EUTHANASIA OF EXPERIMENTAL ANIMALS



EUROPEAN
COMMISSION

This document has been prepared for use within the Commission. It does not necessarily represent the Commission's official position.

A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server (http://europa.eu.int)

Cataloguing data can be found at the end of this publication

Luxembourg: Office for Official Publications of the European Communities, 1997

ISBN 92-827-9694-9

© European Communities, 1997

Reproduction is authorized, except for commercial purposes, provided the source is acknowledged

Printed in Belgium

European Commission

EUTHANASIA OF EXPERIMENTAL ANIMALS

Document

EUTHANASIA OF EXPERIMENTAL ANIMALS

Report prepared for the European Commission by

Mrs Bryony Close
Dr Keith Banister
Dr Vera Baumans
Dr Eva-Maria Bernoth
Dr Niall Bromage
Dr John Bunyan
Professor Dr Wolff Erhardt
Professor Paul Flecknell
Dr Neville Gregory
Professor Dr Hansjoachim Hackbarth
Professor David Morton
Mr Clifford Warwick

EUTHANASIA OF EXPERIMENTAL ANIMALS

CONTENTS

			Page
Preface			1
Acknowledgements			2
1.	Introduction		3
	1.1	Objectives of euthanasia	3
	1.2	Definition of terms	3
	1.3	Signs of pain and distress	4
	1.4	Recognition and confirmation of death	5
	1.5	Personnel and training	5
	1.6	Handling and restraint	6
	1.7	Equipment	6
	1.8	Carcass and waste disposal	6
2.	General comments on methods of euthanasia		7
	2.1	Acceptable methods of euthanasia	7
	2.2	Methods acceptable for unconscious animals	15
	2.3	Methods that are not acceptable for euthanasia	16
3.	Methods of euthanasia for each species group		21
	3.1	Fish	21
	3.2	Amphibians	27
	3.3	Reptiles	31
	3.4	Birds	35
	3.5	Rodents	41
	3.6	Rabbits	47
	3.7	Carnivores - dogs, cats, ferrets	53
	3.8	Large mammals - pigs, sheep, goats, cattle, horses	57
	3.9	Non-human primates	61
	3.10	Other animals not commonly used for experiments	62
4.	References		63
5.	Further reading		71
6.	Euthanasia training materials		89

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 five main sections:

The first section deals with general notes on legislative requirements in Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, general requirements of euthanasia, definitions of terms, and other factors to be considered when killing experimental animals.

The second section 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.

The third section 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 summarising the recommendations for that species.

Section 4 comprises a reference list and a literature recommended for further reading, divided into general and species groups.

Section 5 provides information on audiovisual training materials which 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.

ACKNOWLEDGEMENTS

The authors would like to thank the following people and organisations who provided valuable assistance and comments on the text: Dr J Anderson (Animals (Scientific Procedures) Inspectorate, UK Home Office), Dr N Baudrihaye (European Federation of Pharmaceutical Industries' Associations), Professor J Bourne (Institute for Animal Health, UK), Dr D Forbes (Laboratory Animal Science Association, UK), Professor K Gärtner (Medizinische Hochschule Hannover, Germany), Mr J A Gregory (Institute of Animal Technology, UK), Professor O Hänninen (Secretary General, ICLAS), Mrs R Harrison (UK), Dr F R Homberger (University of Zurich, Switzerland), Mr T D Hornett (Glaxo Research and Development, UK), Dr K Iwarsson (Karolinska institutet, Sweden), Dr T Jeneskog (National Board for Laboratory Animals (CFN), Sweden), Dr M Jennings (Royal Society for the Prevention of Cruelty to Animals, UK), Dr G Mahouy (Institut d'Hematologie, Université de Paris, France), Professor R Murison (University of Bergen, Norway), Mr P Nowlan (University of Dublin, Ireland), Professor C Rehbinder (National Board for Laboratory Animals (CFN), Sweden), Mr A Sainsbury (Institute of Zoology, London), Professor P Schambye (Board of Animal Experiments Inspectorate, Denmark), Dr W Scharmann (Bundesgesundheitsamt, Germany), Professor U Schatzmann (Universität Bern, Switzerland), Dr D Straughan, Dr P Terpstra (CRC Contract Research Center, Belgium), Professor J E van Dijk (University of Utrecht, the Netherlands), Mr D Wilkins (Eurogroup for Animal Welfare), Dr J Wong (Canadian Council on Animal Care).

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 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 (in the context of the experiment) and appropriate for killing the animal with which they are working. As this Directive protects vertebrates, this document will only cover euthanasia of vertebrates. Article 2(l) 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 (eg 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 minimise 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 parthenogenetically 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 recognised 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 reorganisations 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 recognise signs of pain, fear and distress in the relevant species. All personnel must be trained to recognise 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 vocalisations (not always in the human audible range),
- struggling,
- attempts to escape,
- defensive or redirected aggression,
- freezing/immobility response,
- panting,
- salivation,
- urination, defaecation 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 and 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 minimise fear and apprehension must be considered in determining the method of euthanasia. Distress vocalisations, 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 vocalisations 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 vocalisation 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 confirmation 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 minimise 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 minimise 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 tranquillising and immobilising 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 use 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 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 and 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 and Delaney, 1988; Daly and 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 (MAFF, 1991). The animal should remain insensible until exsanguination is performed (Blackmore, 1993). An effective stun may be recognised 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 guinea pigs, 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 specialised 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 and 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 specialised equipment should be used for this method of euthanasia. Alternating electrical current may be used to stun the animals (Breazile and 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 animals' 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, 1991).

Head to tail and head to foot stunning is not acceptable as it does not cause immediate unconsciousness (Breazile and 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 seconds 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 and 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 guinea pigs, 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 1kg if they are sedated or stunned prior to dislocation. Gregory and Wotton (1990) showed that there is not always immediate unconsiousness 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 and 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 anaesthetised 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 and Berntsen, 1986; Andrews *et al*, 1993; Blackmore, 1993; Holson, 1992; Lorden, 1987; Mikeska and 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 seconds after decapitation (Gregory and 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 unecessary. 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 (K. 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 focussing 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 300g) (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; 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 sucessfully used on mice at temperatures of 47-49°C with the animals dying in less than 1 second (Von Cranach *et al*, 1991 (a), 1991 (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 (Berman *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 b.p.< 20-30 mm/Hg).

Inhalational agents.

Inhalational agents are either vaporized or delivered as a gas into chambers or anaesthetic circuits. Chambers used for the 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%. CO_2 stimulates the respiratory centre which may cause anxiety and stress in the animal as well as being aesthetically unpleasant for the observer. CO_2 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). 100% CO_2 may cause severe dyspnoea and distress in consious animals (van Zutphen *et al*, 1993).

100% 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 Mohan 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 and Scott, 1973; Klemm, 1964) and some animals are averse to its pungent odour. Pigs vocalise 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, 1991). Other research indicates that the violent reactions may be after unconsciousness (Andrews *et al*, 1993; Erhardt *et al*, 1989; Forslid, 1986; Mullenax and Dougherty, 1963). Until further research can show that any adverse reactions of pigs is once they are fully anaesthestised, 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 minutes to unconsiousness (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 two weeks old. CO_2 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 and

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.

CO_2 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.

CO_2 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 and Dallaire, 1983). There is little or no distress as there is no smell (Blackmore, 1993; Breazile and 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 vocalisations 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. CO monitors should be installed in the room.

2.1.10 Volatile inhalational anaesthetics.

When using any liquid anaesthetics 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 recognised 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 metabolised 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 and Smith, 1990). The breakdown time in water is about 4 hours, 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 neutralised 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 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 and 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 and Smith, 1990). Quinaldine sulphate may also 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 tranquilliser, 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 of 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 may 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% (200mg/ml) concentration in a dosage of 200mg/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-1-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 very 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 vocalisation 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 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 decapitated prior to all freezing methods as it has been shown that it may take 10-90 seconds 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 is 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 minutes 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 minutes to occur with hyperpnoea for about 10 secs before collapsing (Herin *et al*, 1978; Quine, 1980; Quine *et al*, 1988; Rowsell, 1981; Rowsell, 1990). *It is therefore not an acceptable method unless the animal is anaesthetised.*

2.2.5 Ethanol.

This method, described by Lord (1989, 1991) involves the intraperitoneal injection of 500µl 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 anaesthetised.*

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 and 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, vocalisations, muscle spasms and convulsive seizures (Lumb, 1974). It is also unpleasant for the observer. *It is unacceptable for euthanasia* unless the animal is fully anaesthetised.

2.2.8 Air embolism.

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

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 (eg sinuses, eustachian tubes) expanding and these could cause severe pain and discomfort before the animal becomes unconscious (von Cranach *et al*, 1991). 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 defaecation may occur in the unconscious animal and are aesthetically unpleasant for the observer (Booth, 1978; Hatch 1982). It also may 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 and 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 and 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 specialised 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 and Kitchell, 1969; Green, 1987). If used in a vaporiser, it appeared less irritating (V 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, nephrotoxic and carcinogenic to the operator and other animals. It causes excitement before loss of consciousness (Breazile and 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 minutes 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 anaesthetisation (Summerfelt and 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 a 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 and Smith, 1990)

2.3.16 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 vocalisations 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 80mg/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 defaecation before death (Breazile and 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 acceptablility.

2.3.21 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 euthanising agents.*

3. METHODS OF EUTHANASIA FOR EACH SPECIES GROUP

Section 1 must be consulted before considering this section.

3.1 FISH

There are over 20,000 species of fish with enormously varying lifestyles which makes it very difficult to generalise on methods of euthanasia. The methods listed below are meant as a guide and the operator must assess which is the best method for the species being killed or obtain information from experts. Although fish may not have the same spinothalamic pathways as mammals for pain perception, there is evidence that they do feel pain and should therefore be killed with the same care and consideration.

All fish are sensitive to changes in the physical and chemical parameters of their water (especially temperature, dissolved gas levels, salinity, pH, etc) but some species are much more tolerant of changes in any one of these factors than are others. Therefore unless the species' response is known it is advisable to practise euthanasia in the fish's normal water. If drugs are used the water level should be lowered to ensure rapid sedation but not so much as to distress the fish before the addition of the agent. Dosing is always preferable to injection as the latter technique involves handling the fish and thus induces stress. It may be necessary to fast fish 24-48 hours prior to chemical euthanasia as this will permit more rapid absorption by the gut and minimise the risk of regurgitation which could reduce the effect of the chemicals on the gill lamellae (Brown, 1988). The tanks used should enable the operator to observe the fish and react quickly if there are signs of suffering. It is generally true that cooling will reduce the metabolic and locomotory processes, thus facilitating handling, but it is essential that the normal temperature of the fish and its degree of tolerance be considered. It is also vital to note that in marine fish ice crystals will form in the cells before the sea water freezes, thereby causing the fish extreme pain. In freshwater fish the water will freeze before internal ice crystals will form. However, it must be remembered that cooling does not reduce the ability to feel pain.

