Euthanasia of Animals Used for Scientific Purposes

Edited by

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CONTENTS

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PREFACE

Euthanasia (from the Greek *eu* = well, *thanatos* = death) is defined as the process of inducing a painless death. It is a necessary and accepted procedure in all aspects of veterinary medicine and many aspects of scientific procedures involving animals.

If an animal has to be killed, death must occur with the least fear, anxiety, pain and distress. The method used for euthanasia must either kill the animal very rapidly or instantaneously render the animal unconscious so that death ensues before consciousness is regained. The application of any method must be such as to minimise the impact of any procedure on the welfare of the animal. Consequently, consideration also must be given to how the animal is handled immediately prior to and during euthanasia. Ultimately, for the method to be effective, reliable and humane, the technical competence of the persons involved in all aspects is paramount.

Euthanasia is one of the most commonly performed procedures involving animals used for scientific purposes. Animals need to be killed for various reasons, including the collection of blood and tissues, culling of breeding stock, disposal at the end of an experiment and in those circumstances where animals are experiencing pain and distress which cannot be alleviated.

The aim of this publication is to provide investigators and members of Animal Ethics Committees with details of the current state of information relevant to the euthanasia of animals used for scientific purposes. It is the intention of the writing group that this publication will assist in decisions about the suitability of methods for euthanasia of animals in these circumstances. Emphasis is placed on how to achieve the goals of Reduction and Refinement by choosing that method which is suited to the scientific aims of a study with minimal impact of the procedures on the welfare of the animals.

This publication is not designed as a specific training manual or for use in abattoirs or other animal industries. The Australian Veterinary Association and the Agricultural and Resource Management Council of Australia and New Zealand's Animal Welfare Committee have produced a number of useful documents for this purpose, including Guidelines for Humane Slaughter and Euthanasia (AVA, 1999, AQIS, 1995) and a series of Model Codes of Practice. For further information see the Bibliography and Further Reading sections.

SECTION 1

ANIMAL WELFARE CONSIDERATIONS

1.1 Introduction

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1997) states that when it is necessary to kill an animal,

- *humane procedures must be used. These procedures must avoid distress, be reliable, and produce rapid loss of consciousness without pain until death occurs. The procedures should also be compatible with the scientific or educational aims;*
- *the procedures must be performed only by persons competent in the methods to be used, or under the direct supervision of a competent person. The appropriate means must be readily at hand;*
- *animals should be killed in a quiet, clean environment, and normally away from other animals. There should be no disposal of the carcass until death is established;*
- *dependent neonates of animals being killed must also be killed or provision made for their care; and*
- *when fertilised eggs are used, the method of disposal must ensure the death of the embryo.*

(Sections 3.3.20–3.3.24)

Thus to satisfy the requirements of the Australian Code of Practice there must be objective criteria, if possible, to establish that the method used is humane. Of note, the Code recognises that the skill of the persons involved is important and that the environment in which animals are killed must be considered in order that anxiety and fear are reduced. Further, the code identifies the potential impact of euthanasia methods on research data and areas which require special consideration, such as euthanasia of the foetus, neonate and pouch young.

The method of choice will depend on the species, the individual's needs, and the requirements of the experiment. As it is likely that some methods of euthanasia may distress the persons involved, these feelings must also be taken into account. Nevertheless, the method finally chosen must be the one that causes minimal distress to the animal.

1.2 Minimisation of Pain, Distress, Fear and Anxiety

The basic tenets of the Australian Code of Practice are that (1) animals are used for scientific purposes only when it is justified, weighing the scientific or educational value of the experiment against the potential effects on the welfare of the animal and (2) the impact of any procedure on the welfare of animals must be minimised. These objectives are achieved through the application of the principles of Replacement, Reduction and Refinement—the three Rs proposed by Russell and Burch (1959).

When animals are killed, both the method of euthanasia, particularly the time taken to produce unconsciousness, and how the technique is performed can result in animals

experiencing pain, distress, fear and anxiety. Further, the animal's psychological response to the environment in which it is killed, including interactions with other animals and humans and how it is handled, can result in emotional distress.

The principle of Refinement seeks to reduce to an absolute minimum the amount of pain and distress imposed on animals. Thus, to evaluate the impact of euthanasia methods on animals and to minimise that influence we must critically evaluate the evidence with regard to the effects of each method, taking into account species differences. We need to know how quickly and effectively an animal becomes unconscious and whether or not it experiences pain or distress, be that because of how the method is applied or because of inherent limitations to that method.

The term 'consciousness' is used in two ways in discussions about methods of euthanasia. In one sense it is used simply to mean that the animal is awake, in the other, it refers to the animal's capacity for subjective awareness. There is some confusion in the literature as to the meaning and use of the terms 'consciousness', 'awareness' and 'self awareness' as they relate to animals and often these terms are used interchangeably. Consciousness has been defined as being aware of one's own existence (Gallup, 1985). In its most rudimentary form consciousness is a state where an animal perceives stimuli from the external environment and responds in the normal behaviour of an awake, aware individual: it is a state of sensibility. In a recent symposium devoted to issues of consciousness, cognition and animal welfare (UFAW, 2001), consciousness was described as 'the subjective awareness of some sensory perceptions, emotions and thoughts' (Kirkwood and Hubrecht, 2001) – that is, if an animal has no capacity for consciousness it cannot feel.

The integration of the processes from the reception of the stimulus to the resulting perceptions and affective behaviours—the conscious experience—involves a functional cerebral cortex (Wall, 1992) or the existence of the requisite neuronal systems (Taylor, 2001). Based on neurophysiological and anatomical evidence, the experience of consciousness (sensibility) can be asserted for all vertebrate species and the possibility for invertebrates such as the cephalopods must be considered (Wells, 1978). However, it could be argued that between species there are qualitative differences in conscious experiences which are modulated by the complexity of cognitive and intellectual development of the individual. Across species, to any given stimulus there is a spectrum of responses from simple to sophisticated. In the context of this discussion, these species differences may be reflected in the complexity of behavioural responses and should be taken into account in consideration of the potential negative psychological impact of a situation.

Pain is defined by the International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. The IASP has recently added an explanatory note which is relevant to the assessment of an individual's experience in that the inability to communicate in no way negates the possibility that an individual is experiencing pain. Pain is an aversive sensory and emotional experience which elicits protective motor actions, results in learned avoidance, and may modify species-specific traits of behaviour, including social behaviour (Kitchell, 1987) – this description provides a framework within which a range of pain-

related behaviours can be described. The perception of pain depends upon the activation of designated peripheral receptors and neural pathways which transmit those stimuli to the central nervous system. The motivational-affective component of pain can be modulated by various psychological and environmental stimuli, particularly anxiety and fear (Rose and Adams, 1989; Bateson, 1991). Pain is always a subjective experience and thus an animal's capacity for consciousness is a central question in determining whether or not it can experience pain.

Anxiety and fear are emotional experiences or feeling states. Anxiety involves increased arousal and alertness prompted by an unknown danger that may be present in the immediate environment. Fear can be similarly defined but whereas anxiety is an unfocused response to the unknown, fear is a focused response to a known object or previous experience (AVMA, 1987).

Pain, anxiety and fear will all be manifest as various physiological and behavioural signs of distress - a state in which the animal is unable to adapt to an altered environment or altered internal stimuli (AVMA, 1987). This failure to cope results in a range of abnormal physiological and behavioural responses (Clark *et al.*, 1997a; 1997b) and is accompanied by emotional experiences such as fear, anxiety and depression. Feelings such as fear and anxiety are not only an element of the experiences of pain or distress, but, most importantly, play a major role in modulating the affective experience.

An animal's capacity for consciousness and the impairment of that capacity is a central issue in assessing the impact of euthanasia methods. Unconsciousness is insensibility to all external stimuli. It can be evaluated by the clinical assessment of neurophysiological status and, under experimental conditions, from cortical responses in the EEG (Molony, 1986). Despite the frequent use of euthanasia as a research procedure, to date few studies have critically evaluated the impact of euthanasia methods in these circumstances using these kind of measurements. Although most studies in this regard have been directed towards the development of objective criteria to determine unconsciousness when animals are killed in abattoirs, data are relevant to the research situation.

Cortical function has been studied using electroencephalographic (EEG) and electrocortical (ECoG) measurements. The efficacy of the euthanasia method has been evaluated by either changes to EEG patterns (Blackmore and Newhook, 1982) or cortical responses to visual, auditory or somatosensory stimuli (Daly *et al.*, 1986; Gregory and Wotton, 1988a). These studies have been made in poultry (Gregory and Wotton, 1987, 1988b, 1990; Raj *et al.*, 1991), pigs (Wotton and Gregory, 1986), calves (Blackmore and Newhook, 1981; Gregory and Wotton, 1984), adult cattle (Devine *et al.*, 1986; Daly *et al.*, 1987), sheep (Blackmore and Newhook, 1982; Blackmore *et al.*, 1995) and fish (Kestin *et al.*, 1991). In these studies a range of euthanasia methods were evaluated but most involved physical methods such as stunning and shooting, although several studied methods of inducing hypoxia using either carbon dioxide, argon or nitrogen. In these kinds of studies, a development in data analysis has been the application of computer analysis methods to provide an objective assessment of EEG patterns (Jones *et al.*, 1988) although, as yet, the use of this methodology is not widespread.

In the laboratory setting, there has been limited use of such neurophysiological assessments. Changes in EEG patterns have been used to determine the level of consciousness/unconsciousness and the stage of carbon dioxide narcosis in rhesus monkeys (Mattson *et al.,* 1972) and loss of consciousness in rabbits and dogs following either the use of a captive bolt (Dennis *et al.,* 1988) or T61 administration (Hellebrekers *et al.,* 1990). Interpretation of changes in cortical electrical activity have been pivotal to the current debate concerning decapitation as a method of euthanasia (Mikeska and Klamm, 1975; Vanderwolf *et al.,* 1988; Derr, 1991; Holson, 1992). A review of these papers highlights some of the dilemmas presented in the use of this methodology to assess conscious states and the debate about the interpretation of electrical cortical activity and consciousness. As argued by Daly and colleagues (1987), changes in cortical activity, such as a loss of visual evoked responses, do not represent unconsciousness, but rather indicate an insult to the brain sufficient to cause failure of a primary sensory pathway, and therefore may represent a degree of brain failure inconsistent with sensibility.

A study by Coenen and colleagues (1994) in rats, which sought to correlate changes in electrical brain activity with behavioural and cardiovascular evidence of loss of consciousness and death, indicates one way in which these neurophysiological data can be used to better inform a practical approach to assessing the efficacy of a euthanasia method. Other studies with rats have measured only behavioural and cardiovascular responses to carbon dioxide euthanasia using evidence of cardiovascular collapse as confirmation of death (Kurosawa *et al.,* 1981; Smith and Harrap, 1997). The latter study used a set of key signs similar to those described by Hornett and Haynes (1984), linked to the effects of euthanasia. This is a very useful approach to provide a framework for collection of behavioural data to describe and monitor efficacy of euthanasia methods. The key signs include ataxia, loss of ambulation, loss of recumbency and voluntary movements, evidence of involuntary movements, dyspnoea, respiratory arrest and cardiovascular collapse. Evidence from several experiments suggests that unconsciousness is closely associated with loss of posture. However, a cautionary note is that in some species, such as rabbits and chickens, fear can cause immobility or 'freezing'; although immobile, the animal is conscious.

In consideration of the impact of a euthanasia method on an animal, the other important question is the evidence of pain and distress associated with the use of that method. There are several publications which document an approach to the assessment of pain and distress in animals by the critical evaluation of physiological and behavioural indices (Morton and Griffiths, 1985; Adams, 1988; UFAW, 1989). As noted in the Australian Code of Practice, one must be familiar with the normal behaviour of a species to assess signs of pain or distress. Depending on the species, in circumstances associated with euthanasia, such behaviours will include aggressive and/or abnormal behaviour, abnormal stance or movements, abnormal sounds, altered cardiovascular and/or respiratory function, vomiting and abnormal defecation and urination (Section 3.3.10). Some of these responses can occur in unconscious animals.

The few studies where these kinds of assessments have been used to evaluate the impact of a euthanasia method include the evaluation of air embolism, cervical dislocation and captive bolt in the rabbit (Weisbrod *et al.,* 1984), a comparison of carbon dioxide and halothane in rats (Clifford *et al.,* 1985) and several studies of gaseous euthanasia methods

in rats and mice (Hornett and Haynes, 1984; Blackshaw *et al.*, 1988; Britt, 1989). However, as noted by Britt, the main aim in most of these studies has been to determine when loss of consciousness occurs. However, more recent studies into the effects of carbon dioxide as a method of euthanasia in rats have focused on a critical evaluation of behavioural and physiological data to assess evidence of pain and distress during induction (Smith and Harrap, 1997; Hackbarth *et al.*, 2000).

Grandin (1991, 2000) has described a method of scoring the impact of handling and the efficacy of stunning on the behaviour of animals in abattoirs as an objective way of monitoring animal welfare at slaughter. This kind of approach may be a useful tool, especially in circumstances where a number of animals are killed, to monitor animal welfare and to detect and rectify any problems which may occur.

1.3 Methods of Euthanasia

Methods of euthanasia fall into two broad categories—chemical and physical.

Chemical methods which have been used for euthanasia include:

- Inhalant agents, e.g., ether, halothane, methoxyflurane, isoflurane, enflurane, chloroform, nitrogen, nitrous oxide, carbon dioxide, carbon monoxide, argon, hydrogen cyanide.
- Injectable agents, e.g., barbiturates, chloral hydrate, ethanol, ketamine, magnesium sulphate, potassium chloride, neuromuscular blocking agents.

Physical methods which have been used for euthanasia include:

- penetrating captive bolt pistol;
- gunshot;
- cervical dislocation;
- decapitation;
- electrocution;
- microwave irradiation; and
- stunning with exsanguination.

The method chosen will vary with the species and the purpose for which the animal is being killed but must satisfy the following criteria as set out in the policy on euthanasia of the Australian Veterinary Association (AVA, 1997); namely:

- death without signs of panic, pain or distress;
- minimum time to loss of consciousness;
- reliability and reproducibility;
- simple relatively maintenance-free mechanical equipment;
- minimal environmental impact through contamination;
- minimal emotional effects on the observer and operator; and
- safety for operators and observers.

Methods of euthanasia fall into three broad categories according to their mode of action and how unconsciousness is induced. Unconsciousness is produced by depression of cerebro-cortical function, through (1) the effects of hypoxia or cerebral ischaemia, (2) drugs which depress central nervous system (CNS) function or (3) physical disruption of

brain activity such as electric shock or concussion. Death will occur with the cessation of vital functions due to the effects of hypoxia, ischaemia or drugs. For a detailed discussion of the modes of action of individual methods refer to the 2001 Report of the AVMA Panel on Euthanasia (AVMA, 2001).

To identify potential problem areas, it is helpful to consider the two stages in the effects of a euthanasia method—the animal is rendered unconscious and it dies. Depending on the specific actions of the method used, these stages may occur simultaneously or death occurs after unconsciousness without the animal recovering. In the latter circumstance, the time to onset of unconsciousness may be an issue.

With most methods, both stages (unconsciousness and death) are achieved through the separate or continuous actions of the same agent. The time to onset of unconsciousness and death will depend upon the dose and duration of effect. However, where a method results in unconsciousness and death through different modes of action, animals may be at risk of pain and distress. For example, if the onset of the mechanism which kills the animal precedes the onset of unconsciousness, as when animals experience asphyxia before loss of consciousness.

There are situations where two methods are used for euthanasia. For example, where physical methods of stunning are used to produce unconsciousness, an adjunct method, such as exsanguination, is required to kill the animal. Here again, it is important that the animal does not regain consciousness before death occurs. Important considerations are the duration of the action which causes unconsciousness and how quickly the adjunct method is effected.

Thus to validate the humaneness of a method of euthanasia one needs to know how quickly unconsciousness is produced, whether it is sustained until death ensues and if it occurs before effects such as asphyxia are present. When an adjunct method is necessary to kill the animal, it should be applied quickly.

When using a physical method of euthanasia there are specific issues which must be addressed so as to minimise the risk to the welfare of the animal. Not only must the chosen method be appropriate to the species but the precision in application of the method also is critical. To that end the experience and expertise of the operator, the appropriate use of the device and the mechanical efficiency of the device will determine the efficiency and reliability of that method as being humane. Thus, all physical methods must only be performed by persons who have appropriate skills and mechanical devices must be kept in good working order. Further, to ameliorate the animal's experience of anxiety and fear, consideration should be given to the prior use of sedative or anaesthetic agents before the physical method is applied (AVMA, 1993).

Based on the general criteria outlined above, the suitability of a range of methods as they relate to specific species will be discussed in subsequent sections of this document.

However, an important matter of general concern is how to minimise the impact of those environmental, psychological and social stressors which an animal is likely to experience at the time of euthanasia . Factors such as handling, restraint, unfamiliar

surroundings or animals, and being exposed to the distress of other animals being killed can all cause fear and anxiety.

As a general principle, the Australian Code of Practice requires that animals are not present when euthanasia is performed on another animal. There is a substantial literature which shows that non-stressed rats display negative behaviours and increased adreno-cortical responses when exposed to an environment in which other rats have experienced a stressor (for example, Valenta and Rigby, 1965 ; Mackay-Sim and Laing, 1981). Sadjak and colleagues (1983) have shown an increased adrenocortical response in rats exposed to blood from killed rats. However, there is a view that highly socialised species, such as cattle and sheep, should not be isolated at the time of euthanasia. Anil and colleagues could not find any objective evidence of distress when either sheep (Anil *et al.*, 1996) or pigs (Anil *et al.*, 1997) witnessed the slaughter of conspecifics. In cattle, Grandin (1994) did not observe any aversion to entering an area where other cattle had been slaughtered unless that area was contaminated with blood and saliva from a distressed animal. It is likely that the smell of blood and other excretions, including pheromones and hearing distress vocalisations will cause distress to animals but that the observation of another animal being killed *per se* may not do so. Hence the principle of not exposing animals to this risk or, in those circumstances where isolation stress is of concern, of ensuring that general areas and equipment have been thoroughly cleaned before re-use.

A final note concerning the use of firearms. A detailed description of the use of firearms for euthanasia is beyond the scope of this document. However, firearm licences may be required and these along with information and training should be sought from the appropriate authorities prior to use. Additional information on the appropriate use of firearms is available from species-specific codes of practice published by the Standing Committee on Agriculture and Resource Management (SCARM) of the Agriculture and Resource Management Council of Australian and New Zealand (ARMCANZ) and the Universities Federation for Animal Welfare (UFAW, 1988).

1.4 Special Consideration Involving the Age of the Animal

Special consideration needs to be given to the influence of the age of the animal on the choice and efficacy of euthanasia methods.

The stage of development at which an animal comes under legal protection is theoretically determined by its capacity to experience pain or distress. In Australian animal protection legislation no mention is made of this matter, but in the 1986 United Kingdom *Animals (Scientific Procedures) Act*, provision is made to protect a vertebrate animal in its foetal, larval or embryonic form when:

(a) in the case of a mammal, bird or reptile, half the gestation or incubation period for the relevant species has elapsed; and
b) in any other case, it is capable of independent feeding.

However, Hanson (1987) in a discussion of the rationale for this legislative requirement, noted that this is an arbitrary ruling and is based on little scientific evidence. This did not prevent the European Commission DGX1 Working party (1997) concluding

that the time at which the neural tube develops into functional brain (about 60% gestation) must be taken as the time at which the foetus may perceive pain and should therefore be killed humanely .

The basic question is at what stage of its development can the foetus or neonate experience pain? In species such as sheep and guinea pigs, which are relatively well-developed and responsive at birth, this is likely to be at birth or earlier. It has been suggested that the foetal lamb is potentially capable of perceiving sensory stimuli by 110 days' development (Rawson and Rees, 1993). However, other species, such as the rat, are relatively immature at birth and may not be capable of such sensory perceptions until some days after birth.

