
<td>Moderate</td>
<td>Yes, general</td>
<td>Can lead to pericardial bleeding and cardiac tamponade and should only be used for terminal bleeds.</td>
<td>Diehl (2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Routes of blood collection</td>
<td>Preferred route</td>
<td>Suitable for repeat bleeds</td>
<td>Volume of blood able to be collected</td>
<td>Potential for tissue damage</td>
<td>Anaesthesia required</td>
<td>Comment</td>
<td>Reference</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------</td>
<td>----------------</td>
<td>---------------------------</td>
<td>-------------------------------------</td>
<td>---------------------------</td>
<td>-------------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>Macaque</td>
<td>Saphenous vein</td>
<td>Yes (consider alternating sides)</td>
<td>++(+)</td>
<td>Low</td>
<td>No, although topical local anaesthesia 30-60 mins before the procedure will reduce discomfort</td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.</td>
<td>Diehl (2001), Hem et al (1998), Flecknell et al (1990), Lurrley et al (1990)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Femoral vein</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>No</td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed.</td>
<td>Diehl (2001), Flecknell et al (1990)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cephalic vein</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>No</td>
<td>Consider the use of indwelling catheters for repeated sampling.</td>
<td>Morton et al (1993)</td>
<td></td>
</tr>
<tr>
<td>Marmoset</td>
<td>Saphenous vein</td>
<td>Yes (consider alternating sides)</td>
<td>++(+)</td>
<td>Low</td>
<td>No</td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.</td>
<td>Diehl (2001), Hem et al (1998), Flecknell et al (1990), Lurrley et al (1990)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Femoral vein</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>No</td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed.</td>
<td>Diehl (2001), Flecknell et al (1990)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lateral tail vein</td>
<td>Yes</td>
<td>++(+)</td>
<td>Low</td>
<td>No</td>
<td>Vasodilation may be necessary to promote bleeding by warming the animal. Animals must be monitored for heat stress.</td>
<td>Diehl (2001)</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Routes of blood collection</td>
<td>Preferred route</td>
<td>Suitable for repeat bleeds</td>
<td>Volume of blood able to be collected</td>
<td>Potential for tissue damage</td>
<td>Anaesthesia required</td>
<td>Comment</td>
<td>Reference</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------</td>
<td>----------------</td>
<td>---------------------------</td>
<td>-------------------------------------</td>
<td>----------------------------</td>
<td>---------------------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Ferret</td>
<td>Cranial vena cava</td>
<td>Yes</td>
<td>Yes (consider alternating sides)</td>
<td>+++</td>
<td>Low</td>
<td>No</td>
<td>Proper, humane restraint must be used. Should not be repeated in less than one week.</td>
<td>Diehl (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed.</td>
<td></td>
</tr>
<tr>
<td>Minipig</td>
<td>Marginal ear vein</td>
<td>Yes</td>
<td>Yes (consider alternating sides)</td>
<td>++(+</td>
<td>Low</td>
<td>Yes local infiltration and topical local anaesthesia 30–60 mins before the procedure will reduce discomfort</td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.</td>
<td>Diehl (2001) Flecknell et al (1990)</td>
</tr>
<tr>
<td></td>
<td>Cranial vena cava</td>
<td>Yes</td>
<td>+++</td>
<td>Low</td>
<td>No</td>
<td></td>
<td>Proper, humane restraint must be used. Should not be repeated in less than one week.</td>
<td>Diehl (2001)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Cardiac puncture</td>
<td>No</td>
<td>+++</td>
<td>Moderate</td>
<td>Yes, general</td>
<td>Can lead to pericardial bleeding and cardiac tamponade and should only be used for terminal bleeds.</td>
<td>Diehl (2001)</td>
<td></td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>Yes</td>
<td>Yes (consider alternating sides)</td>
<td>++(+</td>
<td>Low</td>
<td>No</td>
<td>Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.</td>
<td>Diehl (2001) Flecknell et al (1990) Lurley et al (1990)</td>
<td></td>
</tr>
</tbody>
</table>

Note: +, volume in the order of 0.1 mL; ++, volume in the order of 0.1–1.0 mL; ++++, volume in the order of more than 1.0 mL.
REFERENCES


http://www.oie.int/eng/normes/mmanual/A_summary.htm

E ENVIRONMENTAL ENRICHMENT STRATEGIES

WHAT IS ENVIRONMENTAL ENRICHMENT?

Environmental enrichment is an important component of the animal's physical, nutritional and social environment and contributes to meeting their physiological and psychological needs. Environmental enrichment is ‘any measure which promotes expression of natural, species specific behaviours and a decrease in, if not disappearance of, abnormal behaviours. It should be aimed not just at preventing suffering, but at having a positive effect on the physical and psychological well-being’ of the animal (NSW Agriculture Animal Research Review Panel 2003).

WHEN IS ENVIRONMENTAL ENRICHMENT USED?

According to the Australian code of practice for the care and use of animals for scientific purposes (the Code), environmental enrichment must be provided as part of routine animal care:

- Section 4.4.19—‘Animal accommodation should be designed and managed to meet species-specific needs. Pens, cages and containers should ensure animal wellbeing and comfort. Variations to these requirements as part of a project must receive prior AEC approval. The following factors should be taken into account: (i) species-specific behavioural requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy, contact with others of the same species, and environmental enrichment …’
- Section 4.4.25—‘Where possible, animals should be given variety in the composition and presentation of food that is suitable for the species.’

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

It is important to note that different species require different social environments and therefore need different types of environmental enrichment. For example, the social environment of sheep provides enrichment; novelty in the physical environment can be confronting to this species. It is also important to note that environmental enrichment must be undertaken thoughtfully since it can cause unintended harm to animals and introduce confounding variability into research results (Bayne 2005).

Techniques described in the literature rarely highlight the necessity to provide the animals with as natural an environment as possible. The principle of environmental enrichment is to provide a stimulating and varied environment. This means that the provision of environmental enrichment techniques should be random rather than routine. The implementation of environmental enrichment must be part of the daily routine, but its delivery should be unpredictable for the animal. Animals that have appropriate housing, environmental enrichment and compassionate care will provide samples that produce more consistent scientific results (Kurien et al 2004).

The focus of limiting pain and distress has largely been on the procedures performed on animals during an investigation. However, the potential for pain and distress caused by housing conditions that lack suitable environmental enrichment is far greater, because it affects a much greater proportion of laboratory animals in the animal house, not just the experimental groups (Sherwin 2002).

Sterile, barren environments are associated with abnormal, stereotypical behaviours and a reduced variety of normal behaviours. Abnormal or damaging behaviours (such as bar chewing, repetitive circling of the enclosure, wool biting and overgrooming, resulting in hair loss and skin sores) are indicators of reduced wellbeing (Smith and Hargaden 2001, NSW Agriculture Animal Research Review Panel 2003). When a more stimulating, varied environment is provided, animals display a wider range of normal behaviours. For example, when given the opportunity, laboratory mice forage ‘for a wide variety of foods, are physically active, form complex social organisations, build tunnels and construct nests’ (Sherwin 2002).
A complex and enriched environment has a powerful effect on the brain, in particular the cerebral cortex. These influences involve physical, chemical and functional elements—hence the effect on the behaviour, memory and learning ability of animals. Although it was once believed that only the developing brain was susceptible to the influence of environment, it is now recognised that the brain is capable of responding throughout the entire life of an animal (Diamond 2001).

Human–animal interactions are important for the wellbeing of animals, and also affect research outcomes. Animals should be acclimatised to human presence in general, and to the particular individual carers or handlers. To avoid pain and distress, all handling and restraint should be as positive as possible, non-threatening, safe and free from pain and distress.

**SPECIES-SPECIFIC EXAMPLES OF ENVIRONMENTAL ENRICHMENT**

Table E1 summarises some environmental enrichment strategies for rats and mice, cats, ferrets, rabbits, guinea pigs, pigs, sheep, dogs, non-human primates and fish.

**Table E1  Environmental enrichment strategies for animal species used in research**

<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Rats and mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social</td>
<td></td>
</tr>
</tbody>
</table>
|                                  | • Mice and rats are highly social and do best in paired or group housing.  
|                                  | • Aggression can be a problem with males, especially among mice.  
|                                  | • The most successful groups are formed with weanlings who know each other.  
|                                  | • Same-sex groups should be set up before puberty.  
|                                  | • Adding or removing an individual can affect the wellbeing of the whole group.  |
| Human–animal                     |               |
|                                  | • Conduct systematic, gentle, daily handling that is not linked with procedures that may cause stress.  
|                                  | • ‘Gentling’ allows the rat or mouse to become familiar with humans (the animal is allowed to explore its handler and is gently stroked and held).  
|                                  | • Train animals to become used to procedures that have to be repeated. This may involve positive reinforcement or treats after the procedure is completed.  |
| Food                             |               |
|                                  | • Rodents prefer to forage for their food.  
|                                  | • Scattering food, including small quantities of seeds in the substrate, allows foraging and lets the rat or mouse adopt a normal eating posture.  
|                                  | • Scatter seed on top of the pelleted food. Some will fall through the cracks, but most will be trapped between the pellets. The rats learn to manipulate the pellets to access the seeds that fall through. Delivery of the seed is random, but foraging is controlled by the rat.  
|                                  | • Provide small food containers that the animal has to gnaw through to get to the food (eg plastic photographic film containers).  
|                                  | • Provide hard-shelled nuts (eg walnuts, hazelnuts, macadamia nuts), as it takes time to gnaw through the hard shell before the nut is obtained.  
|                                  | • For variety, offer alternative foods in addition to pelleted chow.  |
| Physical environment             |               |
|                                  | • Rats and mice are nocturnal; therefore, they need to be able to hide from light and seek refuge (eg with cardboard rolls, plastic margarine containers etc).  
<p>|                                  | • Enlarge the floor space by providing levels within the cage or by providing items to climb on or exercise in.  |</p>
<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Rats and mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical environment</strong> (continued)</td>
<td></td>
</tr>
</tbody>
</table>
| • When moving about, mice prefer to stay in contact with a wall and away from open spaces. Providing dividers in the cage can make the mouse feel more secure.  
• Cages with solid bottoms allow the use of substrates, which provide opportunities for rodents to dig.  
• A running wheel allows exercise and ‘play’. Mice prefer irregularly shaped wheels or wheels that include hurdles to jump.  
• Provide activity toys, such as climbing ropes, ladders, chains, balls, Kong toys (chew-resistant rubber), Nylaballs (chew-resistant nylon plastic) and Nylabones.  
• Cage lids should have bars to allow for climbing and acrobatic activities.  
• Provide rats with a solid, opaque nest box with a top that the rat can climb onto.  
• When given a choice, rats prefer a nest box to shredded paper.  
• Provide mice with a maze as a surrogate tunnel.  |
| **Olfactory stimulation** |  |
| • Olfactory cues are very important to rodents and are the foundation for development of their social organisation.  
• Aggression in males is common following box cleaning, because territorial scent marking is disrupted. Placing some of the nesting material from the dirty box into the clean box may reduce this.  
• Do not place soiled substrate in the clean box, as this may increase aggression.  
• Rats are natural predators of mice, and the smell of rats will cause a fear response in the mice. Therefore, these two species should not be housed together.  
• Adding shredded paper after cage cleaning reduces the olfactory load and encourages nesting behaviour.  |
| **Provision for natural behaviours** |  |
| • Room lights should be on a day–night cycle, preferably with dimming to imitate dawn and dusk.  
• Provide nesting materials such as tissues, hay or shredded paper, paper towels, paper strips, commercial nesting fibre or wood wool. Mice will build nests with these items with apparent enthusiasm, not just for young but also to hide from light or other stressful stimuli.  
• Substrate must also be provided for rats and mice. Examples include wood shavings, sawdust and pelleted paper.  
• Encourage natural behaviours like digging and tunnelling by providing a substrate that is several centimetres thick.  
• Provide objects to chew, such as wooden blocks with predrilled holes, golf balls or small wooden play balls, softwood blocks, plastic bottles, straw or cardboard tubes. Cardboard tubes also provide shelter and an opportunity to climb.  
• Provide rats with increased structural complexity using shelves, platforms, shelters and ramps.  |

---

* van de Weerd and Baumanns (1995)  
+ Sherwin (2002)  
* Figa (2004)  
* Smith and Hargaden (2001)  
van Loo et al (2001)  
ARAC (2004)  
Patterson-Kane et al (2001)
### Type of environmental enrichment

<table>
<thead>
<tr>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Social</strong></td>
</tr>
</tbody>
</table>
| - Adult cats can be kept in individual cages or in groups.  
  - Sibling cats living together generally get along better than unrelated cats.  
  - Where group housing is used, larger groups allow a relatively stable hierarchy to develop.  
  - Mature females form stable and peaceful groups more readily than do sexually mature males.  
  - Young cats play frequently, and their social development is facilitated by a colony environment that encourages play.  
  - Communication between cats occurs on many levels, through scent marking (urine, faeces, anal glands and scent glands), vocalisation (spitting, growling, snarling, purring, chirping) and posturing.  
  - Cats will take part in mutual grooming and may choose to sleep together. |

| **Human-animal** |
| Cats kept confined will seek additional stimulation from people.  
  - The caregiver is the most important determinant of the cat's wellbeing.  
  - Cats demonstrate a clear preference for human contact over toys.  
  - Periods of time that are not part of the routine feeding and cleaning should be available every day for cats to interact with their caregiver. This may be in the form of talking and patting, or via interaction with a toy.  
  - The most important time for a kitten's socialisation with humans is between 2 and 7 weeks of age. Socialisation should continue throughout the cat's life.  
  - Cats organise their daily routine around their caregiver's activities, so it is important that the caregiver sticks to a routine. Any changes need to be introduced slowly to minimise stress.  
  - Cats that are handled gently and spoken to quietly each day are less timid or aggressive than those that do not receive this attention.  
  - Social contact with humans is particularly important to singly housed cats.  
  - Only calm and gentle staff with an empathy for cats should be selected to look after them. |

| **Food** |
| Cats are carnivores and generally prefer a variety of protein sources.  
  - A variety of food should be offered from weaning to avoid dietary fixation.  
  - The preferred eating pattern is small meals often. Toys or containers with holes in them can be used to hold dry food, providing a play item as well as a snack. |

| **Physical environment** |
| Keeping cats in an environment that encourages a wide range of normal behaviours improves their wellbeing, makes them better candidates for scientific investigation and makes the detection of ill health easier.  
  - Cats housed singly need visual and olfactory contact with other cats.  
  - The enclosure must provide enough space for stretching, exploring, playing, feeding/drinking, resting, scratching and defecating/urinating.  
  - In group-housed cats, the minimum floor space requirement is determined by their socio-spatial needs rather than by bodyweight.  
  - The vertical dimension is very important to cats, and shelving or hammocks at various levels should be provided.  
  - Resting places or retreats for each cat in the group should be provided. If this is not done, cats are likely to sleep in their litter trays. |
<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical environment (continued)</td>
<td>• Elevated resting places that enable the cat to watch its surroundings are preferred. These should be warm, dry and protected on one or two sides. Corners are ideal.</td>
</tr>
<tr>
<td></td>
<td>• A shelf next to a window is also a favoured spot; as cats show a preference for observing the activity in the immediate environment.</td>
</tr>
<tr>
<td></td>
<td>• Scratch posts, an adequate number of litter trays (at least 1 per 2 cats), toys and bedding should be provided.</td>
</tr>
<tr>
<td></td>
<td>• Toys should be changed regularly to stimulate play.</td>
</tr>
<tr>
<td></td>
<td>• Cats spend 14–16 hours a day resting and sleeping, so soft bedding should be provided. Pillows or polyester fleece are preferred. It has been shown that cats that sleep on soft surfaces have longer periods of deep sleep than do cats that sleep on hard surfaces, suggesting that they feel more secure.</td>
</tr>
<tr>
<td></td>
<td>• Hiding is a coping behaviour that cats show in response to potentially stressful situations (including when avoiding other cats). Enclosed boxes or cat beds must be provided for retreat.</td>
</tr>
<tr>
<td></td>
<td>• If a cat has some degree of choice over its physical and social environment, it will develop more effective ways of coping with unpredictable stimuli.</td>
</tr>
<tr>
<td></td>
<td>• Small objects with complex surface texture are the most successful at promoting play.</td>
</tr>
<tr>
<td></td>
<td>• Most cats play alone, so there must be enough space for the cat to play without encroaching on the space of another cat.</td>
</tr>
<tr>
<td></td>
<td>• Having the radio on helps to reduce stress due to unexpected noises.</td>
</tr>
<tr>
<td></td>
<td>• Provide a tray of grass and another tray of dirt.</td>
</tr>
<tr>
<td>Olfactory stimulation</td>
<td>• Scratching provides olfactory stimulation, as the act of scratching deposits scent from the interdigital glands.</td>
</tr>
<tr>
<td></td>
<td>• Scratching posts, rush matting, pieces of carpet and wood should be provided in more than one location.</td>
</tr>
<tr>
<td></td>
<td>• Provide containers of grass or catnip for olfactory stimulation and to help with the elimination of hair balls.</td>
</tr>
<tr>
<td>Provision for natural behaviours</td>
<td>• Provide scratching posts for scent and visual marking of territorial boundaries.</td>
</tr>
<tr>
<td></td>
<td>• Pieces of dry food in containers with holes stimulate pseudo-predatory behaviour.</td>
</tr>
<tr>
<td></td>
<td>• Provide retreats to allow cats to choose solitary time.</td>
</tr>
<tr>
<td></td>
<td>• Cats are good climbers and, given a choice, spend more of their time above ground than at ground level. The provision of ramps, shelves and climbing poles encourages this behaviour.</td>
</tr>
</tbody>
</table>

---

* James (1995)  
* McCune (1995)  
* Rochlitz (2002)  
* Karsh and Turner (1988)  
* De Luca and Kranda (1992)  
* Carlstead et al (1993)  
* Bradshaw and Thorne (1992)  
* Podberscek et al (1991)  
* Hall and Bradshaw (1998)  
* Schroll (2002)
### Social

- Domestic ferrets are social animals and gain physical advantages as well as social benefits if housed in pairs or groups.\(^a\)
- Intact male ferrets will fight during the breeding season, as will intact females. Therefore, for entire ferrets, pairs of male and female are best.\(^i\)
- Ferrets interact with each other; they play, roll and pounce.\(^d\)
- Group-housed ferrets will sleep together.\(^e\)
- Ferrets are less likely to exhibit stereotypical behaviours if they have other ferrets for company.\(^d\)

### Human-animal

- Domestic ferrets are not afraid of humans.
- Ferrets are generally able to handle new environments without fear and with curiosity.
- Ferrets that are handled on a daily basis, gently stroked and picked up are easier to work with than those that do not receive handling.\(^c\)
- Ferrets learn quickly, and with positive reinforcement can be trained to accept procedures that have to be repeated regularly.\(^b\)
- Positive reinforcement may take the form of treats. However, sugary treats or treats high in fibre (such as fruit) should be avoided; dehydrated liver or a similar food should be used.\(^b\)

### Food

- Ferrets are carnivores and have a fast gut-transit rate, necessitating frequent feeds.\(^b\)
- In addition to a balanced ferret (or kitten) chow, ferrets need objects to chew on to keep teeth and gums healthy. These can be pieces of rabbit or lamb complete with bone, or whole fresh dead animals such as rats or mice.\(^a\)^\(^b\)^\(^c\)
- Food products suitable for cat dental hygiene are suitable for ferrets.
- Ferrets will hoard food that they do not consume (for a later snack), so it is important to remove any pieces of leftover bone or carcasses after 24 hours to prevent food poisoning.

### Physical environment

- Lighting should be on a 12-hour light/12-hour dark cycle.\(^c\)
- Light periods can be increased to stimulate oestrus.\(^c\)
- Ferrets prefer a small, enclosed box to sleep in and they become anxious if they do not have one.\(^b\)
- They need nesting material such as straw, shredded paper, or cloth. Cloth should be a close weave so the animals do not get their nails ensnared.\(^a\)^\(^b\)^\(^c\)
- Ferrets urinate and defecate in one area, and so can be trained to use a litter tray. Use non-dusty litter material to avoid respiratory complications.\(^b\)^\(^c\)
- Adequate ventilation and air changes must be provided, as ferrets are prone to respiratory infection.\(^c\)
- Ferrets spend about 20 hours a day asleep. The provision of hammocks gives them alternative sleeping places and increases their effective floor space.\(^a\)^\(^b\)
<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Ferrets</th>
</tr>
</thead>
</table>
| **Physical environment**<br>(continued) | - Toys have been shown to improve ferrets' receptivity to being handled, and need not be sophisticated. Cardboard boxes and plastic shopping bags are a great source of entertainment, but plastic bags should be removed if they become ripped. Other examples of ferret toys are cloth toys for cats or babies, hard plastic balls, and paper bags. Toys should not include latex rubber items intended for dogs or cats.  
  - There is evidence that the greatest benefit is gained from toys if they are changed on a regular basis.a  
  - Ferrets are burrowing animals, and providing cylinders for them to play or rest in (PVC pipe, large mailing tubes or dryer vent tubing) is encouraged. Tunnels can be set up like mazes. This promotes exercise and play and stimulates problem-solving behaviour.d  
  - Floor space can be increased by providing shelving and connecting ramps.  
  - Ferrets do not swing and climb in the same way as rodents, which use the whole surface of a cage as a ‘gym’.b Ferrets prefer to ‘rough and tumble’, grabbing each other by the scruff and wrestling or running and hiding.e Therefore, ferrets require relatively more floor space than rats or mice.d |
| **Olfactory stimulation** | - Ferrets have a well-developed sense of smell, and their olfactory preferences for food are set in the first 3 months of life.a  
  - Territory marking is important, and provision must be made for safe scent marking in the cage.c |
| **Provision for natural behaviours** | - Ferrets maintain instinctive behaviours for play, territory marking and hunting:i  
  - They use urine, stool and anal gland secretions to mark territory by backing up to a vertical surface or dragging their anus on the ground.a An intact male ferret may also rub his abdomen around the perimeter of the cage, leaving skin oil scent on objects. It is important that ferrets have caging that supports this behaviour.c  
  - Provide tunnels, such as cylinders, to allow for burrowing and hiding behaviour.  
  - Provide chow ad libitum to enable snacking—ferrets prefer to eat 8-10 small meals a day.c  
  - Provide an enclosed nest box to allow the opportunity to hide and for sleeping in—a dark box mimics a burrow.abc  
  - Ferrets need room to play-fight and wrestle, and for mutual grooming.abc  
  - Sleeping bags or ‘snooze tubes’ may also be provided for hiding and resting.c  
  - Ferrets are highly inquisitive animals, so toys are important to stimulate their curiosity.abcd |

---

Hillyer and Quesenberry (1997)  
Quesenberry and Carpenter (2004)  
Fox (1998)  
Einon (1995)  
Apflebach (1973)
### Environmental enrichment strategies for Rabbits

<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Rabbits</th>
</tr>
</thead>
</table>
| **Social**                      | Rabbits are a social species, and should be housed in pairs or small groups of compatible individuals wherever possible. Females kept in stable groups are generally compatible, but male rabbits often fight once they reach sexual maturity and must then be separated.  
  Individually housed animals should have visual and olfactory contact with other rabbits.  
  Provide raised areas that permit animals to lie in groups.  
  Rabbits may be housed in pairs with interconnecting cages.  
  |         |
| **Human-animal**                | Human caregivers may be a source of social enrichment.  
  Frequent removal from the cage or enclosure for handling and petting by animal carers is recommended.  
  |         |
| **Food**                        | Stalky hay can be provided on the cage top, in a hay rack or a puzzle bottle that the rabbit must manipulate to get the hay.  
  A variety of food supplements and treats, such as carrot, apple, greens and other vegetables, can be rotated in addition to a balanced formulated ration. Food treats can be scattered throughout the enclosure to promote foraging.  
  |         |
| **Physical environment**        | Plastic or cardboard boxes, sections of PVC pipe or ledges placed 20–30 cm above the floor provide a darkened retreat from others.  
  Raised areas for sitting and lying, such as ledges or overturned boxes, also increase use of the vertical space.  
  The height of the enclosure should permit animals to sit upright.  
  Sturdy, chew-resistant polypropylene plastic balls and dumbbells make good toys, as do suspended stainless steel chain and washer rattles.  
  |         |
| **Olfactory stimulation**       | Avoid the use of strongly scented chemicals and urine from other animals.  
  |         |
| **Provision for natural behaviors** | Non-toxic branches, sticks and cardboard boxes should be provided for gnawing.  
  Nesting boxes should be filled with hay, straw or shredded paper for scratching and burrowing.  
  |         |

---

*a NSW Agriculture Animal Research Review Panel (2003)*  
*b Boers et al (2002)*  
*c Morton et al (1993)*  
*d NHMRC (2004)*
### Type of environmental enrichment

<table>
<thead>
<tr>
<th>Social</th>
<th>Guinea pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Guinea pigs are a social species, and should be housed in pairs or small groups of compatible</td>
</tr>
<tr>
<td></td>
<td>individuals wherever possible. Females kept in stable groups are generally compatible, but</td>
</tr>
<tr>
<td></td>
<td>males often fight once they reach sexual maturity and must then be separated.a</td>
</tr>
<tr>
<td></td>
<td>- Where individual housing is unavoidable, animals should still be in visual, auditory</td>
</tr>
<tr>
<td></td>
<td>and olfactory contact.a</td>
</tr>
<tr>
<td>Human-animal</td>
<td>- Human caregivers may be a source of social enrichment.a</td>
</tr>
<tr>
<td>Food</td>
<td>- Stalky hay that encourages manipulation should be provided in or on the cage top.a</td>
</tr>
<tr>
<td></td>
<td>- Fresh, leafy greens can be given as a supplement to a balanced, formulated ration.a</td>
</tr>
<tr>
<td></td>
<td>- This species is reluctant to eat unfamiliar foods (neophobia), and rapid change of the diet</td>
</tr>
<tr>
<td></td>
<td>can cause digestive upsets. Therefore, introduce only a small amount of a novel food. Once</td>
</tr>
<tr>
<td></td>
<td>the animals are familiarised, variety can be achieved by rotating the food treats.</td>
</tr>
<tr>
<td></td>
<td>- Dried fruit scattered in the bedding encourages foraging.</td>
</tr>
<tr>
<td>Physical environment</td>
<td>Plastic or cardboard boxes and sections of PVC pipe provide a darkened retreat from</td>
</tr>
<tr>
<td></td>
<td>others.a</td>
</tr>
<tr>
<td></td>
<td>- Sturdy, chew-resistant polypropylene plastic balls and dumbbells make good toys.</td>
</tr>
<tr>
<td>Olfactory stimulation</td>
<td>Guinea pigs should be able to maintain olfactory contact with other familiar</td>
</tr>
<tr>
<td></td>
<td>animals.a</td>
</tr>
<tr>
<td>Provision for natural behaviours</td>
<td>Hay, straw or shredded paper should be provided for scratching and burrowing.a</td>
</tr>
</tbody>
</table>

a Reinhardt (2002a)  

b Raje and Stewart (2000)
### Part III  Environmental enrichment strategies

#### Type of environmental enrichment

<table>
<thead>
<tr>
<th>Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Social</strong></td>
</tr>
</tbody>
</table>
| · Pigs are a social species, and should be housed in pairs or small groups of compatible individuals wherever possible. Pigs kept in stable groups are generally compatible; however, avoid disrupting a stable group by adding unfamiliar animals.
| · Provide space and visual barriers to minimise aggressive behaviour.
| · In situations where individual housing is unavoidable, animals should still be in visual, auditory and olfactory contact.
| **Human–animal** |
| · Human caregivers may be a source of social enrichment.
| · Pigs are very sensitive to human behaviour, and positive interactions should include patting, firm stroking and quiet talking.
| **Food** |
| · Use palatable treats when training.
| **Physical environment** |
| · Provide deep litter, such as oat hulls and straw, rather than a concrete floor without bedding.
| · Sticks, stalky hay or straw facilitates manipulation and nest building.
| · Allow opportunities for exercise in addition to the holding pen. Use of a specific exercise pen, or use of aisles between pens, should be offered at least weekly.
| · Avoid close confinement, such as tethering or small pens. Any restraint or close confinement must be well justified.
| · Pigs should be acclimatised to restraint, and slings may be well tolerated.
| · ‘Talk-back radio’ as a background sound can be used to minimise startling.
| **Olfactory stimulation** |
| · Provide olfactory contact with other familiar animals.
| · Provide a clean, manure-free environment and enrichment items.
| **Provision for natural behaviours** |
| · Hay, straw, mushroom compost or shredded paper should be provided for rooting and burrowing.
| · Novelty ‘toys’ are most beneficial when they are new, so the types of items must be rotated frequently.
| · Provide sturdy, chew-resistant polypropylene plastic balls, suspended tyres, rubber hoses or soft cloth strips for chewing.