Overexposure to an anaesthetic is first manifested by a cessation of respiratory movements, followed by spasmodic overextension or flaring of the opercula. These are spaced 15-30 sec apart at first and then at longer intervals. When the interval between spasms is approximately 1 min, cardiac arrest and death follow within a few minutes (Table 1).

Table 1: Stages of loss of consciousness, leading to death in fish (After McFarland and Klontz, 1969).

Level	Designation	Parameter(s)
0	Normal	Reactive to external stimuli; equilibrium and muscle tone normal
1	Light sedation	Slight loss of reactivity to external visual and tactile stimuli; equilibrium normal;
2	Deep sedation	Total loss of reactivity to external stimuli *except* strong pressure; slight decrease in opercular rate; equilibrium normal;
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactive only to strong tactile and vibrational stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; rapid opercular rate; reactive only to deep pressure stimuli
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements very shallow; heart rate very slow
6	Medullary collapse	Opercular movements cease immediately after gasping, followed by cardiac arrest

RECOGNITION AND CONFIRMATION OF DEATH.

Death may be recognised by cessation of respiration (opercular movement) and cessation of heartbeat (palpation). Death should be confirmed by destruction of the brain where possible.

LARVAE

Fish may be classed as oviparous, ovoviviparous or viviparous depending on whether they produce eggs which hatch outside the body, produce eggs which hatch inside the body, or give birth to free-living young (larvae). For simplicity, it is considered that all fish should be protected as soon as they have hatched and the methods of euthanasia recommended for adults are considered acceptable for larvae. Viviparous fish should be treated by immersion or injecting the parent.

ADULTS

Further details of methods may be found in Section 2.

PHYSICAL METHODS

Concussion.

This involves a blow to the back of the head and if carried out by experienced personnel is a humane method of euthanasia. Death must be confirmed by destruction of the brain.

Cervical dislocation.

This is the breaking of the backbone near the head. Small to medium fish are killed by inserting a rod or thumb into the mouth, holding the fish with the opposite hand and displacing it dorsally (Clifford, 1984). It is feasible and effective in small fish, but should be confirmed by exsanguination or destruction of the brain. The stress caused by handling reduces the acceptability of this method. *It is not possible or humane in larger fish.*

Maceration:

Small fish of less than 2cm in length may be humanely killed by placing down a waste disposal unit.

CHEMICAL METHODS.

Agents can be administered by dissolving the chemical in the tank water. Water temperatures often alter the efficacy of the drug and induction is often more rapid at higher temperatures. However, the temperature must not be raised so that it causes any stress to the fish. Drugs may also be administered by intramuscular or intraperitoneal injection. For euthanasia anaesthetic drugs are generally used at double or triple the recommended anaesthetic dose. In all cases, death must be confirmed by destruction of the brain.

Tricaine methane sulphonate (buffered MS-222).

This acts by depression of the central nervous system. This is a benzocaine type drug and is the most effective way to kill most fish. It is soluble in both salt and fresh water. However, it is expensive which may deter some users, especially if they need to kill large numbers of fish. Bicarbonate, imidazole, sodium hydrogen phosphate or sodium hydroxide

should be added to neutralise the water (to pH 7.5) to reduce irritation and tissue damage. It may be used in conjunction with quinaldine or quinaldine sulphate to increase effectiveness.

Benzocaine (ethyl aminobenzoate).

This acts in a similar way to MS-222 but has pH-independent efficacy. However, because it lowers the pH of the water, it should be neutralised to pH 7.5. The breakdown time in water is about 4 hours, thus making this drug acceptable in terms of environmental contamination. As it is insoluble in water benzocaine should first be dissolved in acetone before adding to water.

Etomidate.

It is a potent imidazole-based agent with no analgesic properties. It is highly soluble in water. Stress hormone measurements of fish have indicated that etomidate may give fewer problems than MS-222 (Zwart *et al,* 1989) and it is therefore considered acceptable for euthanasia of fish.

Metomidate.

It is a imidazole-based nonbarbiturate hypnotic agent that has no analgesic properties. Used in overdose, this is considered acceptable for killing most species of fish.

Quinaldine (2-methylquinoline).

This is difficult to obtain in Europe but is commonly used in the USA for humane euthanasia. The recommended dosages for euthanasia vary depending upon species, temperature and water hardness. It accumulates in lipid tissues such as the brain and depresses the sensory centres of the central nervous system. Quinaldine sulphate is also considered acceptable as an effective euthanasia agent for fish.

Halothane.

This may be bubbled through the tank and causes anaesthesia. Death must be ensured by destruction of the brain.

Injectable agents.

Barbiturates may be used but as this requires removal from water and handling, with resultant stress, other methods are preferable. The intraperitoneal route is generally recommended.

METHODS ACCEPTABLE FOR UNCONSCIOUS FISH

Pithing.

For small fish pithing can be employed in which a metal spike is pushed into the top of the head between the eyes and pushed forwards and backwards to destroy the brain and proximal end of the spinal cord. *This method should only be carried out on unconscious animals.* This method is considered acceptable when chemical methods are not appropriate for the study.

Decapitation.

This is possible in small fish but becomes problematic in larger fish. Decapitation of fish should only be carried out under anaesthesia or after stunning as there is some doubt about immediate loss of consciousness. Research on eels showed that the brain was still functioning for up to 35 minutes after decapitation (Verheijen and Flight, 1995) and therefore

the brain should be immediately destroyed. This method is acceptable only if other methods are not considered suitable and under the above constraints. Spinal transection (the neck cut) is also unacceptable except on insensible fish (Flight and Verheijen, 1993).

Exsanguination.

This is not considered acceptable as a method of euthanasia as it is too slow and too difficult to locate the veins, unless the fish is insensible.

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF FISH.

Removal from water causes distress and suffering because of the length of time to unconsciousness. Cooling prolongs this period considerably. This is not an acceptable method of euthanasia of fish (Kestin, 1993; Kestin *et al*, 1991).

Whole body crushing is not considered a humane method of euthanasia.

Electrical stunning could be dangerous for the operator, if an isolated circuit is not used. Electrical stunning does not work with all fish (for example eels) and used alone it is not necessarily lethal (it may only stun larger fish). Alternating current stimulates contraction of skeletal, cardiac and smooth muscle, and it induces tetany, not anaesthesia (Summerfelt and Smith, 1990). Although it is used on fish farms by experts, often to catch fish, it is not considered acceptable in the laboratory situation for euthanasia.

Hypothermia. Putting fish into a freezer or crushed ice prolongs the period of consciousness in fish and does not reduce the ability to feel pain; therefore it should not be used as a method of euthanasia.

Hyperthermia. Fish when put into hot water will press their opercula tightly to the body and thus have a depot of oxygen which prolongs their period of consciousness. Boiling water will cause extreme pain. This method should therefore not be employed to kill fish of any kind.

2-Phenoxyethanol. Its main use is as an antibiotic but is used as an anaesthetic agent as well. It requires large doses to achieve death with a long induction period. Some species exhibit hyperactivity prior to loss of consciousness. It is not considered acceptable for use for euthanasia of fish.

Carbon dioxide. This is not acceptable for euthanasia of fish as it causes intense activity prior to loss of consciousness and is slow acting.

Diethyl ether should not be used because of irritation to the mucous membranes as well as danger to the operator.

Secobarbital and amobarbital both have the disadvantage that induction is too long.

Urethane is carcinogenic and therefore extremely dangerous to the operator.

Chloral hydrate has a long induction period and only acts as a sedative.

Tertiary amyl alcohol causes irritation during induction.

Tribromoethanol is irritant and has a long induction period.

Chlorobutanol has the disadvantage of requiring a broad range of dosages across the different species.

Methyl pentynol causes stress by respiratory arrest.

Pyridines are dangerous to the operator.

Table 1: Characteristics of methods for euthanasia of fish.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
MS-222	++	++	++	++	++	5	Acceptable.
Benzocaine	++	++	++	++	++	5	Acceptable.
Etomidate	++	++	++	++	++	5	Acceptable.
Metomidate	++	++	++	++	++	5	Acceptable.
Concussion	++	+	+	++	-	4	Death to be confirmed.
Maceration	++	++	++	++	+	4	Only for fish less than 2cm in length.
Quinaldine	++	++	++	+	++	4	Difficult to obtain in Europe.
Sodium pentobarbitone	++	++	-	+	++	3	May be useful for large fish, intraperitonal injection.
Cervical dislocation	++	++	+	++	-	3	Not in large fish. To be followed by destruction of the brain.
Halothane	+	+	++	++	++	2	Other methods preferable. Death to be confirmed.
The following methods may only be used on unconscious fish: pithing, decapitation and exsanguination.							
The following methods are not to be used for killing fish: removal from water, whole body crushing, electrical stunning, hypothermia, hyperthermia, 2-phenoxyethanol, carbon dioxide, diethyl ether, secobarbital, amobarbital, urethane, chloral hydrate, tertiary amyl alcohol, tribromoethanol, chlorobutanol, methyl pentynol, pyridines.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.2 AMPHIBIANS

There are many species of amphibians making it difficult to generalise on methods of euthanasia. The skin is thin, protected by a cuticle bearing many mucus glands. This results in it being generally more sensitive to physical and chemical insults than in other vertebrates. Because amphibians are ectothermic and thus accustomed to fluctuations in body temperature, their central nervous system is less sensitive to hypoxia and anoxia. Even when the cranial nerves and brain are deprived of a blood supply these animals are able to respond to stimuli for some time. Although decapitation, by itself, does not produce rapid unconsciousness in the severed heads of amphibians, rapid destruction of the brain does extinguish responses usually thought to indicate consciousness. There is, however, a remarkably intact set of somatic responses to stimuli - long continued body movements, foot withdrawals in response to toe pinching, etc., as well as continued heartbeat in many cases for hours following brain destruction. This continued somatic activity is attributed to:-

1. prolonged tolerance of the spinal cord, peripheral nerves and muscle (smooth, cardiac and skeletal) to hypoxic and hypotensive conditions, and
2. a far greater degree of integration of somatic responses at the level of the spinal cord instead of the brain.

