Recommendations for euthanasia of experimental animals: Part 1

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This report is published in two parts. This first part comprises Sections 1 and 2 of the report, together with a reading list. Section 3 of the report, together with the list of all references cited in both parts and details of training materials, will be published in the January 1997 issue of Laboratory Animals. *Reprints combining both parts of the report will be available from Mrs S E Wolfensohn, Supervisor of Veterinary Services, University of Oxford, Veterinary Services, c/o University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK. (Tel:+44(0)1865-272545, Fax:+44(0)1865-272118, Email: vet@vax.ox.ac.uk).*

Contents to Part 1

Acknowledgements 294

Preface 294

1 Introduction 295
- 1.1 Objectives of euthanasia 295
- 1.2 Definition of terms 295
- 1.3 Signs of pain and distress 296
- 1.4 Recognition and confirmation of death 296
- 1.5 Personnel and training 297
- 1.6 Handling and restraint 297
- 1.7 Equipment 297
- 1.8 Carcass and waste disposal 298

2 General comments on methods of euthanasia 298
- 2.1 Acceptable methods of euthanasia 298
- 2.2 Methods acceptable for unconscious animals 305
- 2.3 Methods that are not acceptable for euthanasia 306

Further reading 309

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Preface

This report has been produced in order to assist personnel concerned with animals used in experiments and for other scientific purposes in assessing which method of euthanasia is the most humane and appropriate for the species of animal that they are using. A brief description of each method is given with reasons for accepting or rejecting them. Details of how to carry out different methods are not provided; these may be found in references cited and in the recommended reading list.

Methods classified as 'acceptable' are those that are considered humane for use on conscious or lightly sedated animals. Other methods may be acceptable only if used on heavily sedated or unconscious animals. In principle, all methods can be used on unconscious animals unless they are unacceptably dangerous to personnel or there is a risk of the animal regaining consciousness before death occurs. Methods included under those 'acceptable for unconscious animals' are those most frequently used in practice. The last category of methods 'not acceptable' are not to be used for the reasons provided in each case.

There are three main sections to this report. Section 1 deals with general notes on legislative requirements of the 1986 Council Directive of the EEC, general requirements of euthanasia, definitions of terms, and other factors to be considered when killing experimental animals. Section 2 provides information on methods of euthanasia used for vertebrates and is divided broadly into acceptable physical and chemical methods, methods acceptable for insensible animals, and those methods not considered acceptable. Section 3 covers each group of species from fish to primates with general information pertaining to the species, including recommendations on embryonic and larval forms. Methods of euthanasia are listed and briefly discussed. At the end of each species section, there is a table summarizing the recommendations for that species.

There are, in addition, comprehensive lists of cited references and literature recommended for further reading (divided into general and species groups), together with information on audiovisual training materials that may be used in training programmes to encourage humane euthanasia practices.

It is recommended that all personnel read Section 1. If information is required about a particular method, this may be obtained in Section 2, and if information is required about a particular species, this may be found in Section 3.

1 Introduction

Animals are killed in laboratories or breeding establishments for various reasons:

- at the end of an experiment or when there might be continuing adverse effects;
- to provide blood and other tissues for a scientific purpose;
- when levels of pain, distress and suffering are likely to exceed the designated level;
- where the health or welfare of the animals are grounds for concern;
- when they are no longer suitable for breeding;
- unwanted stock or those with unsuitable characteristics, for example, type or sex, are not needed.

The Council Directive of 24 November 1986 (Commission of the European Communities 1986) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC) excludes the killing of an animal from the legal definition of an *experiment* (Article 2(d)) if it is carried out using the least painful method accepted in modern practice and in accordance with the scientific purpose of collecting blood and other tissues from the killed animals, therefore leaving these procedures outside the protection of the Directive. This document is designed to assist all those concerned with experimental animals in deciding which method is the most humane and appropriate (in the context of the experiment) for killing the animal with which they are working. As this Directive protects vertebrates, this document will only cover euthanasia of vertebrates. Article 2(1) defines '*humane method of killing*' as the killing of an animal with a minimum of physical and mental suffering, depending on the species.

Whilst this document provides recommendations for the euthanasia of experimental animals, it is strongly recommended that controls and guidelines issued in other EC directives and regulations for the euthanasia of animals be taken into consideration (e.g. Council Directive 93/119/EC (Commission of the European Communities 1993)).

1.1 Objectives of euthanasia

The primary criteria for euthanasia in terms of animal welfare are that the method be painless, achieve rapid unconsciousness and death, require minimum restraint, avoid excitement, is appropriate for the age, species, and health of the animal, must minimize fear and psychological stress in the animal, be reliable, reproducible, irreversible, simple to administer (in small doses if possible) and safe for the operator, and, so far as possible, be aesthetically acceptable for the operator.

1.2 Definition of terms

The word *euthanasia* means a gentle death and should be regarded as an act of humane killing with the minimum of pain, fear and distress.

Consciousness is the state of awareness of a normal animal when it can perceive stimuli from its external environment and respond in the normal behaviour of an awake individual. Unconsciousness will be used to mean insensibility to external stimuli as would be expected in coma or during general anaesthesia. Two main ways of measuring insensibility are to look at the physical responses and responses in the central nervous system at the cortical level.

Pain may be defined as 'an aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species-specific traits of behaviour, including social behaviour' (Zimmermann 1986). Use of the word pain implies a conscious awareness of the stimulus and not an unconscious reflex response.

An *embryo* may be defined as an animal that is developing from a sexually fertilized

or pathenogenetically activated ovum and which is contained within egg membranes or within the maternal body. The embryonic stage ends at the hatching or birth of the young animal (Allaby 1991).

A *foetus* is a mammalian embryo from the stage of its development where its main adult features can be recognized until its birth (Allaby 1991).

A *larva* is considered as the stage during which it is motile and capable of feeding itself, that occurs after hatching from the egg, and prior to the reorganizations involved in becoming adult (Allaby 1991).

1.3 Signs of pain and distress

To ensure euthanasia i.e. a gentle death, it is important to recognize signs of pain, fear and distress in the relevant species. All personnel must be trained to recognize these signs of suffering in the species with which they are working. Assessment of these factors must be based primarily on observations of abnormal behavioural and physiological responses that demonstrate anxiety and fear.