As an example of how this question can be approached, one can consider the evidence for the development of the ability to experience sensory stimuli in the rat. An early stage in the development of the cortex is the generation of its cells which are born between E (embryonic day)11 and roughly the time of birth (E21). The generation of these cells probably continues in the first few days of postnatal life. There are five stages before a functional sensory cortex has developed. The first is the migration of the cells from the neural tube to the anlage cortex. This commences soon after day E11 when the cells are first formed and continues probably up to P(postnatal day)4. Next, the cortex receives connections from the thalamus. Without this step the cortex cannot know what is happening at the periphery. In the case of the visual neocortex, which has been extensively studied, these synapses form from P3-5. Then the internal structure of the neocortex matures. Cells are organised into layers and grow dendrites (receiving processes) and synaptic spines. This maturation is not complete until P12. The final step is for the cerebral cortex to establish connections with surrounding areas of the cortex, setting up the cortical basis of sensory experience. This occurs by P14 (Fitzgerald, 1985).

This development process occurs in all species which are capable of conscious sensory experiences. What differs is the time scale and this will determine the capacity of a particular species to experience sensory inputs. Consequently, in the case of a rat, it seems unlikely that the cerebral cortex can function to register sensory experiences before P12. To allow some margin, rat pups over seven days old can be judged as potentially being able to experience pain and distress and treated accordingly.

Another area where this kind of consideration is important but difficult is with regard to the abilities of marsupial pouch young to have sensory experiences. As a general guide it could be assumed that by the time they are capable of emergence from the pouch they are capable of sensory experiences.

The development of an ability to thermo-regulate begins once the pelage is complete and this ability is mature by the time the young exit from the pouch. Thus it could be assumed that some time during this process the animal's sensory ability has developed such that it is capable of experiencing pain. The time for these processes to occur varies between species. For example, for the tammar wallaby (*Macropus eugenii*), pelage is completed by 120 days and they exit from the pouch at 180 days. The transition in their ability to have sensory responses occurs during that time. Also of note is that the tammar pouch young does not respond to barbiturates before 120 days. By comparison,

pelage is complete in the quokka (*Setonix brachyurus*) at 165 days and in the opossum (*Didelphis virginianus*) by 94 days. For details of marsupial development see Tyndale-Biscoe and Janssens (1988).

There are two other factors which have to be taken into account when choosing a euthanasia method for the foetal or newborn animal—their tolerance to hypoxia and differences in the rate of drug metabolism.

The tolerance of foetal and newborn animals to hypoxia has long been recognised, but, as discussed in a recent review by Singer (1999), the underlying mechanisms have not been extensively studied. As a general guide this tolerance decreases with increasing postnatal age (Adolph, 1969). However, as shown in a study by Glass, Snyder and Webster (1944), there are differences between species and with age. They measured the duration of respiratory movements in rabbits, dogs and guinea pigs when exposed to 100% nitrogen. At the time of birth, breathing continued in rabbits and dogs for 31 minutes and for 6 minutes in guinea pigs. In premature rabbits this was extended to 44 minutes and in the first three weeks post-partum was reduced to 10, 4 then 1.5 minutes for the first, second and third week respectively. Fazekas (1941) showed significant differences in survival times between neonatal rats and guinea pigs exposed to hypoxic conditions; in both species this was significantly greater than adult responses but the species difference was not seen in adults.

The importance of this tolerance to hypoxia when considering the suitability of methods of euthanasia is exemplified in an article by Woolley and Gentle (1988) concerning response to hypoxia in domestic hens. Whilst in adult birds, hypoxia resulted in unconsciousness without evidence of distress before the onset of respiratory failure, in chickens there was a loss of motor control while they were still conscious. Another example of how age effects need to be taken into account when assessing the efficacy of a method of euthanasia is the report of the effects of age on the metabolic and electrical response to decapitation in rats by Zarchin and Mayevsky (1981), who found significantly lower energy consumption and delay in loss of electrocortical responses in both young (14 days) and older (120 days) rats as compared to adults.

Newborn animals also have a diminished capacity to metabolise drugs. Many of the metabolic pathways involved in both primary and secondary drug metabolism do not mature until some days or weeks after birth. For example, newborn mice and guinea pigs do not start to develop the liver enzymes to metabolise drugs such as barbiturates until they are seven days old and this system takes at least 21 days in the mouse and 57 days in the guinea pig to mature (Jondorf *et al.*, 1958). This inability to inactivate drugs through metabolism is probably a major reason for the sensitivity of newborn to barbiturates. A greater sensitivity of the central nervous system to drugs also might be important.

1.5 Signs of Death

Care must be taken to ensure that animals are dead before disposal of the carcase.

The following signs should be used to indicate death:

- *Absence of respiratory movement*. This sign alone is not sufficient as the heart may continue to beat for some time.
- *Absence of heart beat* (determined either by a stethoscope or by palpation of the chest).
- *Absence of pulse.* This can be palpated in the medial aspect of the hind limb in the live animal and is lost after death but is of most use in the larger species, being impossible to discern by palpation in small species such as the rat or mouse.
- *Loss of colour in mucous membranes.* The mucous membranes become pale and mottled, there is no refill after pressure is applied and they become dry and sticky. This again may be more useful in the larger species where the mucous membranes in the mouth are easily accessible.
- *Corneal and palpebral reflexes are lost.* The corneal reflex is elicited when the eyeball is touched and the palpebral reflex is elicited when the eyelids are stroked. The eye should remain open and the lids should not move.
- *Glazing of eyes.* This will occur rapidly after death. The cornea loses its clear moist appearance and becomes opaque, dry and somewhat wrinkled.

1.6 Disposal of Carcases

After death has been verified, the carcase must be disposed of appropriately. This is particularly important for animals such as sheep, cattle, pigs and horses which may be used for human or pet food.

1.7 Personnel Training

It is stressed throughout this document, that whatever the final choice of method, adequate training of staff is vital to ensure that the technique is performed both humanely and effectively. Personnel must have knowledge of and be skilled in how to handle the species involved (UFAW, 1992), how to administer the euthanasia method and how to ascertain that the animal is dead. Personnel must be experienced in how to recognise signs of pain and distress so as to ensure that any unforeseen complications are promptly recognised and dealt with.

These precautions are particularly relevant when physical methods of euthanasia are involved. There are two reasons for this. First, in these circumstances adequate restraint of the animal is important. Second, the accuracy of the delivery of a method of euthanasia is critical to ensure efficacy. Hence, in unskilled hands there is greater risk for animals to experience pain and distress. It cannot be over-emphasised that physical methods must be performed by skilled personnel. Consideration should be given to sedating or anaesthetising the animal first, and at least ensuring that experience is gained initially with anaesthetised animals.

In the development of training programs, it is important to recognise that the killing of animals is emotionally stressful to many people (Owens *et al.,* 1981; Arluke, 1988; Arluke, 1990). The promotion of a caring attitude towards animals is important. However, the emotional tensions of the human-animal bond associated with animals being killed should be addressed in personnel management (Carmack and Becker, 1988). Resolution of these kinds of dilemmas and tensions will promote good animal care, including effective and humane euthanasia (Morrow, 1999).

1.8 Criteria for Recommendations

At the beginning of each section where specific methods for each species are discussed there is a list of methods which are recommended, acceptable with reservations and those which are not acceptable. When possible, scientific references have been used to support these recommendations. However, in many cases it has been necessary to rely on the judgement and experience of the writing group as scientific data are lacking.

When a method is recommended it means that in the opinion of the writing group it is one of the preferred methods for that species. It is humane, is relatively simple to perform and produces rapid unconsciousness and meets all the criteria cited by the AVA (AVA, 1997).

A method which is acceptable but with reservations is one where the method fails to meet all of the AVA criteria, because the method does not produce unconsciousness as quickly as the recommended methods, or the application of the method requires particular skills and training, or there are occupational health and safety considerations. In these circumstances, these methods would be acceptable if in the opinion of the Animal Ethics Committee it was 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 in the opinion of the writing group it is not humane or has other significant problems associated with its use. For example, the use of hydrogen cyanide is not acceptable because it is hazardous to the health of personnel.

SECTION 2

SCIENTIFIC CONSIDERATIONS
Influence of Methods of Euthanasia on Scientific Data

2.1 Introduction

The reproducibility and variability of scientific data collected in an experiment involving animals can be affected by a number of factors, including the way in which animals are killed (Carstensen, 1981, Reilly 1998).

Total variance is a product of analytical, biological and methodological variance (Gartner *et al.,* 1980). Analytical and methodological variance can readily be defined and minimised. The major determinants of biological variance are genetic and environmental factors which can be identified and controlled through appropriate husbandry and management practices (Fox, 1983; Rose, 1994; Harris, 1998). However, what is difficult to identify and to quantify is the biological variance brought about by the interaction of an individual with specific experimental conditions. This may include the influence of biological rhythms, the effects of experimental procedures and the individual's responses to its immediate physical and social environment.

Methods of euthanasia can impact on data variability either directly or indirectly. Specifically, a euthanasia method can alter the qualitative and quantitative characteristics of a parameter, or the circumstances associated with the administration of a method can indirectly modify responses. Even if one cannot substantially change the impact of these effects on measurements, it is useful to recognise their influence, if any, in the interpretation of data and where possible to define and limit these effects on specific measurements.

As a general principle, animals are killed by the most humane method for that species. That condition would only be over-ruled when it is established to the satisfaction of the Animal Ethics Committee that the effects of the euthanasia method on data would compromise the scientific validity of a research proposal. The greater the potential negative impact on well-being, the greater the need to establish scientific value.

In some circumstances, a physical method is chosen because of concerns about chemical agents interfering with subsequent measurements. As noted above, the skills of persons involved are critical because of the greater risk to the animal's welfare using these methods.

Animals are killed to collect blood, tissues and organs for further study and analysis. There is evidence that all euthanasia methods, both chemical and physical, can impact significantly on such measurements. The following examples are not a comprehensive review of all possible complications across a range of species but rather are cited as examples of the diversity of effects which can be seen and the difficulties which may be experienced in minimising such influences. However, it is important to caution against a generalised extrapolation from these examples. What these examples indicate is

that the potential impact of euthanasia methods may need to be validated and defined for a particular set of circumstances and measurements. Clearly, the import of such factors must be evaluated on a case-by-case basis.

2.2 Direct effects of euthanasia techniques on sample analysis

2.2.1 Blood There are a number of reports of the effects of different sampling and euthanasia methods on a range of measurements in blood samples.

Both physical and chemical euthanasia methods modulate the measurements obtained in resting, conscious animals. For example, both decapitation and gaseous anaesthetics produce a significant rise in the levels of circulating noradrenaline and catecholamines when compared with samples from chronically cannulated rats (Carney and Walker, 1973, Depocas and Behrens, 1977; Popper *et al.*, 1977). Milakofsky *et al.*, (1984) analysed amino acid levels and distribution patterns in rat blood obtained by catheterisation or decapitation. They reported a significant artifactual influence due to decapitation in approximately half of the amino acid constituents measured. Similarly, Conahan and colleagues (1985) found that decapitation resulted in higher plasma electrolyte levels in rats and others found a significant increase in plasma ascorbic acid levels after both decapitation and halothane or ether administration (Behrens and Madere, 1979). In all these instances comparisons were made with samples collected from chronically instrumented animals and those samples had been taken at least three days after implantation (Fagin *et al.*, 1983).

One explanation for these effects is a generalised metabolic response secondary to sympathoadrenal release which accompanies handling, decapitation and the use of various anaesthetics including ether and halothane (Depocas and Behrens, 1977; Popper *et al.*, 1977; Roizen *et al.*, 1978). Nevertheless, it is likely that chemical agents, such as anaesthetic drugs, also will have a specific and direct effect on certain measures (Seitz *et al.*, 1973).

The possibility that chronic cannulation itself may be a confounding influence is raised by the work of Laakso and colleagues (1984) who compared the effect on plasma LH and FSH levels when blood was collected from an aortic cannula or after decapitation. They concluded that although gonadotrophin levels were significantly higher associated with decapitation, there may be limitations associated with cannulation. Chronic blood removal was associated with a significant drop in the haematocrit. They hypothesised that chronic stress due to the implanted cannula caused a decrease in gonadotrophin levels.

Since it is widespread practice to use either euthanasia or anaesthesia to assist in blood collection, particularly with small laboratory rodents, there is a need to define that method which is agreed as a 'reference' method, by virtue of the evidence that it has the least impact on a range of biochemical or haematological measures and has minimal impact on the welfare of the animals (Rose, 1990). In an attempt to refine blood collection techniques, to make them more humane, minimise pain and suffering and reduce variability, the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement has produced a useful document describing techniques for removal of blood from laboratory mammals and birds (Anon., 1993).

Several authors have undertaken comprehensive comparative studies to define the impact of a variety of blood collection methods, including physical and chemical methods of euthanasia, on a range of haematological (Archer and Riley, 1981; Bickhardt *et al.*,

1983) and biochemical values in rat serum (Scott and Trick, 1982). These studies illustrate that a wide range of variation occurs and that the magnitude and direction of the responses differs with different methods. Thus the suitability of each method varies with the individual experimental requirements.

Another approach to define and limit variabilities in serum samples associated with methods of euthanasia has been to limit the sympathoadrenal responses using general anaesthetics. Depocas and Behrens (1977) found reduced noradrenaline responses to decapitation when rats were under halothane anaesthesia although halothane, by itself, did have an effect. By contrast, Boehm *et al.*, (1982) found that prolactin levels were higher in blood samples from rats anaesthetised with ether, or anaesthetised with ether and decapitated, compared to prolactin levels in blood from animals killed by decapitation alone.

When Roizen and colleagues (1978) studied the effects of five anaesthetics—cyclopropane, pentobarbital, urethane, chloralose and ketamine—on sympathoadrenal responses to handling and decapitation, all agents prevented or markedly reduced such responses although there was also evidence that the anaesthetic agents had other effects. This conclusion is supported by Scott and Trick's (1982) data where, although anaesthetic agents may in some circumstances have depressed the sympathoadrenal response, the chemical agents still exerted specific and varied effects on biochemical values.

2.2.2 Tissues Both physical and chemical methods of euthanasia can alter tissue histology. The review by Iwarsson and Rehbinder (1993) provides a comprehensive overview of the effects of a range of euthanasia methods on post-mortem findings in rats, mice and guinea pigs. Although many of these effects have long been considered an artefact of tissue preparation this may not be so. For example, Fawell, Thomson and Cooke (1972) showed that oedema of perivascular connective lung tissue of rats, which had been thought to be an artefact, was produced by carbon dioxide euthanasia.

Feldman and Gupta (1976) have shown that some techniques of euthanasia produce histopathologic changes in the tissues of many laboratory species including rats, mice, guinea pigs and rabbits. Each of the methods studied affected the lung tissue to some degree, ranging from mild congestion to marked intramural oedema of pulmonary arteries and alteration of vascular permeability. They determined that some techniques were more suitable than others for particular studies. For example, the use of intraperitoneal sodium thiopentone or carbon dioxide was suitable for pulmonary studies whereas decapitation in mice, rats and guinea pigs, or cervical dislocation in mice was preferable if the abdominal viscera were to be examined. Others have shown that carbon dioxide euthanasia alters lung histology (Britt, 1989) and commonly used euthanasia solutions, including pentobarbital, produced artefacts in lung and kidney tissue of dogs and cats (Port *et al.*, 1978; Prien *et al.*, 1988). Waynforth (1980) reported necrosis of liver parenchymal cells following intraperitoneal pentobarbital sodium and vacuolisation and ground glass lesions were reported in hepatocytes of guinea pigs following carbon dioxide euthanasia (Guttner, 1990). Euthanasia methods can also produce unexpected changes, for example, use of a captive bolt gun can cause shedding of enterocytes from the gut wall (Badaway *et al.*, 1957).

Physical and chemical euthanasia methods can modify the metabolic responses of isolated tissues. A comparative study of the effects of urethane and pentobarbital on the transport of horseradish peroxidase in nervous tissue indicated that, although both agents gave acceptable results, urethane was better in the demonstration of peroxidase transport (Rogers *et al.,* 1980). When compared with decapitation, pentobarbital produced a significant rise in the activity of the dopamine metabolite, dihydroxyphenylacetic acid in selected regions of brain tissue of rats (Zinn *et al.,* 1989). Both pentobarbital and halothane will selectively change the levels of adenosine and guanisine monophosphate activity in various regions of rat brains (Kant *et al.,* 1980). In their study into the effects of carbon dioxide euthanasia on brain tissue, Woodbury and colleagues (1958) found significant shifts in intracellular electrolytes. Malyapa and colleagues (1998) compared the effects of decapitation and carbon dioxide asphyxia on DNA structure in brain cells and although by some markers, such as comet length, no differences were seen, the rats killed by carbon dioxide had more intrinsic DNA damage and showed a wider variation in responses to electromagnetic radiation.

Cervical dislocation, decapitation and various anaesthetic agents can modify the type and order of enzymic activities in liver and heart tissue (Faupel *et al.,* 1972; Seitz *et al.,* 1973; Dutkiewicz and Chelstowski, 1981; Winder *et al.,* 1983; Bhathena, 1992). Both Faupel (1972) and Seitz (1973) looked at the problems associated with sampling liver tissue for the purpose of measuring glycolytic intermediates. All chemical methods tested and cervical dislocation had significant effects on the measured metabolite patterns. They concluded that none of these methods was acceptable for their purposes. In contrast, Winder and colleagues (1983) concluded that when compared with cervical dislocation, carbon dioxide or ether, intravenous barbiturate was the most suitable method for collection of tissues including liver for cyclic AMP assay. However, in his study comparing the effects of decapitation and pentobarbital anaesthesia on plasma and hormone receptors from liver plasma membranes, Bhathena concluded that decapitation was the method of choice (Bhathena,1992). Dutkiewicz and Chelstowski (1981) concluded that decapitation leads to some changes in the functional integrity of the rat heart mitochondria but that ketamine and sodium thiopentone anaesthesia could be used with equal success. Takimoto and Weiner (1979) compared the effects of sodium pentobarbitone with stunning and decapitation on tyrosine hydroxylase activity in the isolated portal vein. Both physical methods resulted in activation of enzymic activity; a response which, based on *in vivo* and *in vitro* evidence, is inhibited by pentobarbital.

In a comparison of the effects of various euthanasia methods, including cervical dislocation, carbon dioxide, halothane and pentobarbital, on immunological parameters Howard and co-workers (1990) showed varying changes to lymphocytic proliferation and cell-mediated lympholysis associated with different methods. For example, whereas pentobarbital anaesthesia followed by cervical dislocation decreased induction of specific cytolytic T-lymphocytes, this method was associated with increased mitogenic proliferation. They concluded that methods of euthanasia do influence certain immunological parameters and consequently selection of a particular euthanasia technique should be given careful consideration.

Not only will there be differences in the effects of chemical and physical agents on particular measurements, but there may be differences between physical methods.

Venkataraman and colleagues (1981) compared acetylcholine content in rat tissues sampled using either the guillotine technique or cervical dislocation. They found that acetylcholine levels in the brain and heart tissues were significantly less after decapitation and attributed these differences to the greater handling stress which they believed was associated with cervical dislocation.

The activity or responses of various isolated tissues or organs can be affected by the method of euthanasia. Segel and Rendig (1982; 1986) reported a difference between the beta adrenergic responses of hearts isolated from rats killed by cervical dislocation compared with those receiving sodium pentobarbitone anaesthesia. Sage, West and Gavin (1985) examined the effects of ether, pentobarbital and alphaxalone on the performance of the isolated beating heart and concluded that pentobarbital was the agent of choice. Both physical and chemical euthanasia methods can modify vascular and intestinal smooth muscle contractility (Butler *et al.,* 1990).