(UFAW/WSPA, 1989)

RECOGNITION AND CONFIRMATION OF DEATH.

Death may be recognised by cessation of heartbeat and respiration and in cases where this is not obvious, it may be confirmed by destruction of the brain.

LARVAE

Tadpoles and newts can be effectively killed by placing in a dish of water with MS-222 or benzocaine (dissolved in acetone). These produce rapid anaesthesia, followed by death.

ADULTS

Further details of methods may be found in Section 2.

When handling these species, it is important to obtain a firm hold, for example by wearing rough textured but non-abrasive gloves or by holding them in coarse material. Cooling to 3-4°C will reduce metabolic and locomotory processes, thus facilitating handling prior to euthanasia. However, it must be remembered that cooling does not reduce the ability to feel pain (UFAW/WSPA, 1989).

PHYSICAL METHODS.

Concussion.

This method, if carried out by a person who is well trained in the technique is an effective and humane way of stunning all amphibians. The hind legs should be grasped and the dorsal surface of the head struck against a hard, solid object. Alternatively, the dorsal surface of the head can be struck with a suitable instrument. Accuracy is essential to ensure immediate unconsciousness and death. Death must be ensured by destroying the brain after concussion.

Microwave.

This is an extremely quick method of euthanasia but must only be carried out by experienced personnel who know exactly where to direct the beam. Only specialised microwave equipment designed for this purpose is to be used. *Under no circumstances should domestic appliances be used for this purpose.* It is not considered as a routine method of euthanasia.

Electrical stunning.

Frogs rendered insensible by electrical means may recover after 10 minutes but if followed immediately by destruction of the brain, it may be considered an acceptable method of euthanasia.

CHEMICAL METHODS.

Drugs are best administered by dissolving them in the water in which the amphibians are placed. This reduces stress from handling and injection.

Tricaine methane sulphonate (buffered MS-222).

This is a quick, non-irritant and humane method of killing amphibians when dissolved in the water into which the amphibians are placed. It is recommended to neutralise the solution with bicarbonate to reduce irritation to the sensitive amphibian skin.

Benzocaine.

Dissolved in the water in which amphibians are placed, benzocaine is an effective drug acting on the CNS quickly and humanely. As benzocaine is not soluble in water, it must be first dissolved in acetone. The solution must be neutralised to avoid irritation as benzocaine reduces the pH.

Sodium pentobarbitone.

This drug, when injected intravenously or intraperitoneally acts quickly on the CNS, rendering the animal unconscious with little distress. However, this should only be carried out by experienced people to ensure the correct site for injection and to reduce handling to a minimum.

T-61.

Intravenously injected, or in frogs, injected into the dorsal lymph sac, this drug is effective and humane for the euthanasia of amphibians.

METHODS ACCEPTABLE FOR UNCONSCIOUS AMPHIBIANS

Pithing ensures a rapid destruction of the brain resulting in immediate unconsciousness. This is a rapid and humane method of killing amphibians if carried out by well trained and experienced operators. *This method should only be carried out on unconscious animals.* In some species it is difficult to bend the head forward to expose the atlanto-occipital space and other methods are preferable in these cases.
Decapitation is acceptable only in insensible amphibians as the time to unconsciousness is unknown because the nervous system is very tolerant of anoxia.

METHODS NOT ACCEPTABLE FOR THE EUTHANASIA OF AMPHIBIANS.

Hypothermia will make the animal torpid but this does not reduce pain. Freezing is not acceptable as the formation of ice-crystals within the body tissues is likely to be extremely painful. Freezing may only be used as a method to confirm death after another method of euthanasia has been used .
Hyperthermia. Amphibians should not be dropped into hot or boiling water as a method of euthanasia as this is extremely painful and inhumane.
Exsanguination and subsequent hypovolaemic shock and anoxia may not render amphibians immediately unconscious, making this an unacceptable method of euthanasia.
Strangulation is considered inhumane, and is not an acceptable method of killing amphibians.
Carbon dioxide may cause irritation to the skin, and induction takes too long, and is therefore not considered an acceptable method.
Ether is irritant to the mucous membranes and because of danger to the operator, it should not be used for killing amphibians.
Chloroform is hepatotoxic and carcinogenic and because of the consequent possible danger to personnel, chloroform should not be used as for euthanasia.
Volatile inhalational anaesthetics are not considered acceptable as they are slow to act and may be irritant to the skin.
Other agents not considered acceptable include **chloral hydrate, ketamine hydrochloride, chlorbutanol, methylpentynol, 2-phenoxyethanol, tertiary amyl alcohol, tribromoethanol, urethane.**

Table 2: Characteristics of methods for euthanasia of amphibians.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
MS-222	++	++	++	++	++	5	Acceptable.
Benzocaine	++	++	++	++	++	5	Acceptable.
Sodium pentobarbitone	+	++	-	+	+	4	Involves handling and intravenous or intraperitoneal injection.
Concussion	++	++	+	++	-	4	Acceptable for use by experienced personnel.
T-61	+	++	-	+	+	3	Involves handling and intravenous injection.
Microwave	++	++	-	+	++	3	Only for small amphibians. Not a routine procedure.
Electrical stunning	+	+	+	-	-	2	To be followed immediately by destruction of the brain.
The following methods are only to be used on unconscious amphibians: pithing and decapitation.							
The following methods are not to be used for killing amphibians: hypothermia, hyperthermia, exsanguination, strangulation, carbon dioxide, diethyl ether, chloroform, volatile inhalational anaesthetics, chloral hydrate, ketamine hydrochloride, chlorbutanol, methylpentynol, 2-phenoxyethanol, tertiary amyl alcohol, tribromoethanol and urethane.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.3 REPTILES

Because reptiles are ectothermic and thus accustomed to fluctuations in body temperature, their central nervous system is less sensitive to a drop in oxygen tension. Even when the cranial nerves and brain are deprived of a blood supply following decapitation, these animals are able to respond to stimuli for some time. Although decapitation, by itself, does not produce rapid unconsciousness in the severed heads of reptiles (Warwick, 1990), rapid destruction of the brain does extinguish responses usually thought to indicate consciousness. There is, however, a remarkably intact set of somatic responses to stimuli - long continued body movements, foot withdrawals in response to toe pinching, etc., as well as continued heartbeat in many cases for hours following brain destruction. This continued somatic activity is attributed to:-

1. prolonged tolerance of the spinal cord, peripheral nerves and muscle (smooth, cardiac and skeletal) to hypoxic and hypotensive conditions, and
2. a far greater degree of integration of somatic responses at the level of the spinal cord instead of the brain.

(UFAW/WSPA, 1989)

Good methods of restraint are important to ensure minimal stress prior to carrying out euthanasia.

Particular care must be taken when handling venomous species, such as many snakes, especially when they are not used to being handled. Padded grasping implements are useful in handling lizards and snakes to ensure a firm but non-damaging restraint. Cooling of most reptiles to 3-4°C will reduce metabolic and locomotory processes (this temperature may kill some tropical species), thus facilitating handling prior to euthanasia. However it must be remembered that cooling does not reduce the ability to feel pain.

In tortoises, turtles and terrapins, retraction of the head and protection by the carapace can cause difficulty for euthanasia. To assist in exposing the head, land tortoises can be placed in shallow, tepid water; large marine species may be put on a frame at 45° head up, inducing neck extension; and soft-shelled species can be placed on their backs to induce neck extension. Rough textured but non-abrasive gloves may be worn when handling aquatic species to facilitate handling.

Effective restraint of the jaws and tail is the key factor to operator safety for restraining crocodilians and this should only be done by experts (UFAW/WSPA, 1989).

RECOGNITION AND CONFIRMATION OF DEATH.

As it is difficult to determine whether reptiles are unconscious or dead, it is recommended that death be confirmed by destruction of the brain. Usually, but by no means always, a lack of pupillary-blink-nictitating membrane responses, except in snakes which do not posses movable eyelids, implies a lack of consciousness. Rigor mortis is a reliable indicator of death as is the prolonged absence of a heartbeat and/or circulation.

EMBRYOS

In the case of reptiles two distinct stages have to be considered: the eggs and the newly hatched individuals. For all practical purposes all newly hatched reptiles can be treated in the same way as adults. As reptiles are born as fully developed individuals (with the exception of not being able to reproduce), killing of the embryos at the egg stage must be carried out in a humane way to account for potentially advanced development. In general eggs may succumb to high and low temperatures but some may withstand freezing. Hypothermia and hyperthermia cannot be considered acceptable because humane death cannot be guaranteed. Drowning is not considered humane as this results in death by anoxia and is slow. Eggs with no embryo may be frozen. Recommended methods would include disruption of the egg and killing of the embryos by in injection of sodium pentobarbitone, anaesthetic overdose or an appropriate physical method to destroy the brain or whole egg or early life form.

ADULTS

As the class Reptilia is varied, it is best to consider three main groups: the snakes and lizards (Squamata); tortoises, turtles and terrapins (Testudines); and crocodiles and alligators (Crocodilia). Larger reptiles may need to be sedated before being killed.

Further details on methods may be obtained in Section 2.

PHYSICAL METHODS.

Captive bolt.

This method may be used within the laboratory situation with relative safety. It is considered an acceptable method for large reptiles but should only be carried out by experts who know exactly where to position the pistol. Care must be taken to ensure that the pistol is well maintained and is of the correct calibre and cartridge length for the species being killed. Good restraint is necessary to ensure humane killing. If the bolt goes through the brain, it should kill the reptile, otherwise it may just stun the animal. Death must be ensured by destruction of the brain.

Concussion.

Small reptiles and those with fine bone structures such as some snakes and lizards may be rendered unconscious by stunning. This involves striking the back of the head of the animal with some hard implement or object, the principle being either to kill the animal outright or to render it unconscious. Ideally, the blow should be given with such force as to cause cessation of brain activity. This must only be performed by people trained and experienced in handling and killing reptiles. Concussion must always be followed by destruction of the brain.

Shooting.

This is an effective method of killing most large reptiles, causing rapid and substantial destruction of the brain. A high level of skill is required in order to hit the brain through the two brain cases found in many reptiles. This method could also be dangerous to the operator and is therefore should only be used in field conditions. A heavy calibre rifle or shotgun of a suitable calibre is necessary for large animals such as adult crocodiles. It is necessary to ensure that the animal does not move its head prior to shooting. The Testudines must have their head exposed and held thus for accurate positioning of the gun.

CHEMICAL METHODS.

Sodium pentobarbitone.