Depending on the species these may include:

- distress vocalizations (not always in the human audible range),
- struggling,
- attempts to escape,
- defensive or redirected aggression,
- freezing/immobility response,
- panting,
- salivation,
- urination, defecation and evacuation of anal sacs,
- pupillary dilatation,
- tachycardia,
- sweating,
- reflex skeletal muscle contractions causing shivering, tremors, or other muscular spasms.

Some of these responses can occur in unconscious as well as conscious animals. Fear may cause immobility or freezing in certain species, particularly rabbits and chickens. This immobility response should not be interpreted as unconsciousness when the animal is, in fact, conscious. In embryos of the last third of their development and very young animals the peripheral as well as the cortical and subcortical components of the pain system are well developed, the neurochemical systems are intact and functional and pain and stressor responses are well documented (Anand & Hickey 1987). Pain may also be associated with deprivation and/or the psychological suffering associated with poor treatment or an inadequate environment.

When assessing the most humane method of euthanasia for any animal, sedation prior to euthanasia may be considered as a method of reducing possible anxiety and distress. However, a factor to consider is that this will involve more handling which in itself may add to the anxiety of the animal, thus negating the purpose of the sedative.

The need to minimize fear and apprehension must be considered in determining the method of euthanaisa. Distress vocalizations, fearful behaviour, and release of certain odours or pheromones by a frightened animal may cause anxiety and apprehension in others. It must be remembered that many vocalizations are at high frequencies and out of the human hearing range. Therefore, whenever possible, animals should not be present during euthanasia of others, especially of their own species. This is particularly important when vocalizations or release of pheromones may occur during induction of unconsciousness. It is also known that the last animal in a group to be removed may become disturbed and so the last two animals may have to be removed together.

1.4 Recognition and confirmation of death

It is essential that all personnel are trained to be able to recognize and confirm death in all the species with which they are working. The most important aspects in recognition of death include cessation of heartbeat and respiration, and absence of reflexes, and in small laboratory animals, the lowering of the body temperature to below 25°C. The method chosen will depend on the species being handled. If there is any doubt about con-

firmation of death, a second method should be used to kill the animal.

1.5 Personnel and training

All methods of euthanasia can be badly performed and therefore personnel carrying out euthanasia on animals must be suitably trained to carry out euthanasia in the most effective and humane manner. Professional advice should be sought.

Training programmes should include courses on the biology of the species to be used, suitable methods of euthanasia for each species and national and European animal welfare regulations. Training must include aspects such as recognition of pain, fear, distress, anxiety, insensibility and death for all species to be used. Detailed courses on methods of euthanasia for each species must be provided, including assessment of the most humane and suitable methods depending on the species and experimental requirements. The operators should be physically capable of carrying out various euthanasia techniques, as well as having sufficient experience in the handling and restraint of the relevant species to minimize distress, fear and anxiety. Courses must include methods to be used to confirm death. Training courses should also cover the functioning and maintenance of the equipment to be used. Competence assessment is necessary at the end of each course.

Experienced personnel who have developed a trusting relationship with the particular animals should be used for euthanasia of these animals as this will minimize stress and anxiety in the animals.

All people performing euthanasia should demonstrate professionalism and sensitivity for the value of animal life. The degree of distress experienced by those people observing or performing euthanasia in any form is dependent on their backgrounds and on their personal philosophies and ethical concerns about using animals in research. The stress of performing euthanasia is magnified when there are strong emotional bonds between personnel and individual animals or when large numbers of animals are killed on a regular basis. The stress experienced by people who regularly perform euthanasia may cause a strong sense of work dissatisfaction or alienation, which might be expressed by absenteeism, belligerence, or careless or callous handling of animals, along with a high turnover rate of personnel. Coping skills for employees should be developed through training programmes. The effects of various agents and methods may be subjective and based on professional judgement, experience and intuition. Some of the reported disadvantages and controversy about certain practices may be based on sentiment and aesthetic considerations rather than on sound scientific data. Some physical methods may be aesthetically unpleasant but quite humane. The choice of method of euthanasia must be based primarily on humane concerns for the animal rather than on the sensitivities of the technician who performs the task or the people who observe the euthanasia. However, the opportunity must be given to personnel to refuse to carry out methods of euthanasia they find personally abhorrent.

1.6 Handling and restraint

As with other procedures applied to animals, euthanasia usually requires some physical control over the animal. The degree of control and kind of restraint needed will be determined by the animal species, breed, size, state of domestication, presence of painful injury or disease, degree of excitement, and method of euthanasia. Suitable control is vital to minimize pain and distress in animals, to assure safety of the person performing euthanasia, and frequently to protect other animals and people. Gentle but firm restraint by a familiar handler, careful handling, stroking, and talking during euthanasia often have a calming effect on many animals. Where capture or restraint may cause pain, injury or anxiety to the animal or danger to the operator, the prior use of tranquillizing and immobilizing drugs may be necessary.

1.7 Equipment

Instruments, equipment and installations used for stunning or killing animals should

be designed, constructed and maintained so as to achieve rapid stunning and death. They should be regularly inspected and cleaned to ensure that they are in a good state of repair and will function correctly at all times. Blood, urine and faeces which could cause anxiety to subsequent animals must be removed.

1.8 Carcass and waste disposal

The possible hazards to humans when animals are known to be carrying a zoonotic agent or were treated with radioisotopes or toxic chemicals must be evaluated and personnel handling such carcasses should take the necessary precautions to protect themselves and others. Care should be taken when disposing of carcasses and other waste (for example water in which agents have been dissolved) that it does not provide any danger to others or the environment. Chemical methods (except carbon dioxide) must not be used on animals destined for consumption or where the carcass may enter the food chain. Operators must ensure that they comply with national and international legislation.

2 General comments on methods of euthanasia

The majority of methods that have been used to kill experimental animals are listed in this section. For those more obscure methods that are not mentioned it should generally be assumed that they are not considered acceptable until they have been carefully assessed under the criteria given in Section 1 and have been considered humane by a qualified person such as a veterinarian or the competent authority. Section 1 must be consulted in conjunction with this section.