---

* Grandin (2002)
* Pearce GP and Paterson AM (1993)
<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Sheep</th>
</tr>
</thead>
</table>
| Social                           | Sheep are a social species, and should be housed in pairs or groups of compatible individuals wherever possible. Females kept in stable groups are generally compatible, but sexually mature males may fight and must then be separated. abc  
- Sheep establish well-defined social hierarchies with other group members, and follow leading animals when moving between locations. Changes to the group disrupt the hierarchy and may result in conflict until a new stable ranking is established. c  
- Where individual housing is unavoidable, animals should be in visual, auditory and olfactory contact with other members of the group. Use of mirrors can reduce isolation stress, but does not fully replace the need for social contact with another familiar sheep. abc |
| Human-animal                     | Familiar human caregivers may be a source of social enrichment. abc  
- Unfamiliar humans are viewed as predators, so contact of unfamiliar people with lambing ewes should be avoided. c  
- Frequent gentle handling and petting by familiar carers promotes acclimatisation to the housing environment. c  
- Rough handling by humans and the sight, smell or sound of dogs should be avoided. d |
| Food                             | This species is reluctant to eat unfamiliar foods (neophobia), and rapid change of the diet can cause digestive upsets. Therefore, introduce only a small amount of a novel food, and increase the proportion over a 2-week period to enable gradual adaptation of the gut micro-organisms without ill effect. a  
- Stalky hay, provided in the enclosure or in a hay rack, facilitates manipulation and normal gut function. a  
- Food rewards such as barley or oats can be of benefit following handling procedures and restraint. c  
- Access to pasture for periods of grazing and exercise should be provided as a supplement to a balanced, formulated ration for intensively housed laboratory sheep. |
| Physical environment             | This species is fearful of unfamiliar surroundings (neophobia) and requires time to adapt to new housing, personnel or laboratory conditions. b  
- Provision of trees, rubbing posts, logs, earthen mounds or plastic barrels increases the display of natural behaviours. b |
| Olfactory stimulation            | Olfactory contact with other familiar sheep should be provided. abc |
| Provision for natural behaviours | Hay and straw should be provided for stimulation of normal chewing and rumination behaviours and correct functioning of the gut. a  
- Animals should have sufficient space, or access to sufficient space, for regular exercise and to permit play behaviours of young animals. |

---

*a Monash University (2005)  
b Hinch and Lynch (1997)  
c Reinhardt and Reinhardt (2002)  
d NHMRC (2004)*
<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Dogs</th>
</tr>
</thead>
</table>
| Social                           | - Dogs are a social species, and should be housed in pairs or small groups of compatible individuals wherever possible.\(^a\)  
- They may be housed in pairs with interconnecting cages.\(^a\) |
| Human-animal                     | - Human caregivers may be a source of social enrichment.\(^a\)  
- Paid or volunteer dog walkers may be used.\(^b\)  
- Frequent removal from the cage or enclosure for handling and petting by animal carers is recommended.\(^a\)  
- Regular contact with a member of the research team is recommended.  
  If this person becomes a familiar, friendly source of contact, the dog will be more confident when handled. The need for contact is especially important for young dogs and for dogs entering the colony for the first time, irrespective of age.\(^c\) |
| Food                             | - Stimulate behaviour by providing some of the following:  
  - rawhide chews, pigs’ ears, dog biscuits\(^b\)  
  - chew bones such as Nylabones, Gumabones\(^b\) or uncooked ox thigh bones  
  - ‘pupsicles’ made of beef stock frozen in dog bowls\(^b\)  
  - food puzzle toys, such as ‘Kong’ (chew-resistant hollow rubber) objects filled with canned dog food or water and then frozen\(^b\)  
  - ball feeders that can be manipulated by the dog to access the dry food contained inside.\(^a\) |
| Physical environment             | - The enclosure should be deep enough to enable retreat.\(^a\)  
- Raised areas for sitting and lying, such as sleeping boards, platforms and hammocks, increase use of the vertical space.\(^a\)  
- The environment can be improved by the addition of:  
  - hammocks\(^b\)  
  - jump boards, ramps\(^c\)  
  - rubber mats, carpets, sheep-hide fleeces.\(^b\)\(^c\)  
- Opportunities for a daily walk should be provided.\(^c\) |
| Olfactory stimulation            | - Olfactory stimulation is very important to this species.\(^a\)  
- Meat-flavoured biscuits and dry foods increase the palatability of a balanced formulated ration or treat, and provide novelty. |
| Provision for natural behaviours  | - Natural behaviours can be facilitated by providing:  
  - rope for tugging, tied to the enclosure wall or door\(^b\)  
  - cardboard boxes for shaking, carrying and chewing\(^c\)  
  - balls, such as tennis balls, basketballs or rubber balls.\(^b\)  
- Chase/fetch/jump toys include:  
  - frisbees  
  - suspended bones  
  - tyre swing.\(^c\) |

\(^{a}\) Hubrecht (2002)  
\(^{b}\) NSW Agriculture Animal Research Review Panel (1999)  
\(^{c}\) NHMRC (1997)  
\(^{d}\) Eisele (2001)  
\(^{e}\) NHMRC (2004)
Type of environmental enrichment | Non-human primates
---|---
Social | While primates in general are social, there is considerable variation in social requirements between species. This variation must be taken into account when planning or assessing management strategies and housing conditions. Non-human primates should be housed in pairs or small groups of compatible individuals wherever possible.

Human-animal | Human caregivers may be a source of social enrichment. Frequent and positive contact with familiar humans facilitates the development of a trustful relationship. Frequent removal from the cage or enclosure for handling by familiar animal carers is recommended in the case of amenable species, such as marmosets. Train macaques to cooperate during procedures by using food rewards.

Food | In the wild, foraging may take up to 70% of the waking hours of non-human primates, so measures should be taken to increase the time the animals spend foraging. Foraging activity can reinforce social behaviour and reduce boredom and is encouraged by, for example, feeding several times a day using forage trays and baskets rather than bowls. Alternatively, biscuit, nut, seed and fruit rations can be placed on the cage mesh roof in a foraging box. Provide high-fibre food items, such as non-toxic tree branches and browse foliage. Willow, banana palms and Ficus spp are recommended, whereas Australian native species with a high content of volatile oils (such as Eucalyptus, Melaleuca and Leptospermum spp) should be avoided. During hot weather, provide 'popsicles' made of fruit and vegetable pieces frozen in juice or water on tongue-depressor sticks, or frozen in food bowls. Foraging may be encouraged by placing whole fruits on the cage mesh roof, and by using a leaf litter substrate to hunt through for seeds or biscuits etc. Non-human primates also enjoy food puzzle toys, such as:

- ‘Kong’ (chew-resistant hollow rubber) objects filled with food, such as food pellets or fruit pieces
- Ball feeders that can be manipulated by the animal to access the dry food contained inside
- Hollow plastic or bamboo-tube forage tubes containing mealworms, dried fruit etc, which the animal must work to find and extract through openings.

Physical environment | The enclosure should be deep or high enough to enable retreat from more dominant members of the group, or from unfamiliar humans. Requirements for vertical and horizontal space differ between species. For example, the provision of vertical space is more important for marmosets and macaques than for baboons. Full use of vertical space can be made by providing perches, climbing frames, nest boxes and vantage points. Raised areas for sitting and lying, such as sleeping boards, platforms and hammocks, also increase use of the vertical space. Other items that can improve the environment include:

- Ropes, non-toxic tree branches or variable-diameter wooden dowel
- Jump boards, ramps
- Non-toxic cardboard boxes for sitting in or on or for tossing (macaques, baboons).

Outside enclosures are important to increase the variety of stimuli. Access to an outside enclosure is recommended for all animals. Provide areas for privacy or retreat by including opaque barriers and nesting boxes within the enclosure.
### Type of environmental enrichment

<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Non-human primates</th>
</tr>
</thead>
</table>
| Olfactory stimulation           | - Use non-toxic herbs and scents on novel objects (eg feathers) for marmosets.  
|                                  | - Use fruit scents and flavours to increase the palatability of a pelleted ration or food treat.  
| Provision for natural behaviours | - Provide for natural behaviours by providing:  
|                                  |   - rope for tugging, tied to the enclosure wall or door*  
|                                  |   - cardboard boxes for shaking, carrying and chewing†  
|                                  |   - balls, stainless steel mirrors, plastic containers, tyres, PVC pipes, milk containers and water tanks‡  
|                                  |   - a water spray or dripping shower (macaques).  
|                                  | - Provide chase/jump toys, such as:  
|                                  |   - suspended branches, ropes, chains  
|                                  |   - tyre swing.  

* NHMRC (2003)  
† NSW Agriculture Animal Research Review Panel (1999)  
‡ Reinhardt (2002b)  
§ Hubrecht (2002)  
¶ NHMRC (2004)

### Type of environmental enrichment

<table>
<thead>
<tr>
<th>Type of environmental enrichment</th>
<th>Fish</th>
</tr>
</thead>
</table>
| Social                          | - Social behaviour and social influences on behaviour can be quite complex, and require investigators and animal care staff to have a good understanding of the species-specific requirements of the animals. For example, many species of flatfish and eel do best in long-term holding when provided with burrow-type environments. In aggressive species, the provision of vertical barriers or shelters may eliminate or curb aggressive behaviour.  
|                                 | - Consideration should be given to population densities, water flow rates or other physical features that may have an effect on social interactions. However, adding complexity to the aquatic environment should be balanced against the need to maintain a high standard of water.  
| Food                            | - Appropriate diet and method of feeding should be tailored to the species used; for example, bottom feeders fed with sinking feed pellets, invertebrates as live prey (eg rotifer and brine shrimp).  
| Physical environment            | - Where the environmental requirements of fish are not well known, as far as possible the holding conditions should be designed to approximate the source environment.  
|                                  | - Examples of methods include: mixed river gravel on the bottom with a mixture of numerous objects such as driftwood, plastic and real plants, plastic tubing and rocks scattered randomly through the tank plus a large power unit to provide a current plus a number of air bubbles.§  
|                                  | - Water quality (eg salinity, alkalinity, hardness) should be tailored to the specific species.  
| Provision for natural behaviours | See above.  

§ Hubrecht (2002)

**Conclusion**

To ensure that animal wellbeing is maintained during the scientific use of animals, the Australian Code of Practice expects investigators to meet animals' behavioural as well as biological needs by providing a safe and enriched environment.
REFERENCES


Part III  Environmental enrichment strategies


http://www.awionline.org/pubs/cq02/cqindex.html


http://www.awionline.org/pubs/cq02/cqindex.html


FOETAL AND EMBRYONIC STUDIES

WHAT ARE FOETAL AND EMBRYONIC STUDIES?

Foetal and embryonic studies are widely used to study foetal and neonatal physiology and pathophysiology, and to validate techniques to correct foetal abnormalities in humans.

See Section 3.3.74–77 of the Australian code of practice for the care and use of animals for scientific purposes (the Code) for more information about foetal and embryonic studies.

The knowledge from foetal and embryonic research is used to improve the survival, health and wellbeing of newborn animals. These animal models are also used to develop knowledge that, by ‘careful extrapolation’, can be applied to other neonatal animals, including humans (Mellor and Gregory 2003).

Studies in animals can also assist in understanding human brain and central nervous system development, and the effects of various genes and environmental toxicants. This research has already shed light on human conditions, including autism, learning disabilities and foetal alcohol syndrome (NRC 2003).

HOW ARE FOETAL AND EMBRYONIC STUDIES DONE?

Embryo studies involve harvesting embryos from a pregnant mother, or developing embryos using in vitro fertilisation techniques.

Foetal surgery is an intervention in which the integrity of the foetus is interrupted. This can involve surgery to allow access to the foetus or the premature delivery of the foetus by caesarean section to facilitate the production of specific pathogen-free progeny.

Access to the foetus can be achieved directly via an abdominal incision in the mother, with exposure of part or all of the foetus through incisions through the uterine wall (Mellor and Gregory 2003). Access may also be gained indirectly by using remote sensing techniques, such as ultrasound or radiological procedures, and by using laparoscopic techniques.

Studies can involve taking samples from the foetus, the foetal sacs, the placenta or the uterus, or applying devices or introducing catheters or instrumentation to the foetus or the placenta.

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

Regardless of the circumstances, the wellbeing of both the foetus and the mother must be considered when unborn animals are subject to surgery or other interventions. Laparotomy with uterine surgery causes significant pain in the mother (Mellor 2004), and can expose the foetus to potentially noxious stimulation. This might be interpreted by some people as potentially painful for the unborn animal.

Where a foetus has been instrumented (eg with catheters or electrodes), subsequent delivery of the foetus, either at full term or following premature labour as a postoperative complication of the surgery, may be impeded because of the presence of the instrumentation. Housing of the pregnant mother in a constrained environment because of the instrumentation of the mother and/or foetus (eg metabolism cage) may also be associated with significant problems if delivery is permitted to proceed while the mother is restrained.
Maternal pain or distress

Laparoscopic techniques
There is some debate about the level of pain that might accompany and follow such procedures. Human patients report a full range of sensations from mild discomfort (mainly due to the inflation of the abdomen) through to severe pain that might continue for several days. In all cases, humans are given at least a sedative analgesic during the procedure. On this basis, it is appropriate to assume (as noted in Section 3.3.4 of the Code) that such procedures will cause pain in animals. Any animals undergoing laparoscopy should receive appropriate medication to achieve good restraint during the procedure and to provide adequate pain management.

Surgery/laparotomy
It is well recognised that surgery and laparotomy cause pain to the mother. If not controlled or managed, pain of such intensity or duration would cause pain and distress to the mother. Some aspects of the management of such pain are discussed below (see ‘How are pain and distress minimised?’).

Foetal pain and distress
Any surgical intervention to the foetus for physiological or pathophysiological studies will expose the foetus to potentially noxious stimulation. Apart from any other considerations, any physical activity of a foetus in response to such manipulation can be disconcerting for those observing it (Mellor and Gregory 2003). However, Mellor et al (2005) argue that the physiological processing of a nociceptive stimulus resulting in such activity and the perception of that stimulus as painful are not the same.

Mellor and Diesch (2006) claim that an animal must be both sentient and conscious for pain and distress to occur. First, the required neural apparatus for sentience must be in place and operational; stimuli must be able to elicit impulse transmission along nerves from sensory receptors to the animal’s brain, and its brain structures must be operationally sophisticated enough to transduce those impulses into perceived sensations. Second, the animal must be conscious to perceive sensations, as unconsciousness nullifies perception. Third, for the conscious animal to suffer, and for its wellbeing to be compromised, the character, intensity and/or duration of the sensations must result in significantly noxious or aversive experiences.

Mellor and Diesch further claim that capacity for sentience in the foetuses of farm animals develops only in the second half of pregnancy. Even then, various neuroinhibitory mechanisms operate in utero to keep the foetus asleep.

There is evidence of long-term effects from the physiological responses to painful stimuli experienced by the foetus. This is the basis for the argument to provide adequate pain relief to the foetus (van de Velde et al 2006).

In regard to euthanasia, the European Commission (reported by Close et al 1997) considers that bird embryos halfway through gestation (when the neural tube has developed into a functional brain) are capable of perceiving pain, and therefore must be destroyed humanely. Mellor and Gregory (2003) report that, when a general anaesthetic is administered to ewes, with enough time allowed for the anaesthetic to act on the foetal lamb, invasive procedures do not seem to cause foetal movements (see also NRC 2003). However, in ewes in which adequate analgesia is achieved through epidural anaesthesia, foetal lambs will respond to stimuli with strong reactions. Such strong behavioural responses have also been noted in rat pups (Mellor and Gregory 2003, NRC 2003).

Whether or not the foetus can experience pain during a surgical procedure, it can still mount a stress response to nociceptive stimuli (Derbyshire 2003) with potentially detrimental consequences. Consequently, effective pain management should be taken into account in those circumstances where the foetus is exposed to noxious stimuli.
HOW ARE PAIN AND DISTRESS MEASURED?

Unless implanted devices can be used to directly measure the wellbeing of the foetus, any signs of pain in the mother should be considered as an important sign of potential pain and distress in the foetus. For information on how pain and distress are measured during foetal and embryonic studies, see the ‘Surgical procedures’ factsheet.

HOW ARE PAIN AND DISTRESS MINIMISED?

There is some discussion in the literature about the pros and cons of analgesic use in foetal surgery (Derbyshire 2003, Myers et al 2003, NRC 2003, Mellor et al 2005, Webster et al 2005). Most of these references discuss the potential problems that might be caused because of differing responses and metabolic processes of foetuses compared with those of older animals. Most of the literature available for examination does not mention the use of analgesic medications for either the mother or the foetus following surgery. The most common description was of induction of anaesthesia for the mother using pentobarbitone, followed by maintenance using halothane with or without nitrous oxide or isoflurane. See Section 3.3.30 of the Code for guidance on the use of analgesics and tranquillising agents.


Effective management of hypothermia also is important in reducing foetal stress. The foetus is more susceptible to hypothermia during surgery and there is need to guard against it. When amniotic fluids are drained, there is a need to replace them with warm fluids.

Where an animal has been instrumented (mother and/or foetus), careful and specific monitoring must be conducted to ensure that premature or natural delivery of the foetus does not proceed unless it occurs in the presence of the investigators. This may include monitoring of intra-uterine pressure from a saline-filled intra-uterine or intra-amniotic catheter, or monitoring of the electromyographic activity from electrodes sewn into the uterine muscle. Strategies must be in place to manage any difficulties that may occur during delivery.

PUBLISHED GUIDELINES AND REFERENCES


G FOOD AND WATER INTAKE MODIFICATION

WHAT IS FOOD AND WATER INTAKE MODIFICATION?

Laboratory animals should be given a nutritionally balanced diet and clean, fresh drinking water (NRC 1995). Often, the animal is supplied with more food and water than is required, allowing it to choose how much or how little it eats or drinks each day (‘ad libitum’ or ‘free food access’ feeding). However, in some research projects, the nutritional value of food is modified, or the quantity, taste or period of access to food or water is changed. Such experimental conditions must be scientifically justified and approved by the animal ethics committee (AEC), as there is potential for adverse effects on animal health and wellbeing.

WHEN ARE FOOD AND WATER INTAKE MODIFIED?

Access to, and the composition of, food and water may be modified for a number of research reasons, and within a number of specialised disciplines. The following is an overview of food and water modification as it is beyond the scope of this document to delve further. See Sections 3.3.11, 3.3.44, 3.3.73 and 5.2.3(iv) of the Australian code of practice for the care and use of animals for scientific purposes (the Code) for more information.

The list that follows gives some examples of the types of research that may involve modification of food and water intake:

- Nutritional research—altering the digestibility and nutrition of the diet to observe the effects on animal growth and productivity.
- Palatability studies—studying preferred and disliked tastes to develop rations that animals will eat more readily (eg for animal production systems), and those that they will not eat (eg for pest animal management systems).
- Research on human diseases where there is a suspected dietary contribution—duplicating the disease effects by manipulating the diet. In these situations, wellbeing concerns are also associated with the disease state being modelled (see Section 4.2, ‘Animal models’).
- Physiological research—studying the regulation of energy (including food intake) and fluid balance, including research on hunger and thirst, obesity, longevity and metabolism.
- Pharmacological research—administering drugs before, after or with food to see how food can affect drug absorption and metabolism.
- Other research—partly or completely restricting food and water intake before anaesthesia, gastrointestinal surgery or transport to reduce the amount of material in the gut and reduce the risk of vomiting or regurgitation.
- Food and water used as rewards to influence behaviour and induce animals to perform tasks.

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

Investigators can restrict food and water intake by limiting the daily quantity available to the animal, limiting the daily period of access of the animal to the food or water, or altering the taste of the food or water.

General issues relating to food and water intake modification for laboratory animals include the following:

- Type and quality of the diet—Use of internationally recognised, balanced diets will reduce variability within and between studies, as well as improve the wellbeing of the animals used.
Form of the diet—Diets can be fed in powdered, liquid or pellet form. Care must be taken with rodents to monitor and clip teeth when using powdered or liquid diets, to avoid overgrowth of incisors. If liquid diets or supplements are provided, care must be taken to ensure the animal does not become dehydrated if the liquid is unpalatable. Water should be available for part of the day to allow animals to rehydrate if needed.

Quality of the diet—The accreditation of the ingredients used and the safety of the diet should be checked.

Important issues to consider when using strategies for restricting food and water intake are described in the following sections. In all cases where the research is likely to impact on an animal’s food or water intake, substantial scientific justification must be provided to the AEC when seeking approval.

**Food restriction**

Food is a source of energy and nutrients, and is comprised of protein, fats, carbohydrates, fibre, vitamins and minerals, and variable amounts of water. Animals eat to meet their requirements for energy and nutrients; however, some species or individuals will eat in excess of their needs if given access to unlimited amounts of food. The surplus intake may be stored in the body (eg fat), or will be removed or excreted from the body (eg surplus protein and minerals).

Unrestricted access to food permits maximal growth and reproduction, but decreases lifespan, increases the incidence of degenerative diseases and neoplasia (cancer), and sometimes increases the risk of obesity (NRC 1995, 1996). Controlling and reducing the food available to an animal is not necessarily harmful if it mimics the variable food availability in the wild. For example, foraging strategies and ‘puzzle feeders’ are used to reduce boredom in non-human primates, zoo animals, dogs and cats (NIMH 2002). Voluntary food intake is influenced by metabolic and energy requirements (growth, exercise, maintenance of normal body temperature) and non-metabolic factors, including the palatability of the food and the amount of work required to obtain it (Toth and Gardiner 2000).

Reducing food intake in the long term (to approximately 80–85% of the amount consumed with free food access) can increase lifespan and reduce the incidence of obesity and of some diseases. However, chronic energy (caloric) restriction alone is stressful for the animal, and will reduce the growth rate of juveniles and reduce bodyweight in adults. Problems may also arise if the food ration is nutritionally incomplete or unbalanced, or if substantial food restriction occurs in growing juvenile animals, whose growth may be permanently stunted if they are chronically underfed or malnourished (NRC 1995, Toth and Gardiner 2000, NIMH 2002). Food restriction, together with other stressors, is associated with gastric ulceration in rats and with death in mice (Toth and Gardiner 2000).

The use of food or fluid rewards (positive reinforcement) is the preferred method for motivating an animal to modify its behaviour or perform specific tasks (NHMRC 2004). Where food restriction is used to motivate animals to perform tasks in psychological studies, the challenge is to balance the severity of the restriction with the need to motivate learning or task performance. Usually, a proportion of the animal’s daily food requirements (eg 10%) is used as a reward (positive reinforcement) during training, and the balance is given at the end of the training session. The animal needs to be trained to perform the task, and also to be trained (gradually) that food or water will become scarce or less frequently available before a training session. This is particularly important in the case of herbivore or ruminant animal subjects, in which rapid changes to the schedule of food or water availability may have a profound impact on gut function and animal health.

Sometimes, food needs to be quite restricted, especially when an animal is learning a difficult task. However, the restriction can be reduced once the task has been mastered (Toth and Gardiner 2000). Section 3.3.44 of the Code states that severe deprivation of food or water must not be used. Therefore, the level of food restriction must be scientifically justified and approved by the AEC.
Complete food deprivation

Some studies use a period of no food intake ('fasting') before anaesthesia or transportation, or at the beginning of food restriction training for animals that have previously had free food access. Short periods (e.g., 24 hours) of no food have no adverse effects in normal, healthy, adult animals (NIH 2005). However, after 48 hours of no food, most animals show signs of physiological and psychological stress (Toth and Gardiner 2000).

The duration of fasting needs to be determined in the specific context of the species and the physiological and health status of the animals involved. For example, sick animals, pregnant ruminants, small metabolically active species such as birds and rodents, and animals that are very young or very old will show signs of physiological stress after only a few hours without food. In contrast, a non-pregnant adult sheep or dog may show no adverse effects after 24 hours without food. Therefore, any proposed period of food deprivation should be carefully assessed for its impact on the animal on a case-by-case basis, and periods longer than 24 hours are not recommended.

Animals undergoing complete food deprivation must be closely monitored. Repeated periods of fasting on the same animal should be restricted to enable full recovery.

Investigators should also restrict the repetition of starvation procedures on the same animal over given periods of time (to allow the animal to fully recover). The regulation and control of such studies should be enforced by regular sample audits of investigators carrying out such procedures (see Sections 3.3.44, 3.3.73 and 3.3.11 of the Code).

Fluid restriction

Animals with unlimited fluid access usually drink more than they need to maintain normal hydration. Fluid intake is influenced by thirst, dryness of the mouth, concurrent feeding, palatability and ease of availability of the fluid, learning and habit (Toth and Gardiner 2000). When water, juices or other fluids are used as a reward, access to water needs to be restricted outside the experimental session. Some studies require the animal to earn its daily fluid requirement during the experimental session (NIMH 2002).

Fluid restriction carries a risk of dehydration and reduced food intake. Acute fluid deficiency may result in rapid loss of more than 15% bodyweight, thirst, dryness of mucous membranes, reduced urine output, reduced food consumption, loss of skin elasticity ('tenting' in response to skin pinch test), lethargy, and shock and cardiovascular collapse (NIH 2005). Dehydration is a common complication that may not be recognised easily at an early stage without close and careful monitoring.

Chronic fluid deficiency results in reduced food intake and loss of bodyweight (Toth and Gardiner 2000, NIH 2005). In young, growing animals, reduced growth rate may be observed rather than loss of bodyweight. Reduced growth rate can be determined by comparison with control animals that have free food access. Loss of bodyweight is a particular concern when it occurs rapidly (over hours or days) or when there is loss of skeletal muscle mass.

Complete fluid deprivation

Some studies use a 24-hour period with no fluid intake before anaesthesia or transportation, or at the beginning of fluid restriction training for animals that have previously had free fluid access. Short periods (up to 24 hours) without fluid have no adverse effects in normal, healthy, adult animals (NIH 2005). However, fluid must not be restricted for longer than 24 hours, and animals must be monitored closely for signs of dehydration. If signs of dehydration are observed, they must be corrected immediately and a free supply of fluid provided.