Sodium pentobarbitone is an effective and humane method of euthanasia in reptiles. The intravenous route can be used by well trained personnel. Where intravenous injection is difficult the intraperitoneal route may be used but it is slower-acting. Injections should not be made intracardially or into the lungs as this is regarded as painful and irritant.

METHODS ACCEPTABLE FOR UNCONSCIOUS REPTILES

Pithing may only be carried out on unconscious animals and by experienced personnel.
Decapitation may only be used if the reptile has been made unconscious by some other methods, such as concussion, as long periods of post-decapitation consciousness have been recorded (Warwick, 1990).

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF REPTILES.

Spinal cord severance. Because of the ability of reptiles to withstand anoxia and cerebral hypoxaemia, spinal cord severance is not an acceptable method of euthanasia. It has been shown that crocodiles may remain conscious for up to 1hr 50 mins after spinal cord severance and other reptiles may remain conscious for similar lengths of time.
Hypothermia will make the animal torpid but will not raise the pain threshold. The formation of ice crystals within the body tissues is likely to be extremely painful. Hypothermia is not acceptable for euthanasia.
Hyperthermia is not considered acceptable as the time to unconciousness is unknown. Boiling water must never be used to kill reptiles.
Exsanguination is not considered humane due to the animal's tolerance of hypoxia.
Chloroform has been used to kill tortoises by injection into the peritoneal cavity with apparently no undesirable effects. Because of the potential trauma to the injected animal and danger to the operator, other methods are considered more acceptable. Chloroform is hepatotoxic and carcinogenic to the operator.
Tricaine methane sulphonate (MS-222) has been used injected intramuscularly in snakes and alligators. There is little information on the humaneness of this method and it is therefore not considered acceptable.
Reptiles are capable of holding their breath for a relatively long period of time and therefore inhalational methods such as **ether, halothane, enflurane, isoflurane and methyoxyflurane** cannot be considered as practicable or humane due to slow induction.
Other agents not to be used for killing reptiles include **CO_2, neuromuscular blocking agents, ketamine hydrochloride** (takes too long to induction), **chloral hydrate and procaine.**

Table 3: Characteristics of methods for euthanasia of reptiles.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
Sodium pentobarbitone	++	++	++	+	++	5	Acceptable, but involves handling.
Captive bolt	++	++	++	+	+	5	Acceptable for large reptiles.
Concussion	+	+	+	++	+	4	To be followed by destruction of the brain.
Shooting	++	++	++	-	+	4	Acceptable only in field conditions.
The following methods are to be used on unconscious reptiles only: pithing and decapitation.							
The following methods are not to be used for killing reptiles: spinal cord severance, hypothermia, hyperthermia, exsanguination, chloroform, MS-222, ether, halothane, methoxyflurane, isoflurane, enflurane, carbon dioxide, neuromuscular blocking agents, ketamine hydrochloride, chloral hydrate and procaine.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.4 BIRDS

Birds have a complex respiratory system comprising the lungs and numerous air sacs with a one-way flow of air. This may influence the rate of absorption of inhalational agents and thus increase their efficiency.

RECOGNITION AND CONFIRMATION OF DEATH.

Death may be recognised by absence of signs of breathing, cardiac arrest and absence of reflexes in the head (i.e. cranial nerve reflexes rather than spinal cord reflexes). Reflexes to be checked would include pinching of wattles or blink reflexes. Death can be ensured by destruction of the brain or ensuring cessation of the heartbeat.

EMBRYOS

Bird embryos from the stage at which the neural tube has developed into a functional brain (>50 % gestation) must be destroyed humanely as they may be capable of perceiving pain from that stage. The most commonly used method of destroying eggs is cooling or freezing. The recommended temperature is <4°C for four hours. Death must be confirmed by decapitation or some other suitable method. In cases where the embryo has been exposed for studies, decapitation is considered an acceptable method of euthanasia as is an overdose of anaesthetic. Disruption of the membranes and maceration (in a macerator designed for the purpose) are also considered as humane methods of killing bird embryos (Bandow, 1987).

ADULTS

Further details on methods may be found in Section 2.

PHYSICAL METHODS.

Cervical dislocation.

Cervical dislocation, if carried out near to the head, causes damage to the lower brain region, resulting in rapid and painless loss of consciousness. This must always be followed immediately by destruction of the brain or section of the major blood vessels in the neck. However, research has shown that visual evoked potentials may remain up to 30 seconds after dislocation which may indicate lack of insensibility (Gregory & Wotton, 1990). Therefore, other methods are considered preferable. This method is not aesthetically pleasant as reflexes remain present for some time. This should not be used for birds of over 3kg or some older birds where the neck is difficult to pull quickly. This method may be used for day old chicks as long as the numbers are kept low to avoid human error due to tiredness (Jaksch, 1981). The wings of the birds should be secured to avoid involuntary flapping (Clifford, 1984).

Maceration.

This method may be used for chicks up to 72 hours old using specialist apparatus which contains rapidly rotating mechanically operated killing blades (Commission of the European Communities, 1993) Only equipment designed for this purpose and complying to the standards of Council Directive 93/119/EC should be used. The blades must turn at > 5000 revs/min. Operators must be trained in the use of such equipment and also have training in the maintenance of the equipment to ensure correct functioning at all times. The capacity and design of the apparatus must be sufficient to ensure that all animals are killed immediately

with no possibility of animals being thrown out by the revolving blades. Chicks must be dropped one at a time down a special chute to reduce the chances of being thrown out by the blades in smaller apparatuses, but larger ones have been designed to take larger number of chicks at a time, without this risk. This method may be aesthetically unpleasant for some operators. *Under no circumstances should domestic appliances be used.*

Concussion.

This is carried out by a hard blow to the head and in small birds (< 250g) this may be done by striking the head on the sharp edge of a table. Although not aesthetically pleasant it is quick and humane if carried out correctly by a person trained and experienced in the technique. This method may also be acceptable for small numbers of day old chicks. Unless sufficient brain damage has been caused resulting in immediate death, this must be followed by destruction of the brain.

Microwave.

Small birds may be killed rapidly and humanely by microwave delivered by specialist apparatus (Zeller *et al*, 1989). *Under no circumstances should domestic appliances be used.* Operators must receive specialist training in this technique to ensure accurate direction of the microwave beam and thus humane death. *This method should not be considered as a routine method of euthanasia.*

Electrical stunning.

Electrical stunning is commonly used in slaughterhouses but is generally *not considered acceptable for use in the laboratory* unless specialised equipment, safe for personnel, under legal controls, is used. The bird must be stunned before cardiac arrest occurs (i.e. the electrodes are placed in such a position that the brain is first affected).

CHEMICAL METHODS.

INHALATIONAL AGENTS.

Carbon dioxide.

This method is used on a large scale for chicks up to 72 hours old (Clifford, 1984). These chicks are young and relatively insensitive to CO_2 so higher doses may be needed than for adult birds. The chicks should be placed in non-porous bags or containers filled with 100% CO_2 for at least 10 minutes using a vapour feed system. A closed system is preferable and quicker in induction than open systems. Care should be taken to avoid overcrowding and the levels of CO_2 must be constantly monitored and maintained. CO_2 may also be used in conjunction with argon and oxygen for poultry (Raj and Gregory, 1994). The CO_2 causes loss of consciousness and the argon causes death by hypoxia. When killing larger birds with CO_2, care must be taken to ensure that the chamber is completely filled prior to putting the birds into it to ensure uniform levels of CO_2 throughout the chamber. Older birds may flap their wings after losing consciousness which may not be acceptable to some operators.

Volatile inhalational anaesthetics.

Air or oxygen must be provided during the induction period. Appropriate gas scavenging equipment needs to be used with all these agents.

Halothane, enflurane, isoflurane.

These agents are considered acceptable for the euthanasia of most birds. They are safe for personnel if used with gas scavenging apparatus and they are effective in causing anaesthesia and euthanasia.

Carbon monoxide.

Carbon monoxide causes rapid death as it combines with the red blood cells in preference to oxygen, thus causing hypoxia. However, it is extremely dangerous to personnel because it is not easily detectable and should only be used by people trained in the technique and with appropriate gas scavenging apparatus. Only the use of commercially compressed CO should be used for euthanasia. Death must be confirmed by physical means.

INJECTABLE AGENTS.

Sodium pentobarbitone.

This is an acceptable method of euthanasia for birds of all ages. Sodium pentobarbitone causes rapid and relatively stress-free death if carried out by experienced personnel. It should be injected intraperitoneally. Some experienced operators may inject it into the foramen magnum at the base of the skull (intracephalic) which is quick.

T-61.

Injected intramuscularly into the pectoral muscles of small birds, T-61 is very effective. It should not be used for larger birds or poultry because it takes some time to act and causes convulsions.

METHODS ACCEPTABLE FOR UNCONSCIOUS BIRDS

Decapitation reduces the blood pressure very quickly which may result in unconsciousness as well as massive trauma across the cord at the level of the brain stem with an ascending and descending effect on neural activity. However, work carried out by Gregory and Wotton (1986, 1990) on chickens shows that there are visually evoked responses for up to 30 seconds after decapitation. Other methods are considered preferable until further research can show that the birds are made immediately insensible.

Pithing is not acceptable unless the bird is fully anaesthetised.

Nitrogen kills birds by anoxia. Day old chicks should not be killed with nitrogen because of their ability to withstand low concentrations of oxygen. The responses by the unconscious bird may be objectionable to personnel. In general the use of nitrogen for the euthanasia of birds is not considered acceptable unless unconscious.

Potassium chloride is cardiotoxic and causes muscle spasms and convulsive seizures, thus making it unpleasant for the operator. It may only be used once the birds are fully anaesthetised.

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF BIRDS.

Neck crushing. The neck of the small bird is pressed against a bar or a specialised set of pliers, designed for the purpose, may be used. It is not known whether neck crushing produces immediate unconsciousness (Gregory & Wotton, 1990). This method is not considered acceptable for euthanasia as there are other more humane methods.