Agents may cause death by three basic mechanisms: (1) hypoxia, direct or indirect; (2) direct depression of neurons vital for life functions; and (3) physical disruption of brain activity and destruction of neurons vital for life (Andrews *et al.* 1993, Lumb & Jones 1984).

Further details for each species group may be obtained in Section 3.

2.1 Acceptable methods of euthanasia

Physical methods

These methods must cause *immediate* loss of consciousness through physical trauma to the brain. They are most useful when pharmacological methods would interfere with the purpose of the experiment. While physical methods may be aesthetically less pleasant for observers and those killing animals, in skilled hands they are quick and certain and possibly the least distressing for the animal. Specialist training is essential for all of these methods. These methods require restraint which may cause extra stress for some animals. If possible the animal should not be killed in the sight or smell of other animals.

2.1.1 Shooting

Shooting in the head to ensure immediate destruction of the brain is an effective and humane way of killing large reptiles and mammals (Australian Veterinary Association 1987, Longair *et al.* 1991). This may be divided into two types: free bullet or captive bolt (with penetration or percussion). The type of weapon used must be selected according to the species to be killed and the environment.

(a) Free bullet Special care must be taken to avoid danger to the operator. All personnel must be trained in these techniques to ensure the correct positioning of the weapon to ensure a direct hit into the brain (Longair *et al.* 1991). Shooting using a free bullet must not be used inside a building because of danger to personnel from ricocheting bullets, but it may be used effectively in the field by skilled marksmen. When the animal can be appropriately restrained, the captive bolt method is preferable as there is less danger to personnel.

A free bullet humane killer is preferred for example, on horses (Blackmore 1985, Dodd 1985, Oliver 1979).

(b) Captive bolt The penetrating captive bolt is an effective tool for rendering many larger animals unconscious (Blackmore & Delaney 1988, Daly & Whittington 1989, Green 1987, Longair *et al.* 1991). Large rabbits and dogs may also be killed in this way (Dennis *et al.* 1988, Holtzmann 1991). However, it is not always effective in large pigs and mature bulls because of the thickness and density of the skull. The purpose of stunning is to render the animal instantaneously insensible to pain by causing concussion (Ministry of Agriculture, Food and Fisheries 1993). The animal should remain insensible until exsanguination is performed (Blackmore 1993). An effective stun may be recognized by the animal collapsing immediately after shooting, with its body and muscles rigid and it should not have a righting reflex. Normal rhythmic breathing should stop, there should be a loss of blink reflex and the eyeball should point outwards and not be rotated into the skull. Effective stunning depends on accurate positioning of the pistol, use of the correct strength of cartridge in relation to the species and size of the animal, the size and speed of the bolt and proper maintenance of the pistol. The site of penetration differs with each species and therefore this method should only be carried out by suitably trained personnel. Appropriate restraint must be used to prevent incorrect positioning of the pistol. The recommended pistol is one with the bolt recessed into the muzzle before firing, rather than one where the bolt extends beyond the muzzle, as the recessed one is likely to generate a higher bolt velocity at impact. The operator should ensure that the bolt retracts to its full extent after each shot and if not, it should not be used again until it has been repaired. The bolt should always be properly cleaned after each use.

2.1.2 *Concussion (stunning)*

This may be carried out by several means depending on the size of the animal. In smaller animals such as small rabbits, newborn kittens and newborn puppies, rats, mice, young guineapigs, hamsters, birds, small reptiles, amphibians and fish (Clifford 1984), a blow on the head may be sufficient to render the animal insensible (Green 1987). Experience and training are essential for the correct choice of method to be used.

In larger animals specialized equipment such as the non-penetrating captive bolt must be used. *The use of the hammer or poleaxe is condemned* as a method of stunning. These methods must always be followed immediately by exsanguination, removal of the heart or destruction of the brain to ensure death. Training is essential for all operators. If not performed correctly, various degrees of consciousness with concomitant pain can ensue. It is difficult to ensure consistency in performance by operators and therefore only a few animals should be killed using this method at any one time. Death must be confirmed in each animal before the next animal is stunned.

High pressure water jet has been successfully used for the stunning of pigs and is an accepted method in Switzerland (Schatzmann *et al.* 1991, 1994).

2.1.3 *Electrical stunning*

This has been used in fish, amphibians, birds, dogs and other carnivores, poultry, pigs (Lambooy & van Voorst 1986, Laursen 1983), sheep, calves, goats and rabbits (Warrington 1974). Horned animals should not be stunned using this method if the horns make it difficult to apply the electrodes accurately. It should not be used in cats due to the high conductivity of their coats (Green 1987). It is not acceptable for use in fish as alternating current stimulates contraction of skeletal, cardiac and smooth muscle and induces tetany, not anaesthesia.

Only specialized equipment should be used for this method of euthanasia. Alternating electrical current may be used to stun the animals (Breazile & Kitchell 1969) but this must be followed by another method to complete death. Alternatively, immediate unconsciousness with cardiac arrest can be caused if electrodes are applied simultaneously to the animal's head and back, but the electrodes must be placed in such a position as to ensure that the current is

directed through the brain in order to produce unconsciousness before cardiac fibrillation (Andrews *et al.* 1993).

The current is usually applied to the animal's head by means of a pair of scissor-like tongs with an electrode at the end of each arm. High voltage stunners are more effective. Animals must be suitably restrained so that the tongs can be accurately applied. The electrodes must span the brain and be applied firmly so that they will not slip out of position when the animal falls to the ground (Ministry of Agriculture, Fisheries and Food 1993).

Head to tail and head to foot stunning is not acceptable as it does not cause immediate unconsciousness (Breazile & Kitchell 1969). Electrodes must not be applied behind the ears or on each side of the neck which would paralyse the animal without rendering it unconscious, resulting in severe pain and suffering. Care must be taken to ensure that the animal does not receive an electric shock before the electrodes are correctly applied, for instance by contact with other animals being stunned or having a wet skin.

The apparatus should have a device which prevents operation if the minimum required current cannot be passed, as well as devices to measure length of time of application, voltage indicators and current level.