Thus there are many examples where methods of euthanasia, both physical and chemical, can modulate a variety of biological measurements in blood, tissues and organs. Whilst the effects of chemical agents may be due to a specific metabolic or pharmacological action, the effects of physical methods are brought about by a non-specific, systemic metabolic response, which may contribute also to the effects of chemical methods. Stress associated with handling is another factor which plays a significant contribution. As illustrated in the report of Venkataraman and colleagues (1981), the degree of handling may influence the impact of different physical methods.

However, the method of euthanasia may not influence the biological system under investigation. For example, Kassay-Farkas and Wyse (1982) showed no difference in the responses of helical strips from the ventral tail artery between rats killed by cervical dislocation or by drugs, Jones and colleagues (1999) found that carbon dioxide euthanasia did not effect biogenic amine levels in the brain and Slott and others showed that the method of euthanasia did not Effect sperm motility in the rat (Slott *et al.,* 1994). Clearly, the need to consider the impact of euthanasia methods will depend on the kinds of measurements to be made.

2.3 Other factors, associated with euthanasia, which can affect data

There are many other factors associated with euthanasia which can influence the variability of measurements, including animal handling, exposure of animals to other animals being killed and the site and timing of samples.

As noted above, handling associated with the application of a euthanasia method can result in sympathoadrenal discharge which will contribute to the possible metabolic disturbances associated with a particular euthanasia method. Simply handling or brief restraint will affect plasma glucose (Besch and Chou, 1971), progesterone (Bruce *et al.,* 1984), catecholamine (Popper *et al.,* 1977; Sadjak *et al.,* 1983) and noradrenaline (Depocas and Behrens, 1977) levels in rats. This effect of handling on measurements is seen in a diverse range of species, not only laboratory species, but farm animals, amphibians (Mbang Kollo and deRoos, 1983) and fish. (Soivio *et al.,* 1976; Pickering *et al.,* 1982). Depocas and Behrens (1977) and Sadjak and co-workers (1983) demonstrated

that when rats were killed either by decapitation or cervical dislocation, handling stress augmented the catecholamine response. Noradrenaline levels were significantly lower when animals were killed by decapitation under halothane anaesthesia than when compared with the effects of handling alone or decapitation (Depocas and Behrens, 1977).

Handling habituation is an alternative to pharmacological modulation of sympathoadrenal activation and avoids the compounding effects of drugs. For example, Corda *et al.,* (1980) have shown marked differences in brain nucleotides in naïve and handling-habituated rats, and Damon *et al.,* (1986) found a significant difference in the response of naïve rats compared to the response of rats acclimatised to caging in studies of nephrotoxicity. Depending on the kind of measurements under investigation, this may be an option to reduce variability.

As reported by Barrett and Stockham (1963) there are many stimuli to which rats are exposed in the day to day routines and procedures of animal facilities which can produce marked increases in plasma corticosterone concentration. The kinds of procedures involved include environmental change, noise, handling, weighing and intraperitoneal injections. Irrespective of the method of euthanasia, these factors variously have the potential to compound the direct effects of euthanasia methods.

There are a number of psychosocial factors which also should be considered. A significant increase in sympathoadrenal activity has been demonstrated when rats are in the presence of other rats being killed or are exposed to the smell of blood from killed rats (Sadjak *et al.,* 1983). However, Gregory (1998) found no change in plasma cortisol levels in pigs or sheep who witnessed another animal being killed.

The sequence in which animals are killed can affect sample analysis. When rats are housed two to a cage and killed sequentially, there are significant differences in measurements of plasma tryptophan and unesterified fatty acid in the rat killed first compared with the second rat (Knott *et al.,* 1977). Other examples of where the order in which animals are killed has affected data include plasma corticosterone (Dunn and Scheving, 1971) plasma protein and blood lactate levels (Bickhardt *et al.,* 1983) and substance P, cholecystokinin and somatostatin levels in brain tissue (Brodin *et al.,* 1994). Further, Knott, Hutson and Curzon (1977) showed that biochemical changes (fatty acid and tryptophan concentrations) are influenced not only by removal of cage mates (intra-group effects) but also by removal of rats from other cages in the same enclosure (intra-chamber effects).

To address ways by which the impact of this complex of stimuli can be defined and reduced, Faupel and colleagues (1972), who were investigating glycolytic intermediates and related compounds in rat liver, suggested a method of killing and tissue sampling which made it possible to avoid or minimise the three main factors falsifying real substrate values: anaesthesia, anoxia and stress. In the measurement of a group of glycolytic intermediates in rat liver preparations, they found that when using a guillotine method to kill the animals, there was least evidence of abnormal metabolism when the rats were killed at the same time of day (morning), in a quiet, slightly darkened room and were removed from their cage some four to six minutes before killing, which was done when

they were resting. The degree of handling, rather than light and noise levels, seemed to be the critical stimulus.

Another strategy to reduce the level of distress of animals may be to kill them in a familiar environment. Of note is the observation of Hackbarth and colleagues who found no signs of distress in rats during the induction of euthanasia using carbon dioxide when the animals remained in their home cage (Hackbarth *et al.,* 2000). This is likely to have been a contributing factor to the low levels of plasma steroids reported when using nitrogen as a method of euthanasia (Holbrook *et al.,* 1980)

Blood sampling techniques *per se* can have a profound effect on sample analysis. Sampling sites, how soon samples are collected after the animal is killed and the timing of samples relative to circadian rhythms and other schedules such as feeding, may be important (Rose, 1990).

There is an extensive literature on the influence of sampling sites on a range of haematological and biochemical measurements. For example, Upton and Morgan (1975) showed that blood collection from the tail compared to the heart gave consistently different values and concluded that the site of removal, as well as the stress produced during blood sampling, can influence a number of parameters in the rat. Similarly, Arola and co-workers (1979) showed significant differences in blood glucose and plasma lactate levels in rat blood collected either from the tail or the neck (after decapitation). These differences were compounded by handling stress. Archer and Riley (1981) compared drugs used for chemical restraint and a number of sites for blood collection and indicated that there was a need for a standardised technique before results could be reliably compared. A comprehensive review of this subject with regard to clinical biochemistry of laboratory animals can be found in Loeb and Quimby (1989).

Other aspects of sampling procedures which need to be considered include the timing of sampling. Both Bickhardt *et al.,* (1983) and Sadjak *et al.,* (1983) concluded that when blood was collected from the necks of rats after decapitation, the sample should be taken within 10 seconds. The other aspect of sampling is the timing of the sample relative to the effect of circadian rhythms (for example, Barrett, 1963) or diurnal rhythms such as feeding patterns (Cayen *et al.,* 1972).

The above findings indicate that the method of euthanasia, as well as the way animals are handled and conditions of sampling, can have a significant effect on the scientific parameters under study. When animals are killed to collect blood, tissues or organs for subsequent measurements, this must be taken into account in order to obtain reliable and reproducible results.

2.4 Choosing a method

The decision as to the most appropriate method of euthanasia will need to be made on a case-by-case basis: the goals of Refinement and Reduction will be achieved by choosing that method which has minimal impact on the welfare of the animal and is suited to the scientific aims.

As a general guide:

1. If the impact of the method on scientific data is not an issue then use a method which is recommended for the species:

2. If, because of the demonstrated influence on scientific data of any method recommended for a species, it is considered justified to use a method which is acceptable with reservations, then take all necessary precautions to minimise any impact on the welfare of the animal;

3. If there is reason to believe a recommended method may influence data but there is no supportive evidence, then the need to use an alternate method should be validated in a pilot study;

4. If the reason for euthanasia is that an animal is experiencing pain or distress which cannot be alleviated, then a rapid and humane death must take priority over the acquisition of data (Australian Code of Practice 1.18);

5. In all circumstances, the influence of environmental and psychosocial factors which can potentiate the experience of pain and distress as well as impacting on data collection, should be minimised;

6. In all circumstances animals should be monitored to
    i. Establish loss of consciousness;
    ii. Ensure unconsciousness persists until death occurs;
    iii. Identify any evidence of pain or distress, and
    iv. Establish the animal is dead.

SECTION 3

RATS AND MICE

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	Carbon dioxide 💣✳	Halothane ☮	Ether ☮🗣 Hydrogen cyanide ☮☹ Carbon monoxide ☮ Nitrogen ☹
Injectable	Pentobarbitone sodium i/p Ethanol i./p		Chloroform ☮
Physical	None recommended	Cervical dislocation 🕮 Possibly 🗣 in animals heavier than 150g (acceptable if stunned or anaesthetised first) Decapitation ☹ 🕮 💣✳ Stunning and exsanguination ☹ 🕮	Microwave irradiation – not yet proven to be humane 💣✳ Decompression. ☹ 💣✳ Asphyxia ☹ 🗣 💣✳ Rapid freezing 🗣 💣✳

💣✳ Requires specialised equipment
☮Occupational Health and Safety Issues
☹ Aesthetically unpleasant
🕮 Training required
$ Expensive
🗣 Inhumane

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

The advantages of inhaled agents are that they can be deployed with minimal handling of the animals, and larger numbers of animals can be killed simultaneously.

Carbon Dioxide

Carbon dioxide is a colourless gas with a soda like flavour. Inhalation of carbon dioxide causes a sense of breathlessness.

100% carbon dioxide was shown to produce collapse in a mean time of 13 seconds in mice, 8 seconds in mature rats and 12 seconds in immature rats, with death (as judged by the absence of cardiac and respiratory function) in 48, 135, and 78 seconds for the same three groups respectively. The induction was accompanied by depression in rats and mild excitement in mice (Blackshaw *et al.,* 1988). Other workers have shown carbon dioxide to have a depressant effect on the central nervous system in rats (Woodbury *et al.,* 1958; Forslid *et al.,* 1986).

Carbon dioxide is available in several forms; food grade, industrial grade or medical grade carbon dioxide, all compressed in cylinders. After passing through a pressure reducing valve it can be piped either into plastic bags enclosing cages of rats or mice, or directly into deep containers into which the animals will be placed. The optimal flow rate appears to be one which displaces approximately 20% of the chamber volume per minute (Hornett and Haynes, 1984). An alternative method of production of carbon dioxide is to place chips of solid carbon dioxide (dry ice) into a beaker of water inside the container in which the animals are to be killed. In the latter case the beaker needs to be covered in such a way that the mice or rats cannot get into it.

Carbon dioxide is included in Schedule 1 of the United Kingdom Animals (Scientific Procedures) Act 1986, as a standard method of euthanasia for rodents and birds used in research laboratories designated under this Act.

Other workers have indicated that exposure to 100% carbon dioxide may cause hypoxia and will also starve the brain of oxygen thus rendering nervous tissue unsuitable if it is to be collected for physiological *in vitro* studies (Urbanski and Kelley, 1991). They recommended the use of 50:50 carbon dioxide:oxygen for sedation but as at least 70% carbon dioxide seems to be optimal to kill animals (Jaksch, 1981), a 70:30 ratio would seem to be more appropriate (UFAW, 1988).

There has been considerable attention paid to the particular aspect of whether it is preferable to place the rodents straight into the container pre-filled with carbon dioxide ('plunge method') or placing them into a container of air and then running in the carbon dioxide at a rapid rate ('the gradual induction method'). A third possibility is to use a carbon dioxide/oxygen mixture e.g., 70% CO_2/30% O_2 .There is no consensus on which of the first two methods is preferable. The European Commission, DGXI – Working party (1996) suggested placing rodents straight into an atmosphere of more than 70% CO_2 , as euthanasia was quicker, but were equivocal in their final recommendation.

Britt (1987) felt both methods caused some stress and that the precharged 'plunge' method was more stressful in rats. Accordingly the 'gradual induction method' was preferred as longer but less stressful. Hewett et al (1993) described similar reactions by animals in both methods, but with neither showing particular distress, but as the 'gradual method' took up to 6 times longer felt there was no advantage to it. Iwarsson and Rehbinder (1993) advocated the CO_2/O_2 mixture as being less stressful, particularly if followed by pure CO_2 after one minute. This was supported by Coenen *et al.,* (1995) and Danneman et al (1997) who felt that there was less indication of agitation and asphyxiation in the 'gradual method'. Danneman et al (1997) concluded that although carbon dioxide can be used in a humane manner, the concentrations that are least likely to cause pain and

distress are associated with the longest time to anaesthesia and death, highest incidence of unwanted side effects and most severe histological changes in the lungs. Smith and Harrap (1997) held the contrary view recommending the 'plunge method' as being quicker but again did not believe that there was evidence of pain or distress in either method. An even more recent publication (Hackbarth *et al.,* 2000) described a method of euthanasing rats in their home cage by the gradual induction method. They concluded from measurements of serum concentrations of glucose, ACTH and corticosterone and behavioural observations that this method led to a rapid death without severe distress and pain and therefore 'seemed to be humane'.

From the above evidence, it would appear that the methods which cause the animals to become unconscious before being asphyxiated (CO_2/O_2 mixture and gradual induction, where oxygen is gradually replaced) are less stressful and are to be preferred. This needs to be weighed against the shorter time to induction when the plunge technique is used. Until there is more conclusive information available the Working Party believes that all three methods are acceptable.

Injectable agents

Pentobarbitone sodium solution

When pentobarbitone sodium is given at a concentration of 60mg/ml (as formulated for inducing general anaesthesia), this is generally regarded as producing quiet induction of unconsciousness and death even when given by the intraperitoneal route at a dose rate of 10–15 mg/100g body weight. It should be noted that the preferred route of administration is intravenous but in rats and mice this is usually not feasible unless the animal is already anaesthetised.

There is some concern about the use of the highly concentrated sodium pentobarbitone solutions (so called 'euthanasia solutions') given by the intraperitoneal route (350-400 mg/ml) as they may produce irritation of the peritoneum and pain prior to unconsciousness due to the solutions' high alkalinity (Wadham *et al.,* 1997). Wadham suggested this problem may be alleviated by adding a fast acting local anaesthetic to the solution prior to use.

Ethanol

Intraperitoneal injections of 0.5 ml to 6–7 weeks old mice produced gradual loss of muscle control, coma and collapse. A 30% concentration resulted in recovery, 50–100% resulted in death, 70% produced death in 161±52 sec. A 100% solution appeared to cause discomfort when injected, but this was eliminated by drying the needle before injection (Lord, 1989, 1991).

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

The advantages of gaseous agents are that they can be deployed with minimal handling of the animals, and larger numbers can be killed simultaneously. However chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel. These agents should always be used in a fume cupboard.

Halothane

A concentration of 4% halothane can produce cardiac arrest in 90 seconds (Green, 1987). Due to a possible hazard to human health it is acceptable with reservations.

PHYSICAL METHODS

Cervical dislocation

Traditionally this method has been used widely in rodents and from the rapid glazing of the eyes that occurs immediately after cervical dislocation, subjectively it would appear to be a quick and humane technique. Green (1987) stated that it was quick and painless, but that excessive bruising of the neck occurred. Iwarsson and Rehbinder (1993) believed that if the method was carried out correctly, it should cause extensive damage to the brainstem and instantaneous unconsciousness. Blackmore (1993), however, felt that death should be confirmed by exsanguination or destruction of the brain. Adequate training is essential and hence the method is acceptable with reservations.

The method involves holding the animal prostrate on a bench with the thumb and forefinger of the operator firmly squeezing the neck behind the head of the animal. The hindquarters of the animal are grasped firmly with the free hand and pulled caudally. An instrument such as the blades of a pair of scissors, or a firm steel rod can be used instead of the thumb and forefinger.

This technique **should not** be used on conscious animals heavier than 150 grams (i.e., adult rats) as the increased musculature in larger animals makes the technique more difficult to perform expeditiously, unless the person undertaking the procedure is very experienced and well trained. Stunning or induction of anaesthesia prior to cervical dislocation would make this technique more acceptable.

Decapitation

This was regarded as the method of choice for central nervous system studies until Mikeska and Klemm (1975) found that low voltage, fast activity (LVFA) electroencephalogram (EEG) waves persisted for an average of 13.6 seconds after decapitation. They believed this indicated a degree of conscious excitement and nociception. Allred and Bernston (1986) disputed the interpretation of the LVFA EEG pattern, claiming that it did not necessarily indicate alertness or arousal. However Klemm (1987) cited work which showed that this type of wave was associated with arousal states and excitement in animals (Klemm, 1969), and in humans (Marcus, 1972). Klemm (1987) also pointed out that in isolated brain preparations, the brain is fully functional and responsive. This was supported by the observation of King *et al.*, (1967) that electroshock applied to the heads of mice within 3 seconds of decapitation produced a 5–7-fold increase in metabolism during the next 3 seconds, based on changes in ATP, P-creatine, glucose

and lactate. These workers also confirmed that LVFA waves persisted for up to 10 seconds in decapitated mice.

Lorden (1987), however, cited Jouvet (1967) who found that the same wave pattern was present during paradoxical (REM) sleep, and Mayevsky (1978) who found that both urethane and pentobarbitone prolonged the period it took for this wave pattern to reach zero. It has also been recorded in association with hypoxia in rats (Meyer and Marx, 1972), and damage to, or anaesthetisation of, the lower brain stem (Batini *et al.,* 1958; Magni *et al.,* 1959; Vanderwolf *et al.,* 1988).

Vanderwolf *et al.,* (1988) produced evidence which may clarify these apparent contradictions. They cited five references indicating that LVFA waves in the neocortex are determined jointly by an ascending cholinergic input from the basal forebrain, and an ascending serotogenic input from the mid-brain. The two pathways can be separated by their atropine sensitivity. In freely moving rats the atropine resistant LVFA waves occur in close correlation with voluntary movements such as walking or turning the head; atropine sensitive LVFA waves are frequently present in animals that are awake but immobile, and can occur during anaesthesia. Except for a component of the LVFA waves occurring during urethane anaesthesia, any LVFA during anaesthesia is of the atropine sensitive type (Vanderwolf *et al.,* 1975).

If a painful stimulus such as a pinch or electric shock to the feet elicits movement of the limbs, head or trunk in an intact rat (as it almost always does), then atropine-resistant neocortical LVFA waves will accompany the movements (Vanderwolf *et al.,* 1975). Although these cannot be elicited under anaesthesia, they can under neuromuscular blockade, which indicates that peripheral feedback from movement is not necessary for their occurrence (Whitshaw *et al.,* 1976). Thus if decapitated rats experience pain, it might be expected that atropine resistant LVFA waves would occur prominently immediately after decapitation. Vanderwolf *et al.,* (1988) demonstrated that the LVFA waves disappear almost immediately when rats are decapitated, and this suggests that there is no awareness of pain during decapitation.

There is evidence that the necessary handling of rats for decapitation increases plasma catecholamine concentrations (Roizen *et al.,* 1978). Because some rats struggle vigorously against the restraint imposed by being held in the guillotine, Vanderwolf *et al.,* (1988) recommended that rats should first be stunned by cervical fracture. Urbanski *et al.,* (1991) also showed that prior sedation with 50:50 carbon dioxide:oxygen alleviated the stress of restraint associated with decapitation.

Derr (1991) believed that unlike cold-blooded vertebrates which were very tolerant to anoxia, in warm-blooded animals the immediate lack of circulation of blood to the brain and subsequent anoxia rendered the head rapidly insensible. Consequently he felt that prior stunning or sedation was unnecessary. A review of the available information was undertaken by Holson (1992), in response to the 1986 AVMA guidelines for euthanasia (AVMA, 1986) which recommended that decapitation should not be used in conscious animals, unless they were first sedated or lightly anaesthetised. In his interpretation of the data Holson (1992) concluded that unconsciousness due to loss of blood pressure and

consequent hypoxia is almost immediate and that the severed head is anatomically and physiologically incapable of perceiving intense pain even if conscious.

In 1993 the American Veterinary Medical Association Panel on Euthanasia issued a recommendation which stated 'Until additional information is available to better define the nature of the persistent EEG activity, this technique should be used in research settings only when scientifically justified by the user and approved by the Institutional Animal Care and use Committee. (AVMA, 1993). The Working Party of the DGXI of the European Commission (1996) indicated that the use of other methods was preferred until further research can show rapid loss of consciousness.