Exsanguination is not an acceptable method of killing birds because the blood clots easily, possibly resulting in incomplete exsanguination.
Decompression (creating a vacuum) induces dypsnoea. After 20 seconds at 60 mm Hg the birds collapse. Because the time to unconsciousness is not known and decompression causes rapid expansion of gases in the air sacs and pneumatic bones which may cause pain, this method is not acceptable in the laboratory situation.
Nitrous oxide requires hypoxic concentrations of up to 100% to be effective and it is slow acting. The bird will convulse after losing consciousness which may deter some operators. It is not acceptable for euthanasia of birds.
Ether and chloroform are not to be used for the killing of birds due to extreme operator danger and irritation to the respiratory passages of the bird.
Cyclopropane is humane and causes rapid anaesthesia, but is flammable and explosive in air. Because of danger to the operator, cyclopropane is not acceptable for euthanasia of birds.
Hydrogen cyanide gas causes rapid and irreversible death by cytotoxic hypoxia but it also causes excitability and distress before death which makes it totally unacceptable.
Other agents not to be used include **methoxyflurane, trichlorethylene, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine alone, and neuromuscular blocking agents.**

Table 4: Characteristics of methods for euthanasia of birds.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable.
T-61	++	++	+	+	++	4	Requires expertise; acceptable for small birds only (< 250g).
Carbon dioxide	++	++	++	++	+	4	Acceptable method, especially for chicks.
Halothane, enflurane, isoflurane	++	++	++	+	++	4	Acceptable.
Maceration	++	++	++	++	-	4	Acceptable for chicks up to 72 hours.
Cervical dislocation	++	++	-	++	-	4	Acceptable for small and young birds (< 250g) if followed by destruction of the brain.
Microwave	++	++	-	++	+	3	To be used by experienced personnel only. Not a routine procedure.
Concussion	++	++	-	++	-	3	Acceptable for birds under 250g.
Carbon monoxide	+	+	++	-	+	2	Danger to operator.
Electrical stunning	+	+	+	-	-	1	Danger to operator. Other methods preferable.
The following methods may only be used on unconscious birds: decapitation, pithing, nitrogen, potassium chloride.							
The following methods are not to be used for killing birds: neck crushing, decompression, exsanguination, nitrous oxide, diethyl ether, chloroform, cyclopropane, hydrogen cyanide gas, trichlorethylene, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine and neuromuscular blocking agents.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.5 RODENTS

Rodents are the mostly commonly used animals for experimental purposes and include mice, rats, hamsters, guinea pigs, gerbils, shrews, and dormice.

RECOGNITION AND CONFIRMATION OF DEATH.

Cessation of respiration and heartbeat, and absence of reflexes are good indicators of irreversible death in rodents. Death may be confirmed by exsanguination or extraction of the heart, evisceration, deep-freezing or decapitation. Personnel must be trained to recognise and ensure death when killing rodents.

EMBRYOS

The time at which the neural tube develops into a functional brain (about 60% gestation) must be taken as the time at which the foetus may perceive pain and should therefore be killed humanely. There is a large variation in degree of development at birth of various rodents. Mice and rats are totally dependent on the nest and have very few layers of neuronal development in the cerebral cortex, whereas guinea-pigs are fully developed and independent at birth.

If a foetus is removed from an anaesthetized mother, and is also insensible, then it may be killed by decapitation or removal of the heart. However, when the foetus is to be removed an increased amount of anaesthetic must be administered to the dam and maintained for longer to ensure that the anaesthetic has crossed the placenta. In many cases inhalational anaesthetics will not anaesthetize a foetus. Foetuses under 4g not anaesthetized prior to removal from the dam may be killed by rapid cooling in liquid nitrogen.

NEONATES

These are newborn rodents up to 10 days old. They may react more like embryos than adults to painful stimuli. They may be killed by decapitation or concussion. Hypothermia may be considered (Phifer and Terry, 1986). CO_2 is not recommended as neonates are more resistant to it, increasing the time to unconsciousness. Further research is necessary to assess which methods are the most humane.

ADULTS

Further details on the methods may be found in Section 2.

PHYSICAL METHODS

When carrying out physical methods of killing rodents, consideration must be given to the careful handling and restraint of the animal prior to killing. Minimal handling and restraint is preferable. Fear and anxiety to the animal may be reduced by prior sedation or handling by familiar people.

Concussion.

This is a quick and humane method of stunning rodents as long as it is performed by experienced and confident operators. This should only be used on rodents of less than 1 kg, above which considerable skill and sometimes a great deal of strength are required to perform it efficiently. Death must always be confirmed.

Cervical dislocation.

This is a commonly used and humane method of killing most small rodents (under 150g (Marshall *et al*, 1994)) as it causes extensive damage to the brainstem resulting in immediate unconsciousness and death. It is more difficult on hamsters and guinea pigs due to their short necks, stronger neck muscles and loose skin over neck and shoulders. In mice and rats the thumb and index finger are placed on either side of the neck at the base of the skull, or, alternatively, a rod is pressed at the base of the skull and then with the other hand, the base of the tail or hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. Larger rodents and older rats should be sedated or stunned prior to dislocation. Death must be confirmed as indicated in the section, *recognition and confirmation of death.*

Decapitation.

The immediate lack of circulation of blood to the brain and subsequent anoxia is thought to render the head rapidly insensible (Derr, 1991). Prior anaesthetization is not generally recommended as this involves further handling and consequent stress to the animal. Other methods are preferable until further research can show immediate unconsciousness. Specialised apparatus should always be used. Care must be taken to ensure that the apparatus is kept clean and the blades are sharp.

Microwave.

This involves the use of specialised apparatus and should only be carried by personnel specially trained in this technique to ensure correct positioning of the beam. When carried out correctly, it is an extremely quick method of killing rodents and therefore very humane. *Under no circumstances should domestic appliances be used for this purpose.* This is not a routine method of euthanasia.

Rapid freezing.

This is carried out by putting the animal into liquid nitrogen. This may only be used for foetuses and small neonates (< 4g) with no fur. Larger or furred animals will not die immediately as it takes some time for the core to become frozen.

CHEMICAL METHODS.

When using chemical methods for euthanasia, death must be confirmed by one of the methods listed above.

INHALATIONAL AGENTS.

Volatile inhalational anaesthetics.

With these agents the rodent is put into an anaesthetic chamber or an appropriate receptacle with gauze or cotton wool soaked in the anaesthetic. Because the liquid state of these anaesthetics are irritant, care must be taken to ensure that the rodent cannot come into contact with the chemical. Air or oxygen must be provided during the induction period.

Halothane, enflurane, isoflurane.

These agents act by depression of the cardiovascular and respiratory systems. They induce anaesthesia and subsequent death. These are all acceptable agents when used with appropriate gas scavenging apparatus.

Carbon dioxide.

It is recommended that a minimum of 70% CO_2 in oxygen or air be used for quick loss of consciousness without hypoxia. This results in rapid anaesthesia followed by death with reduced effects from irritation of the respiratory passages. 100% CO_2 is recommended for guinea-pigs (Noonan, 1994). Only CO_2 from commercially available gas cylinders should be used for euthanasia of rodents. See Section 2 (page 13) for further details.

Carbon monoxide.

Although this is a relatively quick and humane method of killing rodents, because of the danger to the operator it must be used with extreme caution. If chosen, it should be used with appropriate gas scavenging apparatus and only commercially available gas in cylinders is recommended. The rodents must be placed into a container prefilled with at least 6% CO by volume.

INJECTABLE AGENTS.

In larger rodents, where venepuncture is possible without due stress to the animal, intravenous injection is recommended as this results in rapid anaesthesia and death. If venepuncture is not easily performed intraperitoneal injection is preferred, although it takes longer to act and irritation of the peritoneum may occur. Under no circumstances should intrapulmonary or intracardial injection be given unless the animal is fully anaesthetized.

Sodium pentobarbitone.

Injected intravenously or intraperitoneally sodium pentobarbitone acts quickly and humanely to kill all rodents. This is the most acceptable agent for euthanasia. All personnel must be trained in method of injection. Sodium pentobarbitone may cause irritation of the peritoneum, which can be avoided by dilution. Three times the anaesthetic dose is usually recommended (Marshall *et al*, 1994; Noonan, 1994).

T-61.

T-61 acts quickly but must be injected intravenously very slowly, which is not always easy in rodents. It should never be injected by any other route in these species. Prior sedation may be required to assist with restraint during injection. Personnel must be well trained in intravenous injection techniques.

METHODS ACCEPTABLE FOR UNCONSCIOUS RODENTS

Rapid freezing may only be used once the rodent (> 4g) is fully unconscious.
Exsanguination may be used once the rodent is unconscious.
Air embolism may only be used on unconscious rodents as it can be painful.
Potassium chloride is cardiotoxic and potassium chloride causes gasping, vocalisations, muscle spasms and convulsive seizures which make it unacceptable for many operators. It may only be used once the animal is fully anaesthetised.
Ethanol had been used injected intraperitoneally at 70% (Lord, 1989). However, Wallgren & Barry III (1970) state that it is irritant at above 10%, making this unacceptable for euthanasia unless the rodent is unconscious.

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF RODENTS.

Hypothermia. *Under no circumstances should any rodent be killed by placing in a freezer.* Freezing may only be used in insensible animals as a method of ensuring death.
Nitrogen kills rodents by hypoxia making it unacceptable as it takes longer than other agents to achieve unconsciousness. Rats exhibit signs of panic and distress prior to unconsciousness (Hornett & Haynes, 1984).
Nitrous oxide kills by anoxia, is slow, and the rodents show signs of increased activity prior to death, indicating a degree of anxiety making it unacceptable as a method for euthanasia.
Cyclopropane is a humane and quick agent for killing rodents but it is extremely dangerous to the operator and is therefore not considered acceptable.
Ether and chloroform are not to be used under any circumstances for euthanasia of rodents. They are both extremely dangerous to the operator and ether causes irritation to the respiratory passages upon inhalation.
The following agents are not to be used for killing rodents: **decompression, asphyxia, drowning, trichlorethylene, methoxyflurane, hydrogen cyanide gas, strychnine, nicotine, chloral hydrate, magnesium sulphate, curariform drugs and other neuromuscular blocking agents.**

Table 5: Characteristics of methods for euthanasia of rodents.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
Halothane, enflurane, isoflurane	++	++	++	+	++	5	Acceptable.
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable.
Concussion	++	++	+	++	-	4	Acceptable for rodents under 1kg. Death to be confirmed by cessation of circulation.
Cervical dislocation	++	++	+	++	-	4	Acceptable for rodents under 150g. Death to be confirmed by cessation of circulation.
T-61	++	++	-	+	++	4	Only to be injected intravenously.
Carbon dioxide	+	++	++	++	++	4	Acceptable method at >70%.
Microwave	++	++	-	++	+	3	To be used by experienced personnel only. Not a routine procedure.
Decapitation	+	+	+	++	-	2	Other methods preferable.
Carbon monoxide	+	+	+	-	++	2	Danger to operator.
Rapid freezing	-	+	++	++	+	1	Only in small neonates (< 4g).
The following methods may only be used on unconscious rodents: rapid freezing, exsanguination, air embolism, potassium chloride, ethanol.							
The following methods are not to be used for killing rodents: hypothermia, decompression, asphyxia, drowning, nitrogen, nitrous oxide, cyclopropane, diethyl ether, chloroform, methoxyflurane, hydrogen cyanide gas, trichlorethylene, strychnine, nicotine, chloral hydrate, magnesium sulphate and neuromuscular blocking agents.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.6 RABBITS

RECOGNITION AND CONFIRMATION OF DEATH.