The signs of an effective electrical stun are extension of the limbs, opisthotonos (arching of the body and limb spasm), downward rotation of the eyeballs, and a tonic spasm changing into clonic spasm with eventual muscle flaccidity. After 15–20 s the reflexes may begin to reappear and the animal may start to breathe again and therefore another method to ensure death, such as exsanguination, must be carried out immediately (Anil & McKinistry 1991). If the animal is not correctly stunned it may be paralysed whilst remaining fully conscious and is able to feel pain.

2.1.4 Cervical dislocation

This method is used for the euthanasia of fish, poultry, mice, young guineapigs, young rats and neonatal rabbits and newborn kittens and newborn puppies (Clifford 1984, Green 1987, Reilly 1993). It may be used on older rats and rabbits up to 1 kg if they are sedated or stunned prior to dislocation. Gregory and Wotton (1990) showed that there is not always immediate unconsciousness in poultry using this method. Care must be taken to ensure complete separation. If carried out correctly, it should cause extensive damage to the brainstem and instantaneous unconsciousness (Iwarsson & Rehbinder 1993). Death must be confirmed by exsanguination or destruction of the brain (Blackmore 1993).

It may be aesthetically unpleasant for the operator to perform and it is recommended that if the operator is not totally confident of performing this technique quickly and effectively, that they use another method. If possible animals should be sedated or anaesthetized prior to dislocation.

2.1.5 Decapitation

This procedure has been used for killing fish, amphibians, birds, rodents and small rabbits. Decapitation involves the severing of the neck of the animal, close to the head by using a sharp instrument. The use of scissors is discouraged unless they are suited to the species of animal (i.e. have sufficiently long blades) and the pressure is strong enough to sever the neck in one go with ease. Decapitation should be carried out using guillotines designed specially for that purpose to ensure rapid and quick severance in the correct position (Clifford 1984).

There has been much debate over the length of time to loss of consciousness of the decapitated head in both warm and cold-blooded vertebrates (Allred & Berntsen 1986, Andrews *et al.* 1993, Blackmore 1993, Holson 1992, Lorden & Klemm 1987, Mikeska & Klemm 1975, Reilly 1993, Tidswell *et al.* 1987, Vanderwolf *et al.* 1988) and it has been suggested to anaesthetize or sedate the animal first (Smith *et al.* 1986). However, handling and injection of sedatives or anaesthetics prior to decapitation could increase stress prior to euthanasia and is therefore not considered good for the welfare of the animal.

In cold-blooded vertebrates the animals must be stunned or rendered insensible prior to decapitation as they are very tolerant of anoxia (Warwick 1986). In birds research has shown that there may be visually evoked responses for up to 30 s after decapitation (Gregory & Wotton 1990) which makes this unacceptable. In other warm-blooded animals the immediate lack of circulation of blood to the brain and subsequent anoxia is thought to render the head rapidly insensible (Derr 1991) making prior stunning or sedation unnecessary. Use of the puntilla is not acceptable (Commission of the European Communities 1993).

Use of other methods is preferred where possible until further research can show rapid loss of consciousness.

2.1.6 *Maceration*

This method is acceptable for the destruction of chicks up to 72 hours old which often have to be killed in large numbers (Bandow 1987, Commission of the European Communities 1993). *Only macerators designed specifically for this purpose must be used and under no conditions should domestic appliances be used*.

Very small fish (<2 cm long) may be killed by placing down a waste disposal unit (Banister, personal communication 1995).

2.1.7 *Microwave irradiation*

This method is used by neurobiologists as a means to fix brain metabolites without the loss of anatomical integrity of the brain (Moroji *et al.* 1977). Only specialist apparatus (this does *not* include domestic microwave ovens) designed for this purpose is to be used. This involves focusing the microwave beam precisely at a specific part of the brain. It is only to be carried out on small animals such as amphibians, birds, mice, rats and small rabbits (less than 300 g) (Zeller *et al.* 1989). This method requires specialist expertise, but when carried out correctly is humane as death occurs in milliseconds (Andrews *et al.* 1993, Bermann *et al.* 1985, Olfert *et al.* 1993). Care must be taken to ensure correct positioning of the microwave beam but time taken to restrain the animal should be kept to a minimum to reduce stress prior to euthanasia. Whole body radiation has been successfully used on mice at temperatures of 47–49°C with the animals dying in less than 1 s (Von Cranach *et al.* 1991a,b) and is acceptable (Schatzmann, personal communication 1995).

This is not a routine procedure for euthanasia. Care must be taken as this may be dangerous to the operator (Bermann *et al.* 1985).

Chemical methods

Many anaesthetics are used in overdose as euthanasia agents. An anaesthetic is an agent that produces, in a controllable manner, a drug-induced absence of perception of all sensation. It produces unconsciousness, analgesia, and muscle relaxation sufficient to perform procedures painlessly. Indications of anaesthetic overdose include: occurrence of cardiac dysrhythmias; capillary refill time progressively slows to 3 or more seconds; respiration slows, becomes shallow and irregular, becomes diaphragmatic, or may cease; mucous membrane and skin colours may be pale to cyanotic; cardiovascular, central nervous system, musculoskeletal, gastrointestinal, and ocular reflexes are greatly diminished or cease; blood pressure falls rapidly to produce profound hypotension (mean BP < 20–30 mmHg).

Inhalational agents

Inhalational agents are either vaporized or delivered as a gas into chambers or anaesthetic circuits. Chambers used for delivery of inhalants should be properly designed so as to ensure the even distribution of gas and to ensure that the animals are rapidly exposed to a high concentration of the agent. They are valuable for use in many small animals e.g. birds, rodents, cats and small dogs (Smith *et al.* 1986). As rabbits react adversely to gases and show signs of excitation, other methods are preferred (Green 1979). Reptiles and amphibia may hold their breath resulting in a long induction time. Newborn animals are more resistant to hypoxia and may take longer to die: therefore other methods should be used.

It is important to select agents that are not unpleasant to inhale because some can be irritant and therefore be stressful. Agents which produce convulsions prior to unconsciousness are unacceptable for euthanasia. Safety precautions should be taken when administering inhalational agents through the use of appropriate gas scavenging equipment. Death must be confirmed.