Decapitation is generally regarded as being unaesthetic but this Working Party believes that it is acceptable with reservations. They also still recommend that decapitation should be preceded by anaesthesia or stunning.

Stunning plus exsanguination or neck dislocation

Stunning is generally (and traditionally) regarded as producing immediate unconsciousness. Green (1987) stated that stunning and exsanguination is the method of choice when blood is needed without the effects of anaesthetics, but personnel must be well trained. This perhaps is the only indication for this method.

Mice are held by the tail and swung in an arc so that the back of the head only contacts a bench.

Rats are held by the hind quarters and brought downwards quickly so as to strike the back of the head on the edge of the bench. Alternatively the rat can be held in the hand and struck, quickly and forcefully on the back of the head with two fingers.

None of these techniques should be used without prior training.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Ether

This causes marked excitement, as shown by behavioural studies (Blackshaw *et al.*, 1988) and perhaps stress, as shown by marked increases in the plasma concentrations of corticosterone, 18-hydroxy-11-deoxycorticosterone, deoxycorticosterone and aldosterone (Holbrook *et al.*, 1980). It has a high explosive risk and its use is now generally strongly discouraged. It is not acceptable for both health and welfare reasons.

Hydrogen cyanide gas

This is generally recognised as causing rapid unconsciousness, followed by convulsions and involuntary defaecation and micturition. When applied as a gas cyanide is dangerous to personnel and it is not acceptable in this form.

Carbon monoxide

This is believed to cause loss of unconsciousness before the animals become stressed. Collapse and unconsciousness occur within 40 seconds, respiratory failure in 120 seconds and cardiac arrest in 5 to 7 minutes (Green, 1987). The risks to personnel limit its use and it is not generally acceptable.

Nitrogen

Nitrogen produces unconsciousness by inducing anoxia. To achieve euthanasia the concentration of oxygen must be less that 2%. Inhalation produced unconsciousness in rats in 30 seconds and death in 60 seconds (Holbrook *et al.*, 1980). The rats killed in this manner had been handled extensively prior to nitrogen administration. The corticosterone concentrations were only a fraction of those recorded in rats by other authors following rapid decapitation (0.64±0.07 µg/dl, n = 16, vs 5.35 to 16.5 µg/dl, the range from seven other workers). The behavioural studies of Hornet and Haynes (1984) create some concern.

They recorded that rats in a cabinet containing air for two minutes showed early recognition of the nitrogen by making rapid circuits of the cabinet and colliding with the sides. Some would jump as if to escape, hitting the lid with some force. Convulsions were common, and many died with their teeth firmly locked around the grid floor. Whenever nitrogen is used it is important to ensure that the animals are subjected to high concentrations immediately. The animal should be placed into a high concentration of the gas rather than passing gas into the container which holds the animal in air. The gas must not escape from the container as the animals are introduced. The animals must not be breathing when or after they are removed from the nitrogen, otherwise they will rapidly regain consciousness.

Because of the practical difficulties associated with maintaining high levels of nitrogen this method is hard to recommend.

Chloroform

This produces rapid onset of collapse and death in rats and mice, without excitement (Blackshaw *et al.,* 1988). Certain inbred strains of mice, e.g., C3H, are extremely sensitive to the hepatotoxic effects of traces of chloroform vapour in the room environment, resulting in sudden death 24 hours later in male animals. However because of its hepatotoxicity, renotoxicity and suspect carcinogenicity (National Institute of Occupational Health, 1981–1982; Hall and Clarke, 1983), its use is unacceptable.

PHYSICAL METHODS

Microwave irradiation

Microwaves have been used, apparently in the belief that they would quickly destroy the brain. Rats exposed to 1300 watts at 2450 MHz for 2.5 seconds were decapitated, and their plasma and brain concentrations of vasopressin were compared with those of rats decapitated without microwave irradiation (Zbuzek *et al.,* 1983). Irradiated rats had significantly higher plasma concentrations and significantly lower neurohypophyseal and hypothalamic concentrations of vasopression. Sham-irradiated rats, held in the microwave restraint apparatus without being irradiated and then decapitated, also had significantly higher plasma vasopressin concentrations. The authors concluded that the restraint itself for irradiation was stressful. The authors reviewed other work, which suggested that microwave irradiation produced too much between-animal variation to be a useful tool for the study of brain peptides.

This is not a routine method of euthanasia and under no circumstances should domestic appliances be used for this purpose (DGXI EC Working party, 1997).

It has not yet been proven to be humane and is thus not acceptable.

Decompression

In mice, a decompression of 100 mm Hg for three minutes, following a decompression rate of 15 mm Hg per minute (Booth, 1978) is sufficient to kill an animal. The signs in man, as described in pilots flying at high altitudes and exposed to low oxygen levels, vary but include an initial excitement (exhilaration or euphoria) followed by headache, lassitude, sensory dullness, visual impairment, neuromuscular weakness, dyspnoea and loss of

consciousness (Booth, 1978). The hypoxia associated with decompression should not be confused with suffocation from strangulation or asphyxiation in which there is an inability to make breathing movements and a slow build-up of carbon dioxide before unconsciousness occurs.

Objective studies appear to have been made only in the dog (Booth, 1978). When decompression is induced rapidly, respiration becomes deep and rapid for a few seconds; marked abdominal distention occurs immediately due to expansion of gas in the alimentary tract; collapse occurs in about eight seconds, and convulsions in 10 to 12 seconds; decerebrate rigidity may be observed, and then convulsions and quiescence. Lachrymation, salivation and urination usually occur.

From the above description the method would be hard to justify and it is thus unacceptable and not recommended.

Asphyxia

This is not acceptable. In humans, cervical pressure cuffs for 100 seconds produced loss of consciousness first, then convulsions, marked cyanosis, involuntary urination and defaecation, bradycardia and dilation of the pupils (Booth, 1978). Inability to perform breathing movements provokes a sense of breathlessness, and the afferent stimuli which cause this are mediated through the vagus nerve.

Rapid freezing

Green (1987) stated that small animals can be plunged head first into liquid nitrogen after which they become instantly insensible. Serious doubts about the humaneness of this method arise when the report of Allred and Berntson (1986) is considered. They pointed out that the core temperature of an 0.5 gram kidney remains above 30°C for at least 20 seconds after it has been dropped into liquid nitrogen, and that a typical rat brain weighs two grams and is insulated by surrounding skull and muscle.

The method is not acceptable for the above reasons.

SECTION 4

GUINEA PIGS

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	Carbon dioxide 💣✳	Halothane ☮ Nitrous oxide (Must be used with other inhalants) ☮	Ether ☮🗣 Hydrogen cyanide ☮☹🗣 Carbon monoxide ☮ Chloroform ☮
Injectable	Pentobarbitone sodium i/p		No intravenous agents are acceptable
Physical	None recommended	Stunning plus exsanguination ☹ 🕮 Cervical dislocation 🕮	

💣✳ Requires specialised equipment
☮Occupational Health and Safety Issues
☹ Aesthetically unpleasant
🕮 Training required
$ Expensive
🗣 Inhumane

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

Carbon dioxide

Euthanasia by carbon dioxide inhalation is widely used and recommended for killing guinea pigs. This can readily be achieved with industrial or food-grade CO_2, supplied in cylinders. The animals should be immersed in a prefilled container of the gas, rather than displacing the air around the animals with carbon dioxide that is delivered through a tube. By placing the guinea pigs directly into approximately 100% carbon dioxide the period of breathlessness is minimised. With this method unconsciousness is induced in the guinea pigs before there is any significant hyperventilatory stimulus (due to hypercapnea) or nasal irritation (due to carbonic acid production on the nasal mucosa) (Ewbank, 1983; UFAW, 1988). A mixture of 70% CO_2 and 30% O_2 is also recommended, to avoid respiratory stimulus due to hypoxia (UFAW, 1988). Death is due to central depression by the anaesthetic effect of CO_2, as well as from anoxia.

Ensure the cage or bin is not overcrowded (for example no more than six adults in a large garbage bin). Leave the animals inside for at least 20 minutes. Neonatal guinea pigs survive up to 4.5 minutes of anoxia, although this period is reduced to three minutes at one week of age or older (Glass *et al.*, 1944). The best approach is to leave inside until the onset of rigor mortis, or ensure death by exsanguination.

The technique as described above is recommended.

Injectable agents

Barbiturates

When pentobarbitone sodium is given at a concentration of 60mg/ml (as formulated for inducing general anaesthesia) this is generally regarded as producing quiet induction of unconsciousness and death, when given either by the intravenous or intraperitoneal route. An overdose of sodium pentobarbitone, injected intraperitoneally (i/p), is the preferred chemical method for killing guinea pigs as it is difficult to find an easily accessible vein in the conscious guinea pig. The dose is 90 mg/kg body weight.

There is some concern about the use of the highly concentrated sodium pentobarbitone solutions (so called 'euthanasia solutions') given by the intraperitoneal route (350-400 mg/ml) as they may produce irritation of the peritoneum and pain prior to unconsciousness due to the solutions' high alkalinity (Wadham *et al.*, 1997). Wadham suggested this problem may be alleviated by adding a fast acting local anaesthetic to the solution prior to use.

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

The advantages of gaseous agents are that they can be deployed with minimal handling of the animals, and larger numbers can be killed simultaneously. However, chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel and these agents should always be used in a fume cupboard.

Halothane

A concentration of 4% halothane can produce cardiac arrest in 90 seconds (Green, 1987). Due to possible hazard to human health it is acceptable with reservations.

Nitrous oxide

This may be a suitable gaseous agent when used with other inhalants to speed the onset of anaesthesia and produce a smoother induction but it alone does not induce anaesthesia in animals even at 100% concentration (AVMA,1993). It is recommended with reservations due to the hazard to personnel.

PHYSICAL METHODS

All physical methods should be performed only by trained personnel, and consideration should be given to sedating or anaesthetising the animal first (AVMA, 1993). Because of the requirement for careful training, the physical methods are acceptable with reservations.

Stunning plus exsanguination

Stunning by a blow to the head with a blunt instrument is an acceptable alternative. This must be performed by skilled operators (UFAW, 1988) and must be followed by dislocation of the neck or incision and exsanguination. EEG wave activity ceases after an average interval of 20 seconds following incision of the carotid arteries (Rössner and Westhues, 1967).

Cervical dislocation

Cervical dislocation of guinea pigs is usually performed by holding the animal with the fingers of the right hand around the back of the neck with the elbow flexed, then forcefully extending the elbow and flicking backwards with the wrist as the elbow becomes straight.

This technique should not be performed by unskilled operators until it has been learned by practice on dead or anaesthetised animals. This method has been recommended by many authorities (e.g. Green, 1987; UFAW, 1988) although there have been some recommendations against it (FELASA, 1985).

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Ether

Use of inhalation agents can be expensive, irritant, stressful, or dangerous. Ether is all of these and therefore not acceptable.

Hydrogen cyanide gas

This produces violent convulsive seizures before death in some animals and is hazardous to personnel. Its use is therefore unacceptable and it is not recommended because of safety risks.

Carbon monoxide or exhaust gas

8% carbon monoxide produces unconsciousness in 1–2 minutes and death in 5–6 minutes (Ramsey and Eilmann, 1932). Exhaust gas must be filtered through water and a metal gauze. However, the potential risk of this to the operator is unacceptable and the method is not recommended.

Chloroform

Chloroform was previously regarded as humane for guinea pigs (UFAW, 1978) but was not mentioned in the later edition (UFAW, 1988). It is not recommended either by the

AVMA (1993) or the DGX1 European Commission (1997) as it is potentially hepatotoxic, nephrotoxic, and possibly carcinogenic to the operators and it is therefore not acceptable.

Injectable agents

As there are no easy sites for intravenous injection, no injectable agents can be recommended (Lumb, 1974; AVMA, 1993).

SECTION 5

RABBITS

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None recommended	Halothane ☮ Nitrous oxide (must be used with other inhalants) ☮	Chloroform ☮💎☹ Carbon dioxide 💣✳💎☹ Hydrogen cyanide gas ☮☹ Carbon monoxide ☮💣✳
Injectable	Pentobarbitone sodium i/v or i/p	Ketamine with a premedicant such as acetylpromazine or xylazine	Ketamine alone 💎 Magnesium sulphate, Potassium chloride 💎
Physical	None recommended	Stunning and dislocation ☹🕮 Captive Bolt 🕮 💣✳☹ Neck dislocation 🕮 or Decapitation 🕮 💣✳ (should only be used if anaesthetised first)	Neck dislocation without anaesthesia 💎☹ Decapitation without anaesthesia 💎☹💣✳

💣✳ Requires specialised equipment
☮Occupational Health and Safety Issues
☹ Aesthetically unpleasant
🕮 Training required
$ Expensive
💎 Inhumane

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None recommended.

Injectable agents

Barbiturates

When pentobarbitone sodium is given at a concentration of 60mg/ml (as formulated for inducing general anaesthesia) this is generally regarded as producing quiet induction of unconsciousness and death even when given by the intraperitoneal route at a dose rate of 60mg/kg body weight. The preferred route of administration is intravenous and it is recommended that this route should be used when ever possible.

There is some concern about the use of the highly concentrated sodium pentobarbitone solutions (so called 'euthanasia solutions') given by the intraperitoneal route (350-400 mg/ml) as they may produce irritation of the peritoneum and pain prior to unconsciousness due to the solutions' high alkalinity (Wadham *et al.,* 1997). Wadham suggested this problem may be alleviated by adding a fast acting local anaesthetic to the solution prior to use.

PHYSICAL METHODS

None recommended.

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

The advantages of gaseous agents are that they can be deployed with minimal handling of the animals. However chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel and these agents should always be used in a fume cupboard.

Halothane

A concentration of 4% halothane can produce cardiac arrest in 90 seconds (Green, 1987). Due to possible hazard to human health it is acceptable with reservations.

Nitrous oxide

This may be a suitable gaseous agent when used with other inhalants to speed the onset of anaesthesia but it alone does not induce anaesthesia in animals even at 100% concentration (AVMA,1993). It is recommended with reservations due to the hazard to personnel.

Injectable agents

Ketamine hydrochloride

An overdose of ketamine causes extensive muscle contractions and is considered unsuitable as a routine method on its own (Baneux, 1986). However, in an adequately premedicated animal it would be acceptable, i.e., after a sedating dose of acetyl promazine (1 mg/kg) given by the subcutaneous (s/c) or intramuscular (i/m) route, or xylazine (5 mg/kg) given s/c or i/m.

PHYSICAL METHODS

Stunning and dislocation

Dislocation of the neck which simultaneously stuns the animal is acceptable with reservations as a method of killing rabbits, due to the requirement for appropriate training.

The technique is performed by suspending the rabbit by the hind legs, grasping around both hocks with the left hand. When the animal is hanging vertically, a hard sharp blow,

preferably with a blunt metal or heavy wooden bar, is struck immediately behind the skull and ears. Alternatively the rabbit is supported under the chest which allows a downward swing of the bar. The back of the right hand can be used effectively but with a risk of injury to the operator. This method must be performed only by trained operators following practice on an anaesthetised or dead animal. Preferably the rabbit should be sedated first, as it is more difficult to accurately direct the blow if the rabbit is moving or struggling.

Captive bolt

Use of a captive bolt pistol is also acceptable (Dennis *et al.*, 1988; Holtzmann, 1991). Spring operated captive bolt guns are available for rabbits. Care must be taken to ensure that skin-slip over the skull does not spoil the aim of the shot. When performed correctly it results in immediate unconsciousness with no struggling or vocalisation and immediate loss of reflexes and respiration. Thalamic activity on an EEG ceases within one minute. However in the opinion of the writing group the need for training in the use of the captive bolt pistol means that it is acceptable with reservations.

Neck dislocation and decapitation

Both of these techniques are acceptable with reservations but only if the rabbit is already anaesthetised.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Chloroform

Rabbits react adversely to chloroform and so it cannot be regarded as acceptable (UFAW, 1988). Chloroform is also potentially hepatotoxic, nephrotoxic, and possibly carcinogenic to the operators.

Carbon dioxide

Carbon dioxide euthanasia has been recommended (Lumb and Jones, 1973), but Green (1979), Ewbank (1988) and Green (1987) have recommended against using it. This is because in animals larger than guinea pigs there seems to be a delay in the loss of consciousness, together with distress (manifested by restlessness, deep respiration, salivation, pawing at the nose) before they collapse. This distress is believed to result from breathlessness and irritation by carbonic acid production on the nasal mucosa (Ewbank, 1988). However Von Cranach *et al.*, (1991) have recommended the use of 100% carbon dioxide. The method may be acceptable for use on baby rabbits up to three weeks of age, but they have a very high tolerance to anoxia and will recover after 30 minutes of anoxia (Glass *et al.*, 1944).

In the opinion of the writing group the use of carbon dioxide in rabbits is not acceptable.

Hydrogen cyanide gas

This may cause violent convulsions and seizures, and as it is hazardous to use it is not acceptable.

Carbon monoxide and filtered exhaust gas

These are satisfactory, but they are too dangerous to personnel to be acceptable without using specialised equipment. Hence they are not recommended.

Injectable agents

Ketamine hydrochloride
The use of ketamine alone is not acceptable for reasons given above (Baneux, 1986).

Other injectable agents

Magnesium sulphate, potassium chloride, chloral hydrate, strychnine, nicotine, and curariforms are not acceptable as they are not considered humane when used alone (Lumb, 1974; AVMA, 1993).

PHYSICAL METHODS

Neck dislocation alone
Simple dislocation of the neck without simultaneous stunning, whether followed by incision and exsanguination or not is not acceptable, but may be performed in the anaesthetised rabbit.

Decapitation
Decapitation in a conscious rabbit is not acceptable as it would be difficult to restrain the animal adequately to allow the technique to be performed humanely. It also would be aesthetically unpleasant, but could be used in a rabbit which is already anaesthetised.

SECTION 6

DOGS AND CATS

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None recommended	Halothane ☮ Methoxyflurane☮$ Carbon dioxide 💣✵⊗ Carbon monoxide ☮💣✵ Nitrogen⊗	Ether☮ Chloroform ☮🔻 Hydrogen cyanide☮⊗
Injectable	Pentobarbitone sodium i/v or i/p		
Physical	None recommended	Captive Bolt 🕮 💣✵⊗ Free bullet ☮⊗💣✵	Electrocution💣✵🔻⊗ Decompression💣✵🔻?⊗

💣✵ Requires specialised equipment **🕮 Training required**
☮Occupational Health and Safety Issues **$ Expensive**
⊗ Aesthetically unpleasant **🔻 Inhumane**

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None recommended.

Injectable agents

Barbiturates

The most suitable method for killing dogs and cats under nearly all circumstances is the intravenous administration of barbiturates. The first major effect of barbiturates is to produce unconsciousness within seconds, which is followed by respiratory depression. At high dosage levels respiratory arrest is followed by cessation of the heart beat. The drug of choice is sodium pentobarbitone, used at a dose rate of approximately 150 mg/kg.

If the intravenous route is not possible an intraperitoneal injection may be used, although the full effect may not be seen for 15 minutes. When pentobarbitone sodium is

given at a concentration of 60mg/ml (as formulated for inducing general anaesthesia) this is generally regarded as producing quiet induction of unconsciousness and death even when given by the intraperitoneal route.

There is some concern about the use of the highly concentrated sodium pentobarbitone solutions (so called 'euthanasia solutions') given by the intraperitoneal route (350-400 mg/ml) as they may produce irritation of the peritoneum and pain prior to unconsciousness due to the solutions' high alkalinity (Wadham *et al.,* 1997). Wadham suggested this problem may be alleviated by adding a fast acting local anaesthetic to the solution prior to use.

The sedation of dogs and cats before euthanasia has been examined by Ramsey and Wetzel (1998) and Wetzel and Ramsey (1998), respectively. Oral administration of dogs with tiletamine - zolazepam (20mg/kg) and acetyl promazine (2mg/kg) or pentobarbital sodium alone (65mg/kg), consistently induced profound sedation and lateral recumbency. In cats a combination of detomidine (0.5mg/kg) and ketamine (10mg/kg), given orally was effective and reliable in producing sedation.