As with food deprivation, the duration of fluid deprivation needs to be determined in the specific context of the species and the physiological and health status of the animals involved.
Changing the composition of the diet

Diet affects the health of animals in many ways, particularly in ruminant and herbivore species, as the diet has a major influence on the microbial flora of the gut and general gut function. These species will be adversely affected by sudden or extreme changes to the composition of the diet. (For further general information, see Adams and McKinley 1995, Harkness and Wagner 1995, NRC 1995.)

Measuring food and water intake

Accurate measurement of food and water intake often requires animals to be caged or penned individually (e.g., in metabolic cages). In some cases, urine and faeces are collected as part of the assessment of the quantity of food consumed and for chemical analysis. To minimise social isolation when housing animals individually, animals should be able to see, hear and smell others of the same group or species.

Wellbeing issues relating to confinement are raised when the caging restricts the animal’s movement, or where the animal may feel exposed or vulnerable to attack and does not have the option of nesting, hiding or withdrawing. These wellbeing issues may be alleviated by selecting cages of an appropriate size for the animal, providing shaded retreat areas in the cage, or by adjusting room lighting levels. The period of time that animals are closely confined must be well justified, and minimised.

In addition, rodents are coprophagic (i.e., eat their faeces). To reduce coprophagia, rodents are often housed in cages with wire-grid floors where some of the faeces fall through the mesh and become inaccessible to the animal. While wire-based cages will reduce the problem, coprophagic behaviour will still complicate research outcomes of nutritional studies. Also, there are wellbeing issues associated with use of wire-floor cage, such as the increased potential for injury to feet and limbs, and the development of pressure sores over time. Therefore, eliminating coprophagic behavior is not a justification for housing rodents in wire-floored cages.

Behaviour modification

For more information on this topic, see the ‘Behaviour modification’ factsheet.

Food or fluid can be used as a reward, even in well-fed animals (ASAB 2003). Often, however, animals must be hungry or thirsty to work or perform tasks for a food or fluid reward; palatable treats may not be enough incentive if the animal perceives the task to be ‘difficult’ (Toth and Gardiner 2000, NIMH 2002). Two strategies can be used to make the animal hungry or thirsty:

- **Scheduled access**—the animal is given access to food or water for a limited period so that it can consume a daily allowance that maintains normal health and weight. Behavioural testing using a food or fluid reward is conducted at a set time of the day immediately before the scheduled access. It is critical that the animal learns to modify its patterns of food and fluid intake to cope with the reduced period of access, which may be only one or two hours.

- **Restricted access**—the total food or fluid is controlled so that the animal loses weight or gains less weight than littermates (age-matched controls) that have unlimited access to food and water. Typically, the food intake of an animal is measured to establish a baseline, and then a reduced amount of food is provided. The animal loses bodyweight initially, then stabilises at approximately 80–85% of the original baseline bodyweight (NIMH 2002).

These strategies raise concerns as to whether the reduction of food or water intake (‘restriction’) is necessary and can be justified. The manner of food restriction and the animal’s target weight must be carefully considered for the species in question. The age of the animal and the duration or quantity of feeding permitted determine whether the ‘80% rule’ is reasonable for each species.
For example, guinea pigs are less adaptable than rats to scheduled access feeding protocols (Toth and Gardiner 2000). Juveniles of species such as mice, guinea pigs and birds should be allowed to grow until their weight stabilises, after which free food intake may be restricted to 80%. There are some exceptions to this rule, so it is important to research the species thoroughly first. For example, some rat strains grow continuously throughout life, with little or no ‘plateau’, until the animal has become obese (NIMH 2002).

In all cases where the investigator is likely to impact on an animal’s food or water intake, substantial scientific justification must be provided to the AEC when seeking approval.

**HOW ARE PAIN AND DISTRESS MEASURED?**

Monitoring strategies for pain and distress that may develop in an animal when its food and/or water intake are modified should be related to the nature of the modification and the expected effects of that modification on the animal. Some monitoring strategies for common modifications are listed below.

- Monitoring for dehydration: Acute fluid deficiency may result in rapid loss of more than 15% bodyweight, thirst, dryness of mucous membranes, reduced urine output, reduced food consumption, loss of skin elasticity (‘tenting’ in response to skin pinch test), sunken eyes, tremor, lethargy and shock/cardiovascular collapse (NIH 2005). (See ‘Fluid restriction’, above.)

- Monitoring for failure to grow and loss of bodyweight: Chronic (long-term) food or fluid deficiency results in reduced food intake and loss of bodyweight by more than 15% (Toth and Gardiner 2000, NIH 2005). (See ‘Fluid restriction’, above.) In young, growing animals, reduced growth rate may be observed instead of loss of bodyweight. This can be determined by comparison with control animals that have free food access. Loss of bodyweight is particularly a concern when it occurs rapidly (over hours or days) or when there is loss of skeletal muscle mass.

- Monitoring for diarrhoea and bloat: Sudden increases in food intake or changes in diet composition may cause adverse effects, such as diarrhoea (loose faeces), maldigestion or malabsorption of food, bloating (distension of the gut with gas and fluid), dehydration, and shock and cardiovascular collapse.

- Monitoring for general signs of physiological and psychological distress: These include unkempt appearance; abnormal posture; tear production, including red tears or porphyria; teeth grinding; increased or decreased movement; self isolation; aggression; and rapid, open-mouthed or exaggerated breathing.

**HOW ARE PAIN AND DISTRESS MINIMISED?**

General procedures and considerations

Table G1 lists general procedures and points to consider when restricting the diet of animals used in scientific research (NRC 1996, NIMH 2002).
### General procedures

- Restriction must be scientifically justified.
- Motivate behaviour using palatable food rewards (positive reinforcement), such as sweet foods, sultanas and nuts, instead of restriction. (Where sugared food/fluid rewards are used, monitor teeth for caries.)
- Use the least restriction that achieves the scientific objectives. Physiologically, animals tolerate restriction of food better than restriction of water.
- Animals must be monitored, including observation of their behaviour and physiological criteria such as state of hydration and bodyweight.
- In the long term, at least minimal quantities of food and water should be available to maintain the wellbeing of animals. Unless specific protocols require exemptions, laboratory animal species should be allowed at least one nutritionally balanced feed each day. For balanced animal diets, follow the recommendations of the Nutrient Requirements of Domestic Animals series (NRC 1995).
- Record fluid and food intake daily and bodyweight at least weekly.

### Restricted fluid

- Take precautions to avoid acute or chronic dehydration. Intervals of fluid access and total amounts of fluid obtained must be appropriate to the species and the physiological state of the particular animal.
- Fluid control may result in reduced food intake and weight loss over time. Therefore, food should be given immediately after access to fluid in order to maximise food intake. If weight declines, there must be a plan of response to correct this.
- Monitoring daily food intake is a quick way of determining whether there is adequate fluid intake.
- On days with no fluid restriction, animals should receive a period of free access to water.

### Humane endpoints

- Experimental endpoints must include temporary or permanent removal from the research protocol.
- Animals that fail to adapt to the experimental conditions should be excluded.
- Animals that lose more than 10% bodyweight (acutely) or 15% bodyweight (longer term) on restricted food or fluid access protocols should be removed from the research protocol. If an investigator requires more severe weight loss in an animal subject, special justification should be submitted to the AEC. The endpoint criteria for each specific case must be scientifically justified and approved by the AEC.
- Animals showing signs of dehydration, which include thirst, dryness of mucous membranes, reduced urine output, reduced food consumption, loss of skin elasticity ('tenting' in response to skin pinch test), lethargy, or shock/cardiovascular collapse, are to be removed from the research protocol.

**AEC** = animal ethics committee
REFERENCES


http://www.nimh.nih.gov/researchfunding/animals.cfm


http://www.nap.edu/readingroom/books/labrats/

HUMANE KILLING AND EUTHANASIA

WHAT ARE HUMANE KILLING AND EUTHANASIA?

The Australian code of practice for the care and use of animals for scientific purposes (the Code) (NHMRC 2004) defines 'humane killing' as 'the process of killing an animal with minimal pain or distress' and 'euthanasia' as 'the humane killing of an animal in the interests of its own welfare, to alleviate pain and distress'.

Irrespective of the method used, there is a universal principle that pain or distress must be avoided and that the animal experience a rapid loss of consciousness until death occurs (see Sections 3.3.18 and 3.3.23 of the Code; European Commission 1996, 1997; AVMA 2001; Demers et al 2006).

WHEN ARE HUMANE KILLING AND EUTHANASIA USED?

The key difference between humane killing and euthanasia is the reason that the animal is being killed.

Humane killing is used:
- at the end of studies to provide tissues for scientific purposes
- when animals are no longer used for breeding
- when stock are not required (eg unsuitable for particular research purpose).

Euthanasia refers to circumstances where:
- pain, distress or suffering are likely to exceed designated levels and cannot be alleviated promptly (see Section 1.21 of the Code)
- the health or wellbeing of the animal is grounds for concern.

In most circumstances, the same method will be used to kill the animal, whichever the reason. The exception is where animals are killed as part of the experimental protocol; the chosen method should then be compatible with the scientific aims.

SCIENTIFIC CONSIDERATIONS

As a general principle, animals are killed by the most humane method for their species. The method of euthanasia can directly or indirectly affect research results (ANZCCART 2001, ACLAM 2006). Therefore, an alternative method may be used when the animal ethics committee (AEC) agrees that the usual method for the species would affect the validity of the data and compromise the research. In such a case, all necessary precautions must be taken to minimise any impact on the wellbeing of the animal. If it is thought that a recommended method may influence data, but there is no supportive evidence, the need to use an alternative method should be validated in a pilot study.

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

To maximise animal wellbeing, the euthanasia method used must:
- avoid distress and produce rapid loss of consciousness until death occurs
- be reliable, reproducible and irreversible
- be appropriate for the age, species and health of the animal
- require minimum restraint of the animal.
Euthanasia must also:

- be compatible with the objectives of the study
- be simple to administer
- be safe for the operator
- if possible, be aesthetically acceptable to the operator.

**HOW ARE PAIN, DISTRESS, FEAR AND ANXIETY MEASURED?**

An animal’s capacity for consciousness and the effect of the euthanasia process on that capacity are the central issues in assessing the effects of euthanasia methods.

To monitor an animal’s pain, distress, fear and anxiety during the euthanasia process, the investigator must be familiar with the normal behaviour of the species. Depending on the species, behaviours associated with pain, distress, fear and anxiety include aggressive or abnormal behaviour, abnormal stance or movement, abnormal sounds, altered cardiovascular or respiratory function, vomiting, and abnormal defecation and urination.

**HOW ARE PAIN, DISTRESS, FEAR AND ANXIETY REduced?**

The desirable humane endpoint for euthanasia is a painless death. To determine whether a method of euthanasia is humane, the investigator should know how quickly unconsciousness is produced, whether it is sustained until death, and whether it occurs before effects such as asphyxia are present (ANZCCART 2001).

It is therefore essential that investigators can recognise and confirm death in the species they are working with. All the following signs should be used in combination to ensure this (ANZCCART 2001):

- absence of respiratory movement
- absence of heartbeat
- absence of pulse
- loss of colour in mucous membranes
- dilation of pupils and loss of corneal reflexes
- glazing of eyes.

Sometimes, two methods may be used for euthanasia—one resulting in loss of consciousness and the second to ensure subsequent death. If there is any doubt that the animal is dead, a second method should be used.

When using a physical method of euthanasia, the procedure must be appropriate to the species, and the application of the method must be precise. The experience and expertise of the operator and the efficiency of devices used are also critical to the humaneness of the procedure. Personnel must have training and be skilled in handling the animals and in applying the euthanasia method in a way that minimises stress in the animals.

For foetal or newborn animals, two factors must be taken into account when choosing a euthanasia method: such animals are resistant to hypoxia, and they metabolise drugs more slowly. Therefore, two methods acceptable for the species should be combined, and death should be confirmed using the signs described above.

The Code requires that animals should be killed in a quiet, clean environment that is away from other animals where possible (see Section 3.3.20 of the Code).
WHAT METHODS SHOULD BE USED?

Detailed methods of euthanasia for each animal species have been described (European Commission 1996, 1997; ANZCCART 2001; AVMA 2001).

Tables H1–7 summarise appropriate methods of euthanasia according to species and are based on the ANZCCART (2001) document. Methods are listed as ‘recommended’, ‘acceptable with reservations’, or ‘not acceptable’.

When a method is recommended, it is one of the preferred methods. It is humane, is relatively simple to perform and produces rapid unconsciousness.

A method that is acceptable with reservations is one where the method fails to meet all of the above criteria, because:

- the method does not produce unconsciousness as quickly as the recommended methods
- the application of the method requires particular skills and training
- the method is aesthetically unpleasant
- there are occupational health and safety considerations associated with the method.

Methods that are acceptable but with reservations would be acceptable if, in the opinion of the AEC, they were justified by the scientific objective, the person responsible had appropriate skills and training, and due care was given to occupational health and safety.

A method is not acceptable if it is not humane or has other significant problems associated with its use.

The use of carbon dioxide as a euthanasia agent

In the tables below, carbon dioxide (CO₂) is listed as a preferred method; however, recent data (AHAW Panel 2004) have raised the following animal wellbeing issues:

- the risk of compromising animal wellbeing is high, and inherent to CO₂
- the chosen method of administration of CO₂ could further confound or exacerbate this risk.

The risk of compromising animal wellbeing inherent in the use of CO₂ has been raised in recent publications (eg AHAW Panel 2004, Conlee et al 2004, Raj et al 2004) and is supported by evidence from studies involving aversion testing, behavioural observations and physiological responses (eg Raj 1999, Leach et al 2003, Niel and Weary 2006).

At the time of writing, the efficacy and humaneness of CO₂ as a euthanasia agent, and the way it is administered, are matters of debate. A recent meeting at the University of Newcastle (UK) (2006) sought to address these issues. Participants agreed there were issues that needed further research but, in the interim, a gradual fill of the chamber with 100% CO₂ at a rate to fill 20% of the chamber volume per minute was agreed as good practice; a similar recommendation was made in the recent ACLAM (2006) report. The options of adding oxygen to the gas mixture or using a volatile anaesthetic agent before administration of CO₂ as possible modifications to reduce the negative effects of CO₂ are discussed in the report of the Newcastle meeting.
### Table H1  Methods of humane killing and euthanasia in rats and mice

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- carbon dioxide(^a)</td>
<td>- Inhaling:</td>
<td></td>
</tr>
<tr>
<td>- injectable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- pentobarbitone sodium IP</td>
<td>- isoflurane(^{bcde})</td>
<td>- Inhalants:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- ether(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- hydrogen cyanide(^{ef})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- carbon monoxide(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- nitrogen(^i)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- chloroform(^b)</td>
</tr>
<tr>
<td>• Cervical dislocation(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(acceptable if stunned or anaesthetised first; possibly inhumane in animals heavier than 150 g)</td>
<td></td>
<td>- Microwave irradiation (not yet proven to be humane)(^{ce})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Decompression(^{ef})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Asphyxia(^{ef})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Rapid freezing(^e)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Stunning and exsanguination(^{ef})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Decapitation(^{ef})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Euthanasia(^f)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Intraperitoneal; IP</td>
</tr>
</tbody>
</table>

\(^a\) Training required  
\(^b\) Occupational health and safety issues  
\(^c\) Inhumane  
\(^d\) Expensive  
\(^e\) Requires specialised equipment  
\(^f\) Aesthetically unpleasant

### Table H2  Methods of humane killing and euthanasia in guinea pigs

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- carbon dioxide(^e)</td>
<td>- Inhaling:</td>
<td></td>
</tr>
<tr>
<td>- injectable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- pentobarbitone sodium IP</td>
<td>- isoflurane(^{bcde})</td>
<td>- Inhalants:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- ether(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- hydrogen cyanide(^{cf})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- carbon monoxide(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- chloroform(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- no IV agents are acceptable</td>
</tr>
<tr>
<td>• Cervical dislocation(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(injectable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- pentobarbitone sodium IP</td>
<td>- isoflurane(^{bcde})</td>
<td>- Nitrous oxide (must be used with other inhalants)(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- ethanaesthesia(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Euthanasia(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Intraperitoneal; IP</td>
</tr>
</tbody>
</table>

\(^a\) Training required  
\(^b\) Occupational health and safety issues  
\(^c\) Inhumane  
\(^d\) Expensive  
\(^e\) Requires specialised equipment  
\(^f\) Aesthetically unpleasant

\(\text{IP} = \text{intraperitoneal; IV} = \text{intravenous}\)
### Table H3  Methods of humane killing and euthanasia in rabbits

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Inhalant:</td>
<td>- none recommended</td>
<td>- Inhalant:</td>
</tr>
<tr>
<td>- Injectable:</td>
<td>- pentobarbitone sodium IV or IP</td>
<td>- isoflurane&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- nitrous oxide (must be used with other inhalants)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Injectable:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- ketamine with a premedicant such as acetylpromazine or xylazine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- None recommended</td>
<td>Stunning and dislocation&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Neck dislocation&lt;sup&gt;e&lt;/sup&gt; or decapitation&lt;sup&gt;f&lt;/sup&gt; without anaesthesia</td>
</tr>
<tr>
<td></td>
<td>Captive bolt&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N eck dislocation&lt;sup&gt;a&lt;/sup&gt; or decapitation&lt;sup&gt;e&lt;/sup&gt; (only if anaesthetised first)</td>
<td></td>
</tr>
</tbody>
</table>

IP = intraperitoneal; IV = intravenous

- <sup>a</sup> Training required
- <sup>b</sup> Occupational health and safety issues
- <sup>c</sup> Inhumane
- <sup>d</sup> Requires specialised equipment
- <sup>e</sup> Aesthetically unpleasant
- <sup>f</sup> Expensive

### Table H4  Methods of humane killing and euthanasia in sheep and goats

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Inhalant:</td>
<td>- none</td>
<td>- Inhalant:</td>
</tr>
<tr>
<td>- Injectable:</td>
<td>- pentobarbitone sodium IV</td>
<td>- none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- None</td>
<td>Captive bolt&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>Exsanguination&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Electrical stunning and exsanguination&lt;sup&gt;em&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shooting&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

IV = intravenous

- <sup>a</sup> Training required
- <sup>b</sup> Occupational health and safety issues
- <sup>c</sup> Requires specialised equipment
- <sup>d</sup> Aesthetically unpleasant
- <sup>e</sup> Unsure whether technique is humane
Table H5  Methods of humane killing and euthanasia in birds

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· carbon dioxide (chicks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Injectable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· pentobarbitone sodium IP (all birds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· carbon dioxide (adult birds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· methoxyflurane, halothane, isoflurane (chicks and small-to-medium adult birds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· carbon monoxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Cervical dislocation (small and medium-sized birds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Shooting (larger birds)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IP = intraperitoneal

*Training required
\* Occupational health and safety issues
\* Inhumane
\* Expensive
\* Aesthetically unpleasant

Table H6  Methods of humane killing and euthanasia in pigs

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· none</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Injectable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· pentobarbitone sodium IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Inhalant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· carbon dioxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Captive bolt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Electrical stunning and exsanguination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Shooting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Exsanguination without prior stunning</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IV = intravenous

*Training required
\* Occupational health and safety issues
\* Inhumane
\* Requires specialised equipment
\* Aesthetically unpleasant
\* Unsure whether technique is humane
Table H7  Methods of humane killing and euthanasia in fish

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable with reservations</th>
<th>Not acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Skin absorption:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- halothane, tricaine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- methane sulfanate-222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- benzocaine, eugenol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- clove oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Injectable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- sodium pentobarbitone IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(stressful due to removal from water and handling)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Skin absorption:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- carbon dioxide(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Stunning and brain destruction(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Cervical dislocation(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Decapitation/spinal section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(only in stunned or anaesthetised fish)(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Hypothermia and brain destruction(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Cervical dislocation (large fish)(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Decapitation alone(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Removal from water(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Freezing(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Hypothermia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IP = intraperitoneal

\(^a\) Training required
\(^b\) Inhumane
\(^c\) Aesthetically unpleasant
Part III  Humane killing and euthanasia

REFERENCES


PAIN MANAGEMENT: ANAESTHESIA, ANALGESIA AND ANXIOLYTICS

This factsheet describes a range of anaesthetic, analgesic and anxiolytic agents and techniques recommended for many species used for scientific purposes. It is intended to provide broad guidelines only, rather than exhaustive information on the range of agents and regimes currently available and newer agents that are emerging as suitable for use in animals. Investigators are expected to seek further information on specific techniques, agents and interactions that apply to their protocols.

WHAT IS PAIN MANAGEMENT?

Pain can result in significant and undesirable physiological, biochemical and behavioural changes in the animal. Providing effective pain relief can have a dramatic effect on the speed with which animals return to normal after surgical procedures. Pain management is a risk management approach that, when applied to animal pain and distress, involves anticipating, preventing and ameliorating pain. It includes a range of complementary strategies, only one of which is the use of drug therapies. Other aspects include good post-anaesthesia, nursing and husbandry practices. This document will look at anaesthetics, analgesics and anxiolytics.

Anaesthesia involves depression of sensation and motor responses either locally or centrally. Analgesic agents decrease or prevent the perception of pain. Anxiolytic drugs induce a state of relaxation and reduce irritability without resulting in sleep or lack of awareness of surroundings.

WHAT TYPES OF STUDIES USE PAIN MANAGEMENT DRUGS?

As detailed in the Australian code of practice for the care and use of animals for scientific purposes (NHMRC 2004), the use of anaesthetic, analgesic and anxiolytic agents must be suitable for the species, appropriate for the purpose of the study, and consistent with current veterinary and medical practice. Surgical procedures must be performed under appropriate local or general anaesthesia. When an animal is to recover from surgery, effective pain management must be provided (see Sections 3.3.25–3.3.32 of the Code). The pre-anaesthetic preparation of the animal, monitoring of the administration and depth of the anaesthesia, post-anaesthetic monitoring and, importantly, the relief of pain and distress must receive careful attention.

The underlying presumption in the selection and use of a pain management protocol is that, while pain and distress cannot be evaluated easily in animals, investigators and teachers must assume that animals experience these in a manner similar to humans unless there is evidence to the contrary (see Section 1.20 of the Code). Section 1.20 states: ‘Pain and distress cannot be evaluated easily in animals and therefore investigators and teachers must assume that animals experience these in a manner similar to humans unless there is evidence to the contrary. Decisions regarding the animals’ welfare must be based on this assumption.’

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

The crux of animal wellbeing is, as defined in the Code, an ‘animal’s present state with regard to its relationship with all aspects of its environment, both internal and external’. The essential issues of wellbeing relevant to pain management, anaesthesia, analgesia and anxiolytics are dealt with under the following topics:

- Pain management protocol—safe, effective, humane and suitable for the scientific purpose.
- Effective management of anaesthesia—during the pre-anaesthetic period, during anaesthesia, following completion of the procedure and during the recovery of the animal.
- Effective pain management and animal care.
The anaesthetic, analgesic or anxiolytic agent chosen must be safe and humane for the animal, must be safe for humans, and must cause minimal interference with the research protocol.

Many agents are not registered for use in small laboratory animals. This means that extensive evaluation is necessary, not only of the physiological effects and efficacy of the agent as an anaesthetic or analgesic, but also of its histological effects on various tissues (especially those at the injection site) and its effects on research parameters. It is not within the scope of this document to provide information on these aspects of anaesthesia and analgesia. Investigators should consult widely about these issues so that selected regimens are humane and interfere minimally with the overall aims of the research project.

The following factors must be considered before an effective regimen can be selected:

- scientific parameters and goals
- species, strain/breed, age and physiological status of the animal (e.g., pregnancy, health)
- whether the animal is likely to experience pain or distress— to what degree and for what duration
- the plane or depth of anaesthesia required
- whether the procedure is recovery or non-recovery
- the duration of anaesthesia required
- physiological interactions and influence on experimental results of drugs to be used
- humaneness of the technique (e.g., ease of induction, smoothness of recovery)
- side effects of the drugs on the animal (e.g., diarrhoea, vomiting, pica)
- method(s) of administration and dose rates
- experience of investigators with the technique (including a veterinarian with appropriate experience)
- availability of anaesthetic monitoring techniques (including adequate numbers of trained personnel)
- monitoring required during the recovery period
- safety of investigators
- available equipment.

To select a suitable pain management regimen, all the above factors need to be taken into consideration. Figure I1 is an example of a decision-making flowchart that maps this process.
Specific issues to be considered

Variations in response

There are significant variations in response to anaesthetic, analgesic and anxiolytic agents according to the species, strain and sex of the animal. There may also be considerable individual variations between animals of the same strain and sex. It is unwise to extrapolate the effects of an anaesthetic or analgesic agent from one species to another, including humans. Instead, expert advice should be sought when planning a new pain management protocol.

Interactions with research protocol

To minimise potential interactions with the research protocol, the major pharmacological and physiological effects of the various agents should be carefully reviewed (for example, see Milross et al 1996, Thompson et al 2002, Elena et al 2003, Bazin et al 2004 and Bette et al 2004). Errors can occur if an agent's effects are considered only superficially, or if it is assumed that a careful assessment was made by research workers whose publications include details of their anaesthetic or analgesic regimens. Finally, it is important to consider the overall response to
the anaesthesia procedure. The effects of the procedure itself (eg cannulation, surgery), from anaesthetic management (eg hypothermia, poor lung ventilation, poor tissue perfusion) or of inadequate management of postoperative pain may also produce prolonged effects that interact with the research protocol and affect the data obtained.

A N A E S T H E S I A

The suppression of pain perception during the conduct of a procedure can be achieved either by general anaesthesia, or by local or regional anaesthesia.

This section discusses the issues to consider when choosing the most appropriate anaesthetic technique, and describes the steps that are required to effectively manage anaesthesia.

G e n e r a l a n a e s t h e s i a

General anaesthesia involves loss of consciousness, loss of sensation (analgesia) and muscle relaxation. The requirement for depression of reflex activity, muscle tone and the central nervous system will vary with the procedure to be performed. Anaesthesia does not always equate with analgesia. General anaesthesia produces loss of consciousness, preventing the perception of pain while the animal is unconscious. However, noxious stimuli will still be transmitted to, and processed by, the central nervous system. Central hypersensitivity can develop in the spinal cord and brain. Thus, although pain perception is absent while the animal is unconscious, postoperative pain perception can be heightened. Some anaesthetic agents also have analgesic effects, but those that provide poor analgesia (eg pentobarbitone) must not be used without concurrent use of an analgesic agent.

Which technique should be used?

General anaesthesia can be provided:
  - by inhalation of gases or vapours
  - by injection of an anaesthetic or mixture of agents.

Regardless of the method used, ‘induction’ is the period between the animal being conscious and reaching a state of surgical anaesthesia. The ‘maintenance’ period is from when surgical anaesthesia begins, until the onset of the ‘recovery’ period.

I n h a l a t i o n

Anaesthesia using inhalation agents can be induced using an anaesthetic chamber or a face mask, and maintained using a face mask or endotracheal tube. To prevent unwanted exposure of personnel to anaesthetic vapours or waste gases, a method of ‘scavenging’ or removing the waste gases must be in place.