2.1.8 Carbon dioxide

At concentrations above 60%, carbon dioxide acts as an anaesthetic agent and causes rapid loss of consciousness (Green 1987). It is effective and humane for euthanasia of most small animals above 70%. Carbon dioxide stimulates the respiratory centre which may cause anxiety and stress in the animal as well as being aesthetically unpleasant for the observer. Carbon dioxide may form carbonic acid when in contact with the nasal mucous membranes which could produce a fizzy or tingling effect which may be mildly irritating to some species when applied at lower concentrations (Lucke 1979).

In most animals it is recommended to place the animals immediately into $>70\%$ CO_2 where the animals lose consciousness very quickly due to the narcotic effect of the high intake of CO_2 on the brain without causing hypoxia (Blackshaw *et al.* 1988, Forslid *et al.* 1986). One hundred per cent CO_2 may cause severe dyspnoea and distress in conscious animals (van Zutphen *et al.* 1993).

One hundred per cent CO_2 is recommended for chicks up to 72 hours old because they are more tolerant of CO_2. Raj and Gregory (1993, 1994) and Raj *et al.* (1990, 1992) showed that the use of 60% argon in conjunction with CO_2 induces a rapid loss of brain function in turkeys. Older birds may flap their wings when killed under CO_2, even when comatose, which makes it aesthetically less acceptable. Low concentrations of CO_2 (30%) in conjunction with an inert gas is considered acceptable for chickens and turkeys. At this level, the carbon dioxide is not unduly pungent and it acts as an anticonvulsant. It is not recommended for fish as it causes intense activity before loss of consciousness and is slow acting. It should not be used for cats and larger species because it sometimes causes excitement (Glen & Scott 1973, Klemm 1964) and some animals are averse to its pungent odour. Pigs vocalize before losing consciousness, indicating a degree of distress (Gregory *et al.* 1987) and other people have also indicated that it is not humane for pigs (Clifford 1984, Hoenderken 1983, Hoenderken *et al.* 1980, Reilly 1993) despite EC and national slaughter recommendations to the contrary (Commission of the European Communities 1993, Ministry of Agriculture, Food and Fisheries 1993). Other research indicates that the violent reactions may be after unconsciousness (Andrews *et al.* 1993, Erhardt *et al.* 1989, Forslid *et al.* 1986, Mullenax & Dougherty 1963). Until further research can show any adverse reactions of pigs is once they are fully anaesthetized, other methods are preferable. It is not acceptable for other cold-blooded vertebrates as induction is too long. Neonates are particularly tolerant of CO_2 (30–60 min to unconsciousness (van Zutphen *et al.* 1993) depending on maturity at birth (those that are born more mature are less tolerant of CO_2). Therefore this method should not be used in animals less than 2 weeks old. Carbon dioxide should not be used in diving animals such as mink because of their ability to hold their breath.

Research has been carried out examining the possible advantageous effects of adding oxygen to ensure that the animals die from CO_2 narcosis, rather than hypoxia (Iwarsson & Rehbinder 1993). In some species there appears to be a reduction in stress and anxiety, but this is accompanied by a longer induction time (Blackmore 1993). Hewett *et al.* (1993) felt that there was no welfare advantage in using CO_2/O_2 mixtures. It may be difficult to mix gases accurately for routine use.

Carbon dioxide is heavier than air so incomplete filling of a euthanasia chamber may permit tall or climbing animals to avoid exposure to the gas. Therefore the chamber must be prefilled with up to 70% CO_2 before placing the animals in it. However, others feel that it may be better to fill the chamber

once the animals have been placed in it. The chambers must be designed so as to avoid injury to the animals and, if possible, have devices whereby the CO_2 concentration can be easily and accurately measured. Care must be taken to limit the number of animals in a chamber at any one time so as to maintain a constant CO_2 concentration.

Carbon dioxide is non-flammable and non-explosive and therefore presents little hazard to the operator. Fire extinguishers and solid carbon dioxide are not acceptable because of the lowered temperature and the noise created by the extinguisher.

2.1.9 Carbon monoxide

This causes rapid death as it combines with the red blood cells in preference to oxygen, thus causing hypoxia (Chalifoux & Dallaire 1983). There is little or no distress as there is no smell (Blackmore 1993, Breazile & Kitchell 1969, Green 1987, Smith *et al.* 1986). It is not acceptable for use in reptiles because of their low metabolic rate and hypoxic tolerance. It is acceptable for small animals, but in dogs and cats vocalizations and convulsions may occur after unconsciousness making it aesthetically unpleasant. Death should be confirmed by physical means.

Carbon monoxide may be produced by three methods: chemical interaction of sodium formate and sulphuric acid; exhaust fumes from internal combustion engines; and commercially compressed CO gas. Carbon monoxide from petrol engine exhaust is highly irritant to respiratory tissues. To be considered for use in euthanasia, it must be cooled through a water chamber and filtered, using a scrubber unit, in order to remove the various oxides of nitrogen and hydrocarbons, oxygenates of hydrocarbons and carbon particles. Under no circumstances should exhaust from diesel engines be used. Only commercial CO is recommended. The animals should only be introduced into the chamber after it has been filled with a concentration of CO of at least 6% by volume, supplied by a source of 100% CO.

As it is extremely noxious and also dangerous to the operator because it is not detectable, it should only be used in an appropriate gas scavenging apparatus taking extreme care. Carbon monoxide monitors should be installed in the room.

2.1.10 Volatile inhalational anaesthetics

When using any liquid anaesthetic care must be taken to ensure that it is not allowed to come in contact with the animal. Sufficient air or oxygen should be provided during the induction period to prevent hypoxia (Andrews *et al.* 1993). Exposure to trace concentrations of anaesthetic gases is a recognized human health hazard and requires gas scavenging apparatus to be used in the work environment. Volatile inhalational anaesthetics are neither flammable nor explosive.

Halothane Halothane is a commonly used anaesthetic agent for small laboratory animals and is quick acting and stress free in overdose for euthanasia. It has a depressant effect on the cardiovascular and respiratory systems (Green 1987).