Cessation of respiration and heartbeat, and absence of reflexes are good indicators of irreversible death in rabbits. Death may be confirmed by exsanguination or removal of the heart, evisceration, or decapitation. Personnel must be trained to recognise and ensure death when killing rabbits.

EMBRYOS

The time at which the neural tube develops into a functional brain (about 60% gestation) must be taken as the time at which the foetus may perceive pain and should therefore be killed humanely. If an insensible foetus is removed from an anaesthetized mother it may be killed by decapitation or removal of the heart. However, when the foetus is to be removed an increased amount of anaesthetic must be administered to the dam and maintained for longer to ensure that the anaesthetic has crossed the placenta. In many cases inhalational anaesthetics will not anaesthetize a foetus. Those foetuses that are not removed from the dam will die of anoxia when the dam is killed and no further method is necessary to ensure death of the foetus.

NEONATES

These are newborn rabbits up to 10 days old. They may react to painful stimuli more like an embryo than an adult. They may be killed by decapitation and concussion. Further research is necessary to assess which methods are the most humane.

ADULTS

Further details on methods may be obtained in Section 2.

PHYSICAL METHODS

When carrying out physical methods of killing rabbits, consideration must be given to the careful handling and restraint of the animal prior to killing. Minimal handling and restraint is preferable. Fear and anxiety to the animal may be reduced by prior sedation or handling by familiar people.

Concussion.

This is a quick and humane method of stunning rabbits as long is it is performed by experienced and confident operators. This involves striking the base of the head at the top of the neck, in the occipital region. Death must always be confirmed by cessation of circulation.

Cervical dislocation.

This is a humane method of killing rabbits of under 1 kg as it causes extensive damage to the brainstem resulting in immediate unconsciousness and death. It should only be carried out by experienced personnel. Death must always be confirmed by cessation of circulation. Sedation may be necessary prior to dislocation.

Captive bolt.

This method may be useful for large rabbits (over 4 kg) in limited situations (Holtzmann, 1991). Only captive bolts designed specifically for use on rabbits may be used. Personnel must be well trained in order to ensure correct positioning of the weapon. The bolt must penetrate about 3cm into the brain (Holtzmann, 1991). Death must be confirmed by ensuring cessation of circulation.

Decapitation.

Decapitation may be considered a humane method of killing small or young rabbits (under 1 kg) as loss of blood supply ensures rapid loss of consciousness. However, this is not possible in larger and older rabbits where the neck is too thick and strong for quick decapitation.

Electrical stunning.

Only electric tongs designed for this purpose may be used. Care must be taken to ensure that the correct level of current passes directly through the brain to ensure immediate unconsciousness. Death must be confirmed by cessation of circulation.

Microwave.

This involves the use of specialised apparatus and should only be carried out by personnel specially trained in this technique to ensure correct positioning of the beam. It is a quick method of killing small rabbits of under 300g. *Under no circumstances should domestic appliances be used for this purpose.* This is not a routine method of euthanasia.

Rapid freezing.

Foetuses of under 4g may be killed by putting into liquid nitrogen. Larger or furred animals will not die immediately is it takes some time for the core to become frozen.

CHEMICAL METHODS.

When using chemical methods of euthanasia, death must be confirmed by one of the methods listed above.

INHALATIONAL METHODS.

Volatile inhalational anaesthetics.

Rabbits react adversely to all gases (Green, 1979) and other methods are preferable if possible.

With these agents the rabbit is put into an anaesthetic chamber or an appropriate receptacle with gauze or cotton wool soaked in the anaesthetic. Vapours are inhaled until respiration ceases and death ensues. Because the liquid state of these anaesthetics are irritant, care must be taken to ensure that the rabbit cannot come into contact with the chemical. Air or oxygen must be provided during the induction period. Appropriate gas scavenging equipment needs to be used with all these agents.

Halothane, isoflurane, enflurane.

At high concentrations these agents cause rapid anaesthesia and consequent death. These are all acceptable agents when used with appropriate gas scavenging apparatus.

Carbon dioxide.

Large rabbits may become distressed initially whilst still conscious and therefore other methods are considered preferable if possible. 100% CO_2 has been recommended by Von Cranach *et al* (1991a), but may cause distress.

Carbon monoxide.

Although this is a relatively quick and humane method of killing rabbits, because of the danger to the operator it is less acceptable for routine use. If used, it should be used with appropriate gas scavenging apparatus and only commercially available gas in cylinders should be used as fumes from combustion engines are likely to be irritant.

INJECTABLE AGENTS.

In rabbits, where venepuncture is possible via the ear vein (unless damaged) intravenous injection is recommended as this results in rapid anaesthesia and death. Personnel must be trained in the techniques of intravenous and intraperitoneal injection. If venepuncture is not easily performed, intraperitoneal injection is acceptable, although it takes longer to act. Under no circumstances should intrapulmonary or intracardial injection be given unless the animal is fully anaesthetized.

Sodium pentobarbitone.

Injected intravenously, sodium pentobarbitone acts quickly and humanely to kill rabbits. This is the most acceptable agent for euthanasia. Sodium pentobarbitone may cause irritation to the peritoneum which may be avoided by dilution.

T-61.

T-61 acts quickly and humanely but may only be injected very slowly intravenously. It should never be injected by any other route. Prior sedation may be considered necessary to assist with restraint during injection. Personnel must be well trained in intravenous injection techniques. Personnel must take extreme care when handling this agent.

METHODS ACCEPTABLE FOR UNCONSCIOUS RABBITS

Exsanguination may be used to kill rabbits once they are fully unconscious.
Nitrogen kills rabbits by hypoxia and is therefore not considered acceptable for euthanasia of rabbits unless they are unconscious.
Potassium chloride is cardiotoxic and causes gasping, vocalisations, muscle spasms and convulsive seizures which makes is unacceptable for many operators. It may only be used once the animal is fully anaesthestised.
Air embolism by intravenous injection of air at 5-50 ml/kg causes rapid death but as this is accompanied by convulsions, opisthotonos, pupillary dilation and vocalisations, it is not an acceptable method unless the rabbit is fully unconscious.

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF RABBITS.

Hypothermia. Under no circumstances should any rabbit be killed by placing in a freezer.
Nitrous oxide kills by anoxia, is slow acting, and the rabbits show signs of increased activity prior to death, indicating a degree of anxiety. It is therefore not considered an acceptable method.
Methoxyflurane takes too long to act with a large chance of recovery.
Cyclopropane may be a humane and quick method of euthanasia, but is very dangerous to the operator and is therefore not considered acceptable for general use.

Ether and chloroform are not to be used under any circumstances for euthanasia of rabbits. They are both dangerous to the operator and ether causes irritation to the respiratory passages upon inhalation.

Ketamine hydrochloride by intravenous injection causes tonic contractions, accompanied by vocalisations, thus making this unacceptable for euthanasia of rabbits.

Other agents not to be used for killing rabbits include **decompression, asphyxia, drowning, trichlorethylene, hydrogen cyanide gas, hydrocyanic acid, strychnine, nicotine, chloral hydrate, magnesium sulphate, and neuromuscular blocking agents.**

Table 6: Characteristics of methods for euthanasia of rabbits.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable.
T-61	++	++	-	+	++	4	Acceptable. Intravenous injection only.
Cervical dislocation	++	++	-	++	-	4	Acceptable for rabbits under 1kg. Sedation prior to dislocation. Death to be confirmed by cessation of circulation.
Captive bolt	++	++	-	+	+	4	Requires skill. Death to be confirmed by another method.
Concussion	++	+	-	++	-	3	Expertise required. Death to be ensured by another method.
Electrical stunning	++	+	++	-	+	3	Death to be confirmed by another method.
Microwave	++	++	-	++	+	3	To be used by experienced personnel only on small rabbits. Not a routine procedure.
Decapitation	+	+	+	++	-	2	Acceptable for rabbits under 1 kg if other methods not possible.
Halothane, enflurane, isoflurane	++	++	++	+	-	2	Rabbits show signs of distress.
Carbon dioxide	+	+	++	++	+	1	Large rabbits show distress.
Carbon monoxide	+	+	++	-	++	1	Danger to operator.
Rapid freezing	+	+	++	++	+	1	Only in foetuses under 4g. Other methods preferred.
The following methods are only to be used on unconscious rabbits: exsanguination, nitrogen, potassium chloride and air embolism.							
The following methods are not to be used for killing rabbits: hypothermia, decompression, asphyxia, drowning, nitrous oxide, cyclopropane, diethyl ether, chloroform, trichlorethylene, hydrogen cyanide gas, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, hydrocyanic acid, ketamine hydrochloride and neuromuscular blocking agents.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.7 CARNIVORES - DOGS, CATS, FERRETS

RECOGNITION AND CONFIRMATION OF DEATH.

Cessation of respiration and heartbeat, and loss of reflexes are good indicators of irreversible death in carnivores. Death should be confirmed by exsanguination. Personnel should be trained to recognise and ensure death when killing dogs, cats, ferrets or other carnivores.

EMBRYOS

The time at which the neural tube develops into a functional brain must be taken as the time at which the foetus may perceive pain (30% gestation) and therefore should be killed humanely. If an insensible foetus is removed from an anaesthetized mother then it may be killed by decapitation or removal of the heart. However, when the foetus is to be removed an increased amount of anaesthetic must be administered to the dam and maintained for longer to ensure that the anaesthetic has crossed the placenta. In many cases inhalational anaesthetics will not anaesthetize a foetus.

NEONATES

Neonate carnivores should generally be treated as adults. Sodium pentobarbitone is the preferred method, but carbon dioxide, cervical dislocation and concussion may be considered (Hall, 1972) (see Section 2). Operators must be well trained in the physical techniques to ensure that they are correctly and humanely carried out. The animals must be exsanguinated immediately after concussion or cervical dislocation.

ADULTS

Further details on methods are given in Section 2.

PHYSICAL METHODS

In general it is not recommended to use physical methods of euthanasia on carnivores. However, where chemical agents may interfere with the aims of the experiment, the following methods may be used. Restraint of cats for physical methods may be difficult and it is recommended that all animals be sedated prior to euthanasia.