When administering gaseous anaesthesia, use the appropriate anaesthetic machine and vaporiser so that the dose (percentage) of administered anaesthetic can be controlled. An Ayres T-piece or a Bain coaxial circuit is recommended when anaesthetising small animals, to provide a low-resistance, low dead-space circuit. Use of an anaesthetic ‘jar’, where the animal is placed into a chamber containing a pad of gauze or cotton wool soaked in liquid anaesthetic, is not recommended, because the concentration of anaesthetic achieved within the container is unpredictable, and can be dangerously high if potent, easily vaporised anaesthetics are used (eg isoflurane). In addition, direct contact with liquid anaesthetic is extremely unpleasant for the animal, because it is an irritant to mucous membranes.

F a c e m a s k

Face masks are used to induce and maintain anaesthesia, and to provide supplemental oxygen to anaesthetised animals or those recovering from anaesthesia. Mask induction can be used as an alternative to chamber induction. When mask induction is being used, the animal should first become accustomed to breathing 100% oxygen via the face mask. Anaesthetic vapour is
then introduced using an anaesthetic machine, and the concentration is gradually increased from zero up to the appropriate level for the agent used, or until the animal is anaesthetised. The concentration is then reduced to the ‘maintenance’ level (see Table I). Anaesthesia is then maintained using the face mask or following intubation of the animal.

Not all individual animals are comfortable with use of the face mask, the sensations and odours associated with the anaesthetic vapour and gas flow. The response of the animal to the procedure should be monitored. Use of anxiolytic premedicant drugs and training can reduce distress and permit mask induction; however, this may not be suitable for all individuals, and an alternative method of anaesthetic induction should be substituted.

**Anaesthetic chamber**

Anaesthetic chambers are usually made from a plastic or glass box of the type used for aquariums. The animal is placed in the chamber and then anaesthetic gas is introduced at anaesthetic vapour concentrations up to 5%, with high flow rates of oxygen (5 litres or more per minute), until the animal loses its righting reflex. The animal is then taken out of the chamber, and placed in a face mask or intubated. Anaesthetic administration is continued at the maintenance level.

<table>
<thead>
<tr>
<th>Advantages and disadvantages of inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>• relatively simple to administer</td>
</tr>
<tr>
<td>• accurate control over the depth of anaesthesia</td>
</tr>
<tr>
<td>• induction and recovery are rapid</td>
</tr>
<tr>
<td>• provision of oxygen results in higher oxygen concentration in the blood throughout anaesthesia.</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>• specialised equipment is usually needed</td>
</tr>
<tr>
<td>• good ventilation and scavenging equipment are required at all times for safety of personnel.</td>
</tr>
</tbody>
</table>

**Injection**

Manufacturers of anaesthetic agents provide information about suitable routes of administration for their products. For information on methods of administration of injectable anaesthetic agents, see the ‘Administration of substances’ factsheet.

**Intravenous**

In most circumstances, half the calculated dose of anaesthetic is given rapidly (typically over a 5–10 second period), after which additional anaesthetic is given to produce the desired effect. Following IV induction, anaesthesia may be maintained using gaseous agents or continuous IV infusion.

<table>
<thead>
<tr>
<th>Advantages and disadvantages of intravenous anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>• rapid induction of anaesthesia</td>
</tr>
<tr>
<td>• dose administered can be tailored to the individual animal to achieve and maintain the desired depth of anaesthesia.</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>• requires some expertise on the part of the operator</td>
</tr>
<tr>
<td>• good restraint of the animal is essential</td>
</tr>
<tr>
<td>• may be stressful unless animal is premedicated</td>
</tr>
<tr>
<td>• some anaesthetic agents are cytotoxic if administered perivascularly.</td>
</tr>
</tbody>
</table>
Intraperitoneal
When the IP route is used, the onset of action is slower than with IV administration, and the animal will pass through a phase in which it becomes progressively ataxic (‘wobbly’), may exhibit some excitation and hyperactivity, then loses its ability to right itself, and eventually loses consciousness. Anaesthesia then becomes progressively deeper until the pedal withdrawal reflex is lost.

<table>
<thead>
<tr>
<th>Advantages and disadvantages of intraperitoneal anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
</tr>
<tr>
<td>- relatively simple to administer.</td>
</tr>
<tr>
<td>Disadvantages</td>
</tr>
<tr>
<td>- a ‘set dose’ is administered to the animal and, once administered, cannot be removed; because it is impossible to adjust the dose according to the individual animal’s response, inadvertent overdosing and underdosing will frequently occur</td>
</tr>
<tr>
<td>- relatively large doses of anaesthetic must be given to produce the required effect</td>
</tr>
<tr>
<td>- absorption is slow compared with IV administration</td>
</tr>
<tr>
<td>- residual drug effects can persist for a long time, so full recovery can be very prolonged</td>
</tr>
<tr>
<td>- injection of an irritant compound can cause unnecessary pain or discomfort to the animal</td>
</tr>
<tr>
<td>- repeated doses can result in abdominal adhesions.</td>
</tr>
</tbody>
</table>

Subcutaneous
The onset of action is slower when the SC route is used than with other injection routes. The animal will pass through a phase in which it becomes progressively ataxic (‘wobbly’), may exhibit some excitation and hyperactivity, then loses its ability to right itself, and eventually loses consciousness. Anaesthesia then becomes progressively deeper until the pedal withdrawal reflex is lost.

<table>
<thead>
<tr>
<th>Advantages and disadvantages of subcutaneous anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
</tr>
<tr>
<td>- relatively simple to administer.</td>
</tr>
<tr>
<td>Disadvantages</td>
</tr>
<tr>
<td>- some drugs (eg pentobarbitone, thiopentone, ketamine) cannot be administered subcutaneously, as they cause tissue damage and skin sloughing</td>
</tr>
<tr>
<td>- other disadvantages listed for IP injections also apply.</td>
</tr>
</tbody>
</table>

Intramuscular
Intramuscular injections are painful and should be avoided whenever possible. Larger volumes must be administered in multiple sites. The plunger must be withdrawn before injection to ensure that the needle is not in a blood vessel.

<table>
<thead>
<tr>
<th>Advantages and disadvantages of intramuscular anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
</tr>
<tr>
<td>- relatively simple to administer.</td>
</tr>
<tr>
<td>Disadvantages</td>
</tr>
<tr>
<td>- injection of an irritant compound can cause unnecessary pain or discomfort to the animal; there are a number of reports of tissue reactions and myositis with IM administration of some anaesthetic drugs (eg ketamine)</td>
</tr>
<tr>
<td>- in small animals, the injection volume is large compared with the volume of muscle mass used for IM administration; this can result in unnecessary pain or discomfort in the animal and, for this reason, it is recommended that this route be avoided in small rodents</td>
</tr>
<tr>
<td>- other disadvantages listed for IP injections also apply.</td>
</tr>
</tbody>
</table>

16 | GUIDELINES TO PROMOTE THE WELLBEING OF ANIMALS USED FOR SCIENTIFIC PURPOSES
Local anaesthesia

Local anaesthesia involves loss of sensation in a localised area as a result of blockade of the nerve endings by a local anaesthetic. Local anaesthesia can be used to provide anaesthesia during a surgical procedure or to contribute to pain management during the postoperative period.

For minor procedures, such as catheterisation and placement of earbars for stereotaxic procedures, local anaesthetic creams applied to the skin site may be useful. Eye drops containing local anaesthetic can be used to increase the comfort of the animal when examining the surface of the eye.

Local anaesthesia for surgical procedures is most useful when the animal has become accustomed to handling and can be safely restrained. Physical restraint can be used, provided that this can be achieved safely and without causing distress to the animal, or a sedative or tranquiliser can be used. Local anaesthetics such as lignocaine can then be infiltrated into the area that needs to be rendered insensitive (for example, to allow a skin biopsy to be taken), or the drug can be injected around nerve trunks to produce larger areas of anaesthesia. Routes that can be used include epidural, intrathecal, and intra-articular. These are most useful in larger species, such as cat, dog, pig and sheep. Most veterinary anaesthesia texts contain descriptions of these methods.

Local anaesthetic agents injected around the surgical site or nerve trunks can be added to a general anaesthetic regime to reduce pain transmission from the surgical site (see 'Multimodal or balanced analgesia').

Specialised techniques and issues

Reversible anaesthesia

Many injectable anaesthetic regimes involve prolonged recovery periods. This is especially true for rodent anaesthesia, in which anaesthetics administered via the IP or SC route produce on average 30–60 minutes of anaesthesia followed by recovery times of 2–4 hours. During the recovery period, the animals remain susceptible to hypothermia and have some degree of respiratory and cardiovascular depression. These effects can be largely overcome by using reversible anaesthetic regimens.

Antagonists such as naloxone are available for opioids, and the alternative of using partial agonists or mixed agonists-antagonists (eg buprenorphine and butorphanol) has also been well established. The effects of anaesthesia produced by $\alpha_2$-adrenergic agonists such as medetomidine and xylazine can be partially reversed using the $\alpha_2$-adrenoreceptor antagonist, atipamezole.

Neonatal anaesthesia

Anaesthesia of neonatal animals is challenging because they have a reduced capacity to detoxify a wide range of drugs and so their response to anaesthetics can differ considerably from that of adult animals. Prolonged recovery may lead to depletion of liver glycogen stores and result in hypoglycaemia (low blood glucose concentration). Other problems are increased susceptibility to hypothermia, increased possibility of poor pulmonary and circulatory function, and rejection by the mother following the procedure (particularly in rodents). For these reasons, it is preferable to use inhalation anaesthesia (eg isoflurane) so that recovery is rapid and normal feeding is resumed as soon as possible. Neonatal animals usually require a higher concentration of anaesthetic.

Hypothermia for anaesthesia of neonatal rodents

Hypothermia has been recommended for anaesthesia of neonatal rats and mice up to 10–14 days of age. It seems likely that, during hypothermia, the degree of suppression of the peripheral and central nervous system is sufficient to prevent the animal experiencing pain. Disadvantages include increased risk of ventricular fibrillation, tissue hypoxia and metabolic acidosis after rewarming.
Rapid chilling can be achieved by placing the pups into a prepared container, which is then placed on crushed or dry ice, in ice water, or in the freezer compartment of a refrigerator. Placing pups directly onto a cold source may result in tissue necrosis and is not recommended.

The torpor resulting from hypothermia may last up to 10 minutes. Recovery from anaesthesia can be rapid. However, to avoid tissue damage, aggressive rewarming techniques (eg heating pads or lamps) should not be used. An incubator at 33°C for 20-30 minutes is appropriate.

**Effective anaesthetic management**

Effective management of pain will depend on the management of the animal before induction of anaesthesia and performance of the procedure, the monitoring of the effectiveness of the anaesthesia during the procedure, and the management of the animal after the procedure and during its recovery.

**Pre-anaesthesia**

Acclimatise the animal to handling to reduce the effects of stress and the possibility of injury to the animal and personnel during induction. Check that the animal is healthy. Record bodyweight, which will assist in the postoperative monitoring and calculation of drug dose. In some situations, recording of feed and water intake prior to the procedure will assist with postoperative monitoring.

**Fasting**

The period of fasting is species specific, and should be minimised:

- Withhold food (12-16 hours) and water (3-4 hours) for those species that may vomit during induction of anaesthesia (cat, dog, non-human primate). Pigs rarely vomit on induction, although withholding food for 12 hours is common. Ferrets have a fast gut transit time and should only fast for 2-3 hours.

- Pre-anaesthetic fasting of small rodents and rabbits is generally unnecessary, because vomiting during induction does not occur in these species. In addition, fasting in small animals may result in a depletion of glycogen reserves, and the development of hypoglycaemia. Rabbits and rodents are coprophagic (that is, they eat their own faeces), so measures to prevent the ingestion of faeces are necessary if an empty stomach is required for the research protocol. Guinea pigs store food in their cheek pouches and may aspirate this material during induction.

- Large or medium-sized birds (eg ducks, chickens, pigeons) may be starved to reduce the risk of regurgitation of the contents of the crop. Smaller birds should not be fasted for longer than 2 hours, to avoid the risk of inducing hypoglycaemia.

- Opinion varies as to whether ruminants should be starved before induction of general anaesthesia. Fasting and water deprivation may have little effect on the volume of digesta present in the rumen, and on whether or not regurgitation of rumen contents occurs. However, fasting may reduce the incidence of rumenal tympany (‘bloat’) by decreasing the volume of fermentable ingesta. This appears to be a greater problem in animals that are grazing. Even with these precautions, some ruminants will develop rumenal tympany, while others will regurgitate.

**Premedication**

If used, premedication is usually given 15-40 minutes before anaesthetic agents. Premedication agents may include:

- Analgesics given prior to a procedure to inhibit perception of pain, thus providing a degree of prevention of pain, as opposed to pain treatment. These agents can be combined with an anxiolytic drug (eg acetylpromazine–buprenorphine mixture).

- Anxiolytic drugs (eg acetylpromazine, diazepam) in some species may reduce distress and make restraint easier.
Anticholinergic agents (atropine or glycopyrrolate), in some species, will reduce the side effects of many anaesthetic agents, such as the stimulation of respiratory secretions and the parasympathetic stimulation of the cardiopulmonary system. Do not use anticholinergic agents to treat bradycardia (slow heartbeat) if medetomidine has been used, as the combination could lead to adverse cardiovascular effects. Atropine should not be used in ruminants, because of its effects on gastrointestinal activity.

Following the use of an anxiolytic or analgesic, the dose of some anaesthetic agents may need to be reduced.

Anaesthesia

When inducing an animal and monitoring the depth of its anaesthesia, be aware of the stages of anaesthesia. These are outlined in most standard anaesthesia textbooks. The time taken for an animal to pass through each stage will vary with the anaesthetic agent used, and the response of the individual animal.

Induction

Anaesthesia can be induced using inhalation or injectable agents. Whichever technique is chosen, anaesthesia should be administered with appropriate equipment, in a room away from other animals.

Maintenance

Anaesthesia can be maintained using inhalation agents administered via face mask or endotracheal tube, continuous infusion or top-up of injectable agents, or a combination of methods.

Even during brief periods of anaesthesia, it is important to give attention to supporting the animal’s vital body functions.

Following induction, place the animal in a position with its head and neck extended to help ensure that its airway remains unobstructed.

Intubation of the trachea will ensure an adequate airway, particularly with long procedures. This can be achieved in most species; however, familiarity with the species-specific anatomy is essential. Local anaesthetic (lignocaine) spray on the larynx before intubation has been used to prevent laryngospasm, which occurs in some species (eg cats, diving birds, pigs and non-human primates).

If inhalation agents are not used to maintain anaesthesia, provision of oxygen by face mask is advisable to prevent hypoxia.

Hypothermia (a potentially fatal reduction in body temperature) can develop extremely rapidly during anaesthesia, and is among the commonest causes of anaesthetic death. It is a particular problem in smaller animals such as rodents, which have a high surface area to weight ratio and will lose heat rapidly under surgical anaesthesia. Maintain body temperature as close to normal levels as possible. The provision of supplemental heat is recommended (eg thermostatically controlled heating pad, warm hot-water bottle, bubble wrap, aluminium foil and warm fluids). Care should be taken not to overheat or burn the animal when using heating pads or hot-water bottles.

To maintain hydration, the administration of IV or SC infusion of suitable fluids (eg lactated Ringer’s solution, normal saline) is especially important with lengthy anaesthesia or highly invasive surgery. Fluids provided should be warmed so that their use does not contribute to the development of hypothermia.

Under anaesthesia, the animals’ eyes are often open. Therefore, investigators must ensure that the cornea is protected from drying and trauma; for example, by frequent administration of artificial tears or taping eyelids closed.
The position of the animal should be monitored, as bony points on the skeleton create pressure points when in contact with hard surfaces. These points should be padded. It is also important to avoid overstretching or restraint of limbs, as there is risk of damage to nerves and blood vessels. Where possible, allow limbs to lie in a natural anatomical position. When animals are anaesthetised or immobile for long periods, it is recommended that the animal be moved or turned every 20 minutes to promote normal blood flow in the tissues of the lower surface.

Monitoring
Monitor the animal to assess both the depth of anaesthesia and the physiological effects of the drugs.

Anaesthetic depth
The level of monitoring and the techniques used will be determined by the species and the procedure. Investigators must be familiar with species-specific signs of the stages of anaesthesia. As a minimum, monitor the depth of anaesthesia by assessing the presence or absence of reflexes. Monitoring of anaesthetic depth is easier if records are maintained.

Surgical anaesthesia is achieved when the following reflexes and normal muscle tone are lost:
- Righting reflex—animal will not attempt to right itself if placed on its back
- Palpebral blink reflex in response to stroking eyelids
- Withdrawal reflex—flexion of leg when interdigital skin is pinched
- Tail pinch reflex—movement following a firm pinch of the tail (rats and mice)
- Ear pinch reflex—head shaking in response to pinching the pinna (guinea pigs, rabbits and cats)
- Anal sphincter tone
- Muscle tone of the jaw.

Respiratory system
The respiratory system may be monitored by:
- Clinical observations—monitor depth, rate and pattern of respiration (increase in depth and decrease in rate signify anaesthesia)
- Respiratory monitor (eg Ap-Alert)—some instruments may not be sensitive enough to detect apnoea in small species, such as the rat or mouse
- Pulse oximetry—to measure the percentage saturation of arterial blood (cardiorespiratory function)
- End-tidal CO$_2$
- Blood gas analysis.

Respiratory obstruction may be caused by secretions, foreign objects, the tongue or abnormal neck positions. Respiration can be compromised by compression of the thorax by the weight of an arm or equipment.

Cardiovascular system
The cardiovascular system may be monitored by:
- Clinical observations—colour of mucous membranes, capillary refill time, heart sounds and heart rate (stethoscope or oesophageal stethoscope), peripheral pulse quality
- Electrocardiography
- Blood pressure—systemic arterial pressure, central venous pressure.
Body temperature

Body temperature may be monitored using an electronic rectal thermometer or thermocouple, or an ear thermometer, depending on what is appropriate for the species.

Post-anaesthesia

Animals must be observed during recovery from anaesthesia to ensure:

- that the airway is not obstructed
- that body temperature is maintained
- that they do not injure themselves
- that any postoperative pain is adequately controlled.

When gaseous anaesthetics are being used, the animal should be given a minimum of 5 minutes of oxygen (with no anaesthetic agent) at the end of the procedure. This permits time for the anaesthetic agent to be excreted from the lungs, and to ensure adequate oxygenation of tissues before the oxygen supply is withdrawn and the animal breathes only air.

If an endotracheal tube is in place, it must not be removed until the swallowing reflex has returned. Non-ruminant species should be placed on their side with head and neck extended. Ruminants should be propped up on the sternum to minimise the risk of overdistension of the rumen with gas (rumenal tympany or bloat) and of inhalation of regurgitated rumen contents.

The animal’s ability to regulate its body temperature will be reduced until it has recovered from anaesthesia. In its mildest form, hypothermia will lead to a substantial increase in the time taken to recover from an anaesthetic; this in turn can induce a wide variety of metabolic alterations. When more severe, animals become more susceptible to anaesthetic overdose and shock. Therefore, the ambient temperature of the recovery area should be warm (30–35°C for small rodents, 25–30°C for cats and dogs). Supplemental heat may be provided (eg warming lamp, heat pad, incubator), but care must be taken not to overheat the animal. Provision of both heated and non-heated zones in the recovery area will allow the animal to choose its preferred zone following recovery. Monitor both the body temperature of the animal during the post-anaesthesia recovery period, and the temperature of its immediate environment.

Small rodents and other social species should be housed alone during recovery to prevent attack by cage-mates and to prevent disturbance of the other animals. If surgery has been performed, cage bedding should be suitable to prevent wound contamination.

If animals have undergone an invasive procedure, careful monitoring during the postoperative period is essential to assess whether analgesia has been effective, and whether additional analgesia is required. The dose or frequency of administration should be modified according to the needs of the individual animal.

Warmed (37°C) fluids assist in the recovery of the animal. Appropriate fluids (eg normal saline, balanced electrolyte solutions) can be given intravenously (slowly), subcutaneously or intraperitoneally at a dose of up to 3–4% of the animal’s bodyweight.

**ANALGESIA**

Analgesic agents can be broadly divided into two groups: the opioids or narcotic analgesics, and the non-steroidal anti-inflammatory drugs (NSAIDs). There are also other effective analgesic agents that fall outside of this system of classification. Local anaesthetics can also be used to provide postoperative pain relief by blocking all pain sensation from the affected area. In some species, such as ruminants, α2-adrenergic agonists (eg xylazine) may be effective. A balanced analgesic regime may also include the use of N-methyl-D-aspartate (NMDA) receptor antagonists (eg ketamine in subanaesthetic doses), sedatives or tranquillisers.
Analgesic agents can be administered by injection (e.g., IV, SC, IM, IP, epidural and intrathecal routes), orally, or transcutaneously using creams or transdermal patches (fentanyl, codeine, buprenorphine). For further information on methods of administration, see the ‘Administration of substances’ factsheet.

Oral administration can be used for the administration of drugs on a single occasion or on repeated occasions (e.g., ongoing analgesic treatment provided via tablets, or in food, water or jelly). If using oral administration in water or jelly to provide postoperative analgesia, it is advisable to acclimatise the animal to the taste of the jelly or the water containing the analgesic.

Non-pharmacological methods for controlling postoperative or post-procedure pain include acclimatisation of the animal before the procedure. This will decrease anxiety and enhance the effect of concurrently administered analgesic agents. Other non-pharmacological methods include good husbandry, nutritional support, and access to conspecifics for social animals.

Pre-emptive analgesia

In general, it is currently believed that postoperative pain can be controlled more readily if analgesia is provided preoperatively or intra-operatively. This is known as pre-emptive analgesia and refers to the provision of certain types of analgesia, appropriately chosen to target the situation, given prior to incision and continued during the time that nociceptive signals are greatest (about 6–36 hours postoperatively for many surgeries), so that the patient will experience less pain (see review in Kissin 2000). The definition given in Kissin’s review is that pre-emptive analgesia ‘prevents the establishment of central sensitization caused by incisional and inflammatory injuries (covers the period of surgery and the initial postoperative period)’.

Initiating treatment before acute insult is believed to inhibit peripheral and central sensitisation; however, there is still scientific debate on this matter in human medicine. It has been difficult to prove that pre-emptive analgesia is a valid concept in large controlled clinical trials in humans; however, in animal models and some clinical veterinary trials, pre-emptive analgesia is potentially very powerful. Local anaesthetic nerve blockade, spinal/epidural analgesia, opioids, ketamine and NSAIDs are some agents for which a pre-emptive effect on surgical pain has been shown.

Exceptions exist in veterinary medicine, as pre-emptive analgesia has been reported to have some adverse effects under some circumstances. For example, there have been reports of unexpected and unexplained mortality associated with the preoperative administration of buprenorphine to rats anaesthetised with ketamine/mDETomidine. Therefore, for analgesia following surgery or a painful procedure in rats anaesthetised with either ketamine/mDETomidine or ketamine/xylazine, it is recommended that buprenorphine be administered during the post-anaesthetic period, rather than preoperatively.

Care must also be taken with the preoperative administration of NSAIDs, as their action in inhibiting prostaglandin production can result in platelet inhibition and renal dysfunction. Carprofen is the only drug of this class that can be used safely before surgery.

Multimodal or balanced analgesia

Perception of pain arises from a combination of peripheral and central hypersensitivity involving a multitude of pathways, mechanisms and transmitter systems. It is therefore unlikely that a single class of analgesic will completely alleviate pain, irrespective of the dose used. A combination of drugs with different modes of action can be used to produce sequential blocks in the nociceptive pathways, and achieve beneficial additive or synergistic analgesic effects. With this approach, lower doses of any one analgesic agent can usually be used, thereby reducing potential undesirable side effects while improving the control of pain.

For example, opioids can be combined with NSAIDs. The opioid dampens peripheral and central afferent nociceptive transmission. In contrast, the NSAID acts peripherally to decrease the amount of local inflammation and hence the nociceptive information entering the central
nervous system as a result of inflammation. Adding an injectable local anaesthetic (e.g., short-acting xylolcaine, long-acting bupivacaine or mepivacaine) to this regime can provide additional analgesia by blocking transmission in individual nerves in the area of the injection site.

NMDA receptor antagonists can be used to reduce central sensitisation. Sedatives and tranquillisers can also be used to decrease anxiety and stress, which have been shown to heighten responses to pain.

Source: Schofield and Williams (2002)
Monitroring effective analgesia

Because of their individual variation in response to analgesic agents, animals must be monitored carefully during the postoperative period to assess whether or not analgesia has been effective, and whether or not additional analgesia is required. The dose or frequency of administration should be modified according to the needs of the animal. It is difficult to make firm recommendations regarding the routine use of analgesics, the agents to use and the frequency of administration, but the following is a general guide:

- Relatively minor procedure (eg vascular catheterisation)—a single dose of systemic analgesic is administered, either an opioid (eg buprenorphine) or an NSAID (eg carprofen). Alternatively, it may be appropriate in some situations to inject long-acting local anaesthetic (eg bupivacaine or mepivacaine) into the skin and surrounding tissues where pain sensations may arise.
- More invasive surgical procedures (eg laparotomy)—systemic analgesic administration may need to continue for 72 hours. However, this can be variable and should be tailored to the animal’s needs. A common regime is a combination of opioid with an NSAID for 24–36 hours, followed by an NSAID alone for a further 24–36 hours.

ANXIOLYTICS

The major use of anxiolytic agents is to relieve anxiety, which can heighten responses to pain. They are not generally used to produce deep sedation or hypnosis. Their use as a premedicant prior to anaesthesia can result in reduction of apprehension and fear in the animal, and thus reduce resistance to anaesthesia. In this situation, ample time should be allowed to achieve the maximum effect before inducing anaesthesia. Anxiolytic agents can also be included as part of the pain management protocol to reduce pain and distress following a procedure.

There are several major classes of pharmaceutical compounds that can be used as anxiolytics, with considerable overlap in their actions and marked species variations in their effects. Investigators are advised to consult the relevant textbooks and literature with respect to specific agents proposed for use in a research protocol.

SAFE HANDLING OF DRUGS

Many anaesthetic and analgesic agents are controlled (scheduled) drugs with potential for abuse by humans. Legislation governs the acquisition and use of controlled substances such as Schedule 8 (S8) drugs, or drugs of addiction. Records must be kept of their use, and they must be stored in a locked cabinet in a secure area.

Some agents are also associated with significant occupational health and safety issues (eg health risks associated with chronic exposure to halothane, carcinogenic properties of urethane). Investigators must ensure that they are aware of any potential risks associated with the agents they use and take appropriate precautions.

CONCLUSION

To ensure that animal wellbeing is maintained during scientific research, the Code expects investigators to minimise pain and distress and to use procedures and pain control protocols consistent with current medical and veterinary practice. This requires detailed planning of surgical and other potentially painful procedures, a thorough understanding of animal biology, and detailed knowledge of the physiological and pharmacological actions of the anxiolytic, analgesic and anaesthetic drugs proposed for use.

Summaries of some data for selected species (rat, mouse, guinea pig, rabbit, cat, dog, sheep, pig, bird, wallaby, ferret and non-human primate) are provided in Tables II–7 at the end of this factsheet.


### Tables I1–I7: Species-specific dose rates for anaesthetics and analgesics

The following tables summarise some data for selected species (rat, mouse, guinea pig, rabbit, cat, dog, pig, sheep, bird, wallaby, ferret and non-human primate).

Note that this list is not exhaustive and that these dose rates provide only a general guide. Considerable between-strain and between-animal variation occurs. Investigators should consult further about anaesthesia of the species they are using and about techniques and agents.