Enflurane Enflurane is a commonly used anaesthetic agent for small laboratory animals and is quick acting and stress free in overdose for euthanasia (Green 1987). It has a depressant effect on the cardiovascular and respiratory systems. It may be preferred to halothane in situations where drug metabolism or toxicological work is being conducted as very little drug is metabolized in the liver.

Isoflurane Isoflurane is a commonly used anaesthetic agent which is quick acting and stress free in overdose for euthanasia. Isoflurane causes respiratory and cardiovascular depression. However, it has a pungent odour and must therefore not be used on animals which may be able to hold their breath. It is particularly useful where tissues such as liver are to be used for toxicological or microsomal studies as it undergoes no hepatic metabolism.

Agents for aquatic animals for absorption through the skin and gills

2.1.11 Benzocaine (ethyl aminobenzoate)

This agent, dissolved in acetone before adding to tank water, is an effective and

humane method of killing fish and amphibians. It acts by depression of the central nervous system. It has pH-independent efficacy but reduces the pH of the tank water which should therefore be buffered to pH 7.5 to reduce irritation (Brown 1988, Summerfelt & Smith 1990). The breakdown time in water is about 4 h, making this agent environmentally safe and it is safe for personnel. Death should be confirmed by physical means.

2.1.12 Tricaine methane sulphonate (buffered MS-222)

MS-222 is a humane and safe method of euthanasia for fish and amphibians. It has been used for snakes and alligators by intramuscular injection but has a long induction period, thus increasing distress. It acts by depression of the central nervous system. It is soluble in both salt and fresh water but needs to be neutralized with bicarbonate, imidazole, sodium hydrogen phosphate or sodium hydroxide to reduce irritation and tissue damage (Brown 1988). The effectiveness of MS-222 varies with species, size, temperature and water hardness. MS-222 is unstable in sunlight and stock solutions should be stored in brown or opaque bottles. It may be used in conjunction with quinaldine or quinaldine sulphate which is more effective and used in smaller quantities than either agent used alone.

2.1.13 Etomidate and metomidate

These are both non-barbiturate hypnotic agents that act by depression of the central nervous system. They are relatively quick acting and are considered as humane agents for killing fish. They are highly soluble in water (Brown 1988, Summerfelt & Smith 1990).

2.1.14 Quinaldine (2-methylquinoline)

This drug is commonly used for killing fish in a humane way in the United States of America (USA). However it is rarely used and difficult to obtain in Europe. It must first be dissolved in acetone but this has no adverse effects on the animals. It has a relatively long induction time compared to some other agents. Quinaldine accumulates in lipid tissues such as the brain. It depresses the sensory centres of the central nervous system (Summerfelt & Smith 1990). Quinaldine sulphate may also be used for euthanasia of fish.

Injectable agents

Many proprietary mixtures specifically prepared for euthanasia of animals are simply triple strength anaesthetic agents, such as sodium pentobarbitone, but others may have neuromuscular blocking agents incorporated. *It is essential that the animal should become fully anaesthetized before the neuromuscular blocking agents take effect in order to prevent distress to the animal.* Before using any agents for euthanasia, the operator should consult the manufacturer's information leaflet with regard to dosage and route of injection. In general where anaesthetic agents are used, two times the anaesthetic dose produces respiratory arrest, while four times the dose produces cardiac arrest when ventilated artificially. Three times the dose usually causes death quickly and uniformly in non-ventilated animals.

Injection may be administered by various routes. Intravenous administration is preferred because the effect is the most rapid and reliable. Intraperitoneal injection is easier to administer, especially in species where the veins are small and difficult to penetrate but it takes longer to act and may cause irritation and transient pain and distress. Intrapulmonic injection should be avoided because of discomfort to the animals. Oral and rectal routes of administration are inadvisable because of prolonged onset of action, wide range of lethal doses and potential irritation of tissues. Intramuscular and subcutaneous routes must not be used as they take too long to act. The intracardiac route is very painful and penetration of the heart is not always successful on the first attempt; therefore these are not recommended except in insensible animals.

Excitable and vicious animals should be pretreated with a neuroleptanalgesic combination, a tranquillizer, or another depressant. Trained personnel are essential for using these methods.

Care must be taken when disposing of carcasses because of residues in the meat. Care must also be taken to ensure personnel safety.

2.1.15 Barbiturates

These are the most widely used and accepted agents for euthanasia for most animals (Hatch 1982). They include barbituric acid derivatives, oxybarbiturates (sodium pentobarbitone, secobarbital), thiobarbiturates (thiopentone) and various barbiturate mixtures. Sodium pentobarbitone is commonly considered the most suitable agent. They act by depression of the central nervous system and cause cardiac and respiratory arrest. They cause rapid euthanasia with minimal discomfort, depending on the dose of the agent and route of injection (intravenous is preferred as it is quickest). In some countries barbiturates may only be obtained under licence.

Sodium pentobarbitone This is generally used either by intravenous or intraperitoneal injection of 18% (200 mg/ml) concentration in a dosage of 200 mg/kg for euthanasia. Intravenous injection results in quicker death but the intraperitoneal route may be simpler to perform in many species, thus reducing the stress caused by handling. However, sodium pentobarbitone may cause irritation of the peritoneum which can be avoided by diluting the drug. Intracardiac injection may only be used on a fully anaesthetized animal as this is very painful and is therefore not considered acceptable. Intracephalic injection (foramen magnum) is effective on large birds such as poultry, but requires specialist skills.

2.1.16 T-61

This agent combines a local anaesthetic (tetracaine HCL), a hypnotic agent and curariform drug (*N*-2-(m-methoxyphenyl)-2-ethylbutyl-l-gamma-hydroxybutyramide (20%), 4,4′-methylene bis-cyclohexyltrimethyl ammonium iodide (0.5%) and tetracaine hydrochloride (0.5%) in aqueous solution with formamide). It must only be injected intravenously and slowly as it is otherwise painful. In small birds it may be injected into the pectoral muscle, but it is not suitable for poultry. The animal must be sedated prior to administration of T-61.