Captive bolt.

Captive bolts designed specially for the purpose of killing animals of this size may be used. Personnel must be trained in these techniques to ensure correct positioning of the pistol and immediate death. Death must be confirmed by cessation of circulation by exsanguination.

Shooting.

Shooting of carnivores using a free bullet is only acceptable under field conditions when no other method can be used. Only specialised marksmen should be used.

Electrocution.

Ear clips are attached to ensure that the current flows directly through the brain and death is confirmed by passing the current through the heart. There are two phases: stunning with 500 volt shock between the ears, followed by a lethal shock at 1 kilovolt passing from the ear to hindleg. Cats should not be killed by electrocution due to the high conductivity of their coats. Only specially designed apparatus should be used for this purpose and personnel must be well trained in this technique. Equipment must be regularly checked and maintained to

ensure correct voltage. Death must be confirmed by one of the methods in the section *recognition and confirmation of death.*

CHEMICAL METHODS

In general, chemical methods of euthanasia are preferred for all dogs, cats, ferrets and foxes. It may be preferable to sedate the animal prior to euthanasia to reduce stress and anxiety.

INHALATIONAL METHODS.

Volatile inhalational anaesthetics.

These include halothane, isoflurane and enflurane. These are all acceptable methods of euthanasia for carnivores. Appropriate gas scavenging apparatus should be used to prevent operator exposure.

INJECTABLE AGENTS.

If possible injection should be given intravenously in order to achieve rapid anaesthesia and euthanasia with minimal stress.

Sodium pentobarbitone.

Injected intravenously, this agent provides rapid and humane euthanasia. Intracardiac and intrapulmonary routes of injection should not be used as they are extremely painful, unless under full anaesthesia. All personnel must be trained in these techniques.

Secobarbital/dibucaine.

Secobarbital is a short-acting analogue of thiamylol sodium. Dibucaine is a highly toxic local anaesthetic causing rapid loss of consciousness, loss of respiration and cardiac arrest (Herschler *et al*, 1981; Wallach *et al*, 1981).

T-61.

This agent is very effective but must only be injected very slowly intravenously. Animals must be sedated prior to administration. It may cause convulsions in the unconscious animal, which may be aesthetically unpleasant.

METHODS ACCEPTABLE FOR UNCONSCIOUS CARNIVORES

Exsanguination may be used to kill carnivores once they are unconscious.
Dislocation of neck may be used on small animals under anaesthetic. Death must always be confirmed by one of the methods listed above.
Potassium chloride may be used to kill unconscious carnivores.

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF CARNIVORES.

Striking of chest of cats has been suggested as a method of euthanasia but this is not considered as humane and is not to be used under any circumstances.
Decompression has been used as a method of euthanasia in the USA and Japan. It probably causes much anxiety and stress to the animals and they may experience pain due to the

expansion of air in the sinuses and other body cavities. It is not considered acceptable for euthanasia of carnivores.

Although **carbon dioxide** causes cats to become unconscious within 1 minute, they move about the cage, licking, sneezing and trying to climb out, indicating that it may be stressful. The animals also convulse which makes this method aesthetically unpleasant for the operator. This is not considered acceptable as a method of euthanasia for carnivores except for neonates.

Carbon monoxide at concentrations above 6% is a relatively quick method of euthanasia and is recommended for the killing of mustelids (Commission of the European Communities, 1993). However, it causes convulsions and vocalisations which may still be in the conscious phase (Chalifoux and Dallaire, 1983). Because of this and the danger to the operator, it is not considered acceptable for experimental animals.

Nitrogen causes unconsciousness in dogs and cats in 1-2 minutes with hyperpnea for about 10 seconds before collapse. After collapse there are vocalisations, opisthonos, convulsions and gasping. Kittens and puppies are resistant to anoxia; they fall unconscious but fail to die. This is not an acceptable method.

Ether and chloroform are not acceptable methods of euthanasia due to irritation of the respiratory passages and danger to the operator.

The following agents are also not to be used for killing carnivores: **drowning, concussion (adults), decapitation, asphyxia, strangulation, nitrous oxide, hydrogen cyanide gas, cyclopropane, methoxyflurane, trichlorethylene, air embolism, hydrocyanic acid, chloral hydrate, strychnine, nicotine, magnesium sulphate, and neuromuscular blocking agents.**

Table 7: Characteristics of methods for euthanasia of dogs, cats, ferrets, foxes.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
Sodium pentobarbitone	++	++	-	+	++	5	Acceptable. Intravenous injection.
T-61	++	++	-	+	+	4	Acceptable but only by slow intravenous injection under sedation.
Secobarbital/dibucaine	++	++	-	+	++	4	Acceptable. Intravenous injection.
Halothane, isoflurane, enflurane	++	++	+	+	++	4	Acceptable.
Captive bolt	++	++	-	++	+	3	To be followed by exsanguination.
Electrocution	++	++	-	-	-	3	Use only special equipment. To be followed by exsanguination.
Concussion	++	++	+	++	-	2	Only to be used on neonates. To be followed by exsanguination.
Shooting	++	++	-	-	-	1	Acceptable only in field conditions when other methods not possible by specialised marksmen.
The following methods are acceptable for unconscious carnivores: exsanguination, neck dislocation and potassium chloride.							
The following methods are not to be used for killing carnivores: decompression, decapitation, drowning, strangulation, asphyxiation, air embolism, striking chest of cats, carbon monoxide, carbon dioxide, methoxyflurane, nitrogen, nitrous oxide, trichlorethylene, hydrocyanic acid, diethyl ether, chloroform, hydrogen cyanide gas, cyclopropane, chloral hydrate, strychnine, nicotine, magnesium sulphate and neuromuscular blocking agents.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.8 LARGE MAMMALS - PIGS, SHEEP, GOATS, CATTLE, HORSES

Personnel using and having to kill any large mammal must receive specialised training in the handling, restraint and techniques of euthanasia of these animals. It is important to avoid actions which may increase the animals' awareness of the unusual situation. The animal is best killed in a familiar environment.

All operators are recommended to obtain and read EC (Council Directive 93/119/EC) (Commission of the European Communities, 1993) and national regulations on slaughter methods which cover most of these animals. It may be necessary to take the animals to approved slaughterhouses where specialised equipment is available for humane euthanasia of these animals. Euthanasia may have to be carried out by a person who has been trained and holds a certificate under national slaughter legislation or by a veterinarian with appropriate training.

RECOGNITION AND CONFIRMATION OF DEATH.

Cessation of respiration and heartbeat, and loss of reflexes are good indicators of irreversible death. Death should be confirmed by exsanguination. Personnel should be trained to recognise and ensure death when killing these animals.

EMBRYOS

The foetuses of these large mammals are well developed at birth and therefore considerable care must be taken to ensure that they are killed humanely if removed from the uterus. The time at which euthanasia should be considered should be from the time at which the neural tube develops into a functional brain and they may thus be able to feel pain (>30% gestation). Foetuses may also be large and in general any method used on an adult is considered acceptable.

NEONATES

Because large mammals are born in an advanced stage of development, they should be treated as adults.

ADULTS

Further details on methods are given in Section 2.

PHYSICAL METHODS.

The animals must be suitably restrained in adequate devices to ensure that the animal remains still and calm so that the method of euthanasia is accurate and quick. Personnel should be quiet and handle the animals with care so as to reduce stress and anxiety in the animals.

Captive bolt.

The use of the captive bolt is the most acceptable physical method for large mammals and is preferable to free bullets because of operator safety. Penetrative captive bolts are preferred. Personnel must be well trained in the use of captive bolt pistols to ensure correct positioning for the species being killed (Universities Federation for Animal Welfare, 1989). Care must be taken to ensure that the correct size bolt and cartridge are used and that the weapon is kept clean and maintained in good working order. It is not recommended to use captive bolt pistols on adult pigs and mature bulls because of their thick skulls. Death must be confirmed immediately by exsanguination or pithing through the hole made by the bolt.

Free bullet humane killers.

A humane killer firing a free bullet is an efficient method of killing horses, mules, donkeys and old or hard-headed animals (Blackmore, 1985; Dodd, 1985; Oliver, 1979). Extra care must be taken as it is not as safe as the captive bolt and it is therefore considered acceptable only under field conditions. All personnel must be trained in these methods to ensure correct positioning of the weapon and that the correct calibre of weapon is used. It must be noted that the position of the weapon differs with each species and whether the animal is horned or not. The animals tend to slump forwards when shot and therefore the operator must take care to avoid personal damage. Operators must ensure that the weapon is kept well maintained so that the chance of misfiring is minimised. Death must be confirmed immediately by exsanguination or pithing through the hole made by the bullet.

Shooting.

Shooting using a free bullet should only be done in field conditions when no other method can be used. Only specialised marksmen should carry this out.

Concussion.

This should be done using a mechanically operated instrument which administers a blow to the skull without fracture of the skull. It must be noted that the position for the application of percussion stunners differs from that for captive bolt pistols. Death must be ensured by immediate exsanguination (within 20 seconds of stunning) (Blackmore, 1979).

Electrical stunning.

This method should only be carried out in slaughterhouses where specialised equipment is available for the animals being killed. It is commonly used for stunning pigs, sheep, calves and goats. Tongs should be applied on each side of the head between the eye and ear to span the brain. Moist sponge rubber pads fitted to the tongs are considered undesirable when dealing with high voltages. Sheep and goats should be shaved in the region of the tongs to ensure good electrical contact. It should not be used where horns make it difficult to position the tongs correctly. Care must be taken to ensure that the animals cannot receive electrical shocks from contact with other animals, from wet surfaces, or from accidental contact with the tongs. Head only or head to back stunning across the head are both acceptable methods as they both ensure immediate unconsciousness. Animals must be exsanguinated immediately after stunning to ensure death. Personnel must ensure that the correct voltage and current is used for the species of animal being killed.

CHEMICAL METHODS.

INHALATIONAL METHODS.

Volatile inhalational anaesthetics.

Halothane, isoflurane and enflurane may be used with an anaesthetic mask for lambs and kids.

Carbon dioxide.

Carbon dioxide has been used to kill pigs. The pigs are placed into large chambers that are previously filled with CO_2 gas to above 70%. Specialised equipment only must be used. Death must be confirmed by exsanguination. Because pigs tend to show signs of stress other methods are considered preferable. CO_2 must not be used on any other large animal.

INJECTABLE METHODS.

Personnel must be trained in intravenous injection techniques and handling and restraint of the animals. Animals should be suitably restrained and/or sedated prior to euthanasia.