The following abbreviations are used in these tables:

- **PO** = per os (administered by mouth)
- **SC** = subcutaneous (administered by an injection under the skin)
- **IM** = intramuscular (administered by an injection into a muscle)
- **IP** = intraperitoneal (administered by an injection into the abdominal cavity)
- **IV** = intravenous (administered by an injection into a vein).

#### Table I1: Inhalation anaesthesia: induction and maintenance concentrations of inhalation anaesthetics in laboratory animals

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Induction (%)</th>
<th>Maintenance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>4</td>
<td>1–2.5+</td>
</tr>
<tr>
<td>Enflurane</td>
<td>3–5</td>
<td>1–3</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>5–8</td>
<td>2.5–4</td>
</tr>
<tr>
<td>Halothane</td>
<td>4</td>
<td>1–2</td>
</tr>
</tbody>
</table>

Note: The potency of inhalation agents is indicated by the minimum alveolar concentration (MAC 50) value. This value is the alveolar concentration of an anaesthetic required to block the response to a specified stimulus in 50% of a group of animals. The lower the MAC value, the lower the concentration required, so the more potent the anaesthetic (Flecknell 1996). Investigators should seek information on the MAC value of the inhalation agent proposed for use.

#### Table I2: Injectable anaesthesia in rodents and rabbits

Note that these dose rates provide only a general guide, and should be tailored to the specific procedure and animal strain/breed. Considerable between-strain and between-animal variation occurs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat</th>
<th>Mouse</th>
<th>Guinea pig (light anaesthesia only)</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alphaxalone (Alfaxan CD®)</td>
<td>10–12 mg/kg IV</td>
<td>10–15 mg/kg IV</td>
<td>40 mg/kg IP</td>
<td>6–9 mg/kg IV</td>
</tr>
<tr>
<td>Alpha-chloralose (non-recovery only)</td>
<td>55–65 mg/kg IP</td>
<td>-</td>
<td>70 mg/kg IP</td>
<td>80–100 mg/kg IV</td>
</tr>
<tr>
<td>Alpha-chloralose + urethane (non-recovery only)</td>
<td>50–60 mg/kg IP + 500–800 mg/kg IP (administer urethane 20–30 min prior to alpha-chloralose)</td>
<td>-</td>
<td>-</td>
<td>80 mg chloralose IV + 400–800 mg/kg urethane IV</td>
</tr>
<tr>
<td>Drug</td>
<td>Rat</td>
<td>Mouse</td>
<td>Guinea pig</td>
<td>Rabbit</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------------------------------</td>
<td>----------------</td>
<td>------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Fentanyl/medetomidine</td>
<td></td>
<td>N ot recommended</td>
<td>-</td>
<td>8 µg/kg IV + 330 µg/kg IV 1 mg/kg IV + 1 mg/kg IV (nalbuphine)</td>
</tr>
<tr>
<td>Reverse medetomidine with atipamezole, and fentanyl with an opioid</td>
<td>300 µg/kg IP + 300 µg/kg IP 1 mg/kg SC or IP + 2 mg/kg SC or IP (butorphanol)</td>
<td>80 mg/kg IP</td>
<td>-</td>
<td>47.5 mg/kg IV</td>
</tr>
<tr>
<td>Inactin</td>
<td>80–100 mg/kg IP</td>
<td>80 mg/kg IP</td>
<td>-</td>
<td>47.5 mg/kg IV</td>
</tr>
<tr>
<td>Ketamine + acepromazine</td>
<td>75–80 mg/kg IP + 2.5 mg/kg IP</td>
<td>100 mg/kg IP + 5 mg/kg IP</td>
<td>125 mg/kg IP or SC + 5 mg/kg IP or SC</td>
<td>50 mg/kg SC + 1 mg/kg SC</td>
</tr>
<tr>
<td>Ketamine + diazepam</td>
<td>75 mg/kg IP + 5 mg/kg IP</td>
<td>100 mg/kg IP + 5 mg/kg IP</td>
<td>100 mg/kg IP or IM + 5 mg/kg IP or IM</td>
<td>25 mg/kg IM + 5 mg/kg IM</td>
</tr>
<tr>
<td>Ketamine + medetomidine</td>
<td>75 mg/kg IP + 0.5 mg/kg IP</td>
<td>75 mg/kg IP + 1 mg/kg IP</td>
<td>40 mg/kg IP + 0.5 mg/kg IP</td>
<td>25 mg/kg SC + 0.5 mg/kg SC</td>
</tr>
<tr>
<td>Reverse medetomidine with atipamezole</td>
<td>1 mg/kg SC or IP</td>
<td>1 mg/kg SC or IP</td>
<td>1 mg/kg SC</td>
<td>1 mg/kg SC or IV</td>
</tr>
<tr>
<td>Ketamine + midazolam</td>
<td>75 mg/kg IP + 5 mg/kg IP</td>
<td>100 mg/kg IP + 5 mg/kg IP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ketamine + xylazine</td>
<td>75–100 mg/kg IP + 10 mg/kg IP</td>
<td>80–100 mg/kg IP + 10 mg/kg IP</td>
<td>40 mg/kg IM + 5 mg/kg SC or IM</td>
<td>35 mg/kg IM + 5 mg/kg IM, O R 10 mg/kg IV + 3 mg/kg IV</td>
</tr>
<tr>
<td>Reverse xylazine with atipamezole</td>
<td>1 mg/kg SC or IP</td>
<td>1 mg/kg SC</td>
<td>1 mg/kg SC</td>
<td>1 mg/kg SC or IV</td>
</tr>
<tr>
<td>Methohexitone</td>
<td>10 mg/kg IV</td>
<td>10 mg/kg IV</td>
<td>31 mg/kg IP</td>
<td>10 mg/kg IV</td>
</tr>
<tr>
<td>Pentobarbitone(^a)</td>
<td>40–50 mg/kg IP</td>
<td>40–50 mg/kg IP</td>
<td>37 mg/kg IP</td>
<td>30–45 mg/kg IV</td>
</tr>
<tr>
<td>Propofol</td>
<td>10 mg/kg IV</td>
<td>26 mg/kg IV</td>
<td>-</td>
<td>10 mg/kg IV</td>
</tr>
<tr>
<td>Tiletamine/zolazepam</td>
<td>40 mg/kg IP</td>
<td>80 mg/kg IP</td>
<td>40–60 mg/kg IM (sedation)</td>
<td>-</td>
</tr>
<tr>
<td>Tribromoethanol (avertin)(^c)</td>
<td>-</td>
<td>240 mg/kg IP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tiletamine/zolazepam + medetomidine</td>
<td>-</td>
<td>-</td>
<td>40 mg/kg IM + 0.5 mg/kg IM</td>
<td>-</td>
</tr>
<tr>
<td>Thiopentone(^b)</td>
<td>30 mg/kg IV</td>
<td>30–40 mg/kg IV</td>
<td>-</td>
<td>15–30 mg/kg IV</td>
</tr>
<tr>
<td>Urethane(^a)</td>
<td>1000–1500 mg/kg IP</td>
<td>-</td>
<td>1500 mg/kg IV or IP</td>
<td>1000–2000 mg/kg IV or IP</td>
</tr>
</tbody>
</table>

\(^a\) Urethane is carcinogenic, and should only be used for non-recovery anaesthesia.

\(^b\) Pentobarbitone as part of a euthanasia solution may be used in non-recovery anaesthesia only. These euthanasia solutions are not anaesthetic grade in purity, and often contain other ingredients. Anaesthesia grade pentobarbitone is available.

\(^c\) Tribromoethanol solutions are not commercially available and must be prepared according to a published method. Due to its irritancy, tribromoethanol is not suitable to be used for anaesthesia by IP injection more than once, as to do so results in anaesthetic mortalities.

Table 13  Injectable anaesthesia in other laboratory animals

Note that these dose rates provide only a general guide, and should be tailored to the specific procedure and animal strain/breed. Considerable between-strain and between-animal variation occurs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cat</th>
<th>Dog</th>
<th>Pig</th>
<th>Sheep</th>
<th>Bird</th>
<th>Wallaby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-chloralose (non-recovery only)</td>
<td>70 mg/kg IP or 60 mg/kg IV</td>
<td>80 mg/kg IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alphaxalone (Alfaxan CD®)</td>
<td>9–12 mg/kg IV</td>
<td>12–18 mg/kg IM</td>
<td>-</td>
<td>6 mg/kg IM, then 2 mg/kg IV</td>
<td>2–3 mg/kg IV (adult) 6 mg/kg IV (lamb)</td>
<td>-</td>
</tr>
<tr>
<td>Azaperone (sedation)</td>
<td>-</td>
<td>-</td>
<td>2–8 mg/kg IM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ketamine + acepromazine</td>
<td>20 mg/kg IM + 0.11 mg/kg IM</td>
<td>-</td>
<td>33 mg/kg IM + 1.1 mg/kg IM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ketamine + diazepam</td>
<td>7 mg/kg IM + 0.08 mg/kg IM or SC</td>
<td>2.5–7.5 mg/kg IM + 0.05 mg/kg IM</td>
<td>10 mg/kg IM + 0.08 mg/kg IM</td>
<td>10–15 mg/kg IM + 0.5–2 mg/kg IM</td>
<td>10–15 mg/kg IM + 2 mg/kg IM + 4 mg/kg IV + 1 mg/kg IV</td>
<td>20–40 mg/kg IM + 1–1.5 mg/kg IM</td>
</tr>
<tr>
<td>Reverse medetomidine with atipamezole</td>
<td>10 mg/kg IM + 0.2 mg/kg IM</td>
<td>-</td>
<td>10–15 mg/kg IM + 0.5–2 mg/kg IM</td>
<td>10–15 mg/kg IM + 2 mg/kg IM</td>
<td>10–15 mg/kg IM + 2–4 mg/kg IV + 1 mg/kg IV</td>
<td>-</td>
</tr>
<tr>
<td>Ketamine + medetomidine</td>
<td>7 mg/kg IM + 5 mg/kg IV or SC</td>
<td>2.5–7.5 mg/kg IM + 0.05 mg/kg IM</td>
<td>10 mg/kg IM + 0.08 mg/kg IM</td>
<td>10–15 mg/kg IM + 0.5–2 mg/kg IM</td>
<td>10–15 mg/kg IM + 2 mg/kg IM</td>
<td>-</td>
</tr>
<tr>
<td>Ketamine + xylazine</td>
<td>22 mg/kg IM + 1.1 mg/kg IM or SC</td>
<td>5 mg/kg IV + 1–2 mg/kg IV or IM</td>
<td>-</td>
<td>4 mg/kg IV + 0.2 mg/kg IV</td>
<td>10–30 mg/kg IM + 2–6 mg/kg IM</td>
<td>3 mg/kg IM + 2–3 mg/kg IM</td>
</tr>
<tr>
<td>Reverse xylazine with atipamezole</td>
<td>4–8 mg/kg IV</td>
<td>5 mg/kg IV</td>
<td>4 mg/kg IV</td>
<td>-</td>
<td>10 mg/kg IV</td>
<td>-</td>
</tr>
<tr>
<td>Methohexitone</td>
<td>4–8 mg/kg IV</td>
<td>5 mg/kg IV</td>
<td>4 mg/kg IV</td>
<td>-</td>
<td>10 mg/kg IV</td>
<td>-</td>
</tr>
<tr>
<td>Pentobarbitonea</td>
<td>20–30 mg/kg IV</td>
<td>20–30 mg/kg IV</td>
<td>20–30 mg/kg IV</td>
<td>30 mg/kg IV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Propofol</td>
<td>5–8 mg/kg IV</td>
<td>5–7.5 mg/kg IV</td>
<td>2.5–3.5 mg/kg IV</td>
<td>4–5 mg/kg IV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tiletamine/ zolazepam (Zoletil®)</td>
<td>7.5 mg/kg IM + 2–4 mg/kg IM (restraint) 6–8 mg/kg IM (light anaesthesia)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20–30 mg/kg IM 2.5 mg/kg IV (for induction)</td>
<td>-</td>
</tr>
</tbody>
</table>
Part III  Pain management

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cat</th>
<th>Dog</th>
<th>Pig</th>
<th>Sheep</th>
<th>Bird</th>
<th>Wallaby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiletamine/ zolazepam + xylazine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 mg/kg IM + 0.5 mg/kg IM</td>
</tr>
<tr>
<td>Reverse xylazine with atipamezole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50–400 µg/kg IM</td>
</tr>
<tr>
<td>Thioptene</td>
<td>10–15 mg/kg IV</td>
<td>10–20 mg/kg IV</td>
<td>6.6–25 mg/kg IV</td>
<td>10–15 mg/kg IV</td>
<td>-</td>
<td>20 mg/kg IV</td>
</tr>
<tr>
<td>Urethaneb (non-recovery)</td>
<td>750 mg/kg IV</td>
<td>1500 mg/kg IV</td>
<td>1000 mg/kg IV</td>
<td>-</td>
<td>1000 mg/kg IV</td>
<td>-</td>
</tr>
</tbody>
</table>

* Pentobarbitone as part of a euthanasia solution may be used in non-recovery anaesthesia only. These euthanasia solutions are not anaesthetic grade in purity and often contain other ingredients. Anaesthesia grade pentobarbitone is available overseas and is scheduled S8 if imported into Australia.

* Urethane is carcinogenic and should only be used for non-recovery anaesthesia.


Table 4  Analgesia in rodents and rabbits: non-steroidal anti-inflammatory drugs (NSAIDs)

Note that these dose rates provide only a general guide, and should be tailored to the specific procedure and animal strain/breed. Considerable between-strain and between-animal variation occurs. It is therefore essential to assess the analgesic effects in each individual animal.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat</th>
<th>Mouse</th>
<th>Guinea pig</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin (acetylsalicylic acid)</td>
<td>100 mg/kg PO</td>
<td>120 mg/kg PO</td>
<td>80–90 mg/kg PO</td>
<td>100 mg/kg PO</td>
</tr>
<tr>
<td>Carprofen</td>
<td>5 mg/kg SC 12–24-hourly</td>
<td>5 mg/kg SC 24-hourly</td>
<td>2.5 mg/kg SC 24-hourly</td>
<td>1.5 mg/kg PO 12-hourly 4 mg/kg SC 24-hourly</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg/kg PO 24-hourly</td>
<td>8 mg/kg PO 24-hourly</td>
<td>2 mg/kg PO 24-hourly</td>
<td>-</td>
</tr>
<tr>
<td>Flunixin</td>
<td>2.5 mg/kg SC 12–24-hourly</td>
<td>2.5 mg/kg SC 12–24-hourly</td>
<td>-</td>
<td>1.0 mg/kg SC 12–24-hourly</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>15 mg/kg PO 24-hourly</td>
<td>30 mg/kg PO 24-hourly</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2 mg/kg PO frequency unknown</td>
<td>1 mg/kg PO frequency unknown</td>
<td>8 mg/kg PO frequency unknown</td>
<td>12.5 mg/kg PO frequency unknown</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5 mg/kg SC, PO 24-hourly</td>
<td>-</td>
<td>-</td>
<td>3 mg/kg SC 24-hourly</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1 mg/kg SC, PO 24-hourly</td>
<td>1–2 mg/kg SC</td>
<td>-</td>
<td>0.2 mg/kg SC 24-hourly Up to 3 days</td>
</tr>
<tr>
<td>Paracetamol (acetaminophen)</td>
<td>200 mg/kg PO 24-hourly</td>
<td>200 mg/kg PO 24-hourly</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>3 mg/kg PO 24-hourly</td>
<td>3 mg/kg PO 24-hourly</td>
<td>6 mg/kg PO 24-hourly</td>
<td>-</td>
</tr>
</tbody>
</table>

Note that these dose rates provide only a general guide, and should be tailored to the specific procedure and animal strain/breed. Considerable between-strain and between-animal variation occurs. It is therefore essential to assess the analgesic effects in each individual animal.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat</th>
<th>Mouse</th>
<th>Guinea pig</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.01–0.05 mg/kg</td>
<td>0.05–0.10 mg/kg</td>
<td>0.05 mg/kg SC</td>
<td>0.01–0.05 mg/kg SC,</td>
</tr>
<tr>
<td></td>
<td>SC, IV 8–12-hourly</td>
<td>8–12-hourly</td>
<td>8–12-hourly</td>
<td>IM or IV 6–12-hourly</td>
</tr>
<tr>
<td></td>
<td>0.10–0.25+ mg/kg PO</td>
<td>8–12-hourly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butorphanol</td>
<td>1.0–2.0 mg/kg SC</td>
<td>1.0–2.0 mg/kg SC</td>
<td>–</td>
<td>0.1–0.5 mg/kg IV 4-hourly</td>
</tr>
<tr>
<td></td>
<td>2–4-hourly</td>
<td>4-hourly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>2–5 mg/kg SC</td>
<td>2–5 mg/kg SC</td>
<td>2–5 mg/kg SC</td>
<td>2–5 mg/kg SC or IM</td>
</tr>
<tr>
<td></td>
<td>4-hourly</td>
<td>4-hourly</td>
<td>or IM 4-hourly</td>
<td>4-hourly</td>
</tr>
<tr>
<td></td>
<td>Sustained release</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>formulation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.8 mg/kg SC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-hourly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>1–2 mg/kg IM</td>
<td>2–4 mg/kg IM</td>
<td>1–2 mg/kg IV, IP or IM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-hourly</td>
<td>4-hourly</td>
<td>4-hourly</td>
<td>4-hourly</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>5–10 mg/kg SC</td>
<td>5–10 mg/kg SC</td>
<td>–</td>
<td>5–10 mg/kg SC, IM, IV 4-hourly</td>
</tr>
<tr>
<td></td>
<td>3–4-hourly</td>
<td>3–4-hourly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pethidine</td>
<td>10–20 mg/kg SC</td>
<td>10–20 mg/kg SC</td>
<td>10 mg/kg SC or IM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>or IM 2–3-hourly</td>
<td>2–3-hourly</td>
<td>2–4-hourly</td>
<td>2–3-hourly</td>
</tr>
</tbody>
</table>

*a Do not use injectable form of buprenorphine for oral preparation as it is too bitter (see below).
*b Morphine—sustained release formulation. In N,O-carboxymethylchitosan (NOCC) and chitosan (Tasker et al 1997). 


Oral administration of buprenorphine in rats

Volker et al (2000) has described the following method for oral administration of buprenorphine in rats using jelly; acclimatise the rats to consumption of jelly over several days or weeks. Dissolve 85 g of jelly crystals in 250 mL of boiling water. Place aliquots of 4 mL of jelly liquid in ice-block moulds for refrigeration. Rats will accept berry, orange, lime and strawberry flavours.

When analgesia is required, 3 buprenorphine sublingual tablets (Temgesic, Reckitts and Coleman, 0.2 mg/tablet) are crushed into the base of each ice-block mould and moistened with 0.5 mL warm water, prior to the addition of 3.5 mL of warm jelly (total 4 mL). The jelly disks are set at 4–8°C. For acute pain, the number of disks given to each animal is calculated on a dose rate of 2 mg/kg.
Table 16  Analgesia in other laboratory animals: non-steroidal anti-inflammatory drugs (NSAIDs)

Note that these dose rates provide only a general guide, and should be tailored to the specific procedure and animal strain/breed. Considerable between-strain and between-animal variation occurs. It is therefore essential to assess the analgesic effects in each individual animal.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cat</th>
<th>Dog</th>
<th>Pig</th>
<th>Sheep</th>
<th>Bird</th>
<th>Wallaby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin (acetylsalicylic acid)</td>
<td>10–25 mg/kg PO every 48 hours</td>
<td>10–25 mg/kg PO 8–12-hourly</td>
<td>10 mg/kg PO 4-hourly</td>
<td>50–100 mg/kg PO 6–12-hourly</td>
<td>5–10 mg/kg 8–24-hourly</td>
<td>-</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2-4 mg/kg SC or IV once 2 mg/kg PO for 4 days, then every other day</td>
<td>4 mg/kg IV or SC on induction, then 2.2 mg/kg 24-hourly IV, SC or PO</td>
<td>2-4 mg/kg IV or SC 24-hourly</td>
<td>1.5-2.0 mg/kg IV or SC 24-hourly</td>
<td>0.5-2 mg/kg 24-hourly</td>
<td>2-4 mg/kg IV, SC, PO 24-hourly</td>
</tr>
<tr>
<td>Flunixin</td>
<td>1 mg/kg SC, PO single dose</td>
<td>1 mg/kg IV or SC single dose 1 mg/kg PO, daily for up to 3 days</td>
<td>1-2 mg/kg IV or SC 24-hourly</td>
<td>2 mg/kg, IV or SC 24-hourly</td>
<td>-</td>
<td>1 mg/kg IV, IM, SC</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>-</td>
<td>10 mg/kg PO 24-hourly</td>
<td>-</td>
<td>-</td>
<td>1 mg/kg 12-hourly</td>
<td>-</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2 mg/kg SC, once daily for up to 3 days 1 mg/kg PO, once daily for up to 5 days</td>
<td>2 mg/kg SC, once daily for up to 3 days 1 mg/kg PO, daily for 5 days</td>
<td>3 mg/kg IM once only</td>
<td>1-2 mg/kg 24-hourly</td>
<td>1 mg/kg SC, PO 24-hourly</td>
<td>-</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.2 mg/kg SC, PO, then 0.1 mg/kg daily</td>
<td>0.2 mg/kg IV, SC or PO, then 0.1 mg/kg PO daily.</td>
<td>-</td>
<td>-</td>
<td>0.1-0.3 mg/kg 24-hourly</td>
<td>-</td>
</tr>
<tr>
<td>Paracetamol (acetaminophen)</td>
<td>Contraindicated — liver toxicity</td>
<td>15 mg/kg PO 6-8-hourly</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note that these dose rates provide only a general guide, and should be tailored to the specific procedure and animal strain/breed. Considerable between-strain and between-animal variation occurs. It is therefore essential to assess the analgesic effects in each individual animal.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cat</th>
<th>Dog</th>
<th>Pig</th>
<th>Sheep</th>
<th>Bird</th>
<th>Wallaby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.005–0.01 mg/kg SC or IV 4–8-hourly</td>
<td>0.005–0.02 mg/kg IM, SC, IV 4–8-hourly</td>
<td>0.01 mg/kg IM or IV 6–12-hourly</td>
<td>0.005–0.010 mg/kg IM or IV 4-hourly</td>
<td>0.01–0.05 mg/kg IM or IV 4-hourly</td>
<td>0.01 mg/kg SC 12-hourly</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>0.4–0.8 mg/kg SC or IM 2–4-hourly</td>
<td>0.2–0.4 mg/kg SC or IM 2–3-hourly</td>
<td>0.1–0.3 mg/kg IM 4-hourly</td>
<td>0.5 mg/kg IM, SC 2–3-hourly</td>
<td>1–4 mg/kg IM 6-hourly</td>
<td>-</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.1–0.2 mg/kg SC, IM, IV 4-hourly</td>
<td>0.1–1.0 mg/kg SC, IM, IV 4–6-hourly</td>
<td>0.2–1.0 mg/kg IM 4-hourly</td>
<td>0.2–0.5 mg/kg IM 4-hourly*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>0.3–0.5 mg/kg SC, IM, IV 3-hourly</td>
<td>0.3–0.5 mg/kg SC, IM, IV 3–4-hourly</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>0.05–0.40 mg/kg SC, IM, IV 2–4-hourly</td>
<td>0.05–0.22 mg/kg IM, SC, IV 2–4-hourly</td>
<td>0.02 mg/kg IM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>1–4 mg/kg IM, IV 2–3-hourly</td>
<td>1–4 mg/kg IM or IV 2–4-hourly</td>
<td>2 mg/kg IM or IV 4-hourly</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pethidine</td>
<td>3.5–10.0 mg/kg SC or 10–15 mg/kg SC 2–3-hourly</td>
<td>3.5–10.0 mg/kg IM or 10–15 mg/kg SC 2–3.5-hourly</td>
<td>2 mg/kg IM or IV 2–4-hourly</td>
<td>2 mg/kg IM or IV 2-hourly</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.002–0.003 mg/kg IV bolus, repeated every 20–30 mins, or by continuous infusion (0.002–0.003 mg/kg/hr) Transdermal: 25 µg/hr every 3 days</td>
<td>0.001–0.005 mg/kg IV bolus, repeated every 20–30 mins, or by continuous infusion (0.003–0.010 mg/kg/hr) Transdermal: 3–10 kg: 25 µg/hr 10–20 kg: 50 µg/hr 20–30 kg: 75 µg/hr &gt;30 kg: 100 µg/hr Transdermal: 50–100 µg/hr applied 8–12 hrs prior to surgery. Reapply every 72 hours. Transdermal: 2 µg/kg/hr applied 24 hrs prior to surgery. Remove after 72 hours.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Although this is the suggested frequency, the effects at this dosage are unknown.

Further reading


Further reading

Recommended texts

Educational resources and websites
Flecknell P. Digital veterinary anaesthesia images and videos.
http://www.digires.co.uk/
International Veterinary Information Services (IVIS).
http://www.ivis.org/home.asp
http://www.cvm.okstate.edu/Courses/vmed5412/default.htm
The Virtual Anesthesia Machine.
http://vam.anest.ufl.edu/index.html

REFERENCES

Polyclonal Antibody Production

What are polyclonal antibodies?

Antibodies are proteins with special properties that are produced by the immune system in response to the body being exposed to foreign material. They play a critical role in the defence mechanisms of the body. Antibodies belong to the class of protein known as immunoglobulins. Antibodies are termed monoclonal when they are specific for one so-called binding site or antigenic determinant on a foreign material (the antigen). They are termed polyclonal when their specificity extends to multiple binding sites or antigenic determinants on the antigen. The production of polyclonal antibodies is the normal response of the immune system to an antigen in the whole animal. In the laboratory setting, monoclonal antibodies are produced through specialised techniques. Although there is increasing use of monoclonal antibodies in research, there are many circumstances where polyclonal antibodies are more appropriate. Further discussion of the rationale for choosing a particular method, and animal wellbeing implications, is provided by Hendriksen and Hau (2003) and The National Academies (2005).

When are polyclonal antibodies used?

High-titre, high-affinity polyclonal antibodies are used in a variety of experimental and diagnostic situations to identify and locate specific molecules, such as in immunohistochemical and immunofluorescence procedures, immunoelectron microscopy, ELISA (enzyme-linked immunosorbent assay) assays and Western blots.

How are polyclonal antibodies produced?

The production of polyclonal antibodies involves administering a foreign antigen (such as a protein, a glycoprotein, a lipopolysaccharide or a peptide) to an animal to induce an immunological response that will result in the production of antibodies specific to that antigen. An adjuvant is usually administered with the antigen to enhance the immunological response. Some weeks after the antigen/adjuvant is administered, blood is collected and the antibody is then separated in the laboratory.

To optimise the collection of antibodies, small blood samples should be taken after the initial immunisation to assess the level of antibody production. This will determine whether booster immunisation is required and ensure that adequate levels of antibody have been produced before blood is collected to harvest the antibody.

The procedure is most often carried out on rabbits (Stills 1994), but other laboratory animals such as rats, mice and guinea pigs are used in particular circumstances. Sheep, goats and, occasionally, horses can be used if large volumes of antibody are required.

The protocol for the production of an antibody should be tailored to the specific characteristics of that antibody. The protocol will be determined by a number of factors, including:

- the biological and chemical qualities of the antigen
- the route chosen to administer the antigen
and, if an adjuvant is used:

- the type and volume of adjuvant
- the ratio of antigen to adjuvant.
Although the production of polyclonal antibodies is widely practised, there are few studies looking at the effects of various immunisation protocols on the antibody response. As a general guide, Palmer et al (1997) recommend at least 4 weeks between initial immunisation and a booster injection, with two or three booster injections. They also advise allowing a 3-4-month period before the final booster, which can produce a significant increase in antibody titre associated with the induction of immunological memory.