There has been concern that the curariform drug may cause cessation of respiratory activity before the onset of unconsciousness (Barocio 1983, Baumans *et al.* 1988, Eikmeier 1961, Quin 1963, Lumb *et al.* 1978, Rowan 1986) therefore causing distress to the animal, but Hellebrekers *et al.* (1990) showed that loss of consciousness and loss of muscle activity in rabbits and dogs occurred simultaneously, therefore making this an acceptable agent for euthanasia. The muscle relaxant prevents the terminal gasp reported for the barbiturates, thus making it more acceptable for the observer. In some dogs there is vocalization and muscular activity. This is not a conscious response but may be aesthetically unpleasant. It is not a controlled drug in many countries and may therefore be easier to obtain than barbiturates. In other countries, such as Sweden, it is not available.

2.2 Methods acceptable for unconscious animals

2.2.1 Pithing

This is an effective way of killing some fish, amphibians and reptiles. This is carried out by inserting a sharp needle through the foramen magnum into the base of the brain to ensure quick brain destruction. If not carried out correctly and quickly, the animal will remain conscious with subsequent pain and distress. The animal must first be rendered unconscious by stunning or anaesthesia. This method should only be carried out by competent personnel.

2.2.2 Rapid freezing

Rapid freezing has been used to minimize enzyme activity for subsequent biochemical estimations of tissues. Techniques involve: (a) immersion of the intact animal into liquid nitrogen; (b) decapitation and immediate immersion of the head into liquid nitrogen; (c) freeze blowing; (d) *in situ* freezing; and (e) funnel freezing. The animals must be fully anaesthetized, rendered insensible or decapi-

tated prior to all freezing methods as it has been shown that it may take 10–90 s to freeze the deep structures due to the poor thermal conductivity of the tissues surrounding the brain. It is acceptable only under specific circumstances when experimental design requires such treatment in very small animals such as embryos or neonatal rodents and rabbits (Green 1987, Van Zutphen *et al.* 1993). Personnel carrying out this technique must be well trained and specialist equipment is required.

2.2.3 Exsanguination

Exsanguination should only be carried out after the animal has been rendered insensible by another method because of the stress associated with extreme hypovolaemia and the pain of incising the deeper blood vessels. An animal must not be exsanguinated in sight or smell of other animals, using a different room if possible. *This is not an acceptable method of killing birds* because of the tendency for the blood to clot and thus result in incomplete exsanguination and therefore inadequate euthanasia. It is also *not acceptable for reptiles and other cold-blooded vertebrates* because of their slow metabolic rate and hypoxic tolerance.

2.2.4 Nitrogen/argon

Nitrogen or argon displaces O_2 and produces death by hypoxia. At 39%, rats become unconscious only after 3 min and show signs of panic and distress (Andrews *et al.* 1993). It causes unconsciousness but not death in young animals. In dogs and cats unconsciousness takes 1–2 min to occur with hyperpnoea for about 10 s before collapsing (Herin *et al.* 1978, Quine 1980, Quine *et al.* 1988, Rowsell 1981, 1990). *It is therefore not an acceptable method unless the animal is anaesthetized*.

2.2.5 Ethanol

This method, described by Lord (1989, 1991) involves the intraperitoneal injection of 500 μ1 70% ethanol into mice. Ethanol causes depression of the central nervous system. The mice show a gross loss of muscle control before becoming comatose, followed by respiratory arrest. Irritation of the peritoneum may occur. Wallgren and Barry III (1970) state that it is irritant at concentrations of above 10% w/v and that the mortality is due to unspecific trauma. *It is not acceptable for euthanasia of vertebrates unless they are anaesthetized.*

2.2.6 Chloral hydrate

This acts by very slow depression of the central nervous system. *It is not acceptable for use by itself* as it lacks analgesic effects, is very slow to take effect, causes aesthetically objectionable animal movements, large volumes are required and it causes irritation of the peritoneum (Breazile & Kitchell 1969, Hatch 1982). It may be used for large animals, intravenously, under anaesthetic (Lumb 1974) or in combination with magnesium sulphate and sodium pentobarbitone (Olfert *et al.* 1993).

2.2.7 Potassium chloride

The potassium ion is cardiotoxic. Potassium chloride causes gasping, vocalizations, muscle spasms and convulsive seizures (Lumb 1974). It is also unpleasant for the observer. *It is unacceptable for euthanasia unless the animal is fully anaesthetized*.

2.2.8 Air embolism

This involves intravenous injection of 5 to 50 ml/kg air. It has occasionally been used in rabbits (Weisbrod *et al.* 1984). It may be accompanied by convulsions, opisthotonos and vocalizations (Hatch 1982). It is a very painful and unreliable method and *is not acceptable unless the animal is fully anaesthetized*.

2.3 Methods that are not acceptable for euthanasia

2.3.1 Decompression/vacuum

This method acts by inducing cerebral hypoxia. There may be adverse physical effects due to trapped gases in the body cavities (e.g. sinuses, eustachian tubes) expanding and these could cause severe pain and discomfort before the animal becomes

unconscious (Von Cranach *et al.* 1991a). There is also a chance of failure of equipment, resulting in rapid recompression with severe pain and distress to the animals. Bloating, bleeding, vomiting, convulsions, urination and defecation may occur in the unconscious animal and are aesthetically unpleasant for the observer (Booth 1978, Hatch 1982). It may also take some time before unconsciousness (Barber 1972). For these reasons, decompression is *not acceptable as a method of euthanasia*.

2.3.2 Hypothermia

Hypothermia involves the killing of animals by placing them in very cold temperatures such as deep freezers. Hypothermia is known to act as an anaesthetic agent to a certain extent (Phifer & Terry 1986). *However, it is not an acceptable method of euthanasia for any animal*. Deep freezers may only be used to ensure death once the animal is fully unconscious and unlikely to recover (Summerfelt & Smith 1990).

2.3.3 Hyperthermia

Raising the temperature in order to kill animals has been suggested for some cold-blooded vertebrates which will die above their critical temperatures which may be only a few degrees above their normal activity range but *this is not acceptable. Animals must never be dropped into boiling water as it causes intense pain and a slow death*.