The technique is recommended.

PHYSICAL METHODS

None recommended.

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

Halothane, methoxyflurane

Occupational exposure is a hazard with all anaesthetic vapours and staff should be protected from exposure to levels greater than two ppm, hence these gases are recommended as acceptable with reservations.

Anaesthetic vapours at high concentrations may be irritating and there may be an excitement phase during induction. Halothane or methoxyflurane can be used satisfactorily to euthanase young dogs and cats, particularly when used in an anaesthetic chamber into which the gases are gradually piped.

Under some circumstances feral cats, trapped in a cage, may be euthanased by an anaesthetic gas introduced into a plastic bag placed around the cage.

Carbon dioxide

Carbon dioxide is non-flammable and non-explosive and has been extensively used for routine euthanasia of small animals. As an anaesthetic agent for the dog, 40% CO_2 gave a quick induction (1–2 minutes) with no struggling, retching or vomiting (Leake and Waters, 1929). Little work has been reported on CO_2 euthanasia in the dog (Carding, 1977),

although it is used in animal shelters for puppies. Carding (1968) reported a combination of 40% CO_2 and 3% CO was satisfactory when used in two dogs.

In the cat, CO_2 has been used quite extensively for euthanasia. A report by Glenn and Scott (1973) indicated that with concentrations of CO_2 of more than 60%, loss of consciousness occurred within 45 seconds and respiratory arrest within five minutes. There was some excitement before loss of consciousness, but in no case was it either prolonged or marked. Simonsen *et al.,* (1981) tested 70% CO_2 + 30% O_2. The oxygen was added to reduce the effects of hypoxia, prior to the onset of narcosis and anaesthesia. They came to the conclusion that the mixture was inferior to exhaust fumes, causing some discomfort during the initial phase of about 30 seconds. The time required for euthanasia may be considerably extended in immature animals.

Hence carbon dioxide is acceptable with reservations for use in dogs and cats.

Carbon monoxide

Carbon monoxide has been widely used throughout the world for killing unwanted dogs and cats. It rapidly causes histotoxic anoxia. Unconsciousness and death occur very quickly without pain or appreciable discomfort. A concentration of 6% causes unconsciousness within 30 seconds and death within one minute in dogs (Blood, Johnston and Blackwood, 1972). Using pure CO, Challifoux and Dallaire (1983) noted vocalisation and agitation, possibly in the conscious phase and associated with changes in the EEG, which were followed by rapid cerebral death. Premedication with acetylpromazine largely prevented these behavioural changes (Dallaire and Challifoux, 1985).

In cats, Simonsen and Thordal-Christensen (1981) showed that carbon monoxide derived from exhaust fumes had little effect on behaviour in the initial phase of euthanasia (yawning, trembling and staggering) which lasted about 32 seconds; the total process taking about 60 seconds. They recommended it as a satisfactory agent for euthanasia.

Carbon monoxide gas is available in exhaust fumes from petrol engines (idling) after cooling and removal of particles, as a compressed gas or from the reaction of sodium formate on sulphuric acid. However, this is a highly dangerous gas, odourless but lethal at 3% and it should be treated with great caution.

Because of the hazard to personnel, the use of carbon monoxide is acceptable with reservations.

Nitrogen

Nitrogen is inert, colourless and odourless and constitutes 78% of normal atmospheric air. Rapid replacement of O2 by N2 to give 98–99% N2 within 45 seconds caused unconsciousness in dogs within 40 seconds; the EEG became isoelectric by 80 seconds and arterial blood pressure was undetectable within approximately 20 seconds. Although the induction of unconsciousness was quick and painless, this was followed by a period of yelping, gasping, convulsions and muscular tremors, which may disturb some observers (Herin *et al.,* 1978). Although the manner of death may be aesthetically objectionable, Carding (1977) recommended N2 for euthanasia of dogs, on the grounds of the rapid loss of consciousness and the early appearance of an isoelectric EEG.

The writing group accordingly recommend it as acceptable with reservations.

Very young animals are not euthanased rapidly and may survive a N2 atmosphere for up to 30 minutes. This method is therefore unsuitable for dogs under four months of age (Glass *et al.*, 1944).

PHYSICAL METHODS

Dogs can be declared dangerous because of attacks on livestock and people, and orders may be made for their destruction. Feral cats are destructive of fauna and following trapping euthanasia is indicated. In both these situations physical methods such as the use of a captive bolt pistol are appropriate. In the case of mercy-killing, a physical method may be desirable but it can only be recommended as acceptable with reservations. Whenever possible, dogs and cats should be killed with an overdose of an anaesthetic.

Captive bolt

A functional cerebral cortex is necessary to feel pain, and this is assessed by measuring the EEG and the auditory evoked potential (AEP), to provide measures of changes in cortical and brainstem activity. Dennis *et al.*, (1988) showed that there was motor collapse and loss of corneal reflex following the use of a captive-bolt pistol in dogs. Within 15 seconds of firing the pistol, EEG activity was isoelectric and organised AEP activity above the medulla ceased. It was concluded that cerebral death occurred immediately and that this was a humane method of euthanasia.

Dogs: A captive bolt pistol or free-bullet pistol should be placed against the head at the intersection of lines drawn from the lateral corner of each eye to the base of the ear on the opposite side. The barrel should be aimed at the medulla oblongata (spinal cord) and the trigger pulled. It is essential that the animal be as relaxed and quiet as possible.

Cats: A captive bolt pistol can be used only if the animal is still. It should be applied to the top of the head, between the ears and pointing straight down.

Free Bullet

The free bullet has been a traditional method in rural Australia to kill domestic dogs and sometimes cats. No experimental data are available for the technique and the likelihood of success will depend largely on the experience and skill of the operator (Longair *et al.*, 1991). The use of firearms (free bullets) is generally prohibited in urban areas and when used in rural areas the method presents a potentially dangerous hazard for the operator and any bystanders. Free bullets are acceptable only in the complete absence of injectable euthanasia agents when they may be used as a last resort for euthanasia.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Ether and chloroform

Ether is flammable and highly explosive and chloroform is hepatotoxic. Their use is therefore contra-indicated and neither can be regarded as acceptable.

Hydrogen cyanide gas

Hydrocyanic acid has a long history as a lethal agent for animals and humans. It is extremely rapid in action and causes death by histotoxic anoxia. Cyanide in its gaseous form may cause ataxia followed by convulsions, but there is no evidence that the effects are painful. Nevertheless, it is aesthetically objectionable and extremely dangerous to the operator and cannot be recommended as a satisfactory method of euthanasia in the dog and cat.

PHYSICAL METHODS

Electrocution

This was used for many years in the UK as a simple, rapid and effective method of euthanasia for dogs and cats and is still used in parts of Asia. It is essential to pass the current through the brain to produce an instantaneous stun and loss of consciousness. An electric current passed between front and rear limbs or between the neck and feet causes prompt ventricular fibrillation, with unconsciousness occurring only after fibrillation has occurred. (Roberts, 1954). Ayala-Guerrero *et al.*, (1994) investigated electrocution as a method of euthanasia in dogs. The precise positioning of the electrodes on the skin of the occipital protuberance and near the insertion of the tail were important along with wetting of the contact areas with electrolyte. They claimed animals exhibited a minimum of suffering and the method was appropriate for the euthanasia of dogs.

Nevertheless the occurrence of fibrillation before unconsciousness makes the method unacceptable for dogs and cats (UFAW, 1988).

Decompression

Decompression leads to hypoxia and in some humans this causes excitement, exhilaration and euphoria, followed by headache, sensory dullness and dyspnoea before loss of consciousness. Acute hypoxia may result in unconsciousness without prior warning (Booth, 1978). Sudden decompression to 30 mm Hg causes rapid and marked abdominal distension in the dog, which collapses with convulsions in a few seconds. Salivation and urination usually occur. However, decompression at a rate of 1200 m per minute for 10 minutes (to 12000 m or approximately 140 mm Hg) rapidly induces unconsciousness without these side effects.

In cats, decompression to 15000 m (100 mm Hg) has been recommended at a rate of 600 m per minute, with maintenance of this pressure for three minutes (Barber, 1972).

Carding (1977) considered that decompression was not sufficiently studied to recommend it as an acceptable form of euthanasia, although it was an efficient way of killing large numbers of animals. Particular concern was shown about possible distress in young, sick and aged animals and the lack of data on the variability of response in both dogs and cats. Von Cranach et al (1991) stated that the method could result in severe pain and discomfort before the animal became unconscious due to expansion of trapped gases in body cavities such as the sinuses. Accordingly this method is not acceptable.

Currently Under Revision

SECTION 7

HORSES

SPECIAL PRECAUTIONS

It should be stressed that untrained personnel should not attempt to euthanase a horse both for reasons of their own safety and for the safety and welfare of the horse. Euthanasia should not be attempted unless there is at least one other experienced assistant to assist in restraint of the horse.

Hence all methods which are recommended are acceptable with reservations.

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None	None	
Injectable	None	Pentobarbitone with either xylazine or succinyl choline 🕮 Chloral hydrate + magnesium sulphate + sodium pentobarbitone 🕮	Potassium chloride 🙀 unless already anaesthetised Guaiphenesin alone 🙀 Mephenesin alone 🙀 Succinyl choline alone 🙀
Physical	None	Shooting 🕮 💣✇☹ Captive Bolt 🕮☹💣	

💣 Requires specialised equipment **🕮 Training required**
✇Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **🙀 Inhumane**

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

None recommended.

Injectable agents

Care should be taken with the disposal of the carcase after death if chemical means are used, particularly if the animal is to be used in the human or pet food industry.

Currently Under Revision

Barbiturates

Horses may be satisfactorily euthanased by an i/v injection of succinyl choline (9 mg/50 kg) followed immediately by sodium pentobarbitone i/v at a dose of 50 ml of a 400 mg/ml solution given while standing, followed by another 50 ml once recumbent. Sodium pentobarbitone alone may result in unacceptable excitement prior to unconsciousness. Premedication by i/m xylazine (1–2 mg/kg) may be necessary in the case of nervous or vicious animals. Only well-trained personnel should attempt euthanasia of a horse by means of i/v succinyl choline and barbiturate.

Schneider and Zwickauer (1997) reported that in Germany the commonly used euthanasia procedure in horses is the application of alpha-2-agonists for sedation followed by an overdose of barbiturate.

Chloral hydrate, magnesium sulphate and sodium pentobarbital

This combination was introduced as an intravenous anaesthetic for horses (Lumb and Jones, 1984) with the advantage that it did not cause excitement during induction and there was complete immobilisation during anaesthesia. Administered as an overdose it could be used as an euthanasia agent in horses.

Chloral hydrate was much used as an anaesthetic in horses but has been replaced by more effective agents. Magnesium sulphate does not depress the central nervous system and causes death by asphyxia resulting from complete neuromuscular block. Use of these drugs separately is not acceptable

PHYSICAL METHODS

Shooting or Captive Bolt

Although horses can be euthanased using anaesthetics, shooting or a captive bolt pistol are often used in emergency or field situations.

A captive bolt pistol may be used. According to UFAW (1988) a free bullet (0.32 calibre) should be used to humanely destroy a horse. The exact placement of the pistol and suitable restraint are essential for successful euthanasia as well as for protection of the operator. The brain is in the upper part of the head and it is necessary to shoot just above the intersection of a diagonal line taken from the base of each ear to the medial canthus of the opposite eye (AQIS Operational Guidelines for the Welfare of Animals at Abattoirs and Slaughterhouses 2nd edition 1995.). If used correctly, the horse drops dead immediately and no further action is necessary. It is important that the head should be still. Giving the horse a bowl of oats to eat will often ensure this (UFAW, 1987).

NOT ACCEPTABLE

CHEMICAL METHODS

Injectable agents

Potassium chloride

This compound does not have any anaesthetic action and its use should be restricted to anaesthetised animals where it is an efficient agent for causing cardiac arrest, otherwise its use is not acceptable.

Guaiphenesin, mephenesin or succinyl choline

Immobilising drugs such as these should only be used when standard methods of restraint are impractical, impossible or dangerous or when manual capture and restraint may cause pain and injury through struggling and anxiety. They cause no loss of consciousness and to prevent death by suffocation, euthanasia by physical or chemical means should be performed immediately. On no account should these drugs be used alone.

Buelke (1990) reported that 200 mg of succinyl choline given i/m, followed immediately after collapse by i/v sodium pentobarbitone/phenytoin gave excellent euthanasia in a group of 10 unbroken horses.

SECTION 8

CATTLE

SPECIAL PRECAUTIONS

It should be stressed that untrained personnel should not attempt to euthanase adult cattle both for reasons of their own safety and for the welfare of the animals. In addition, euthanasia should not be attempted unless there is at least one other experienced person available to assist in restraint. Hence there is no recommended method, only methods that are acceptable with the indicated reservations.

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None	None	
Injectable	None	Pentobarbitone sodium 🕮	Magnesium sulphate alone 🗣 Potassium chloride alone 🗣 Guaiphenesin alone 🗣 Mephenesin alone 🗣 Succinyl choline alone 🗣
Physical	None	Captive Bolt 🕮☹💣* Shooting 🕮 💣*☮☹	

💣* Requires specialised equipment **🕮 Training required**
☮Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **🗣 Inhumane**

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

None recommended.

Injectable agents

Care should be taken with the disposal of the carcase after death if chemical means are used. There should be no opportunity for the carcase to be diverted for human consumption or for pet food. Burial or incineration on site may be satisfactory. If the

carcase is to be removed from the site it should be defaced by the use of multiple knife incisions followed by staining with brilliant black ink.

Barbiturates

Calves may be killed by an injection of sodium pentobarbitone (concentrated euthanasia solution, 325 - 400 mg/ml) given intravenously, via the jugular vein. A dose rate of 1ml/2kg body weight is recommended. It is important to ensure that the animals are dead (check for the absence of eye reflexes and heart beat and appearance of glazed eyes) following injection and before disposing of the carcase.

In adult cattle large volumes may be necessary and consequently prior catheterisation of the jugular vein will make administration easier and safer for both animal and operator. A dose of up to 100 ml of pentobarbitone in concentrated form (400mg/ml), may be necessary in adult cattle. Excitable or nervous animals should be sedated before euthanasia. Xylazine at a dose rate of 20mg/100kg (1ml /100kg of a 20mg/ml solution) given by the intramuscular route will provide moderate sedation (Lumb and Jones, 1996).

PHYSICAL METHODS

Although cattle can be euthanased using anaesthetics, shooting or a captive bolt pistol are more often used.

It has been demonstrated that captive bolt stunning followed by exsanguination is a suitable method of euthanasia. This applies to both penetrating (Finnie, 1997) and non-penetrating (mushroom-head) captive bolt pistols (Finnie, 1995). The exact placement of the pistol and suitable restraint of the animal are essential for successful euthanasia as well as for protection of the operator. The recommended position of the bolt on the skull is at the convergence of lines connecting the ear base and ocular medial canthus (AQIS Operational Guidelines, 1995; Finnie, 1997). Stunning should be followed by an immediate collapse and respiration should cease (Fricker and Riek, 1981). Check for the absence of a corneal reflex (Shaw, 1989) and a glazed appearance of the eyes. Reflex kicking is normal but the animal should not make any co-ordinated attempts to rise. The heart may continue beating for several minutes. Gregory and Shaw (2000) describe procedures for checking the effectiveness of captive bolt stunning of cattle.

Some disadvantages of the method are:

- reflex movements may be aesthetically objectionable;
- mechanical damage to portion of the brain;
- capital cost of the captive bolt pistol;
- operator must be experienced and confident;
- blood catecholamine levels may be increased (Rulofson et al 1988); and
- captive bolt stunning devices may be ineffective if they are used infrequently and not cleaned and maintained after each use.

The method is humane and acceptable but with reservations.

Shooting

Shooting is suitable only if the operator has an appropriate firearms licence and has had considerable experience with both firearms and animals. Accordingly the writing group

feels the technique is only acceptable to euthanase cattle used for scientific purposes when other methods are likely to be more dangerous or inhumane.

NOT ACCEPTABLE

CHEMICAL METHODS

Injectable agents

Potassium chloride

This compound does not have any anaesthetic action and its use should be restricted to anaesthetised animals where it is an efficient agent for causing cardiac arrest, otherwise its use is not acceptable.

Guaiphenesin, mephenesin or succinyl choline

Immobilising drugs such as these should only be used when standard methods of restraint are impractical, impossible or dangerous or when manual capture and restraint may cause pain and injury through struggling and anxiety. They cause no loss of consciousness and to prevent death by suffocation, euthanasia by physical or chemical means should be performed immediately. **On no account should these drugs be used alone.**

Magnesium sulphate

Magnesium sulphate has three actions; neuromuscular blockade, cardiac irregularities and anaesthesia. Depending on the route of administration it is suspected that cardiac arrest and neuromuscular blockade can precede anaesthesia (Lumb and Jones, 1996). It is not recommended as a euthanasia agent.

SECTION 9

SHEEP AND GOATS

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None	None	
Injectable	Pentobarbitone sodium 🕮		
Physical	None	Captive Bolt 🕮☹💣 Electrical stunning and exsanguination 💣 🕮 Shooting 🕮 💣☮☹	Exsanguination alone 👎**?** ☹

💣 Requires specialised equipment **🕮 Training required**
☮Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **👎 Inhumane**

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None recommended.

Injectable agents

Care should be taken with the disposal of the carcase after death if chemical means are used. There should be no opportunity for the carcase to be diverted for human consumption or for pet food. Burial or incineration on site may be satisfactory. If the carcase is to be removed from the site it should be defaced by the use of multiple knife incisions followed by staining with brilliant black ink.

Barbiturates

The normal i/v injection route for sheep and goats is via the jugular vein. Shearing (or clipping with scissors) the area of the neck over the vein facilitates the procedure. The animal may be restrained either by an assistant standing astride, or to one side of it, or by positioning the animal on its rump and keeping it vertical in a sitting position. The required volume of barbiturate should be injected reasonably rapidly. For sodium pentobarbitone, a

dose of 80mg/kg is usually sufficient. With sodium pentobarbitone solutions (60mg/ml) approximately 50 ml will be required for a 40 kg sheep. It is advantageous to use euthanasia solutions (sodium pentobarbitone 300 – 400 mg/ml) as the injection volume will then be approximately 10 ml for a 40 kg sheep. It is important to ensure that the animals are dead (check for the absence of eye reflexes and heart beat and appearance of glazed eyes) following injection and before disposing of the carcase.

The possible disadvantages of the procedure include:

- operator training, experience and confidence required; and/or
- gross changes in body chemistry.

ACCEPTABLE WITH RESERVATIONS

PHYSICAL METHODS

Captive bolt stunning

It has been demonstrated that both penetrating and non-penetrating captive bolt stunners are suitable for euthanasia of sheep (Finnie *et al.,* 2000). The correct locations on the skull are illustrated in the AQIS Operational Guidelines (1995). Upon firing, the animal should immediately collapse to the ground and respiration should cease (Fricker and Reik, 1981). Check for the absence of the corneal reflex (Shaw, 1989) and a glazed appearance of the eyes. Reflex kicking is normal but the animal should not make any co-ordinated attempts to rise. The heart may continue beating for several minutes.

When performed correctly, captive bolt stunning causes immediate and permanent insensibility (Blackmore and Delaney, 1988).

Some possible disadvantages of the method are:

- reflex movements may be aesthetically objectionable;
- mechanical damage to portion of the brain;
- capital cost of the captive bolt pistol;
- operator must be trained, experienced and confident;
- shedding of epithelial cells from intestinal mucosa (Badawy *et al.,* 1957); and
- captive bolt stunning devices may be ineffective if they are used infrequently and not cleaned and maintained after each use.

The method is humane and is acceptable but with reservations.

Electrical stunning and exsanguination.