**Route of administration**

There is no conclusive evidence as to the optimal route to administer an antigen (Hanly et al 1995, Palmer et al 1997). Various routes are used—intravenous (IV), intramuscular (IM), subcutaneous (SC), intraperitoneal (IP) and intradermal (ID)—the aim being to broadly distribute the antigen to lymphoid tissue to maximise the immune response. Intravenous administration will facilitate systemic distribution; when other routes are used, multiple injections can be used to more widely distribute the antigen. Based on the potential to deliver antigen efficiently to the lymphatic system, the intradermal route using multiple sites is considered to be the method of choice, although this view is disputed. Also, there is a risk of infection or abscess formation at the site of any antigen/adjuvant injection. Consequently, where possible, the site that provides easiest access for monitoring should be selected.

Due to the high impact of foot-pad injection on the wellbeing of animals, special justification must be provided to the animal ethics committee (AEC) before this route is used. In those circumstances where foot-pad injection is justified, consideration must be given to supportive treatment to minimise the impact.

Oil adjuvants should not be given by either the intravenous or intraperitoneal route.

There will also be special cases in which the study requires the harvesting of stimulated immunological cells from lymphoid tissues, mostly lymph nodes. In these circumstances, a small dose of antigen can be injected directly into a lymph node or the spleen or, alternatively, into the foot pad, where lymphatic drainage into adjacent lymph nodes provides a mechanism to harvest immunologically stimulated cells.

For a detailed discussion of the rationale behind the choice of various injection routes, see Hanly et al (1995). However, the choice of a route for administration of the antigen will also be influenced by the choice of adjuvant. This is most often the limiting factor in decisions concerning the route and the volume of antigen/adjuvant injected at any site.

**Choice of adjuvant**

Adjuvants, which are a common component of human and veterinary vaccines, enhance and prolong the immune response to an antigen. In many situations, this effect is essential to obtain high antibody titre, as many of the antigens used produce a weak immunogenic response. Adjuvants, of which many are available, generally fall into the categories of mineral oils, aluminium salts, saponins, peptides, bacterial toxins or a combination of these (eg Freund’s complete adjuvant [FCA], ISCOMS, montanides).

The injection of most adjuvants will cause transitory and acute pain. All adjuvants are associated with an enhanced inflammatory response, and some produce pathological lesions—complications that cause pain and have significant animal wellbeing implications (discussed below). The nature and severity of the side effects of adjuvants will limit how they are used. Consequently, the choice of adjuvant will depend on the efficacy of the antibody response to a particular antigen, balanced against minimising the potential negative effects of the adjuvant (for a detailed discussion of the action of various adjuvants, see Stills 1994; Jennings 1995; Hanly et al 1995, 1997; The National Academies 2005).
WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

The notion that polyclonal antibody production can have a significant impact on the wellbeing of animals has led organisations (such as the National Institutes of Health in the United States and the Home Office in the United Kingdom) to issue guidelines on producing antibodies. In 2000, the Dutch Inspectorate for Health Protection and Veterinary Public Health published a code of practice for immunising laboratory animals and identified many immunisation procedures in the moderate–severe pain and distress categories.

Four aspects of the procedure have the potential to cause pain and distress:
- inflammation and pain associated with the route of delivery of the antigen/adjuvant
- pathological complications associated with dispersal of injected material
- anaphylaxis associated with booster immunisations
- the bleeding protocol (ie frequency and volume) used to harvest antibodies.

The use and choice of adjuvant are major factors in the level and severity of these complications, with FCA most often implicated. However, the value of FCA is its potency as an immunostimulant, so while less severe reactions may be seen with other adjuvants, this is often at the expense of lower or inadequate antibody responses. The use of FCA is associated with painful acute inflammation at the injection site (Amyx 1987), and there is substantial evidence of ongoing pathology, with granulomatous lesions evident in various internal organs (Broderson 1989). The negative impact of these lesions on the wellbeing of rabbits has been questioned, with no evidence of changes to a range of physiological and behavioural measures (Halliday et al 2000). However, there is anecdotal evidence of a transient decrease in food intake in sheep in the first 10–14 days after FCA injection (ANZZCART 1998). Recent commentary suggests that, when the proper protocols are used in preparing and administering FCA/antigen (see below), its use in polyclonal antibody production may not be as problematic from an animal wellbeing perspective as previously thought (HSUS 2005).

The route of administration also affects the type of complications that can occur. Although both the intravenous and the intraperitoneal routes enable rapid distribution of antigen, both carry a risk of anaphylactic shock with booster injections, and the choice of suitable adjuvants is limited—oil adjuvants induce peritonitis and are contraindicated for intravenous use. Absorption is rapid when the intramuscular route is used, as the muscle is well vascularised, but oil adjuvants will act as a depot, with inflammation of the surrounding muscle and possible abscess formation that can spread along interfascial planes and may cause nerve damage. The location of the depot within a muscle may lead to some of these complications being overlooked until they are well advanced. The subcutaneous route allows the inflammatory process to be monitored, although absorption of the antigen is slow. Intradermal administration results in efficient processing of the antigen, but the use of oil adjuvants can lead to ulceration. Foot-pad injections of antigen/adjuvant are obviously an area of special concern. See also the ‘Blood collection’ factsheet.

HOW ARE PAIN AND DISTRESS MEASURED?

In addition to the usual criteria for monitoring animal wellbeing, when animals are being used for polyclonal antibody production, special attention needs to be paid to inflammation, abscess formation or ulceration of the injection site.
**HOW ARE PAIN AND DISTRESS MINIMISED?**

The key strategies to minimise the impact of production procedures on animal wellbeing are:

- ensuring that the ratio of antigen to oil adjuvant does not exceed 1:1, to reduce the probability of adverse reactions
- aseptic preparation, which is essential to avoid the risk of abscess formation
- choosing the adjuvant that will provide adequate antibody titre with minimum side effects—a pilot study may assist in this decision
- choosing the site for injection of the antigen/adjuvant mixture that will optimise antibody production and minimise the risk of complications
- choosing the species that will provide adequate amounts of antibody with the least impact on the animals’ wellbeing—for example, depending on the volume of the antibody required, using a large animal such as a sheep or horse so as to use fewer animals or using oral immunisation of chickens to harvest antibodies from eggs (Hau and Henriksen 2005).

From various guidelines, the consensus is that subcutaneous and intradermal are the routes of choice for the primary injection in rabbits (CCAC 2002, Leenaars et al 2002, HSUS 2005), and that the impact of immunisation is reduced by using multiple sites with a reduced volume at each site. Subcutaneous and intramuscular injections are recommended for other species (Table J1). However, only the muscle in one leg should be used in small animals.

Oil adjuvants should not be used for intramuscular injection in rodents, because they can cause painful and potentially fatal emboli. The intravenous or intraperitoneal routes for oil adjuvants should also not be used. In those special cases where a foot-pad injection is justified on scientific grounds, only one foot should be injected, and special nursing is required. Foot-pad injection should not be given to rabbits.

<table>
<thead>
<tr>
<th>Table J1</th>
<th>Maximum volumes for injection of antigen with depot adjuvant for different animal species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td><strong>Maximum volume per site</strong></td>
</tr>
<tr>
<td>Mouse</td>
<td>100 µL</td>
</tr>
<tr>
<td></td>
<td>50 µL</td>
</tr>
<tr>
<td>Guinea pig, rat</td>
<td>200 µL</td>
</tr>
<tr>
<td>Rabbit</td>
<td>250 µL</td>
</tr>
<tr>
<td></td>
<td>25 µL</td>
</tr>
<tr>
<td>Sheep, goat</td>
<td>500 µL (250 µL per site if multiple sites)</td>
</tr>
<tr>
<td>Chicken</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

ID = intradermal; IM = intramuscular; SC = subcutaneous


Future advances in methods to administer antigens via the oral or intranasal route and the use of chickens to harvest antibodies from egg yolks are likely to provide opportunities to refine methods of antibody production (HSUS 2005).
REFERENCES


K  SURGICAL PROCEDURES

WHAT IS A SURGICAL PROCEDURE?

A surgical procedure is one that requires the incision of living tissue. In the scientific setting, the type of procedure will depend upon the scientific purpose but can range from a superficial cutdown to the penetration and exposure of a body cavity or extensive tissue dissection.

WHEN ARE SURGICAL PROCEDURES USED?

Surgical procedures are performed for a variety of reasons but, most often, to:

- collect tissues as biopsy material for laboratory analysis
- insert chronic vascular catheters to
  - enable blood collection from animals; for example, when multiple samples are required over a period of days or weeks, or where there is limited access to superficial vessels, or the protocol requires sampling specific organs or sites
  - monitor arterial or venous blood pressure
  - infuse substances either over a period of time or to target specific sites
- implant catheters or devices to collect other body fluids such as bile, pancreatic secretions, lymph, cerebrospinal fluid or gastrointestinal contents, or to administer substances to a specific site
- implant electrodes to record or stimulate specific sites in neurophysiological studies
- implant devices, such as telemetry probes for chronic physiological and behavioural monitoring or miniature pumps to deliver substances at a predetermined rate over a period of time
- transplant organs either in the same (autograph) or different (xenograph) species at the same (orthotopic) or different (heterotopic) site in the recipient animal
- create an experimental model to study a physiological or pathological process, eg models of coronary ischemia or nerve injury
- assess the safety and efficacy of devices for implantation into humans, eg heart valves, ventricular assist devices, orthopaedic implants
- develop and evaluate novel surgical techniques for human use.

Surgical procedures are also used in professional training. Such activities are covered by specific guidelines published by the NHMRC and the NSW Animal Research Review Panel (see below).

WHAT IS INVOLVED?

In the research setting, surgical procedures may involve a novel technique or the adaptation of methods, most often from human surgery, for use in laboratory animals. Except in studies pertaining to veterinary medicine, the kind of surgical procedures performed are not related to the normal care and husbandry of the species. Consequently, the notion of 'experimental surgery' recognises these differences and the special circumstances involved, which may, for example, require the adaptation of techniques to meet species differences in anatomy and physiology, or the modification of surgical instruments and monitoring equipment. Most notably, equipment and techniques have been adapted from methods used in humans for use in the rat and, with the development of transgenic species, are being adapted for use in the mouse (see, for example, Messier et al 1999).

There is a significant body of literature that describes the general principles involved in experimental surgery (for example, Lumley et al 1990), as well as publications that detail surgical
techniques in specific species or particular surgical procedures, such as the implantation of catheters or the development of animal models. (Please refer to Further reading at the end of this section for additional information.)

Any surgical procedure must be performed using anaesthetic techniques that are appropriate for the type of procedure and the species involved. Depending on the aims of the study, at the completion of the surgical procedure, animals are either allowed to regain consciousness (recover) or not (non-recovery). In the latter case, the animal must be euthanased at the end of the procedure without regaining consciousness.

When an animal is to recover from a surgical procedure, as in veterinary or human medicine, special precautions are taken to ensure that the risk of complications, such as postoperative pain or infection, are minimised. In the veterinary setting, surgical procedures involving farm animals such as sheep and pigs are limited, and the level of risk is low. However, when these species are used as models of the human situation, standards used in human medicine are applied.

There are situations in agricultural and wildlife studies where procedures have to be performed in the field. In these circumstances, some compromise may be needed, which necessitates careful consideration by the investigator and the animal ethics committee (AEC) to balance the level of risk to the animal against the justification for the procedure to be conducted in these circumstances.

WHAT ARE THE ESSENTIAL ANIMAL WELFARE ISSUES TO CONSIDER?

The nature of surgical procedures places the wellbeing of an animal at significant risk, most often associated with one or more of the following:

1. Inadequate pain management can be a problem either during or after a procedure.

2. Complications can occur during or immediately following surgery, in particular
   - blood loss due to tissue trauma or inadequate control of bleeding resulting in compromised tissue perfusion and oxygenation and, if severe, leading to cardiovascular collapse
   - dehydration due to uncompensated fluid loss during surgery, which will be exacerbated by the exposure and drying of tissues, restricted fluid intake prior to surgery and reduced voluntary intake in the postoperative period
   - hypothermia due to the impairment of thermoregulation by anaesthetic agents (Benazon 1974), which is a major risk in small rodents that have a large surface area relative to body mass and a high metabolic rate (Flecknell 1996); this is a common cause of mortality in the peri-operative period and is associated with prolonged anaesthetic recovery, impaired cardiovascular function (Waynforth and Flecknell 1992) and impaired immune function (Ben-Eliyahu et al 1999)
   - hypoxia and poor tissue perfusion (i) as a consequence of decreased blood volume, dehydration, acid-base imbalance or hypothermia or (ii) associated with inadequate respiratory function, either as a side effect of anaesthetic agents, or airway obstruction, or poor body positioning that may obstruct the trachea or restrict respiratory movement
   - metabolic disturbances due to activation of the hypothalamic-pituitary-adrenal (HPA) axis and associated changes in cellular function, with altered glucose and protein metabolism resulting in hyperglycaemia and negative nitrogen balance, which, even following minor procedures, can last for several days; fluctuations or instability in cardiovascular and respiratory function resulting in acid-base and electrolyte imbalances with accompanying metabolic disturbances will further exacerbate the effects of poor tissue perfusion and delay recovery
   - cardiovascular and/or respiratory failure, which are risks during surgical procedures and in the immediate postoperative period, not only due to the potential complications listed above, but also because many anaesthetic agents have significant and specific depressive effects on both systems--a risk that is exacerbated by poor management of the anaesthetic dose.
3. Postoperative infections can include infection and breakdown of the wound caused by a failure in aseptic techniques; or result from excessive tissue trauma, poor haemostasis, implanted devices, electrodes or catheters, all of which can be a nidus for infection. Hypothermia (Ben-Eliyahu et al 1999) and anaesthetic agents (Lockwood et al 1993) modulate the immune response and increase infection risk following surgical procedures.

4. Delayed postoperative recovery can result from anaesthetic overdose or the prolongation of the effects of the anaesthetic agents, and be associated with impaired organ function and drug metabolism caused by poor tissue perfusion and hypoxia.

5. Delayed wound healing or breakdown can result from one or more of the following
   - infection
   - poor tissue viability associated with poor tissue perfusion or excessive tissue damage caused by (i) poor tissue handling, (ii) failure to maintain adequate blood supply, or (iii) dehydration of tissues during surgery
   - poor apposition of organs or tissues during closure
   - poor choice of suture materials and/or method, which impairs tissue perfusion and may result in poor apposition of tissues and a greater risk of the animal accidentally removing the sutures (note: inflammation of the wound site will increase this risk)
   - impaired healing due to suppressed immune function, either as part of a deliberate intervention (for example, when an animal is immunosuppressed following organ transplantation or has suppressed immune function due to genetic selection or manipulation) or associated with peri-operative complications such as hypothermia.

6. Complications with implanted catheters or devices are most often are due to
   - (i) the development of an infection either at the site of the implant where the catheter or device is the source of a systemic infection, or (ii) a skin infection developing at the point of exit of a catheter or lead, which may result in a systemic infection by tracking down the catheter or device or a subcutaneous tunnel, or (iii) the systemic introduction of a pathogen during the flushing of catheters
   - the leakage of gastrointestinal contents around an external fistula causing suppuration of the surrounding skin
   - catheters, electrodes or implanted devices being dislodged by the animal, or its cage-mates, resulting in major haemorrhage, tissue trauma, contamination of the abdominal cavity by gastrointestinal contents or secretions, septicaemia and, possibly, death due to haemorrhagic or septic shock
   - leakage of gastrointestinal contents, pancreatic secretions or bile into the abdominal cavity causing peritonitis
   - failure of vascular catheters due to thrombosis or infection
   - damage to organs, such as the kidney, due to infarction by thrombi released from the implant
   - blockage or infection of biliary or pancreatic catheters, which, due to the nature of the secretions, results in cholecystitis and impaired liver function or acute pancreatitis
   - the size, weight or site of implantation of catheters and devices impacting on an animal’s normal activities and, when implanted in body cavities, impacting on the function of vital organs.

7. Consequences of specific surgical procedures will be determined by each procedure. For example, in the case of transplantation, there is an increased risk of infection due to immunosuppression and significant consequences if there is failure of the transplanted organ. When surgical techniques are used to induce specific conditions, the expected consequences will be determined by that condition; for example, acute liver failure, cardiac ischemia or renal hypertension.
8. Social isolation may be required during recovery from anaesthesia to prevent aggression from other members of a social group. However, in some cases, ongoing isolation may be required to prevent damage to the surgical site/catheter/instrumentation or implants.

HOW ARE PAIN AND DISTRESS MEASURED?

Detailed descriptions of the identification of pain behaviours, species-specific responses and methods to measure pain have been discussed elsewhere in this document (see Section 2.4).

Following a surgical procedure, the pattern of behaviours that indicate an animal is experiencing pain will depend on the species, the degree of tissue trauma associated with a particular surgical procedure and the surgical site. There are reports describing postsurgical pain behaviours in a range of species following different surgical procedures; for example, laparotomy in the rat (Roughan and Flecknell 2003) and castration in lambs (Thornton and Waterman-Pearson 1999). Also, a number of papers report the development of a ‘pain scale’, based on behavioural and clinical criteria, to assist in the assessment of postoperative pain. Stasiak and colleagues (2003) have recently published a review of pain scales and describe pain scales that they have developed and evaluated for use in orthopaedic surgery in a range of species, including sheep, birds and rabbits.

Postoperative infections also lead to sickness behaviour, which is an adaptive response to infection associated with activation of the cytokine system (Dantzer 2001, Johnson 2002). Animals show depressed motor activity, reduced food and water intake, decreased exploration of their physical and social environment, impaired memory and cognitive abilities and, particularly in young animals, decreased social interactions.

HOW ARE PAIN AND DISTRESS MINIMISED?

General principles

The potential risk to the wellbeing of the animal from any surgical procedure and the consequent issues that need to be addressed are recognised in the Australian code of practice for the care and use of animals for scientific purposes (the Code), which includes special sections on anaesthesia and surgery (3.3.25–32), postoperative care (3.3.33–38), implanted devices (3.3.39) and organ and tissue transplantation (3.3.40). The Code emphasises the importance of:

- the development and revision of pain management plans
- selecting anaesthetic and analgesic agents appropriate to the species and the procedure
- monitoring the depth of anaesthesia and management of the side effects of anaesthesia
- using aseptic procedures in all recovery procedures
- the competence of those involved in all aspects of the process, especially in the administration and monitoring of the anaesthetic and performance of the surgical procedures.

Personnel involved

A 1993 report by the Council on Research of the AVMA (Brown et al 1993) discusses the elements that are important to achieve a successful outcome when surgery is conducted for scientific purposes. This report highlights the central importance of the skills and knowledge of those involved and ‘the need for them to be familiar with the anatomy, physiology, pharmacology, anaesthesia and basic care of the species undergoing surgery’. This report also emphasises the importance of surgical technique to maintain tissue viability by good tissue-handling techniques, effective haemostasis, maintenance of blood supply to tissues, asepsis, accurate tissue apposition, proper use of surgical instruments and equipment and expeditious performance. Appropriate handling of tissues helps reduce postsurgical pain and, together with effective haemostasis, reduces the risk of postoperative infections.
Experimental surgery will often involve the use of novel techniques or will adapt surgical methods that have been used in another species. In these circumstances, when the surgeon is not familiar with the procedure in a particular species, or with the anatomical approach or the feasibility of the proposed new or novel procedure, to minimise surgical complications and to develop and review postoperative management strategies, the following steps are proposed:

1. perform an anatomical dissection using cadaver specimens to become familiar with the anatomical landmarks, to evaluate the feasibility of the proposed procedure and the optimal surgical approach, and to identify surgical risks

2. perform the surgery as a non-recovery procedure in a sufficient number of animals to be confident of being able to manage the animal through the recovery stage; this step will also enable an evaluation of the anaesthetic technique and supportive therapies that will best maintain physiologic stability during the surgical procedures

3. develop a postoperative management plan based on the predicted consequences and risks

4. conduct a pilot study that allows for the recovery of a limited number of animals

5. review and revise surgical and anaesthetic procedures, and pain and postoperative management plans.

Attending a course and working with an experienced mentor are recommended for people who need to acquire skills in basic surgical techniques. Simulation models can be used to practise suture techniques and placement of catheters. Several CD programs, listed below, are a helpful introduction to the general principles involved in good surgical practice.

Effective pain management

For any surgical procedure, pain management involves effective pain control both during and after the procedure.

The choice of suitable anaesthetic and analgesic techniques can significantly reduce the incidence of postoperative complications (Bonnet and Marret 2005). Due to the variety of procedures performed in a range of species, the importance of choosing the anaesthetic and analgesic agents that are appropriate for the procedure and the species cannot be overemphasised. It is important to recognise, when translating a surgical technique from one species to another, that species differences in drug responses and effects mean that the methods for anaesthesia and pain management that are appropriate for one species may not be so in another. A detailed discussion of the use of anaesthetic and analgesic drugs is provided in the ‘Pain management’ factsheet of this document.

An understanding of the neurological responses to injury is important in the development of an effective pain management plan for surgical procedures. Unlike the treatment of pain in many other circumstances, the surgical procedure initiates the pain response, meaning that it is possible to control the development of that response de novo. We now have a better understanding of the plasticity of the neurological responses to pain and of how the initial response translates into changes in neurotransmitters in the spinal cord that can sustain the sensation of, and sensitivity to, pain beyond the period of the injury. Consequently, one approach to pain management in surgical procedures is to block the transmission of the initial pain stimulus (pre-emptive analgesia) (for detailed discussion, see Flecknell and Waterman-Pearson 2000). Although widely practised in both human and veterinary medicine, a recent report to the NHMRC by the Australian Society of Anaesthetists has questioned the efficacy of this strategy.

There is a substantial body of literature on the pharmacology of analgesic agents in laboratory species, including the rat, mouse, guinea pig, rabbit, pig and sheep, but very few have critically evaluated the efficacy of analgesics in the management of postoperative pain following...

It is important that investigators and members of AECs appreciate that there is no simple prescriptive method for effective pain management following surgical procedures in the research setting; protocols need to be customised for the particular circumstances and must be subject to ongoing evaluation. This is a rapidly evolving area, and investigators should refer to the literature to keep informed of new developments.

Aseptic technique

Aseptic surgery has been defined as ‘surgery performed in ways and by means sufficiently free from micro-organisms so that appreciable infection or suppuration does not develop’ (Brown et al 1993). The standard methods to achieve this are described in detail in surgical texts (eg Lumley et al 1990), and the importance of these methods to the successful outcome of surgical procedures in the research setting is emphasised in guidelines such as those produced by the AVMA (Brown et al 1993) and LASA/UFAW (1989).

The Code is unequivocal: aseptic procedures must be used when it is intended to recover an animal from a surgical procedure (3.3.29).

For some years, there was a view that aseptic procedures were not necessary when recovery procedures were performed on rodents. However, the report by Popp and Brennan (1981) challenged this notion, as have more recent publications such as that by Bradfield et al (1992); the case for aseptic surgery in rodents is well argued by Cunliffe-Beamer (1993). However, in a recent literature review, Cooper et al (2000) concluded that good surgical technique was as important as asepsis in the prevention of postsurgical infections in rodents.

Specific guidelines on the use of aseptic techniques in rodents have been published by the NIH (2005). The comprehensive, online publication by Brown and Hoogstraten-Miller (2004), which incorporates a training CD developed by the NIH, is an excellent resource for the practical application of these methods for rodent survival surgery.

Although, in general, the use of aseptic procedures is not necessary for non-recovery procedures, in light of the length of procedures in many neurological studies, the recent report by the National Academies of Science (National Research Council 2003) has recommended aseptic procedures should be considered in these kind of studies.

The elements of aseptic technique involve:

- the conduct of surgical procedures in a designated area that has been disinfected—for large animals, this will usually require a dedicated facility, but for small rodents it may be a designated work area
- the preparation of the surgical site to minimise the risk of entry of bacteria into the wound—this will usually involve removal of hair, fur or wool in the immediate vicinity of the intended surgical wound and cleaning and disinfecting that area
- the surgeon and others in the vicinity of the operative field wearing protective clothing, masks and head cover
- the surgeon and surgical assistants performing a surgical scrub and using sterile surgical gowns and gloves (gloves only may be used in rodent and field surgery)
- the surgical site being draped with sterile drapes to create a sterile ‘field’ around the site; a double drape method is used for major surgery of the abdominal or thoracic cavity or when surgery of the viscera is involved
- sterile instruments and packs being used
only sterile instruments, drapes, packs and gloves coming into contact with the surgical site
sterile surfaces kept dry to avoid moisture contaminating the surgical area.

An appendix to the NIH guideline (2005) gives a comprehensive guide to the appropriate disinfectants and sterilisation methods for use in aseptic surgery.

Prevention and management of peri-operative complications

In any procedure involving the administration of an anaesthetic, with or without surgery, the clinical fitness of the animal is a major determinant of an uneventful recovery. In circumstances involving perturbations to physiological and metabolic homeostasis, pre-existing clinical or subclinical disease can undermine effective management strategies.

The clinical health of all animals should be checked some days before surgery is planned; special attention should be given to signs of compromised respiratory or cardiovascular function or of intercurrent infection. Also, where the procedures are likely to compromise their ability to respond to infections (e.g., immunosuppression), animals should be screened for subclinical infections.

The effects of transportation, and the introduction of animals to new facilities, social groups and personnel, on the stress response (with attendant physiological, biochemical and behavioural changes) are well documented (van Ruiven et al 1996). Surgical stress will exacerbate these changes, and will not only impair the animal’s ability to maintain homeostasis during the surgical procedure, but will increase the risk of postsurgical infections by compromising immune function. A period of acclimatisation should be allowed, to ensure the animal has recovered from these stressors before surgery is scheduled. This time will vary with the circumstances, but is usually a minimum of 10–14 days for laboratory-bred animals and can be some weeks for farm species.

Table K1, at the end of this factsheet, summarises the major risks in the peri-operative period, the possible causes of each and remedial actions. The range of complications is broad, and the risks will be determined by the procedures undertaken, the competence of the person administering and managing the anaesthetic and the skills and competence of the surgeon. However, as indicated in the table, a number of risk factors can act in concert to influence complications.

As a general rule, the key strategies to prevent or manage these complications are:
- effective management of anaesthesia to ensure an adequate plane of anaesthesia with minimal physiological disturbance
- effective pain management during and after surgery
- effective aseptic procedures
- good surgical technique to ensure tissue viability
- supporting maintenance of body temperature and cardiovascular and respiratory function
- providing fluid and nutritional support to maintain metabolic homeostasis
- prevention of postsurgical infection
- effective postsurgical care to promote rapid return to physiological homeostasis and tissue repair.

Particularly in rodents and young animals, prevention of hypothermia can be a major determinant of a successful outcome. Rembert et al (2004) have recently reviewed methods used to support thermal homeostasis in rodents. Prevention of dehydration in these species is also important; as demonstrated by Hampshire et al (2001), fluid therapy promotes recovery and a more rapid return to homeostasis.
Control of postoperative infections

Anaesthesia and surgery will modulate the immune response (Salo 1992). Aseptic procedures and good surgical technique are critical to minimising the risk of postoperative infections. As a general guide, prophylactic use of antibiotics is not recommended (Brown et al 1993, Morris 1995), and they should be reserved for circumstances where there has been a likely breakdown in aseptic technique. When antibiotic therapy is indicated, care should be taken to choose the appropriate agent and, particularly where treatment is ineffective or infection is an ongoing problem in a procedure or in a facility, bacterial cultures and sensitivity testing should be performed (see Morris 1995).