2.3.4 Drowning/removal from water

Drowning is not a humane method of euthanasia for any vertebrate as it is slow and causes severe stress and anxiety from hypoxia. Removal from water for gilled vertebrates is not acceptable (including tadpoles) (Kestin *et al.* 1991).

2.3.5 Neck crushing

This is a method sometimes employed to kill birds. The neck of a small bird is pressed against a bar or specialized pliers or bone calipers may be used. However, this only results in paralysis from destruction of the spinal cord and does not damage the brain with possible subsequent remaining consciousness with pain, fear and distress. This method is *not acceptable for the euthanasia of birds or any animal*.

2.3.6 Strangulation

This is not an acceptable method of killing any animal due to the time taken to unconsciousness, and the pain, undue anxiety and stress that it would cause.

2.3.7 Nitrous oxide

Hypoxic concentrations of almost 100% are necessary to achieve euthanasia and it is slow acting therefore causing unnecessary stress. The animal will convulse after losing consciousness which reduces the acceptability to the observer. *It is not an acceptable euthanasia agent*. However, it may be used with other agents to speed the onset of anaesthesia.

2.3.8 Cyclopropane

Cyclopropane is a humane method of euthanasia for most laboratory animals as it produces rapid and deep anaesthesia. However, it is flammable in air and explosive in oxygen which makes it dangerous to the operator which reduces its acceptability as a general agent for euthanasia.

2.3.9 Ether (diethyl ether)

Ether is irritant to the mucous membranes and at high concentrations traditionally found in closed containers and jars, it may be stressful to the animals as it elevates catecholamines (Blackshaw *et al.* 1988, Breazile & Kitchell 1969, Green 1987). If used in a vaporizer, it appeared less irritating (Baumans, personal communication 1995). It markedly elevates some blood chemicals (e.g. glucose) under high concentrations. It is dangerous to the operator because of its explosive properties. *It is not an acceptable method of euthanasia*.

2.3.10 Chloroform

Chloroform acts by depression of the central nervous system and causes cardiac and respiratory failure. *This is not acceptable as a euthanasia agent* as it is hepatotoxic, ne-

phrotoxic and carcinogenic to the operator and other animals. It causes excitement before loss of consciousness (Breazile & Kitchell 1969). Trace concentrations carried to breeding centres have been shown to seriously interfere with breeding programmes in rodents (Green 1987).

2.3.11 *Methoxyflurane*

Methoxyflurane is a commonly used anaesthetic agent but is very slow acting and there is a high chance of full recovery even after 20 min of overdose. It is difficult to obtain in Europe.

2.3.12 *Trichlorethylene*

Because trichlorethylene is mainly an analgesic agent and produces only light anaesthesia, *it is not acceptable as an agent for euthanasia*. It is carcinogenic, causes hypercapnia and is dangerous to the operator.

2.3.13 *Hydrogen cyanide gas*

Hydrogen cyanide gas blocks oxygen uptake causing respiratory difficulties and violent convulsions before the onset of unconsciousness and death (Hatch 1982). It is also very dangerous to the operator. *It is not acceptable for the euthanasia of any animal.*

2.3.14 *2-Phenoxyethanol*

This agent is designed as a fish antibiotic but given in large enough doses, it can kill. Dosage levels must be high and death may be slow, thus increasing distress for the fish. It causes hyperactivity in some fish prior to anaesthetization (Summerfelt & Smith 1990). It has a very long time of chemical breakdown in water which makes it difficult to dispose of as it would be dangerous to the environment, killing bacteria in sewage systems if poured down the drain. *It is not acceptable for euthanasia of fish*.

2.3.15 *Urethane*

Animals may be placed in a 1–2% solution of urethane. It is commonly used as an anaesthetic. However, it is *very* carcinogenic and because of this potential danger to the operator and problems of safe disposal, *it is not acceptable* (Summerfelt & Smith 1990).

2.3.16 *Neuromuscular blocking agents*

Neuromuscular blocking agents and other agents that do not induce loss of consciousness prior to death are not to be used for euthanasia under any circumstances.

2.3.17 *Ketamine*

Ketamine is not considered acceptable as a sole agent for euthanasia as large volumes would be necessary. Extensive convulsions and vocalizations in rabbits make it aesthetically unacceptable (Baneux *et al.* 1986, Reilly 1993). Used in conjunction with xylazine, it may be acceptable.

2.3.18 *Sedatives*

Because of the large volumes that would be necessary to cause death, *sedatives are not acceptable as euthanasia agents*.

2.3.19 *Magnesium sulphate*

This has been used with or without sodium pentobarbitone at 80 mg/kg. It is a neuromuscular blocking agent and myocardial depressant, not a central nervous system depressant (Hatch 1982, Olfert *et al.* 1993). Large volumes are required and the animals may exhibit muscle spasms, convulsive seizures, vocalization, gasping breaths and defecation before death (Breazile & Kitchell 1969). The animal remains conscious until the brain succumbs to anoxaemic anoxia. It lacks analgesic or anaesthetic effects and therefore is *not an acceptable agent by itself*.

2.3.20 *Other injectable anaesthetics*

Euthanasia may be induced with many other agents (e.g. alphaxolone/alphadolone, propofol) but because these agents have a relatively wide safety margin, high doses would be required, reducing their acceptibility.

2.3.21 *Other agents*

Other agents not to be used include nicotine (produces serious side effects before death) and strychnine (excites the central nervous system and the animal remains conscious and in excruciating pain until it dies of suffocation) (Hatch 1982, Lumb 1974).

2.4.22 *Agents administered orally*

Agents have been added to drinking water for mass euthanasia of some animals as may

occur in some large scale breeding establishments. There is always a risk of some animals not receiving an adequate dose, and the time to act is generally slow. These substances are dangerous to the operator and are *not acceptable for use as agents for euthanasia*.

2.3.23 Narcotic analgesics

Opiate derivates such as morphine and etorphine are central nervous depressants as well as analgesics. Overdosage causes death by depression of the respiratory centres in the medulla. There is a large variability of reactions by different species: some species are rendered maniacal by large dosages of these drugs. Because there is not much information on the humaneness of these drugs, they are *not acceptable as euthanizing agents*.

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