In research facilities where there is a slaughter room and the carcasses are to be used for meat or carcase appraisal or for food, electrical stunning followed immediately by exsanguination is an acceptable form of euthanasia when applied correctly. However it is acceptable with reservations due to the need for specialised equipment and appropriate training.

Shooting

Shooting is suitable only if the operator has an appropriate firearms licence and has had considerable experience with both firearms and animals. Accordingly the writing group feels the technique is only acceptable to euthanase sheep and goats used for scientific purposes under exceptional circumstances.

NOT ACCEPTABLE

PHYSICAL METHODS

Exsanguination

It is suggested that AECs approve this method only where there is a specific research need which prevents the use of the acceptable methods. The non-availability of a captive bolt pistol is not sufficient justification to use this method.

A long sharp knife (minimum length 14 cm) is essential. If wool growth is excessive shear the neck. After making a transverse incision high up on the neck immediately check that the carotids/jugulars on both sides have been severed. A captive bolt pistol should be immediately available and should be used if difficulties arise.

If bilateral severance of vessels occurs, onset of insensibility is rapid (Newhook and Blackmore, 1982), but with unilateral severance the time to loss of brain responsiveness may be prolonged (Gregory and Wotton, 1984). Thus, in the hands of a skilled operator, the method is probably humane but if the technique used is less than perfect, an inhumane situation can easily develop. In addition, the combination of reflex kicking and loss of blood is likely to be aesthetically objectionable.

SECTION 10

PIGS

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None	None	
Injectable	Pentobarbitone sodium 🕮		Carbon dioxide 💣?
Physical	None	Captive Bolt 🕮☹💣* Electrical stunning and exsanguination 💣* 🕮 Shooting 🕮 💣*☮☹	Exsanguination without prior stunning 💣

💣* Requires specialised equipment **🕮 Training required**
☮Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **💣 Inhumane**

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None recommended.

Injectable agents

Care should be taken with the disposal of the carcase after death if chemical means are used. There should be no opportunity for the carcase to be diverted for human consumption or for pet food. Burial or incineration on site may be satisfactory. If the carcase is to be removed from the site it should be defaced by the use of multiple knife incisions followed by staining with brilliant black ink.

Barbiturates

The ear vein is the normal site of injection but this vein is not easy to use without training. Sodium pentobarbitone (60 mg/ml at a dose of 5 ml/2 kg) is satisfactory for small animals but for larger animals euthanasia solutions (sodium pentobarbitone, 300–400 mg/ml at a dose of 1 ml/2 kg) should be used. For inexperienced operators, prior sedation of the animal by i/m injection of azaperone ('Stresnil' 40mg/ml azaperone, 1-2ml/20kg) 30 minutes before attempting an i/v injection would assist. It is important to ensure that the

animals are dead (check for the absence of a eye reflexes and heart beat and appearance of glazed eyes) following injection and before disposing of the carcase.

ACCEPTABLE WITH RESERVATIONS

PHYSICAL METHODS

Captive bolt stunning

The pistol should be positioned at the intersection of imaginary lines drawn from each eye to the opposite ear. The captive bolt should be directed through the skull at right angles to the skull. (AQIS, 1995). This method is very satisfactory with piglets but problems may arise with larger animals due to the presence of large frontal sinuses. For adult animals a captive bolt pistol constructed for use with cattle, not sheep, together with the appropriate cartridges is desirable.

Captive bolt stunning devices may be ineffective if they are used infrequently and not cleaned and maintained according to the manufacturer's instructions after each use. The disadvantages are that there may be very vigorous reflex kicking and there is mechanical damage to the brain. The method is acceptable with reservations concerning the need for well trained staff.

Electrical stunning and exsanguination

In research facilities where there is a slaughter room and the carcasses are to be used for meat or carcase appraisal or for food, electrical stunning followed immediately by exsanguination is an acceptable form of euthanasia when applied correctly. However it is acceptable with reservations due to the need for specialised equipment and appropriate training.

Shooting

Shooting is suitable only if the operator has an appropriate firearms licence and has had considerable experience with both firearms and animals. Blackmore *et al.,* (1995) suggested that buckshot fired from a 12 gauge shotgun may be suitable for the euthanasia of large mature pigs (and also of other domestic livestock).

The writing group feels that shooting is only acceptable to euthanase pigs used for scientific purposes under exceptional circumstances.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Carbon dioxide

There is still some discussion about the humaneness of this method, which is used extensively in some countries for the stunning of animals in abattoirs. It does appear that the concentration of CO2 used may be an important factor in determining the time of onset of insensibility (Blackmore and Delaney, 1988). Although carbon dioxide stunning is used

extensively in abattoirs, doubts are still being expressed with regards to its welfare implications. (Raj and Gregory, 1995; Raj and Gregory 1996; Raj *et al.,* 1997, Jongman *et al.*; 2000). For this reason, the writing group does not wish to endorse the technique at this time.

PHYSICAL METHODS

Exsanguination without prior stunning

This is not acceptable.

SECTION 11

NON-HUMAN PRIMATES

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None	Carbon dioxide 💣✇ (**marmosets only**)	
Injectable	None	Pentobarbitone sodium 🕮 ✇	
		Ketamine hydrochloride 🕮 ✇ followed by pentobarbitone sodium	
		Alphaxalone/alphadalone 🕮 ✇ followed by pentobarbitone sodium **(marmosets only)**	
Physical	None		

💣 Requires specialised equipment **🕮 Training required**
✇Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **Inhumane**

SPECIAL PRECAUTIONS

It should be stressed that untrained personnel should not attempt to euthanase non-human primates both for reasons of their own safety and for the safety and welfare of the animal. In addition euthanasia should not be attempted unless there is at least one other experienced assistant to assist. Hence all methods which are acceptable are with reservations.

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

Carbon dioxide

Marmosets can be euthanased by piping CO_2 into a dark plastic bag. The method is rapid, simple and causes minimal distress to the animal (UFAW, 1987). This technique should not be used with the larger non-human primates as the time to unconsciousness will be slow and animals may show signs of distress (manifested by restlessness, deep respiration, salivation, pawing at the nose) before they collapse. This distress is believed to result from breathlessness and irritation by carbonic acid production on the nasal mucosa (Ewbank, 1983).

Injectable agents

Barbiturates

Euthanasia can be achieved by i/v administration of 60 mg/kg sodium pentobarbitone (UFAW, 1987). However, to ensure the safety of personnel, all primates must be appropriately physically and/or chemically restrained prior to attempting a intravenous injection.

Larger primates should be restrained with a squeeze cage to facilitate handling and injections. Smaller primates (marmosets, squirrel monkeys) can be held by hand, but protective leather gloves should be used.

Ketamine hydrochloride given by the intramuscular route initially will provide sufficient chemical restraint to allow the barbiturate to be given safely by the intravenous route with minimal stress to the animal. The dose of ketamine hydrochloride recommended for chemical restraint in macaques is 10–30 mg/kg, in squirrel monkeys 25 mg/kg and marmosets 20 mg/kg.

Marmosets can also be chemically restrained by the use of alphaxalone/alphadalone (Saffan) at a dose of 18mg/kg administered into the quadriceps muscle of the hind leg. As the volume is relatively large it may be necessary to divide the dose and inject into both legs (Clarke, 1994). Following chemical restraint the animal can then be killed by an overdose of barbiturate.

The barbiturate overdose can be given by the intracardiac (i/c) route. However, this must only be done in animals that are already anaesthetised.

NOT ACCEPTABLE

PHYSICAL METHODS

None are recommended.

Currently Under Revision

SECTION 12

AUSTRALIAN MAMMALS

This section outlines possible euthanasia techniques for use in Australian mammals. Some background information concerning likely mature body weight, temperament and handling is also provided.

It is often preferable to euthanase by an overdose of anaesthetic whilst anaesthetised for other procedures, but if this is not possible then some alternative techniques are provided.

General information on identification, husbandry and body weight can be found in Evans (1982), Strachan (1982), Lavery (1985), NHMRC (1990) and Graves *et al.*, (1990).

Dingo (Order Carnivora)

Dingos (*Canis familiaris*) can be killed in the same ways as dogs. (See Section 6, Dogs and Cats.) However, it may be necessary to restrain them chemically, either with ketamine (40 mg/kg i/m) or 'Leptan'((fentanyl/droperidol, 1 ml/7 kg) before administering an overdose of barbiturate by the intravenous route.

The body weight of an adult dingo is about 15–20 kg.

Bats (Order Chiroptera)

Fruit bats/blossom bats (Family Pteropodidae)

***Pteropus policephalus* – Grey-headed flying-fox** (mature body weight 700 g (male))
***P. scapulatus* – Little red flying-fox** (mature body weight 350 g (male))
***P. alecto* – Black flying-fox** (mature body weight 700 g (male))

Ghost bats (Family Megadermatidae)

***Macroderma gigas* – Ghost bat** (mature body weight 150 g)

The weights given are those of captive animals, which are likely to be heavier than those in the wild, except for *P. scapulatus* which might be lighter as this species does not adapt to captivity. Females may be up to 50–100 g lighter than males depending on the season.

Little red flying foxes are shyer than larger varieties and are stress sensitive.

Bats require careful handling to prevent injury to the operator from biting and clawing and to prevent damage to the legs or wings. It may be easiest to cover them with a cloth or wrap them in a towel to keep the wings together.

Prior sedation with ketamine (80–120 mg/kg i/m) will then allow an overdose of barbiturate (60mg/ml) given by the i/p route. Carbon dioxide may be used in a chamber.

Marsupials (Order Marsupialia)

Marsupial insectivores and carnivores (Family Dasyuridae)

***Antechinus stuartii* – Brown marsupial mouse, Brown antechinus** (mature body weight 20–35 g)
***Antechinus flavipes* – Yellow-footed marsupial mouse, Yellow-footed antechinus** (mature body weight 34–56 g)
As these species are a similar size and shape to rodents, the same techniques used for rodents are applicable.

Chemical methods: Intraperitoneal barbiturate (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice).

Gaseous methods: Halothane, isoflurane, methoxyflurane and carbon dioxide.
Physical methods would probably be difficult and are not recommended.
Care needs to be taken as these animals are prone to bite.

***Dasycercus cristicauda* – Crest-tailed marsupial rat, Mulgara** (rat/mouse size)

Use techniques as for Antechinus species.

***Dasyuroides byrnei* – Byrne's marsupial rat, Kowari** (mature body weight 100–200 g)

This carnivorous marsupial should be held firmly around the shoulders and jaws with the thumb and forefinger to prevent biting.

The same techniques suggested for Antechinus should be used for these animals.

***Dasyurus hallucatus* – Little northern native cat, Northern quoll** (mature body weight up to 750g)
These animals will also bite and should be handled with gloves and the head held firmly.

Similar techniques as used for Antechinus would be suitable.

***Dasyurus viverrinus* – Eastern quoll** (mature body weight 800–1400 g)

This is a small nocturnal carnivorous, insectivorous species which is reasonably amenable to handling, particularly when placed in a small bag. The head should be covered and the animal handled firmly.

Similar techniques as used for Antechinus would be suitable. If the operator is skilled, the marginal ear veins may be used for i/v barbiturate administration.

***Dasyurus maculatus* – Tiger cat, Tiger quoll** (mature body weight 4–7 kg)

Reasonable to handle when done regularly and confidently. A handling bag is often useful.

It could be treated in the same way as a small cat. For example, an anaesthetic chamber could be used if not easy to handle or an overdose of barbiturate could be given either i/p or i/v. (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in section 3, Rats and Mice).

Other techniques as recommended for cats could also be used.

***Sminthopsis crassicaudata* – Fat-tailed dunnart**, Fat-tailed marsupial mouse (mature body weight 12–20 g)
***Sminthopsis macroura*– Stripe-faced dunnart**, Darling Downs marsupial mouse (mature body weight 16–18 g)

This tiny insectivorous marsupial should be killed in the same way as a mouse, for example, by inhalation of carbon dioxide or i/p barbiturate (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in section 3, Rats and Mice).

Kangaroos and wallabies (Family Macropodidae)

Small Macropods (1–4 kg body weight)
***Aepyprymnus rufescens* – Rufous bettong or Rufous rat kangaroo** (mature body weight 3–3.5 kg)
***Bettongia penicillata* – Brushtailed bettong or Brushtailed rat kangaroo** (mature body weight 1.3 kg)
***Lagorchestes conspicillatus* – Spectacled Hare-wallaby** (mature body weight 4 kg)
***Potorous tridactylus* – Long-nosed potoroo** (mature body weight 1–1.2 kg)
***Setonix brachyurus* – Quokka** (mature body weight 3–4 kg)

These species are difficult to handle and are easily stressed and injured during capture. Both hands are needed for restraint; therefore a second person is needed for any injections. They can be restrained in cloth bags. A hand-held net can be used to catch the animal, which is then euthanased by i/p barbiturate or transferred to a hessian sack and transported for euthanasia elsewhere. (See reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice).

If the operator is skilled the overdose of barbiturate can be given by the intravenous route. These animals are often best restrained in sacks, so that one of the lateral caudal tail veins, the cephalic vein in the forearm or the lateral saphenous or tarsal vein in the hind limb can be made accessible.

The anaesthetic chamber may also be used to deliver an overdose of anaesthetic gases or carbon dioxide. Alternatively, once animals are asleep an overdose of barbiturate can be given i/v or i/c. They can be chemically restrained with ketamine (15 mg/kg) + xylazine (5 mg/kg) i/m or zolazepam (5 mg/kg) + tiletamine (5 mg/kg) i/m prior to overdosage with barbiturate (pers. com. G. Phelps).

Medium-sized Macropods (4–10 kg body weight)

***Macropus eugenii* – Tammar wallaby or Dama wallaby** (mature body weight 4.5–8.4 kg)
***Macropus parma* – Parma wallaby** (mature body weight 3.2–5.9 kg)
***Petrogale penicillata* – Brushtailed rock-wallaby** (mature body weight 6 kg)
***Petrogale xanthopus* – Yellow-footed rock-wallaby** (mature body weight 6 kg)
***Thylogale billardierii* – Red-bellied pademelon, *Thylogale stigmatica* – Red-legged pademelon, *Thylogale thetis* – Red-necked pademelon** (mature body weight 3.8–7.0 kg)

The medium-sized macropods are best restrained in a sack to lessen injury to the animal and to the operator, prior to administration of an overdose of barbiturate via the lateral caudal tail vein. Alternatively, the cephalic vein in the forearm or the lateral saphenous or tarsal vein in the hind limb can be used.

If initial restraint is needed, this can be achieved with xylazine (0.2 mg/100 g) and ketamine (3 mg/100 g) i/m. Other dosages have also been recommended, ketamine (15 mg/kg) + xylazine (5 mg/kg) i/m or zolazepam (5 mg/kg) + tiletamine (5 mg/kg) i/m prior to overdosage with barbiturate (pers. com. G. Phelps).

Two people may be required for the restraint of larger animals, one to hold the tail and hind legs and the other to hold the head and forearms while the animal is on the ground. An open-weave hessian bag is most useful for handling.

Large Macropods (body weight greater than 10 kg)

***Macropus giganteus* – Eastern Grey kangaroo** (mature body weight 32–66 kg)
***M. fuliginosus* – Western Grey kangaroo, M. rufus – Red kangaroo, M. robustus – Wallaroo** (mature body weight 30–70 kg)
***Macropus rufogriseus, M.r. bankianus* – Redneck wallaby, *M.r. rufogriseus* – Bennett's wallaby** (mature body weight 15 kg)
***Wallabia bicolor* – Swamp wallaby or Blacktailed wallaby** (mature body weight 13–17 kg)

A large male can be dangerous to the handler, especially from the claws of the hind feet.

An overdose of barbiturate via the tail vein, cephalic vein or the lateral saphenous or tarsal vein in the hind limb can be used once the animal has been restrained on the ground. This will require at least two people. However, prior sedation with diazepam (0.5–1 mg/kg) given s/c may be useful. Alternatively they can be chemically restrained with ketamine (15 mg/kg) + xylazine (5 mg/kg) i.m or zolazepam (5 mg/kg) + tiletamine (5 mg/kg) i/m prior to overdosage with barbiturate (pers. com. G. Phelps).

In experienced hands a more humane method is to shoot the animal. While the chest shot is recommended by kangaroo harvesters, at close range a shot which damages the brain ('head shot') would be preferable, but as with all physical methods training is imperative.

Numbat (Family Myrmecobiidae)

***Myrmecobius fasciatus* – Numbat** (mature body weight 450 g)

These animals can be killed using the same chemical and gaseous methods as for mice and rats.

Marsupial mole (Family Notoryctidae)

***Notoryctes typhlops* – Marsupial mole** (mature body weight 40–70 g)

These tiny animals can be handled and killed using the same chemical or gaseous methods as for mice and rats.

Bandicoots (Family Peramelidae)

***Isoodon macrourus* – Northern brown bandicoot, Brindled bandicoot, Short-nosed bandicoot** (mature body weight 1110 g (female)–2100 g (male), some may reach up to 3–4 kg)
***Isoodon obesulus* – Southern brown bandicoot, Short-nosed bandicoot** (size: similar to **Isoodon macrourus**)

With gentle handling bandicoots are reasonable to manage and the same techniques of euthanasia used for the cat or guinea pig can be used. For example, halothane/O_2 can be administered in a chamber or by mask or an overdose of barbiturate can be given i/p (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice).

Pygmy possums (Family Burramyidae)

***Acrobates pygmaeus* – Feathertail glider, Pygmy glider** (mature body weight 50 g)
***Cercartetus concinnus* – Western pygmy possum** (mature body weight 13 g)
***Cercartetus lepidus* – Little pygmy possum** (mature body weight 7 g)
***Cercartetus nanus* – Eastern pygmy possum, Pygmy possum** (mature body weight 24 g)

These tiny animals should be killed in the same way as mice or rats, i.e., euthanasia by carbon dioxide or anaesthetic vapour or, if readily handled, by an overdose of barbiturate given i/p (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice).

Physical methods would probably be difficult to achieve humanely unless the animals could be restrained adequately.

Ringtail possums and gliders (Family Petauridae)

***Gymnobelideus leadbeateri* – Leadbeater's possum** (mature body weight 150 g)
***Petaurus breviceps* – Sugar glider** (mature body weight 130 g)

***Petaurus breviceps papuanus* – Papuan sugar glider**
***Petaurus norfolcensis* – Squirrel Glider**
***Pseudocheirus peregrinus* – Common ringtail possum, Grey ringtail possum** (mature body weight 900 g)
***Petauroides volans (Schoinobates volans)* – Greater glider** (mature body weight 1.3 kg)

These possums can be aggressive and need to be handled with care. The best approach may be to place the animal in a hessian sack or cloth bag and place this in an anaesthesia chamber. Anaesthetic vapour or carbon dioxide can be given to induce unconsciousness, after which an overdose with barbiturate is given either i/p or i/v (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice). Quiet animals can be restrained by using one hand to hold the animal's head and the other to hold the tail and hind legs. A barbiturate overdose can then be given i/p.

Brushtail possum, Cuscus (Family Phalangeridae)

***Trichosurus vulpecula* – Common brushtail possum** (mature body weight 3 kg)
***Trichosurus caninus* – Mountain possum** (mature body weight 3 kg)
***Phalanger maculatus* – Spotted cuscus** (mature body weight 1.5–3.6 kg)

Possums can be aggressive and as they are capable of inflicting severe bites and scratches they should always be handled with care. They are best handled during daylight hours when they are most likely to be sleepy. Injections can be given by the intramuscular route with the animal asleep in a suspended sack, by passing the needle through the sack after selecting an appropriate point by viewing through the open mouth of the sack. With practice they can be restrained by grasping firmly around shoulders, tail base and rump, but probably should be placed in a sack if the animal needs to be examined.

They are similar in size to a small cat and can be treated accordingly.

The anaesthetic chamber is a simple way of killing these animals with the minimum of stress. The animal is placed in a hessian sack before putting into the chamber.