Implanted catheters or devices present a major risk to the development of postoperative infections. This risk is greater when catheters or electrodes are externalised through a skin wound, but can also be caused by:

- inadequate sterilisation of the implant
- excessive tissue trauma
- inadequate positioning of the device
- excessive tissue reaction due to poor biocompatibility of the implant materials.

Some materials used in implants may be more susceptible to the development of infections than others (see, for example, Sherertz et al 1995). Data from the manufacturer may be helpful in identifying and preventing the risk of infection with an implanted device.

Every time IV catheters are flushed, there is a risk of introducing an infection. Studies measuring bacterial colonisation of vascular catheters in rats and pigs have shown an infection rate of 67% within 7 days of implantation (Sampath et al 2001). Once introduced, bacteria colonise the surface of the catheter and become a source for ongoing infection; trauma to the blood vessel caused by catheter tips can be another source of ongoing infection. Careful attention to catheter placement and aseptic methods will reduce these risks; impregnation of the catheter lumen with antiseptic, such as chlorhexidine, can be used to reduce bacterial colonisation of the catheter wall (Sampath et al 2001). Particularly where these procedures are common, development of a management plan to prevent infections in vascular catheters (see Pearson 1996) would be worthwhile.

One approach to reduce the incidence of infections associated with vascular catheters, especially in large animals, has been the use of subcutaneous vascular access ports (VAP) (see Swindle et al 2005 for detailed review). Chuang et al (2005) showed a significant reduction in morbidity using VAPs. These ports have also been used in rats (Paulose and Dakshinamurti 1987).

The other major issue when using chronic vascular catheters is the development of thromboemboli at the catheter tip. The thrombogenicity of the catheter material and injury to the vessel wall in the catheter placement will predispose towards thrombus development and thus limit the usefulness of the catheter. Thrombi may become infected and so be an ongoing source of infection, or, by shedding microemboli, can cause damage to tissues and organs (Danneman et al 1988). Maintenance of the catheter by regular flushing, and closure with a heparin/saline ‘plug’, will reduce the incidence of thrombus development. Treatment to reduce the thrombogenicity of the catheter surface, for example, by bonding heparin to the catheter material, will also decrease thrombus development (Freeman et al 1990).

Finally, it is important to protect any external catheters or devices from accidental removal. Restricting the animal’s ability to move around is one option and may be necessary when the catheters and leads need to be connected to equipment. However, in some circumstances, for example, a chronic infusion, it may be possible to secure the catheter in a way that allows the animal to move around (see Gay 1986). In most situations, external catheters, when not in use, can be protected under a dressing, wrap or jacket that will allow the animal normal movement. However, it is important to protect the dressing from protruding sharp objects in the cage or from being chewed by other animals.
SCIENTIFIC ISSUES

The primary scientific goal following any surgical procedure is for the animal to recover with minimal disturbance to its physiological state, except where a defined pathology is intended. In either circumstance, minimising the complications from the procedure will promote this outcome.

The selection of anaesthetic and analgesic agents in terms of their possible effects on data collection is an important consideration (Thurmon and Benson 1987, Heavner 1994, Bazin et al 2004). The potential confounding effects of analgesic agents on animal models have been a matter for debate. Of note are two recent publications that have validated the efficacy of postoperative analgesics without affecting the development of models of neuropathic pain (Stewart and Martin 2003) or renal ischemia (Deng et al 2000).

It also is important to validate the time required for the animals to recover from a surgical procedure and achieve physiological homeostasis and, when procedures involve chronic implants, whether the implant confounds data collection. Studies following abdominal surgery or vascular catheterisation in rats have indicated 3–5 days should be allowed following these procedures (Fagin et al 1983, Heindorff et al 1990), whereas Tornatzky and Miczek (1993) reported that it took 10–14 days to return to physiological baseline following telemetry implants.

Implantation of catheters and devices can have further subtle or unforeseen effects on data collection. For example, drugs may bind to the catheter material and thus affect pharmacokinetic data (Chindavijak et al 1988); implantation of mini-osmotic pumps is associated with altered thyroid function in rats (Wyatt et al 1995), and transient elevation of corticosteroids in mice but without changes to their immune response (Rowland et al 1990); and telemetry implants have long-term effects on autonomic responses in rats (Einstein et al 2004). These studies draw attention to the potential for implanted devices to confound data and the need to validate such effects when used in particular studies.

At the completion of a study involving experimental surgery, a postmortem examination should be undertaken to identify any surgical complications and to validate the position and patency of catheters and electrodes. This is important to monitor and review procedures and identify opportunities to modify techniques.

CONCLUSIONS

Steps that reduce or minimise the magnitude and duration of metabolic perturbations associated with surgical stress and postoperative complications support scientific and animal welfare goals and promote the principles of Refinement and Reduction. A summary of strategies to minimise risks to animal wellbeing is listed in Table K1.

The complexity and range of issues involved in surgical procedures require careful evaluation to identify risks, to develop strategies to minimise or manage those risks and to develop a pain management plan. A pilot study may be necessary to inform this process. Planning should also include an assessment of the availability and suitability of facilities and equipment, and of the skills, knowledge and experience of the people involved. Once a management plan has been formulated, ongoing review will identify opportunities to refine methods and procedures.
### Table K1  Minimising surgical risks to animal wellbeing

<table>
<thead>
<tr>
<th>Major risks</th>
<th>Possible causes</th>
<th>Remedial actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>Choice of anaesthetics or analgesics</td>
<td>- Select agents that are appropriate for the species and type of procedure</td>
</tr>
</tbody>
</table>
|             | Inadequate pain management | - Develop a pain management plan  
|             |                              | - Monitor and assess efficacy |
| Poor blood flow (poor tissue perfusion) | Blood loss | - Use good surgical technique and management of haemostasis  
|             |                              | - Monitor heart rate and capillary fill  
|             |                              | - Maintain circulating blood volume with blood or replacement fluids  
|             |                              | - Replace blood loss > 10% of circulating volume |
| Anaesthetic overdose—cardiac depression | | - Closely monitor depth of anaesthesia, cardiovascular function and tissue perfusion, and adjust anaesthetic dose  
|             |                              | - Administer cardiac stimulants if necessary |
| Hypothermia | | - See ‘Hypothermia’ below |
| Dehydration | | - Maintain hydration with IV or SC warm physiological solution (eg Ringer’s lactate solution or 4% dextrose saline--intra-operatively 10 mL/kg/hr; daily maintenance 40–80 mL/kg/24hr)  
|             |                              | - Keep exposed tissues moist with warm, saline-soaked swabs |
| Acid-base and electrolyte imbalance | | - See ‘Metabolic disturbances’ below |
| Poor oxygenation | Hypoxia due to respiratory depression caused by anaesthetic overdose | - Monitor depth of anaesthetic, respiratory rate and colour of mucous membranes  
|             |                              | - Administer oxygen  
|             |                              | - Provide mechanical respiratory support  
|             |                              | - Reduce anaesthetic dose  
|             |                              | - Administer respiratory stimulant  
|             |                              | - If surgery completed, administer agent to reverse anaesthetic, if applicable |
| Hypoxia due to airway obstruction | | - Check patency of airway  
|             |                              | - Remove any mechanical obstruction, eg excess mucus, blood or foreign body  
|             |                              | - Check body position to ensure respiratory movement is not restricted or airway obstructed |
| Tissue hypoxia linked to poor tissue perfusion | | - See ‘Poor blood flow’ above |
| Hypothermia | Anaesthetic agents | - Monitor body temperature during surgery |
| Exposure of body cavity or tissues to cold room air (major problem in animals with high surface area to bodyweight ratio and/or high metabolic rate) | | - Limit exposure to cold surfaces esp. when anaesthetised; lie animal on insulating material (eg bubble wrap) and provide heating source (eg warming blanket during surgery and recovery)  
|             |                              | - Keep exposed tissues warm and moist  
|             |                              | - Place animals in a warm environment during recovery |
| Use of cold parenteral fluids | | - Administer warm fluids |
| Inspiration of cool air/gases | | - Humidify and warm inspired air if possible |
## Part III  Surgical procedures

<table>
<thead>
<tr>
<th>Major risks</th>
<th>Possible causes</th>
<th>Remedial actions</th>
</tr>
</thead>
</table>
| Metabolic disturbances     | Surgical stress activates the HPA axis, resulting in glycogenolysis and hyperglycaemia, and affects protein metabolism, leading to negative nitrogen balance | - Acclimatise animals to facilities and personnel to reduce activation of stress response  
- Monitor and manage factors likely to exacerbate surgical stress response, especially tissue damage and pain  
- Provide nutritional support to minimise glycolysis during surgery and postoperative recovery  
- Monitor water and food intake post surgery  
- Promote metabolic homeostasis by maintaining normothermia and adequate tissue perfusion and oxygenation  
- Minimise tissue ischemia during surgery  
- Minimise blood loss and dehydration  
- Monitor and correct acid–base and electrolyte imbalances during surgery and recovery |
| Poor recovery              | Delayed recovery from anaesthetic due to overdose, or impaired drug metabolism associated with hypothermia, reduced tissue perfusion and impaired organ function | - Monitor depth of anaesthesia to avoid overdose  
- Monitor and manage potential complications from anaesthesia, especially body temperature and cardiovascular and respiratory function |
| Hypothermia                 | - See ‘Hypothermia’ above                                                                                                                  |                                                                                                                                                 |
| Poor tissue perfusion       | - See ‘Poor blood flow’ above                                                                                                               |                                                                                                                                                 |
| Poor oxygenation           | - See ‘Poor oxygenation’ above                                                                                                              |                                                                                                                                                 |
| Dehydration                 | - See ‘Dehydration’ above; also need to ensure adequate hydration in postoperative period—monitor if parenteral fluids needed                   |                                                                                                                                                 |
| Postoperative infection     | - See ‘Postoperative infection’ below                                                                                                        |                                                                                                                                                 |
| Wound failure              | - See ‘Wound failure’ below                                                                                                                 |                                                                                                                                                 |
| Self trauma                 | - Choose an anaesthetic with smoother recovery properties  
- Improve monitoring and provide appropriate pain relief  
- Improve housing                                                                 |                                                                                                                                                 |
| Social stress               | - Ensure auditory, visual and olfactory contact with other animals                                                                               |                                                                                                                                                 |
| Postoperative infection     | Breakdown in aseptic technique                                                                                                                | - Review and revise procedures and compare with published standards  
- Ensure sterilisation of catheters and devices is validated                                                                 |
| Poor surgical technique     | - Review and revise procedures; implement training if necessary                                                                             |                                                                                                                                                 |
| Peri-operative hypothermia  | - See ‘Hypothermia’ above                                                                                                                   |                                                                                                                                                 |
| Poor tissue perfusion       | - See ‘Poor blood flow’ above                                                                                                                |                                                                                                                                                 |
| Implanted catheter or device or exit site infected | - Effective sterilisation of implant; aseptic procedures during catheter maintenance                                                          |                                                                                                                                                 |
| Pre-existing disease        | - Clinical screening prior to surgery                                                                                                         |                                                                                                                                                 |
### Part III Surgical procedures

<table>
<thead>
<tr>
<th>Major risks</th>
<th>Possible causes</th>
<th>Remedial actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound failure</td>
<td>Poor surgical technique</td>
<td>• Review procedures to ensure gentle tissue handling, effective haemostasis, maintenance of tissue perfusion and correct methods and materials for wound closure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure aseptic techniques are used for any recovery procedures or in the maintenance of intravascular catheters</td>
</tr>
<tr>
<td>Postoperative infection</td>
<td></td>
<td>• See ‘Postoperative infection’ above</td>
</tr>
</tbody>
</table>

HPA = hypothalamic-pituitary-adrenal axis; IV = intravenous; SC = subcutaneous

### Further reading

Guidelines


Recommended texts


Education resources

DASIE (Dog Abdominal Surrogate for Instructional Exercises): A laminated fabric and polyurethane model designed and constructed to resist cutting, and to hold sutures in a manner similar to normal tissues. Used for practising aseptic technique, instrument handling, suturing and ligation. Available from DASIE International. Email: dasieinternational@hotmail.com

Digital Material for Trainers: A series of 12 digital video CDs covering handling, procedures, anaesthesia and surgery for common laboratory animals. Three CDs include interactive course notes. Purchasing information available at: http://www.digires.co.uk/index.html


Principles of Surgery: This website from the University of Pennsylvania School of Veterinary Medicine includes videos of suture patterns and techniques. http://calvet.upenn.edu/projects/surgery/index.htm

Training in Basic Biotechnology for Laboratory Mice: National Human Genome Research Institute, Office of Laboratory Animal Medicine, National Institutes of Health. Request for copies should be sent to: rodent-cd@mail.nih.gov

Training in Survival Rodent Surgery: A CD-ROM that has been developed by the NIH Animal Research Advisory Committee to assist in the development of proper surgical skills. It has three elements: simple suture patterns, rodent survival surgery and special considerations for aseptic surgery in transgenic mice. Copies can be requested by emailing: rodents-cd@oc.nih.gov

REFERENCES


Wyatt I, Coutts CT, Foster PM, Davies DT and Elocme CR (1995). The effect of implantation of osmotic pumps on rat thyroid hormone and testosterone levels in the plasma, an implication for tissue ‘S’ phase studies. Toxicology 95:51-54.
TOXICOLOGY

WHAT IS TOXICOLOGY?

Toxicology is the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such effects.

WHY DO TOXICOLOGY TESTING?

Toxicology testing can identify potential adverse health effects or demonstrate the safety of new chemicals and products, thereby providing the basis for safeguarding human and animal health (Stokes 2000). Such testing is increasingly important for risk analysis. Ecotoxicology testing may be required by legislation to characterise hazards and for environmental risk assessment (APVMA 2005).

Regulatory authorities need to balance concerns for animal wellbeing with the need to obtain toxicological information. Toxicology is an increasingly international field that includes a wide variety of organisations concerned with the development and validation of alternative tests (Evans 2000).

EXAMPLES OF TOXICOLOGICAL STUDY METHODS

There are many types of toxicology studies. Some examples are listed here:

- acute toxicity—the toxicity produced by a test substance when administered in one or more doses not exceeding 24 hours
- subchronic toxicity—the test substance is administered daily at a single high dose level for at least 90 days
- chronic toxicity—the test substance is administered for at least 6 months in rodents, with variable requirements in other species
- irritation—the evaluation of substances that produce irritation, for example, to the eye or skin
- carcinogenicity studies—the evaluation of a substance’s potential to cause cancer
- teratology studies—the determination of a substance’s potential to cause abnormal development and the production of congenital anomalies
- gene toxicity (mutagenicity)—testing the ability of a substance to induce genetic mutation
- ecotoxicology studies—testing the risk of substances to the ecosystem.

WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

The essential animal wellbeing considerations are to:

- consider using alternatives to animals
- minimise unnecessary use of animals by using a statistically designed study that addresses the regulatory requirements
- minimise pain and distress when animals are essential to the study.

Consider the use of alternatives to animals

There is ample scope for application of the 3Rs (Replacement, Reduction and Refinement) in the use of animals in toxicology studies. Increasingly, validated alternatives to sentient animals are being used in toxicity testing. Validation is defined as the process by which the reliability and relevance of a procedure are established for a particular purpose (Fentem and Balls 1997).
In Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) website states that studies involving animals should be conducted with the minimum number of animals necessary to allow valid conclusions to be drawn. Applicants are encouraged to submit data obtained from in vitro assay systems, or from alternative methods that use fewer animals, as a means of facilitating validation of alternative methodologies and reducing the number of animals used in toxicity testing (APVMA 2005).

To find possible alternatives, investigators should refer to relevant databases before starting toxicology studies. Roi (2005) discusses the European Centre for the Validation of Alternative Methods (ECVAM) Data Base service on Alternative Methods (DB-ALM). DB-ALM is based on extensive literature reviews, including ECVAM in-house information, and provides its information as evaluated data sheets. The current online version refers to 3000 registered users from 65 countries.

A number of websites may also be useful. In 1997, the Johns Hopkins Center for Alternatives to Animal Testing (CAAT) launched Altweb, the Alternatives to Animal Testing website. Altweb was created to serve as an international central reference point for information, resources and news about alternatives, and is freely available to all users (Howard 2005). Langley (2000) felt that alternatives in toxicology had evolved rapidly in the previous decade and that the process of method development, prevalidation, and validation had reached the level of international consensus.

The National Library of Medicine (USA) provides an annotated bibliography on alternatives to animal testing, which incorporates access to nine different databases related to toxicology.

**Design the study to address the regulatory requirements**

To minimise unnecessary animal use, it is essential to ascertain the regulatory requirements specified by the authorities, including those for test types, specified target species, route of administration and statistical parameters. As an example, the following is an excerpt from the APVMA toxicity testing requirements (APVMA 2005):

To permit assessment of the acute toxicology of a chemical to exposed humans, studies in animals should examine the most likely route(s) and form(s) of exposure. They should be performed with both the active constituent and the products to be marketed in Australia. Acute oral toxicity studies should be performed in at least one mammalian species. Rat is the preferred rodent species for oral studies. Acute dermal and inhalation studies in at least one species are also required. LD₅₀ and LC₅₀ values are normally not required and estimates of the lethal dose using alternative procedures are sufficient for hazard classification purposes. Reports should include details of the observed toxic signs, reason(s) for death and other data which will enable assessment of acute toxic potential. For skin and eye irritation studies, the rabbit is an acceptable species but properly validated alternatives to the usual in vivo test would be suitable. Eye irritation tests may be unnecessary in the case of substances or formulations where chemical or physical properties suggest this form of toxicity is likely, e.g. pH above 11.5 or below 2. A skin sensitisation study is also required to test for possible hypersensitivity reactions to the chemical. Guinea pigs are normally used for sensitisation studies.

**Minimise pain and distress**

When alternatives are not available or are not permitted by regulatory authorities, there is a strong potential for toxicity studies in animals to be accompanied by clinical signs of pain and distress. Within the legislative constraints, minimisation of pain and distress should be a prime requisite. Toxicity testing regulations usually only allow for the treatment of pain and distress in animals if the treatment does not interfere with the study. As a result, animals in

---

9 [http://altweb.jhsph.edu/](http://altweb.jhsph.edu/)
such studies are rarely treated with analgesics because of potential confounding effects. It is therefore essential that proposed protocols should always have clearly defined endpoints describing when animals should be removed from the study for humane reasons, and should include written criteria to determine when animals can be removed, treated or euthanased. Death as an endpoint should be avoided in all circumstances unless it is a mandatory legislative requirement. The identification and development of detectable biomarkers may serve as early endpoints. These should be linked to the mechanism or mode of action of toxins and may be clinical, pathological, physiological or behavioural. Such biomarkers could be observable, such as behaviour, or measurable, such as body temperature and blood pressure, or make use of serum chemistry (Stokes 2000). Advances in non-invasive telemetry allow ECG and arterial blood pressure data to be recorded with minimal impact on the animal (Prior 2005). Poon and Chu (1998) considered that, from a toxicological research perspective, humane endpoints should be based on tests that cause a minimal amount of pain and distress to the animals, that detect treatment effects at dose levels that do not produce excessive pain and distress, and that detect and monitor early signs of pain and distress. They found that urinary biomarkers could provide sensitive early indicators of perturbation; the collection of urine can be non-invasive.

To identify the earliest decision point for successful completion of a study or to determine criteria for the humane killing of an animal, it may be useful to implement pilot studies (Koeter and Goldberg 2000).

New and revised test methods, and approaches that incorporate humane endpoints, are being considered and adopted by national and international testing authorities. These methods and approaches must be adequately validated before they are adopted, and it must be established that they will provide better or equivalent information for use in risk assessment.

REFERENCES


Howard C (2005). Altweb, the alternatives to animal testing web site: a global clearing house of information about the 3R's. In: Abstracts, 5th World Congress on Alternatives and Animal Use in the Life Sciences, 111.


TUMOUR INDUCTION

WHAT IS TUMOUR INDUCTION?

Tumours can be induced in laboratory animals by:

- transplantation of specific types of tumour cells, most often of human origin
- exposure to carcinogenic substances or treatments
- infection with tumour-inducing viruses.

With the advent of genetically modified animals, strains can be selected for spontaneous development of specific tumours.

WHY INDUCE TUMOURS?

Tumours are induced to investigate either the biology of a specific tumour (eg factors that regulate growth or the development of metastases) or to evaluate the efficacy of various treatments to inhibit or reverse tumour growth and/or metastatic development.

WHAT IS INVOLVED?

The procedure most often used involves grafting tumour cells onto the host animal. Depending on the cell type and the purpose of the study, this may involve transplanting the cells either orthotopically (ie into the tissue or organ of origin) or ectopically, most often into the subcutaneous space in the flank. Except in those cases where the tumour cells are given intravenously or implanted subcutaneously, transplantation will involve a surgical procedure.

To enhance the uptake of the graft, the immunological status of the host may be modulated either by whole-body irradiation or by the use of immunosuppressive agents. Animals that are immunologically compromised, such as athymic nude mice, SCID mice and RAG knockout mice, are also used. A combination of these strategies may be used.

When tumours are induced by exposing animals to carcinogenic substances or treatments (eg exposure to ultraviolet light in studies of melanoma), the level and frequency of treatments will depend on the agents of interest. Animals may receive single or multiple exposures and may be held in isolation or restrained for prolonged periods.

There are also animal models based on tumours that develop spontaneously. However, maintaining a breeding colony of such animals is usually very difficult. With the advent of transgenic strains, these kinds of animal models are only used in special circumstances. One of the unintended consequences of the development of a transgenic strain may be a high incidence of spontaneous tumours that are not evident in the parent stock.

Once a tumour graft is established, a wide variety of studies may be undertaken. This may involve administering biomarkers or metabolic modulators to study the regulation of cell development and migration, harvesting tumour cells at various stages of development for further in vitro analysis, or evaluating various therapeutic regimes.

As highlighted in recent reviews, there is a trend to use orthotopic models. Although more difficult to manage than subcutaneous implants, these models facilitate the study of the interactions between tumour cells and host tissues. These data are most relevant to understanding tumour growth, the development of metastases and the efficacy of therapeutic strategies that are specific to the host tissue (Killion et al 1999). Similarly, transgenic and knockout animal models provide a more accurate model of cellular and molecular events and are increasingly being used in cancer studies (see, for example, Rosenberg and Bortner 1998).
WHAT ARE THE ESSENTIAL ANIMAL WELFARE ISSUES TO CONSIDER?

The major impact on the wellbeing of animals in tumour-inducing studies is associated with:

- the development and biology of the tumour
- side effects of therapeutic agents
- the consequences of surgery
- side effects of immunomodulatory treatments, such as irradiation.

Adverse effects, in particular, tumour size, pain and malnutrition, will vary with tumour type. Depending on the site of implantation, the growth of the tumour may affect an animal’s mobility (e.g. when implanted in the flank) or, especially with orthotopic transplants, result in major physiological complications (e.g. bowel obstruction in colon cancer). Further, the development and spread of metastases into major organs such as the liver or lungs can result in major organ failure.

Recent neurochemical studies have indicated that tumours are associated with a persistent pain state that differs from inflammatory or neuropathic pain (Honore et al. 2000). However, it has been suggested that because of the lack of association with host tissues, pain levels are significantly less with ectopic grafts than with orthotopic grafts (Wallace 2000). In the case of the latter, animals are likely to experience pain in a manner similar to humans.

Cachexia, a state of severe malnutrition, is concomitant with tumour development. Although there is some decrease in food intake, significant metabolic changes are the primary reason for the weight loss, muscle wasting, anaemia and anorexia seen with this condition; the severity of these effects varies with different animal models (Emery 1999). Other factors can contribute to weight loss during tumour development in animal models. These include, in particular, the effects of radiotherapy, with an associated decrease in food consumption, and in the absorption of nutrients if the gastrointestinal tract is damaged during treatment (Yatvin and Gerber 1970); the attenuating effect of a tumour on the metabolic response to surgery, with changes in gut permeability (de Blaauw et al. 2003); and decreased food intake associated with the side effects of chemotherapeutic agents.

Potential complications from the surgical procedure can affect tumour development and have implications both for the interpretation of data and for the impact of the procedure on the wellbeing of the animal. Hypothermia, a common surgical complication, suppresses immune function and host resistance to tumour metastasis (Ben-Eliyahu et al. 1999); the choice of anaesthetic agent can affect tumour grafting (Milross et al. 1996); and postoperative pain management can affect tumour development (Page et al. 2001).

The specific side effects of chemotherapeutic agents also need to be taken into consideration. These may include the immediate effects of nausea or loss of appetite, and more long-term effects, including weight loss and pathological changes such as impaired liver or kidney function.

HOW ARE PAIN AND DISTRESS MONITORED?

The UK Co-ordinating Committee on Cancer Research guidelines (UKCCCR 1997) provide a comprehensive discussion of how animal wellbeing should be monitored in animals that have been subjected to the induction of tumours and, in particular, how monitoring strategies are integral to achieving humane endpoints.
In these studies, particular attention must be given to:

- the development of the tumour and the impact of that development on mobility, body systems and organs
- body condition, including bodyweight and state of hydration
- evidence of pain or distress associated with the development of the tumour

Care should be taken when interpreting changes in an animal’s bodyweight, as on its own it may not be a reliable indicator. With the growth of the tumour, the tumour tissue weight increases, and sometimes fluid accumulates in body tissues (oedema) and body cavities (e.g., ascites in the abdomen). These may mask significant weight losses in the body; hence the need to interpret such changes in a critical way, taking into consideration other factors such as body condition and behaviours.

The value of new imaging techniques such as bioluminescence (Soling and Rainov 2003), contrast-enhanced ultrasound (Delorme and Martin 2006), volumetric computer tomography (Greschus et al. 2005) and video microscopy (Kan and Liu 1999) to monitor tumour growth and metastatic spread is evident. However, these should be supplemented with specific measures of organ function to evaluate changes and inform the establishment of endpoints.

**HOW CAN PAIN AND DISTRESS BE MINIMISED?**

The Australian code of practice for the care and use of animals for scientific purposes (the Code) (Sections 3.3.65–68) sets out the general principles that need to be taken into account when seeking to minimise pain and distress in these kinds of studies. Tumour type and size should be considered in the context of the animal’s overall body condition when monitoring for adverse health conditions.

The protocol chosen should be compatible with the objectives of the study and have the least risk to the animal; in those circumstances where the choice is unclear, a pilot study should be undertaken.

To minimise the potential impact of tumour-inducing procedures on animals, planning for such studies should consider:

- the site of implantation or method of induction of the tumour
- the known biology of the tumour—growth rate, invasiveness, potential for ulceration, development of metastases, production of cachectic factors
- establishment of the earliest endpoint that will provide an answer—tumour size, weight loss etc
- the separate toxic effects of anticancer therapies, if these are being investigated
- in therapy trials, setting out endpoints that will, as far as possible, be compatible with a reliable assessment of the efficacy of the therapy.

Strategies to minimise the impact of a given protocol will need to be specifically developed in the context of each study, taking into account the impact of each of the factors above.

An essential element of these studies is the setting of humane endpoints to limit the impact on the wellbeing of the animals, using evidence-based criteria that should be tailored for specific research protocols. There is extensive discussion of how to develop endpoints, with a number of case studies in the United Kingdom Co-ordinating Committee on Cancer Research guidelines (UKCCCR 1997; see also Clarke 1997 and Wallace 2000; for specific examples, see Redgate et al. 1991 and Aldred et al 2002).

The recent development of imaging techniques that enable the in vivo monitoring of tumour growth and metastasis is an important advance in refining and managing the impact of tumour development and in setting accurate endpoints in terms of growth, metastasis and efficacy of therapeutic interventions (Weissleder 2002, Soling and Rainov 2003, Serganova and Blasberg 2006).
Pain management is particularly challenging, not only because of the confounding factors associated with an animal model in which the immunological system is compromised to achieve the research objectives, but also because of the special nature of the pain associated with cancer development. To date, there have been few critical studies into the efficacy of analgesics in these kinds of animal models. Both van Loo et al (1997) and Roughan et al (2004) describe behavioural evidence of pain in animals in which tumours have been implanted. While van Loo and colleagues did not find any effect with buprenorphine, Roughan et al found some evidence of efficacy with carprofen and meloxicam, but concluded that a more critical set of criteria needed to be established to validate effective pain management.

The effects of cachexia are difficult to manage, but it is important to ensure that animals are receiving a nutritionally adequate and varied diet. If necessary, animals should be given dietary supplements that are readily absorbed and treats that can stimulate appetite. It is also important to reduce the effects of other causes of weight loss. For example, there is evidence that prophylactic use of parenteral fluids significantly decreases the morbidity associated with radiotherapy (Smith et al 1999).

Other aspects to consider include:

* experimental design, to ensure that the minimum number of animals is used (highlighted in a recent review by Clarke 1997)
* animal care and management (Schiffer 1997, Wallace 2000)
* housing and management of animals (Riley 1981, and the subsequent review by Fitzmaurice 1988)
* diet and animal housing procedures (Haseeman et al 2003)
* social stress, particularly the effects of isolation on immune function and tumour growth (eg Hoffman-Goetz et al 1992, Stefanski 2001) and the potential for such influences to confound the interpretation of the efficacy of chemotherapy (eg Kerr et al 1997, Giraldi et al 2000).

REFERENCES


WILDLIFE RESEARCH

NOTE: Before starting any wildlife research, investigators must ensure that adequate standard operating procedures (SOPs) are in place and that adequate training has been provided to all personnel involved.

WHAT IS WILDLIFE RESEARCH?

Studies involving wildlife cover a range of situations, from studies in the field to those in laboratories, and involve free-living native, non-indigenous or feral mammals, birds, reptiles, amphibians, fish and cephalopods. These animals may either be captive bred or from free-living populations.

See Section 5 of the Australian code of practice for the care and use of animals for scientific purposes (the Code) for special considerations relating to wildlife research.

WHY DO WILDLIFE RESEARCH?

Wildlife studies are undertaken to obtain information about the biology of a species; the characteristics of, and influences on, the viability of wildlife populations; interactions between species; and the relationship between a species and its habitat. Data from such studies are essential for the effective management and conservation of wildlife populations and to assess the risk of human activities on the viability of free-living populations. Studies involving wildlife are also important in our understanding of evolutionary biology, and some captive-bred populations are used as models in biomedical research (eg studies in reproduction using marsupials, Tindale-Biscoe and Janssens 1988).

WHAT IS INVOLVED IN WILDLIFE RESEARCH?

Wildlife studies can involve a diverse range of activities, including observation of animals’ behaviours in their native habitats or the laboratory; capture and release in the field; sampling of blood, body fluids and tissues; recording of physiological or behavioural responses to changing environmental or social conditions; and breeding in captive environments. In both the field and the laboratory, animals may experience a range of potential physiological and psychological stressors. Studies may also involve the manipulation of habitat and social groupings and the alteration of predator-prey relationships, reducing an animal’s ability to control and make choices about its situation. Further, investigations into methods to control feral animals may involve the use of baits or other means to kill animals.

Field studies may simply involve observing animals, but will often include capture with a trap or net, measurements of such things as bodyweight and size, and the collection of specimens such as hair, saliva, blood or stomach contents. Before being released, the animals may be identified by a tag or marker or have a tracking device fitted. These procedures may necessitate the sedation or anaesthetisation of the animals. In these types of studies, animals are not usually transported from where they are captured, but there may be circumstances in which animals are taken to a field laboratory and held for some days before release. Some field studies may involve killing animals to obtain biological specimens.

Laboratory studies will involve either animals that have been captured in the wild, transported to the laboratory and held for the period of the study, or animals from a captive breeding program. The decision to conduct studies in the laboratory may be determined by the need to undertake more invasive measurements, which require the close monitoring of the animal over an extended period, the need to have better control over the conditions of the study (for example, in predator-prey studies), or the need to make detailed observations of an identified group of animals over an extended period. At the completion of laboratory studies, animals that have been captured may be returned to their natural habitats or become part of a captive breeding program.
WHAT ARE THE ESSENTIAL ANIMAL WELLBEING ISSUES TO CONSIDER?

Two key elements set wildlife studies apart from other kinds of research involving animals:
- Wildlife species are not accustomed to interactions with or handling by humans.
- Many studies are conducted in the field, where it is not possible to achieve the same level of control of conditions as in the laboratory.

Any human interaction with wildlife has the potential to interfere with the balance between an animal and its physical and social environment, and thus potentially threaten the animal’s wellbeing. In the field, humans can disrupt social groupings, behaviours, and relationships within and between species. The health and wellbeing of individuals or populations also can be put at risk by unintentional disturbance of habitats or interference in predator–prey relationships, with increased risk of predation.

All aspects of human intervention with wildlife, including capture, handling, restraint, transport, marking, specific sampling procedures and the use of tracking devices, will affect their wellbeing and can disrupt normal behaviours and group dynamics. Because of their lack of familiarity with humans, there is a risk that wildlife will be physically injured when handled.

The design and management of a captive environment, including the physical structure, environmental variables and social experiences, will have a significant impact on the health and wellbeing of wildlife.

The survival of animals that are released back to their natural habitat can also be significantly affected by human intervention. An animal is less able to protect itself if it is stressed following capture and sampling procedures or has not fully recovered from the effects of sedative or anaesthetic agents. It can be more easily identified by predators if it has an identification mark or tag or a device attached. Attached devices, such as radio collars, also put animals at a greater risk of being accidentally trapped or injured. Released animals may not readily be accepted back by their cohort, may be less able to seek adequate shelter and food, and may be more easily identified by predators.

Other factors to consider are:
- pathogens and parasites carried by animals can be a hazard for other animals, and captive animals may bring these into the environment
- animals that have habituated with people have major problems when released
- observational studies can upset animals, particularly at reproduction time
- capture and immediate release studies can potentially cause major disruption (eg to young animals in den).

The potential risk of human intervention to non-target species and populations must also be taken into consideration.

HOW CAN PAIN AND DISTRESS BE MONITORED?

While access to wildlife in laboratory conditions enables frequent monitoring of their health and wellbeing and the impact of various procedures, this is not usually possible in the field. However, during long-term field studies, monitoring morbidity and mortality within a population may provide some useful information to assess the impact of human interventions.
HOW CAN PAIN AND DISTRESS BE MINIMISED?

Considering the wide range of species involved in wildlife studies and the diversity of circumstances and procedures, the investigator must use knowledge and skill to ensure that the most appropriate methods are chosen to meet the needs of the species and the experimental design. If the available information is inadequate or unreliable, a pilot study should be considered in order to minimise the impact of proposed procedures and develop strategies to manage possible complications.

The study design should be informed by current knowledge of the physiological and behavioural needs of the target species. Particular care needs to be taken in the selection of methods for capture and, when necessary, for transport, housing and husbandry, as well as those for procedures such as blood and tissue sampling, identification, anaesthesia, sedation and euthanasia.

Investigators should be aware that there are other sources of information on wildlife handling, husbandry and other procedures, in addition to the academic environment. For example, wildlife parks, zoos and wildlife veterinarians may be useful when planning research projects. A number of guidelines for field studies with various wildlife species have been published (see below). These should be consulted for specific procedures and species.

The New South Wales Animal Research Review Panel has published guidelines to address specific animal wellbeing concerns in wildlife work, such as the use of pitfall traps and the conduct of wildlife surveys. These documents discuss how the principles of the 3Rs (Replacement, Reduction and Refinement) can be applied to wildlife research, and recommend methods for the capture of various species and the design and management of traps to minimise the risk to target and non-target species. Further, Australian Mammals: Biology and Captive Management (Jackson 2003) is a valuable resource on the care of animals in captivity.

Because of the nature of field work, the careful management of traps and appropriate emergency procedures are critical in minimising the impact of such studies on the wellbeing of animals. Trap management includes the selection of the trap most suited to the species, so that animals are caught with minimum risk and not exposed to environmental extremes or predators, and monitoring of the trap to ensure that animals are not held longer than necessary. Planning for emergencies is also very important in field work. For example, plans need to be in place for managing or euthanasing injured animals, and for managing traps during inclement weather or in the absence of the person responsible for monitoring.

As noted above, methods for identifying animals in their natural habitats and applying tracking devices can place them at risk. A comprehensive discussion of the animal wellbeing aspects of various methods used for marking amphibians, reptiles and marine mammals has been published by the New Zealand Department of Conservation (Mellor et al 2004).
Further reading

Guidelines


http://www.mammalsociety.org/committees/commanimalcareuse/98acucguidelines.pdf


http://www.coe.int/animalwelfare


http://www.nmnh.si.edu/BIRDNET/GuideToUse/guidelines_use.html?Operation=ENTER+HERE+%7E+English

REFERENCES


APPENDIX: PROCESS REPORT

BACKGROUND

The Guidelines to promote the wellbeing of animals for scientific purposes: The assessment and alleviation of pain and distress in research animals 2008 is a revised edition of the Ways of minimising pain and distress in animals in research: Practical information for research scientists and animal experimentation ethics committees 1994. In developing and issuing these guidelines, the National Health and Medical Research Council (NHMRC) and its principal committees are obliged under the National Health and Medical Research Council Act 1992 (Section 13) to release draft guidelines for public consultation.

In November 2000, the NHMRC decided to revise the 1994 document. The Animal Welfare Committee sought advice from animal care practitioners as to the appropriateness, accuracy and usefulness of the 1994 edition. Based on the information received, a working group with members comprising of two Animal Welfare Committee members, researchers and experts from institutions on animal care authorities, was formed to update and further develop the guidelines. Between 2000 and 2004 the working group sought information and advice through public consultation on the content of the document which includes several new sections. A technical writer was engaged in November 2004 to facilitate this development.

The guidelines were distributed for public consultation 25 January 2006. Consultations closed on 20 April 2006, 32 submissions were received, 29 of which were considered as part of the revision. Those not formally considered were received outside the consultation period.

The guidelines were considered by the Council of the NHMRC at its 166th session. At that session the Council agreed to advise the CEO that the final document should be issued. The guidelines were issued in May 2008.

Working Group
Mrs Elizabeth Grant AM (Chair) Animal Welfare Committee
Professor Margaret Rose University of New South Wales
Dr Simon Bain Australian National University
Dr Lynda Bonning Baker Heart Research Institute
Dr Denise Noonan Monash University
Dr Mary Bate University of Newcastle
Dr Steven Atkinson University of New England
Dr David Adams Animal Welfare Committee

NHMRC Staff
Mrs Wendy Fahy November 2000-March 2007
Ms Louise Hemsley June 2007- May 2008
Ms Michelle Waterford December 2007- May 2008

Technical Writer
Dr Malini Devadas Biotext Pty Ltd
Dr Carolyn Weiler Biotext Pty Ltd
INDEX

A
abbreviations, xi
abnormality, signs of, 29–30
acclimatisation of animals, 22, K:7
accommodation for animals, 23–4
actions to be taken, 33
acute blood loss, see blood loss
adjuvants, A:6, J:2–4
administration of substances, A:1–10, J:2
AECs, see animal ethics committees
Altweb, L:2
amphibians, 37
anaesthesia, I:4–7, I:9, I:15–18
hypothermia following, K:10
induction of, I:9
surgical procedures, K:5–6
anaesthetic chambers, I:5
anaesthetic creams, D:2–3, I:7
analgesia, I:11–14
foetal studies, F:2
surgical procedures, K:5–6
anaphylaxis, J:3
animal ethics committees
approval by, 43–4
involvement in monitoring, 34–5
reporting obligations, 46–7
animal models, 19–20
animal welfare, ix
animal wellbeing
checklist for, 49
defined, 5–6, ix
effect on outcomes, 13–14
impact of studies on, 29
key principles, 3–4
minimising surgical risk, K:10–12
strategies to promote, 45–7
animals
administering solutions to, A:6–7, A:9
assessing condition of, 45–6
choice of, 20–1
necessity for use, 17–18
antagonists to anaesthesia, I:7
antibiotic therapy, K:8
anticholinergic agents, I:9
antigens, J:1–5
anxiolytics, I:14
approval for new research, 43–4
aseptic technique, A:7, K:6
assessing pain and distress, 28–35
Australian Code for the Responsible Conduct of Research, 35
Australian Code of Practice for the Care And Use of Animals for Scientific Purposes, 3
Australian Pesticides and Veterinary Medicines Authority, L:2
autonomic nervous system, 8–9
aversive stimuli, B:2
awake-behaving neuroscience studies, B:2

B
balanced analgesia, I:12–13
behaviour
and choice of animal, 20
environmental enrichment and, E:1
behaviour modification studies, B:1–6, G:4–5
behavioural indicators
of distress, 7–8
of pain, 11, 30
of wellbeing, 6, E:1
sickness behaviour, K:4
binary score-sheet system, 34
biological sample collection, C:1–6, D:1–11
biomarkers, L:3
biomedical studies, 19
birds
analgesia, I:20–1
humane killing, H:6
injection anaesthesia, I:17–18
signs of pain or distress, 37
bloat, G:5
blood collection, D:1–11
blood loss, D:3, D:5
after surgery, K:2, K:10
blood vessels, D:1
body temperature monitoring, I:11,
see also hypothermia
bodyweight loss, see weight loss
brain, environmental effects on, E:2
breed, choice of, 20
buprenorphine, I:12, I:19

c
 cachexia, M:2, M:4
Canadian Council on Animal Care guidelines, 32
capillary tube method of urine collection, C:2
captive environments, N:2
carcinogenicity studies, L:1
cardiovascular monitoring, I:10, K:2
catheters
blood collection via, D:1
infections from, K:8
surgical implanting, K:1
cats
analgesia, I:20–1
blood collection from, D:8
environmental enrichment, E:4–5
injection anaesthesia, I:17–18
signs of pain or distress, 37
cattle, signs of pain or distress, 38
causes of pain, 9–10
central sensitisation, 10, I:4
cerebrospinal fluid, C:2, C:4
checklist for animal wellbeing, 49
choice, behaviour modification by, B:1
chronic blood loss, D:5
circadian rhythms, 6, 29
clinical signs, 28
Co-ordinating Committee on Cancer Research (UK), M:2–3
CO2 euthanasia, see carbon dioxide euthanasia
collaboration between investigators, 17,
see also team approach
care of animals, restraint of animals
conjunctival samples, C:3
continuous administration, A:5
coprophagy, G:4
CSF, C:2, C:4
cystocentesis, C:3
d cameraphagy, G:4
death, ix
debilitating illness, B:2
dehydration, B:4, G:3
after surgery, K:2, K:10–11
during anaesthesia, I:9
monitoring for, G:5
diabetes, G:1
diet, 23, G:4, see also drinking water; food
distress, 7–8
behaviour modification studies, B:4
definition, ix
during substance administration, A:8
maternal, F:2
predicting, 26–8
sample collection, C:5
documentation of monitoring strategy, 33–4
drugs
behaviour modification by, B:1
safe handling of, I:14
Dutch Inspectorate for Health Protection and Veterinary Public Health, J:3
E
ectotoxicology studies, L:1
injection anaesthesia, I:17–18
exploratory studies, 19, 24–5
extrapolation from human to animal, 30
eye irritation tests, L:2
F
face mask anaesthesia, I:4–5
faeces
collecting, C:4
eating of, G:4
fasting, G:3, I:8
FCA, J:3
ferrets
blood collection from, D:10
environmental enrichment, E:6–7
foetal studies, F:1–4, H:2
field studies, N:1
fish
environmental enrichment, E:14
humane killing, H:7
signs of pain or distress, 38
fluid administration, I:11
fluid restriction, G:3, G:6
food
as a reward, B:2
for dogs, E:12
for ferrets, E:6
for fish, E:14
for guinea pigs, E:9
for pigs, E:10
for primates, E:13
for rabbits, E:8
Index

For sheep, E:11
intake modification, G:1–7
restricted access to, G:2, G:6
substance administration in, A:1
used for environmental enrichment, E:2, E:4
foot-pad injection, J:2–3
Freund’s complete adjuvant, J:3

G
gavage, A:1–2
general anaesthesia, I:4
general principles, 3–4
genetic variability
   and choice of animal, 20
   naturally occurring, 19
   transgenic strains, M:1
genital tract secretions, C:2
goats
   antigen injections, J:4
   humane killing, H:5
   signs of pain or distress, 41
growth failure, B:4, G:5
guinea pigs
   analgesia, I:18–19
   antigen injections, J:4
   blood collection from, D:10
   environmental enrichment, E:9
   humane killing, H:4
   injection anaesthesia, I:15–16
   intravenous administration, A:3
   signs of pain or distress, 38

H
hazards, 26
health of animals, 20
heart blood collection, D:1
hormonal system, 8–9
horses, signs of pain or distress, 39
housing of animals, 23–4
human—animal interactions, E:2
   with cats, E:4
   with dogs, E:12
   with ferrets, E:6
   with guinea pigs, E:9
   with pigs, E:10
   with primates, E:13
   with rabbits, E:8
   with rodents, E:2
   with sheep, E:11
   with wildlife, N:2
humane endpoints, 31–2
   dietary restrictions, G:6
   tumour induction, M:3
humane killing, ix, H:1–8
husbandry, 23–4
hydration, see dehydration; drinking water;
fluid restriction
hypothermia
   after surgery, K:2, K:10
   anaesthetic effects of, I:7–8
   during anaesthesia, I:9, I:11
   during surgery, K:7
   foetal studies, F:3
   from tumour induction, M:2
hypoxia, K:2, K:10

I
imaging techniques, M:3
immune system
   modulations of, M:1
   post-operative recovery, K:8
   responses to stress, 9
implants
   complications following surgery, K:3
   effects on outcomes, K:9
   infections from, K:8
   surgical procedures, K:1
induced neurological deficits, B:3
induction of anaesthesia, I:9
infections after surgery, K:3, K:8, K:11
inflammatory response, 10, D:3, J:2
inhalation anaesthesia, I:4, I:15
injections, A:2–4
   anaesthesia, I:5, I:15–18
   techniques, A:7–8
intervention points, 31–2
intradermal administration, A:4, J:2
intramuscular administration, A:3–4, I:6
intraperitoneal administration, A:3–4, I:6
intravenous administration, A:2–4, I:5
invasiveness of procedures, I:3
isolation, B:2, K:4
IV administration, A:2–4, I:5

J
Justification principle, 4

K
key principles, 3–4
koalas, 39

L
laboratory wildlife studies, N:1–2
laparoscopy, F:2
laparotomy, F:1–2
laws, 3
local anaesthesia, I:7
lymphoid harvesting, J:2
Index

M
macaques, blood collection, D:2, D:9
macropods
analgesia, I:20–1
injection anaesthesia, I:17–18
signs of pain or distress, 39
managing risks, 26–7
marmosets, blood collection, D:9
maximum injectable volumes, A:4
measurement
food and water intake, G:4
pain and distress, F:3, G:5
pain scale, K:4
metabolic cages, C:2–3
metabolic disturbances, K:2, K:11
methods, 25
mice
analgesia, I:18–19
antigen injections, J:4
blood collection, D:2, D:7
environmental enrichment, E:2–3
humane killing, H:4
injection anaesthesia, I:15–16
intravenous administration, A:3
signs of pain or distress, 39
milk collection, C:1, C:3–4
minimising pain and distress, 28–35, B:4–5
minipigs, see pigs
mismothering, A:7
mobility loss, B:4
monitoring
anaesthesia, I:10
analgesia, I:14
behaviour modification studies, B:4
checklist for, 34
documentation of strategy, 33–4
during substance administration, A:8
frequency of, 33
pain and distress, 28–35
strategies for, 45
volume of blood collected, D:4
monoclonal antibodies, J:1
multimodal analgesia, I:12–13
mutagenicity testing, L:1
mutillation, D:2
rabbis, E:8
rodents, E:3
sheep, E:11
naturally occurring genetic variation, 19
needle size, A:4, D:3
negative signs, 34, 45
neonates
anaesthesia for, I:7–8
humane killing, H:2
rejection risk, A:7
nerve pathways involved in pain, 10
neuroendocrine responses, 8–9
neurological deficits, B:1
new research, 17–41
newborn animals, see neonates
‘no abnormalities detected’, 34, 45
non-human primates, 40, E:13–14
non-steroidal anti-inflammatory drugs,
I:12, I:18, I:20
normality, species-specific, 29
NSAIDs, I:12, I:18, I:20
nutritional research, G:1

O
oil adjuvants, J:2, J:3, J:4
olfactory stimulation
cats, E:5
dogs, E:12
ferrets, E:7
guinea pigs, E:9
pigs, E:10
primates, E:14
rabbits, E:8
rodents, E:3
sheep, E:11
opioid analgesia, I:19, I:21
oral administration, A:1–2, A:4, I:12
orthoptic models, M:1
osmotic minipump, A:5
oxygen, euthanasia using, H:3
oxytocin, C:4

P
pain, 9–11
analgesic actions, I:13
behaviour modification studies, B:4
definition, ix
during substance administration, A:8–9
from tumours, M:2
management of, I:1–24, K:5–6
maternal, F:2
measurement scale, K:4
minimising, K:10
predicting, 26–8
sample collection, C:5
surgical procedures, K:2
tumour induction, M:4
palatability studies, G:1
pathological changes, 19
peri-operative complications, K:7
permanent indwelling venous catheter, A:5
personnel, see also training
involved in surgery, K:4–5
solution administration by, A:7, A:9
pharmacological research, G:1
physical environment
enrichment of, E:2–5
for dogs, E:12
for ferrets, E:6–7
for fish, E:14
for guinea pigs, E:9
for pigs, E:10
for primates, E:13
for rabbits, E:8
for sheep, E:11
physiological indicators
of distress, 7–8, B:4
of pain, 11, B:4
of wellbeing, 6
research into, G:1
physiological solutions, A:6
pigs
analgesia, I:20–1
blood collection, D:2, D:10
environmental enrichment, E:10
humane killing, H:6
injection anaesthesia, I:17–18
signs of pain or distress, 40
pilot studies, 26
platypuses, 40
polyclonal antibody production, J:1–5
positive reinforcement (rewards), B:1, G:2
possums, 40
post-anaesthesia, I:11
post-operative recovery, K:3, K:8
pre-anaesthesia, I:8
pre-emptive analgesia, I:12, K:5
predicting pain and distress, 26–9
predictive studies, 19, 25
premedication, I:8–9
primates, 40, E:13–14
process report, process report 1
protocols
pain management, I:2–3
planning, 17–41
required, 24
publishing requirements, 36
punishments, B:1

R
rabbits
analgesia, I:18–19
antigen injections, J:4
blood collection, D:2, D:8
environmental enrichment, E:8
humane killing, H:5
injection anaesthesia, I:15–16
intravenous administration, A:3
signs of pain or distress, 40
radiotherapy, M:2
rats
analgesia, I:18–19
antigen injections, J:4
blood collection, D:2, D:7
environmental enrichment, E:2–3
humane killing, H:4
injection anaesthesia, I:15–16
intravenous administration, A:3
signs of pain or distress, 41
Reduction principle, 3–4
Refinement principle, 3–4
refrigerated solutions, A:6
regulations, 3
Replacement principle, 3–4
reporting requirements, 25, 46–7
reptiles, signs of pain or distress, 41
respiratory monitoring, I:10
Responsibility principle, 4
restraint of animals, A:8, B:3–4,
see also confinement
restricted access to food and water, G:4–5
retrobulbar sinus, D:1
reversible anaesthesia, I:7
reviews of monitoring strategy, 46
rewards, B:1, G:2
risk management, 26–7
rodents, see also guinea pigs; mice; rats
analgesia, I:18–19
aseptic technique for surgery, K:6
environmental enrichment, E:2
humane killing, H:4
injection anaesthesia, I:15–16
neonatal rejection, A:7

S
safe handling of drugs, I:14
saliva collection, C:1, C:3
sample collection, C:1–6, D:1–11
sample size, 25
scheduled access to food and water, G:4–5
scientific activities
approval for, 43–4
experimental design, 24–5
general principles, 3–4
planning, 17–41
scientific outcomes
anaesthesia effects and, I:3–4
blood collection and, D:5
methods of euthanasia, H:1
surgical procedures and, K:9
wellbeing and, 13–14
Index

semen collection, C:2, C:4
sheep
  analgesia, I:20–1
  antigen injections, J:4
environmental enrichment, E:11
foetal studies, F:2
humane killing, H:5
injection anaesthesia, I:17–18
signs of pain or distress, 41
signs, defining, 29–31, see also monitoring
skin sensitisation studies, L:2
social enrichment
  for cats, E:4
  for dogs, E:12
  for ferrets, E:6
  for fish, E:14
  for guinea pigs, E:9
  for pigs, E:10
  for primates, E:13
  for rabbits, E:8
  for rodents, E:2–3
  for sheep, E:11
  stress induced by, M:4
social variables, behaviour modification by, B:1–3
sourcing animals, 21
species, choice of, 20
species-specific behaviours
  housing requirements, 23
  signs of pain or distress, 37–41
  wellbeing and, 6
species-specific procedures
  anaesthesia, I:15–21
  blood collection, D:7–10
  IV administration, A:3
standard operating procedures, 47
starvation procedures, G:3
statistical analysis, 24–5
strain, choice of, 20
stress, 7–8, B:1
students, supervising, 35
subcutaneous administration, A:3–5, I:6
subcutaneous vascular access ports, K:8
submitting proposals, 43–4
substance administration, A:1–10
supervision of students, 35
surgical procedures, K:1–15
  anaesthesia during, I:1
  tumour induction, M:2

T
  team approach, 33, see also collaboration
    between investigators
tear collection, C:3
teratological studies, L:1
thrombosis, D:3, K:3, K:8
toxicology, L:1–4

tracheal injury, A:2
training
  for surgery, K:4–5
  in substance administration, A:7
  of observers, 33, 35
transgenic strains, M:1
transplants, K:1, M:1
transporting animals, 21–2
trapping, N:3
tumour induction, M:1–6
turtles, signs of pain or distress, 41

U
  urine collection, C:1–3, C:6, L:3

V
  vaginal secretions, C:4
VAPs, K:8
vascular catheters, K:8
volume of blood collected, D:4
volume of injectate, A:4, A:9

W
  wallabies
    analgesia, I:20–1
    injection anaesthesia, I:17–18
water, see drinking water
weight loss, B:4, G:3
  from tumour induction, M:2
monitors for, G:5
wellbeing, see animal wellbeing
wildlife research, B:3, N:1–4
wound healing, K:3, K:12