An overdose of barbiturate can be given i/p, (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice) either as a single procedure or used once the animal is unconscious in the anaesthetic chamber. Alternatively they can be chemically restrained with ketamine (15 mg/kg) + xylazine (5 mg/kg) i/m or zolazepam (5 mg/kg) + tiletamine (5 mg/kg) i/m prior to overdosage with barbiturate (pers. com. G. Phelps). The i/c route can be used in anaesthetised animals.

Oral potassium cyanide delivered either as a paste in glucose +cinnamon bait or as encapsulated tablets is also recommended.

Honey possum (Family Tarsipedidae)

***Tarsipes rostratus* – Honey possum** (mature body weight 10 g)

These animals should be killed using similar chemical or gaseous methods for mice.

Koala (Family Phascolarctidae)

***Phascolarctos cinereus* – Koala** (mature body weight 5.1–6.5 kg)

These animals need to be restrained carefully as they have very sharp claws and powerful jaws. They can be restrained by holding the nape of the neck with the other hand flat under rump, or the animal can be held in a sack. Alternatively they can be restrained in a hessian bag with the person restraining the animal kneeling astride the animal, an arm can then be withdrawn and the cephalic vein used to administer an overdose of barbiturate (pers. com. G. Phelps).

Sedation can be achieved by ketamine (10 mg/kg)/xylazine (5 mg/kg) i/m. Koalas can be anaesthetised using a mask and then an overdose of barbiturate can be given i/p, i/v or i/c.

Wombats (Family Vombatidae)

***Vombatus ursinus* – Common wombat** (mature body weight 26 kg)
***Lasiorhinus latifrons* – Hairy-nosed wombat** (mature body weight 19–32 kg)

These animals bite and scratch but can be restrained in a hessian bag. Grasp the animal under its forearms from behind and lift the animal into the bag or use a strong hand-held catching net for restraint. Some workers feel initial sedation is essential before attempting to handle these powerful animals, which can inflict severe wounds. In this case darting may be necessary. Initial sedation can be achieved via the i/m route (e.g. 'Saffan' (0.5–1 ml/kg) or xylazine/ketamine (5 mg/kg and 10 mg/kg respectively) or by the use of a mask to induce anaesthesia. Once asleep an overdose of barbiturate can be given i/v (radial vein on medial aspect of forearm or cephalic vein) or i/c.

Monotremes (Order Monotremata)

Platypus (Family Ornithorhynchidae)

***Ornithorhynchus anatinus* – Platypus** (mature body weight 1–2 kg)

Platypus can be picked up by the tail but beware of venomous spurs on the hindlegs of the male. There are no readily accessible veins. The animal can be sedated prior to euthanasia if required using ketamine/xylazine i/m at a dose rate of 10–20 mg/kg ketamine and 1–2 mg/kg of xylazine. The steroid anaesthetic 'Saffan' can be given i/m at 0.5 ml/kg for immobilisation.

Once immobilised, an overdose of barbiturate can be given i/p. However, a single i/p overdose of barbiturate (at a dose rate of 80–150 mg/kg) can be used without initial sedation if the animal can be handled readily. (See reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice).

Echidna (Family Tachyglossidae)

Tachyglossus aculeatus – Short-beaked echidna (mature body weight 2–7 kg)

There are no easily accessible veins and the animal is most readily killed in an anaesthetic chamber supplied with halothane, methoxyflurane or carbon dioxide as detailed for other species. Alternatively an overdose of barbiturate (80–150 mg/kg) can be given i/p (see reservations associated with use of concentrated sodium pentobarbitone solutions by the intraperitoneal route in Section 3, Rats and Mice).

Seals and sea-lions (Order Pinnipedia)

Eared seals (Family Otariidae)

***Arctocephalus forsteri* – New Zealand fur seal** (mature body weight 180–200 kg (male), 90 kg (female))
***Arctocephalus pusillus* – Australian fur seal** (mature body weight 218–360 kg (male), 80–110 kg (female))
***Arctocephalus tropicalis* – Subantarctic fur seal** (mature body weight 140 kg (male), 50 kg (female))
***Neophoca cinerea* – Australian sea-lion** (mature body weight 300 kg (male), 80 kg (female))

Earless seals (Family Phocidae)

***Hydrurga leptonyx* – Leopard seal** (mature body weight 270 kg (male), 300 kg (female))

Seals and sea-lions may be immobilised with ketamine (1–2 mg/kg i/m or 4–6 mg/kg when given alone) and diazepam (0.2 mg/kg i/m) given with a 100 x 1.65 mm (16 gauge) needle. Seals are best netted first whilst sea-lions can be injected via projectile syringes at close range. An overdose of barbiturate can then be given intravenously. In Otariid (eared) seals the caudal gluteal vein is the preferred site whilst in Phocid (earless) seals the extradural intravertebral sinus is readily located.

Shooting is an effective means of humane killing, again with the proviso that personnel should be well trained. A free bullet pistol or revolver, a .22 calibre rifle at close range or a shot gun at point-blank range should be used. The site is side of the head, mid-way between the eye and the ear (where the skull is thinnest). More details are available in the Guidelines from the Antarctic Animal Care and Ionising Radiation Usage Ethics Committee (1990).

Rodents (Order Rodentia)

Rats and Mice (Family Muridae)

***Hydromys chrysogaster* – Eastern water rat** (mature body weight 600–750 g)
***Notomys alexis* – Spinifex hopping mouse, Dargawarra** (mature body weight

100–200 g)
***Pseudomys australis* – Plains rat** (mature body weight 100–200 g)
***Rattus colleti, R. fuscipes* – Bush rat, *R. leucopus* – Cape York rat, *R. lutreolus* – Swamp rat, *R. sordidus* – Sordid rat, *R. tunneyi* – Pale field rat, *R. villosissimus* – Long-haired or Plague rat** (mature body weight 60–300 g)

All of these species can be handled and killed by the same methods as laboratory mice and rats.

It is not advisable to handle these animals by the tail, as it will often break off.

SECTION 13

BIRDS (Class Aves)

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	Carbon dioxide (chicks)	Carbon dioxide ⊗ (adult birds) Methoxyflurane, halothane, isoflurane (Chicks and small/medium adult birds) ☮$	Carbon monoxide 💣
Injectable	Pentobarbitone sodium 🕮 - All birds		
Physical	None	Cervical dislocation 🕮 (chicks, small and medium sized birds only) Shooting 🕮 (large birds only)	Cervical dislocation 🕯 (large birds) Decapitation 🕯

💣* **Requires specialised equipment**
☮**Occupational Health and Safety Issues**
⊗ **Aesthetically unpleasant**
🕮 **Training required**
$ Expensive
🕯 **Inhumane**

Embryos/Eggs

It is believed that by 50% of the gestation period the neural tube has developed sufficiently to allow perception of pain. Accordingly bird embryos older than this must be killed humanely. The most commonly used method of destroying eggs is to cool or freeze them (<4(C for 4 hours). Death must then be confirmed by decapitation or some other suitable method. Decapitation or an overdose of anaesthetic is an acceptable method of euthanasia for an embryo exposed for studies (European Commission, 1997).

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

Chicks

Carbon dioxide

Carbon dioxide has been used on a large scale for chicks up to 72 hours old (Clifford, 1984). However, as with all immature animals they tend to be more resistant to the effects of anoxia and so higher concentrations are required than for adults. The chicks should

remain in chambers filled with 100% CO_2 for at least 10 minutes. Care should be taken to avoid overcrowding.

Injectable Agents

All birds

Barbiturates

Sodium pentobarbitone (anaesthetic solution 60mg/ml at a dose rate of at least 80mg/kg) can be given by the intraperitoneal route in all birds and is quick and relatively stress free if performed by experienced operators. The intravenous route can also be used in larger birds by experienced operators.

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Inhaled agents

Chicks

Halothane, methoxyflurane, isoflurane.

All of these anaesthetic agents may be used to euthanase chicks but they are hazardous to the health of operators and must be used only with adequate scavenging equipment. Hence their use is acceptable with reservations.

Small/medium birds (under 3kg)

Carbon dioxide and carbon dioxide/argon oxygen

Carbon dioxide may be used in adult birds but care must be taken to ensure that the chamber is filled prior to putting the birds into it to ensure that there are uniform levels in the chamber. The excessive flapping following loss of consciousness in older birds is aesthetically unpleasant and hence this method is acceptable with reservations. Carbon dioxide induces gasping and breathlessness before the loss of consciousness.

Carbon dioxide has been used in conjunction with argon and oxygen for poultry (Raj and Gregory, 1994).

Halothane, methoxyflurane, isoflurane.

All of these anaesthetic agents may be used to euthanase small/medium birds but they are hazardous to the health of operators and must be used only with adequate scavenging equipment. Hence their use is acceptable with reservations.

PHYSICAL METHODS

Chicks

Cervical dislocation

Cervical dislocation may be used for day old chicks as long as the numbers killed by this method are kept low to avoid human error due to tiredness. (Jaksch, 1981). However, in light of work by Gregory and Wotton (1990), who reported that cervical dislocation does

not immediately affect visual evoked responses in the brain and it does not appear to have a concussive effect, it is acceptable with reservations.

Small/medium birds (under 3kg)

Cervical dislocation

Cervical dislocation has been the preferred method of euthanasia for small to medium-sized birds (Green, 1979; UFAW, 1987. As indicated above it does not immediately affect visual evoked responses in the brain and it does not appear to have a concussive effect (Gregory and Wotton, 1990). When it is used the legs are taken in the left hand and the head held between the first two fingers of the right hand with the thumb under the beak. A sharp jerk with each hand, pulling the head backward over the neck will break the spinal cord and the carotid arteries. The method is not aesthetically pleasant however, as reflexes remain present for some time. The method is thus acceptable with reservations and its use should probably be reserved for occasions when other methods are not available.

Large birds

Larger birds such as emus are best killed by shooting in the head, however as specialised equipment and training are required this method is only acceptable with reservations.

NOT ACCEPTABLE

CHEMICAL AGENTS

Inhaled agents

All birds

Carbon monoxide

Carbon monoxide causes rapid death but as it is extremely dangerous to operators it is not acceptable in chicks, small/medium birds or large birds.

PHYSICAL METHODS

Large birds

Cervical dislocation

In birds larger than 3 kg and some older birds it is very difficult to pull the neck quickly and hence this method is not acceptable.

Decapitation

Gregory and Wotton (1990; 1987) examined the effects of decapitation and neck dislocation on visual evoked responses in the brain before and after the head was disconnected from the body. In many of the birds the evoked responses immediately after disconnection were identical to those beforehand. It was suggested that if these methods were humane they would have to act in a similar manner to concussion. When birds were concussed the evoked responses were immediately lost and they failed to return. The conclusion was that when used in the prescribed manner neither decapitation nor neck dislocation caused an immediate disruption of neurotransmission. There is a serious risk that the birds are not rendered instantaneously insensible and therefore decapitation alone is not acceptable.

SECTION 14

REPTILES (Class Reptilia)

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant	None		Halothane, isoflurane methoxyflurane 💣☹
Injectable	Pentobarbitone sodium 🕮 - all reptiles, but small crocodiles only.		
Physical		Stunning + destruction of the brain – snakes, lizards 🕮 ☹ Captive bolt - large crocodiles 🕮 💣☮	Hypothermia (cooling and freezing) – all reptiles 💔 Decapitation alone 💔☹

💣 Requires specialised equipment **🕮 Training required**
☮ Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **💔 Inhumane**

Reptiles are poikilothermic animals (cold blooded) and hence their body temperature will fluctuate with the external temperature. The differences in their metabolism, respiration and tolerance to cerebral hypoxia mean that techniques used for homeothermic animals are not always appropriate for use in reptiles (AVMA, 1993). For instance, decapitation by itself does not produce rapid unconsciousness in the severed heads of reptiles, and thus its use in conscious reptiles is not recommended (Warwick, 1986). However decapitation followed quickly by destruction of the brain (pithing), extinguishes responses usually thought to indicate consciousness (Warwick, 1990). This information has led to the recommendation that decapitation should only be used if the reptile has been rendered unconscious by other methods, such as concussion (European Commission, 1997).

There may be persistence of somatic responses even after brain destruction (body movements, heartbeat). This is believed to be due to the tolerance of the spinal cord, peripheral nerves and muscle to hypoxia and hypotension and to the greater degree of integration of somatic responses at the spinal cord level rather than in the brain in reptiles and amphibians, compared with homeothermic animals (UFAW/WSPA, 1989). The AVMA (1993) has therefore indicated that double pithing following decapitation in some poikilotherms is necessary to destroy both the brain and the spinal cord. The anatomical features of some species however, preclude the effective use of this method. In addition pithing requires dexterity and skill and should only be done by trained operators.

Cooling (3–4°C) will reduce a reptile's metabolism and reduce locomotion and hence will facilitate handling, however it should be remembered that cooling does not reduce the ability to feel pain.

The Working Party of the DGXI of the European Commission (1997) stated that hypothermia (cooling followed by freezing) was not acceptable for euthanasia purposes in any animal as there may be an initial period of discomfort due to ice crystal formation, both on the skin and within the body. Referring to Summerfelt and Smith, 1990 they suggested that deep freezers should only be used to ensure death once the animal was fully unconscious and unlikely to recover. The ice crystal formation may also limit the usefulness of any tissues obtained from the animal for subsequent microscopic studies (Canadian Council on Animal Care, 1984)

The method chosen for euthanasing a reptile will depend upon species, size and any hazard presented by physical restraint for performance of the procedure. The use of inhalation anaesthetic agents (halothane, isoflurane and methoxyflurane) can present difficulties in reptiles, as they are able to hold their breath for long periods and consequently induction can be protracted. Euthanasia either by injectable agents or by physical methods is thus preferable. More details can be obtained in the reviews by Cooper *et al.*,, (1989) and Frye (1991).

TORTOISES AND TURTLES (ORDER CHELONIA)

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None

Injectable agents

Barbiturates

The AVMA (1993) and the European Commission (1997) recommended that sodium pentobarbitone was an effective and humane method of euthanasia in reptiles, at a dose rate of 60mg/kg. The intravenous route was recommended where possible, particularly when using highly concentrated solutions of sodium pentobarbitone (euthanasia solutions, 325-400mg/ml) otherwise the intraperitoneal route could be used. If the intraperitoneal route of injection was to be employed then it may be preferable to use the sodium pentobarbitone solution formulated for anaesthetic purposes (60mg/ml) to reduce the incidence of pain on injection. Injections should not be intracardiac or into the lungs as this may be painful and irritant (European Commission, 1997).

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Halothane, isoflurane, methoxyflurane

The use of inhalation anaesthetic agents can present difficulties in reptiles, as they are able to hold their breath for long periods and consequently induction can be protracted. In addition, chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel. Consequently the use of these agents for euthanasia purposes in turtles and tortoises is not acceptable.

PHYSICAL METHODS

Hypothermia

UFAW (1987) recommended immobilising the animals by covering with crushed ice and then subsequently killing them by deep freezing. However, this is likely to cause an initial period of discomfort, due to ice crystal formation and is not now an acceptable method (AVMA, 1993).

Decapitation alone

Decapitation by itself does not produce rapid unconsciousness in the severed heads of reptiles, and thus its use in conscious tortoises or turtles is not acceptable (Warwick, 1986). Decapitation should only be used if the reptile has been rendered unconscious by other methods, such as concussion (European Commission, 1997).

SNAKES AND LIZARDS (ORDER SQUAMATA)

Snakes (Sub-order Serpentes)

Handling of snakes, particularly venomous ones should only be attempted by adequately trained and experienced operators. Cooling (3-4°C) will facilitate handling of snakes. However, it should be remembered that cooling does not reduce the ability to feel pain.

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Injectable agents

Barbiturates

The AVMA (1993) and the European Commission (1997) recommended that sodium pentobarbitone was an effective and humane method of euthanasia in snakes, at a dose rate of 60mg/kg. The intravenous route was recommended where possible, particularly when using highly concentrated solutions of sodium pentobarbitone (euthanasia solutions, 325-400mg/ml), otherwise the intraperitoneal route could be used. If the intraperitoneal route of injection was to be employed then it may be preferable to use the sodium

pentobarbitone solution formulated for anaesthetic purposes (60mg/ml) to reduce the incidence of pain on injection. Injections should not be made intracardiac or into the lungs as this may be painful and irritant (European Commission, 1997).

ACCEPTABLE WITH RESERVATIONS

PHYSICAL METHODS

Stunning + destruction of brain

A sharp blow just behind the head followed by decapitation and destruction of the brain is recommended for euthanasia in some snakes, particularly those with fine bone structures (Green, 1979; European Commission, 1997). However, as the technique requires appropriate training and experience the method is acceptable with reservations.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Halothane, isoflurane, methoxyflurane

The use of inhalation anaesthetic agents can present difficulties in reptiles, as they are able to hold their breath for long periods and consequently induction can be protracted. In addition chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel. Consequently the use of these agents for euthanasia purposes in snakes is not acceptable.

PHYSICAL METHODS

Hypothermia

UFAW (1987) recommended immobilising the animals by covering with crushed ice and then subsequently killing them by deep freezing. However, this is likely to cause an initial period of discomfort, due to ice crystal formation as discussed above and is not now an acceptable method (AVMA, 1993).

Decapitation alone

Decapitation by itself does not produce rapid unconsciousness in the severed heads of reptiles, and thus its use in conscious snakes is not acceptable (Warwick, 1986). Decapitation should only be used if the snake has been rendered unconscious by other methods, such as concussion (European Commission, 1997).

LIZARDS (SUB-ORDER LACERTILIA)

Australian lizards vary in body weight from less than 20 g to 10 kg. The larger species (e.g., lace monitor) bite and scratch and should be handled by an experienced operator.

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Injectable agents

Barbiturates

The AVMA (1993) and the European Commission (1997) recommended that sodium pentobarbitone was an effective and humane method of euthanasia in lizards, at a dose rate of 60mg/kg. The intravenous route was recommended where possible, particularly when using highly concentrated solutions of sodium pentobarbitone (euthanasia solutions, 325-400mg/ml) otherwise the intraperitoneal route could be used. If the intraperitoneal route of injection was to be employed then it may be preferable to use the sodium pentobarbitone solution formulated for anaesthetic purposes (60mg/ml) to reduce the incidence of pain on injection. Injections should not however be made intra-cardially or into the lungs as this was regarded as painful and irritant (European Commission, 1997).

ACCEPTABLE WITH RESERVATIONS

PHYSICAL METHODS

Decapitation + destruction of the brain

A sharp blow just behind the head followed by decapitation and destruction of the brain was recommended for euthanasia of some lizards with fine bone structures (Green, 1979; European Commission, 1997). However, as this technique requires training and experience it is only acceptable with reservations.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Halothane, isoflurane, methoxyflurane

The use of inhalation anaesthetic agents can present difficulties in reptiles, as they are able to hold their breath for long periods and consequently induction can be protracted. In addition chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel. Consequently the use of these agents for euthanasia purposes in lizards is not acceptable.

PHYSICAL METHODS

Hypothermia

UFAW (1987) recommended immobilising the animals by covering with crushed ice and then subsequently killing them by deep freezing. However, this is likely to cause an initial period of discomfort, due to ice crystal formation as discussed above and is not now an.acceptable method. (AVMA, 1993).

Decapitation alone

Decapitation by itself does not produce rapid unconsciousness in the severed heads of reptiles, and thus its use in conscious lizards is not acceptable (Warwick, 1986). Decapitation should only be used if the lizard has been rendered unconscious by other methods, such as concussion (European Commission, 1997).

CROCODILES AND ALLIGATORS (CROCODILIA)

Handling and restraint of these animals should only be undertaken by trained and experienced personnel. In the larger animals, effective restraint of the jaws and tail is the key to ensuring operator safety (UFAW/WSPA, 1989).

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None

Injectable agents

Small crocodiles only

Barbiturates

The AVMA (1993) and the European Commission (1997) recommended that sodium pentobarbitone was an effective and humane method of euthanasia in reptiles, at a dose rate of 60mg/kg. The intravenous route was recommended, but as this will be difficult in these animals the intraperitoneal route should be used instead. If the intraperitoneal route of injection is to be employed then it is preferable to use the sodium pentobarbitone solution formulated for anaesthetic purposes (60mg/ml) to reduce the incidence of pain on injection. Injections should not however be made intra-cardially or into the lungs as this was regarded as painful and irritant (European Commission, 1997).

ACCEPTABLE WITH RESERVATIONS

PHYSICAL METHODS

Large Crocodiles

Captive Bolt

The European Commission (1997) has recommended the use of the captive bolt in the laboratory situation for large reptiles, but this should only be carried out by experts who know where to position the pistol, and who are experienced in the handling and restraint of large crocodiles. Further information on the appropriate location for captive bolt penetration is available from Cooper *et al.,* (1989). Because of the need for training, specialised equipment and hazards to personnel associated with handling these dangerous animals this method is only acceptable with reservations.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Halothane, isoflurane, methoxyflurane

The use of inhalation anaesthetic agents can present difficulties in reptiles, as they are able to hold their breath for long periods and consequently induction can be protracted. In addition chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel. Consequently the use of these agents for euthanasia purposes in crocodiles is not acceptable.

PHYSICAL METHODS

Hypothermia

UFAW (1987) recommended immobilising the animals by covering with crushed ice and then subsequently killing them by deep freezing, however this is likely to cause an initial period of discomfort, due to ice crystal formation as discussed above and is not now an acceptable method (AVMA, 1993).

Decapitation alone

Decapitation by itself does not produce rapid unconsciousness in the severed heads of reptiles, and thus its use in conscious crocodiles is not acceptable (Warwick, 1986).

SECTION 15

AMPHIBIANS

FROGS, TOADS, NEWTS AND SALAMANDERS (Class Amphibia)

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical **Inhalant** **Injectable** **Skin Absorption**	None Pentobarbitone sodium 🕮 MS-222, benzocaine, chloral hydrate		Halothane, isoflurane methoxyflurane ☮👎
Physical	None	Stunning and decapitation 🕮☹ Stunning followed by pithing 🕮☹	Hypothermia (cooling and freezing) Decapitation alone 👎☹

💣* **Requires specialised equipment** 🕮 **Training required**
☮**Occupational Health and Safety Issues** **$ Expensive**
☹ **Aesthetically unpleasant** 👎 **Inhumane**

Amphibians are poikilothermic animals (cold-blooded) and hence their body temperature will fluctuate with the external temperature. The differences in their metabolism, respiration and tolerance to cerebral hypoxia mean that techniques used for homeothermic animals are not always appropriate for use in amphibians (AVMA, 1993). Warwick's work in reptiles (1986) indicated that decapitation by itself does not produce rapid unconsciousness in the severed heads, and it is likely that the same will apply to amphibians. However, decapitation followed quickly by destruction of the brain (pithing), extinguishes responses usually thought to indicate consciousness (Warwick, 1990). Consequently the European Commission (1997) recommended that decapitation should only be used if the amphibian has been made unconscious by other methods, such as concussion.

There may be persistence of somatic responses even after brain destruction (body movements, heartbeat). This is believed to be due to the tolerance of the spinal cord, peripheral nerves and muscle to hypoxia and hypotension and to the greater degree of integration of somatic responses at the spinal cord level rather than in the brain in reptiles and amphibians, compared with homeothermic animals (UFAW/WSPA, 1989). The AVMA (1993) has therefore indicated that double pithing following decapitation in some poikilotherms is necessary to destroy both the brain and the spinal cord. The anatomical features of some species, however, preclude the effective use of this method. In addition, pithing requires dexterity and skill and should only be done by trained operators.

Cooling (3–4°C) will reduce an amphibian's metabolism and reduce locomotion and hence will facilitate handling. However, cooling does not reduce the ability to feel pain.

The European Commission (1997) stated that hypothermia (cooling followed by freezing) was not acceptable for euthanasia purposes in any animal as there may be an initial period of discomfort due to ice crystal formation, both on the skin and within the body. Referring to Summerfelt and Smith (1990) they suggested that deep freezers should only be used to ensure death once the animal was fully unconscious and unlikely to recover. The ice crystal formation may also limit the usefulness of any tissues obtained from the animal for subsequent microscopic studies (Canadian Council on Animal Care, 1984).

The skin of amphibians is thin and protected by a cuticle bearing many mucus glands and is thus more sensitive to physical and chemical damage. Anaesthetic agents are readily absorbed through the skin, either as vapours or dissolved in water. When euthanasing amphibians they can be placed either in an anaesthetic chamber into which the anaesthetic vapour is piped or into water in which the anaesthetic is dissolved, thus reducing stress associated with handling. (AVMA, 1993). The European Commission (1997) however felt that euthanasia by absorption of volatile anaesthetic agents in an anaesthetic chamber was unacceptable as the agents were slow to act and may be irritant to the skin. They recommended the use of anaesthetic agents dissolved in water.

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None

Injectable agents

Barbiturates

The AVMA (1993) and the European Commission (1997) recommended that sodium pentobarbitone was an effective and humane method of euthanasia in amphibians, at a dose rate of 60mg/kg. The intravenous route was recommended where possible, particularly when using highly concentrated solutions of sodium pentobarbitone (euthanasia solutions, 325-400mg/ml) otherwise the intraperitoneal route could be used. If the intraperitoneal route of injection was to be employed then it may be preferable to use the sodium pentobarbitone solution formulated for anaesthetic purposes (60mg/ml) to reduce the incidence of pain on injection. Injections should not however be made intra-cardially or into the lungs as this was regarded as painful and irritant (European Commission, 1997).

Skin Absorption

Chloral hydrate

For euthanasia of frogs and toads, Tyler (1995) recommended placing the animal in a container holding a 2–3 mm layer of a 3% solution of chloral hydrate. This is absorbed through the ventral skin and the animal dies in a relaxed state within a few minutes. Care should be taken to ensure that the animal is dead.

MS-222, benzocaine

Tricaine methane sulphonate (buffered MS-222) or benzocaine dissolved in water can be used in a similar fashion to chloral hydrate to produce a non-irritant quick and humane method of killing amphibians (European Commission, 1997). Both the benzocaine (which must be dissolved in acetone first) and the MS-222 reduce the pH of the solutions in which they are dissolved. It is therefore recommended that the solutions should be neutralised with bicarbonate before placing the animal into either of them, so as to reduce possible irritation to the skin.

ACCEPTABLE WITH RESERVATIONS

PHYSICAL METHODS

Stunning and decapitation/Stunning and pithing

Decapitation is not recommended for amphibia unless the animal has first been stunned by a blow to the head or neck. Stunning followed by pithing is also recommended. However, the operator must be well trained and experienced in the method (European Commission, 1997) and consequently it is acceptable with reservations.

NOT ACCEPTABLE

CHEMICAL METHODS

Inhaled agents

Halothane, isoflurane, methoxyflurane

The use of inhalation anaesthetic agents in amphibians is not recommended either by inhalation or skin absorption as induction will be slow and the vapours may cause irritation to the skin. In addition chronic exposure to low levels of anaesthetic gases is regarded as an occupational health and safety risk to personnel. Consequently the use of these agents for euthanasia purposes in amphibians is not acceptable.

PHYSICAL METHODS

Hypothermia

UFAW (1987) recommended immobilising the animals by covering with crushed ice and then subsequently killing them by deep freezing. However, this is likely to cause an initial period of discomfort, due to ice crystal formation as discussed above and is not now an acceptable method. (AVMA, 1993)

Decapitation alone

As discussed above it is possible that in a similar situation to reptiles, decapitation by itself does not produce rapid unconsciousness and thus its use in conscious amphibians is not acceptable (Warwick, 1986).

SECTION 16

FISH

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical **Inhalant** **Injectable** **Skin Absorption**	Halothane, MS-222, benzocaine, eugenol, clove oil	Sodium pentobarbitone (stressful due to removal from water and handling)	Carbon dioxide 🗣
Physical		Stunning + brain destruction 🕮 ☹ Cervical dislocation 🕮 ☹ Decapitation/Spinal section (only in stunned or anaesthetised fish) 🕮 ☹	Cervical dislocation (large fish) 🗣 Decapitation alone 🗣 Removal from water 🗣 Hypothermia/Freezing 🗣

💣* Requires specialised equipment **🕮 Training required**
☮Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **🗣 Inhumane**

Despite the differences in neural pathways between fish and mammals, the neuroanatomy of fish and their complement of neurotransmitters suggests that fish can feel pain (Gregory, 1999) and this must be taken into account when fish are being euthanased.

To reduce stress associated with handling, euthanasia of fish can be effectively achieved by the addition of a euthanasia or anaesthetic agent to the water in which the fish is normally held. However death should be confirmed by destruction of the brain.

RECOMMENDED TECHNIQUES

CHEMICAL METHODS

Inhaled agents

None

Skin Absorption

Halothane

Halothane may be bubbled through the tank of water to anaesthetise and kill fish but death should be confirmed by destruction of the brain.

MS-222, benzocaine, etomidate

An overdose of tricaine methane sulphonate (MS-222) (Martin, 1995), benzocaine or etomidate (European Commission, 1997) may be placed in the aquarium. However, death should be confirmed by destruction of the brain when chemical methods are used.

Eugenol, clove oil

The use of eugenol or clove oil poured into the water has also been recommended.

ACCEPTABLE WITH RESERVATIONS

CHEMICAL METHODS

Injectable agents

As injection of drugs involves removal of the fish from water and handling this is likely to be stressful and consequently other methods of administering drugs are preferable

Barbiturates

Sodium pentobarbitone can be administered by the intraperitoneal route but due to the stress associated with removal from the water and handling, this method is only acceptable with reservations.

PHYSICAL METHODS

Stunning + brain destruction

Stunning by means of a blow to the back of the head, followed by destruction of the brain is another humane method of euthanasia but does require training and experience and consequently is acceptable with reservations.

Cervical dislocation

Cervical dislocation can also be used in small fish, whereby the backbone is broken near the head but again requires some training and experience. It may also be stressful due to the handling required (Clifford, 1984). It is not acceptable in larger fish.

Decapitation

Decapitation or spinal transection should only be carried out in already anaesthetised or stunned animals as there is some doubt that decapitation alone is followed by immediate loss of consciousness. (Flight and Verheijen, 1993; Verheijen and Flight, 1995).

NOT ACCEPTABLE

CHEMICAL METHODS

Skin Absorption

Carbon dioxide

Carbon dioxide may also be bubbled through the tank of water but as this causes hyperactivity before the loss of consciousness this may indicate that there is some irritation or distress and so is not acceptable.

PHYSICAL METHODS

Removal from water, hypothermia

Removal of the fish from water in order to kill it is not an acceptable method of euthanasing fish because of the length of time for the fish to become unconscious (Kestin *et al.,* 1991). This period will be prolonged if the fish are cooled at the same time and hence hypothermia (cooling followed by freezing, or direct freezing) is also unacceptable.

SECTION 17

INVERTEBRATES

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical **Inhalant** **Injectable** **Skin Absorption**	Carbon dioxide bubbled into water (crustaceans and molluscs).MS222 in water (crustaceans, cephalopods). Should be followed by destruction of brain or ganglia to ensure death. Clove oil in water (crabs)	Injection with xylazine or pentobarbitone (crabs) followed by placing in boiling water. 🕮	
Physical		Cooling followed by freezing or spinal section (crustaceans) 🕮 ☹ Brain destruction or decapitation without prior anaesthesia (cephalopods) 🕮 ☹	

💣 Requires specialised equipment **🕮 Training required**
☮Occupational Health and Safety Issues **$ Expensive**
☹ Aesthetically unpleasant **🗡 Inhumane**

Crustaceans

Green (1979) suggested that crustaceans could be immersed in ethanol, magnesium sulphate (7%) or saturated carbon dioxide solutions before placing in boiling water to kill them. Immersion in MS-222 may also be used, but death needs to be confirmed.

Crabs may be injected with xylazine (70 mg/kg) or pentobarbitone (250 mg/kg) before placing in boiling water (Oswald, 1977). Placing crabs in a solution of 0.125ml/L clove oil for 60 minutes is said to provide a humane way of killing crabs (Gardner, 1997).

Crustaceans can be cooled and then deep frozen. Alternatively they can be cooled in a salt/ice slurry for 20 minutes before sectioning to destroy ganglia or placing in boiling water.

Molluscs and other aquatic invertebrates

Green (1979) suggested the use of sodium pentobarbitone, amylocaine or tricaine in the aquarium, or placing in water saturated with carbon dioxide.

Methods of anaesthesia and euthanasia in marine invertebrates including gastropods and molluscs are discussed by Runham *et al,*. (1965), Meier-Brook (1976) and Smaldon and Lee (1979).

The UFAW Handbook on the Care and Management of Cephalopods in the Laboratory (1991) provides further details of euthanasia methods for octopus and squid. They recommend the use of terminal anaesthesia by placing the animal in an anaesthetic solution until respiration stops (approximately 5–10 minutes) followed by physical destruction of the brain. However, if the animal must be killed without anaesthesia the following method is recommended but requires skill and practice. The animal should be placed on a smooth surface to which the arms and suckers will adhere, the mantle and viscera can then be gently pulled to stretch the body. The cranium is located between the eyes and using a sharp scalpel the brain can be bisected with a cut downwards and forwards and is followed by two lateral cuts to sever the brain from the optic lobes, further transverse cuts will disconnect the brain from the major peripheral nerves. Squids can be killed by decapitation, cutting between the head and mantle.

Additional information on euthanasia of insects and crustacea may also be found in Cooper (1990).

SECTION 18

ANAESTHETIC DRUGS—GENERIC NAMES, TRADE NAMES AND MANUFACTURERS

Many of the drugs listed below are classed as Schedule 4 (or equivalent) substances under the relevant legislation in Australian States and Territories and as such are restricted drugs available on prescription only. Fentanyl and Pethidine are Schedule 8 (or equivalent) substances and as such are dangerous drugs. Schedule 8 drugs are strictly controlled and have stringent recording requirements. Potential users should determine availability prior to use.

Acetylpromazine:	'Promex 2' 2 mg/ml acepromazine. Apex Laboratories Pty Ltd, 61 Chivers Road, Somersby, NSW 2250 'A.C.P. 2 Injection' 2 mg/ml acepromazine. Delvet Pty Ltd, 7 Kelray Place, Asquith NSW 2077.
Alphaxalone/ Alphadalone:	'Saffan' Alphaxalone 9 mg/ml. Alphadalone acetate 3 mg/ml. Schering-Plough Animal Health, 71 Epping Road, North Ryde, NSW 2113.
Azaperone:	'Stresnil' 40 mg/ml azaperone. Boehringer Ingelheim Pty Ltd, Vetmedica Division, 85 Waterloo Road North Ryde, NSW 2113.
Diazepam:	'Valium' diazepam 10 mg in 2 ml. Roche Diagnostics, 31 Victoria Avenue, Castle Hill, NSW 2154. 'Pamlin Injection' diazepam 5mg/ml. Parnell Laboratories (Aust) Pty Ltd, 6 Century Estate, 476 Gardeners Road, Alexandria NSW 2015.
Fentanyl/ Droperidol	'Innovar – Vet' (Mallinckrodt, USA) or 'Leptan' (Parnell) each ml contains fentanyl 0.4mg and droperidol 20mg. Parnell Laboratories (Aust) Pty Ltd, 6 Century Estate, 476 Gardeners Road, Alexandria NSW 2015.
Halothane:	'Halothane M&B' Merial Australia Pty Ltd, 6/79 George Street, Parramatta, NSW 2150.
Isoflurane:	'Isoflo' Abbott Australasia, Captain Cook Drive, Kurnell, NSW 2231.
Ketamine:	'Ketamav 100' ketamine hydrochloride 100mg/ml. Mavlab Pty Ltd, 33 Rowlands Street, Slacks Creek, Qld 4127. 'Ketavet 100' ketamine hydrochloride 100mg/ml. Delvet Pty Ltd, 7 Kelray Place, Asquith NSW 2077.
Methoxyflurane:	'Penthrane' Medical Developments Aust. Pty Ltd. 7/56 Smith Road Springvale, Vic 3171
Pentobarbitone:	'Nembutal' 60 mg/ml pentobarbitone sodium. Merial Australia Pty Ltd, 6/79 George Street, Parramatta, NSW 2150.

Pentobarbitone:	‘Euthanasia Solution’ 320 mg/ml pentobarbitone sodium. Apex Laboratories Pty Ltd, 61 Chivers Road, Somersby, NSW 2250. ‘Euthanasia Fort Solution’ 400mg/ml pentobarbitone sodium. Apex Laboratories Pty Ltd, 61 Chivers Road, Somersby, NSW 2250. ‘Lethabarb’ 325mg/ml pentobarbitone sodium. Virbac (Australia) Pty Ltd, 15 Pritchard Place, Peakhurst, NSW 2210
Thiopentone:	‘Pentothal Veterinary Sterile Powder’ Vials 5 gm. Merial Australia Pty Ltd, 6/79 George Street, Parramatta, NSW 2150.
Tiletamine/ Zolazepam:	‘Zoletil 50’ Tiletamine 150 mg, Zolazepam 150 mg. ‘Zoletil 100’ Tiletamine 250 mg, Zolazepam 250 mg. Virbac Australia Pty Ltd, 15 Pritchard Place, Peakhurst, NSW 2210
Xylazine:	‘Xylazil-20’ xylazine (as hydrochloride) 20mg/ml. Ilium Veterinary Products, 98 Long Street, Smithfield, NSW 2164. Xylazil-100’ xylazine (as hydrochloride) 100mg/ml. Ilium Veterinary Products, 98 Long Street, Smithfield, NSW 2164. ‘Thiazine 50’ xylazine (as hydrochloride) 50mg/ml. RWR Veterinary Products, 299 Castlereagh Road, Agnes Banks, NSW 2753

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Further reading

Australian Code of Practice for the Care and use of Animals for Scientific Purposes, 6th Edition, 1997 . The 6th edition is sponsored by the National Health and Medical Research Council (NHMRC), the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Research Council (ARC), and the Australian Vice-Chancellors Committee (AVCC). It is available from the Australian Government Publishing Service, Canberra or at the following website:

HYPERLINK - http://www.health.gov.au/nhmrc/research/awc/code.htm

Guidelines for Humane Slaughter and Euthanasia in the Australian Veterinary Association Members Directory and Policy Compendium (1997), published by the Australian Veterinary Association, 134-136 Hampden Road, Artarmon, NSW 2064

Model Codes of Practice for the Welfare of Animals produced by the Standing Committee on Agriculture and Resource Management, Animal Welfare Committee and endorsed by the Agriculture and Resource Management Council of Australia and New Zealand,
including:

The Pig
The Domestic Fowl
Road Transport of Livestock
Rail Transport of Livestock
Air Transport of Livestock
Livestock and Poultry at Slaughtering Establishments
Sea Transport of Livestock
Animals at Saleyards
The Goat
The Sheep
Intensive Husbandry of Rabbits
The Farming of Deer
Destruction or Capture, Handling and Marketing of Feral Livestock Animals.

These Model Codes of Practice are available from CSIRO Publications, 314 Albert Street, East Melbourne, Victoria 3002.

AQIS (1995) Australian Quarantine and Inspection Service Operational Guidelines for the Welfare of Animals at Abattoirs and Slaughterhouses, Second Edition, Australian Government Publishing Service, Canberra.

Euthanasia of Amphibians and Reptiles. (1989) Report of a Joint Universities Federation of Animal Welfare/World Society for the Protection of Animals Working Party. Potters Bar; UFAW. This report is available from ANZCCART, Adelaide.

Captive Bolt Pistols

Captive-bolt pistols are available from: Food Processing Equipment, 878 Main North Road, Pooraka, SA 5095. Tel. 08-8262-5300.