Sodium pentobarbitone.

Injected intravenously, sodium pentobarbitone provides rapid euthanasia. Large volumes may be required for larger mammals and this may be made easier by insertion of a catheter into the jugular vein (Andrews *et al*, 1993). Alternatively lower volumes of highly concentrated solutions may be used, but it must be recognised that this poses a greater danger to the operator. Excitable or nervous animals must be sedated prior to injection with sodium pentobarbitone.

Quinalbarbitone/nupercaine.

This proprietary mixture causes rapid and humane death in horses. This should be injected intravenously over a period of 5-8 seconds. It is not available in some countries.

T-61.

T-61 is also an effective agent for euthanasia of large mammals by slow intravenous injection only. It may be necessary to sedate excitable or nervous animals initially.

METHODS ACCEPTABLE FOR UNCONSCIOUS LARGE MAMMALS.

Exsanguination may be used to kill unconscious mammals.
Chloral hydrate may be used intravenously, on unconscious mammals, or in conjunction with magnesium sulphate and sodium pentobarbitone.
Potassium chloride may be used to kill unconscious mammals.

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF LARGE MAMMALS.

Carbon monoxide. Some animals, including pigs, show signs of severe excitation and vocalisations, sometimes before unconsciousness, at high levels of carbon monoxide. It is not an acceptable agent for euthanasia.
The following agents are also not acceptable for the euthanasia of large mammals: **methoxyflurane, trichlorethylene, strychnine, nicotine, magnesium sulphate, thiopentone sodium, ketamine hydrochloride, curariform drugs and other neuromuscular blocking agents.**

Table 8: Characteristics of methods for euthanasia of large mammals.

AGENT	RAPIDITY	EFFICACY	EASE OF USE	OPERATOR SAFETY	AESTHETIC VALUE	1-5 OVERALL RATING	REMARKS
Sodium pentobarbitone	++	++	-	+	++	5	Acceptable by intravenous injection.
Quinalbarbitone/nupercaine	++	++	-	+	++	5	Effective for horses intravenously.
Captive bolt	++	++	+	+	+	5	To be followed by exsanguination.
Free bullet humane killer	++	++	+	-	+	5	To be followed by exsanguination. In field conditions only.
T-61	++	++	-	+	++	4	Acceptable by intravenous injection.
Electrical stunning	++	++	+	-	-	4	Use only specialised equipment. To be followed immediately by exsanguination.
Shooting	++	++	-	-	-	2	Only in field conditions by a specialised marksman.
Concussion	++	+	-	+	+	2	To be followed immediately by exsanguination.
Halothane, isoflurane, enflurane	+	+	+	+	+	2	Recommended for lambs and kids.
Carbon dioxide	+	+	++	++	+	1	Only for use with pigs at >70%.
The following methods are acceptable only on unconscious large mammals: exsanguination, chloral hydrate and potassium chloride.							
The following methods are not to be used for killing large mammals: carbon monoxide, methoxyflurane, trichlorethylene, strychnine, nicotine, magnesium sulphate, thiopentone sodium, ketamine hydrochloride, neuromuscular blocking agents.							

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended.

3.9 NON-HUMAN PRIMATES

Personnel handling primates should be specially trained for these purposes. It is preferable that if primates have to be killed, that this be carried out by someone familiar to them in order to reduce stress and anxiety. For all larger primates, sedation (e.g. ketamine) should be administered prior to euthanasia.

Cessation of heartbeat and respiration, and absence of reflexes may be considered as good indicators of death.

EMBRYOS

All foetuses in which the neural tube has developed into a functional brain must be killed humanely. Foetuses are sometimes required for experimental purposes but the mother is rarely killed to allow removal of the foetus from the uterus. These foetuses may be killed by overdose of anaesthetic or physical methods after anaesthetization.

ADULTS

The only recommended method for killing primates is by overdose of anaesthetic. Sodium pentobarbitone injected intravenously is the most acceptable agent. Exsanguination under inhalation anaesthesia is also considered acceptable, but this must be followed by perfusion.

Infants of some small species such as marmosets may be difficult to inject and this requires specialist expertise.

3.10 OTHER ANIMALS NOT COMMONLY USED FOR EXPERIMENTS

As vertebrate animals vary so much in size and physiology, the method chosen to kill any animal not included above should be chosen from those methods for animals that are most similar biologically. Advice should be obtained from a veterinarian. In general, an overdose of sodium pentobarbitone injected intravenously may be considered as a humane method of killing most animals. It is advisable in most cases to sedate the animal prior to euthanasia.

4. REFERENCES

Allaby, M., 1991. *Concise Oxford Dictionary of Zoology,* Oxford University Press, Oxford, UK.

Allred, J.B., Berntson, G.G. 1986. Is euthanasia of rats by decapitation inhumane? *The Journal of Nutrition* **116(9):** 1859-1861.

Anand, K.J.S., Hickey, P.R. 1987. Pain and its effects in the human neonate and fetus. *New England Journal of Medicine* **317:** 1321-1329.

Anil, M.H., McKinistry, J.L. 1991. Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *The Veterinary Record* **128:** 106-107.

Andrews, E.J., Taylor Bennett, B., Clark, J.D., Houpt, K.A., Pascoe, P.J., Robinson, G.W., Boyce, J.R. 1993. Report of the AVMA Panel on Euthanasia. *Journal of the American Veterinary Medical Association* **202:** 229-249.

Australian Veterinary Association. 1987. Guidelines on humane slaughter and euthanasia. *Australian Veterinary Journal,* 6 November: 4-7.

Bandow, J.H. 1987. *The humane disposal of unwanted day old chicks and hatchery eggs in the poultry industry.* Report for the Canadian Federation of Humane Societies, Ontario, Canada.

Baneux, P.J.R., Garner, D., McIntyre, H.B., Holshuh, H.J. 1986. Euthanasia of rabbits by intravenous administration of ketamine. *Journal of the American Veterinary Medical Association* **189(9):** 1038-1039.

Barber, B.R. 1972. Use of a standard autoclave for decompression euthanasia. *Journal of the Institute of Animal Technicians* **23(3):** 106-110.

Barocio, L.D. 1983. Review of literature on use of T-61 as an euthanasic agent. *International Journal for the Study of Animal Problems* **4(4):** 336-342.

Baumans, V., Hellebrekers, L.J., Bertens, A.P.M.G, Hartman, W. 1988. Evaluation of T-61 as an euthanasia agent in rabbits and dogs. *26th Scientific Meeting of the Society for Laboratory Animal Science*, Basel, 13-15 Sept, 1988.

Berman, E., King, J.B., All, J., Carter, H.B., Rehnberg, B., Stead, A.G. 1985. Lethality in mice and rats exposed to 2450 MHz circulatory polarized microwaves as a function of exposure duration and environmental factors. *Journal of Applied Toxicology* **5(1):** 23-31.

Blackmore, D.K. 1979. Non-penetrative percussion stunning of sheep and calves. *The Veterinary Record* **105:** 372-375.

Blackmore, D.K. 1985. Energy requirements for the penetration of heads of domestic stock and the development of a multiple projectile. *The Veterinary Record* **116:** 36-40.

Blackmore, D.K. 1993. Euthanasia; not always eu. *Australian Veterinary Journal* **70(11):** 409-413.

Blackmore, D.K., Delaney, M.W. 1988. *Slaughter of Stock. A Practical Review and Guide.* Publication No 118, Veterinary Continuing Education, Massey University, Palmerston North, New Zealand.

Blackshaw, J.K., Fenwick, D.C., Beattie, A.W., Allan, D.J. 1988. The behaviour of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. *Laboratory Animals* **22(1):** 67-75.

Booth, N.H. 1978. Effect of rapid decompression and associated hypoxic phenomena in euthanasia of animals: a review. *Journal of the American Veterinary Medical Association* **173(3):** 308-314.

Brown, L.A. 1988. Anesthesia in fish. *Veterinary Clinics of North America: Small Animal Practice* **18(2):** 317-330.

Breazile, J.E., Kitchell, R.L. 1969. Euthanasia for laboratory animals. *Federal Proceedings* **28:** 1577-1579.

Chalifoux, A., Dallaire, A. 1983. Physiologic and behavioral evaluation of CO euthanasia of adult dogs. *American Journal of Veterinary Research* **44(12):** 2412-2417.

Clifford, D.H. 1984. Preanesthesia, anesthesia, analgesia, and euthanasia. In: *Laboratory Animal Medicine* (ed J.G.Fox, B.J.Cohen, F.M.Loew): 527-562. Academic Press, Orlando, Florida.

Commission of the European Communities. 1986 *Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisons of the Member States regarding the protection of animals used for experimental and other scientific purposes.* 86/609/EEC. ISSN 03780 6978.

Commission the European Communities. 1993. *Council Directive on the protection of animals at the time of slaughter or killing.* 93/119/EC. No L 340/21.

Daly, C.C., Whittington, P.E. 1989. Investigations into the principal determinants of effective captive bolt stunning of sheep. *Research in Veterinary Science* **46:** 406-408.

Dennis, M.B.Jr., Dong, W.K., Weisbrod., K.A., Elchlepp, C.A. 1988. Use of captive bolt as a method of euthanasia in larger laboratory animal species. *Laboratory Animal Science* **38(4):** 459-462.

Derr, R.F. 1991. Pain perception in decapitated rat brain. *Life Sciences* **49(19):** 1399-1402.

Dodd, K. 1985. Humane euthanasia. I. Shooting a horse. *Irish Veterinary Journal* **39:** 150-151.

Eikmeier, H. 1961. Erfahningen mit einem neuen Präparat zur schmerzlosen Tötung von Kleintieren (T-61). *Die Blauen Hefter für den Tierarztliche,* H.L. 536-537.

Erhardt, Von W., Ring, Chr., Kraft, H., Schmid, A., Weinmann, H.M., Ebert, R., Schläger, B., Schindele, M., Heinze, R., Lomholt, N., Kallweit, E., Henning, M., Unshelm, J., Berner,

H., Blümel, G. 1989. Die CO_2-Betäubung von Schlachtschweinen aus anästhesiologischer Sicht. *Deutsche Tierärztliche Wochenschrift* **96:** 92-99.

Flight, W.G.F., Verheijen, F.J. 1993. The 'neck-cut' (spinal transection): not a humane way to slaughter eel, *Anguilla anguilla* (L.). *Aquaculture and Fish Management* **24:** 523-528.

Forslid, A., Ingvar, M., Rosen, I., Ingvar, D.H. 1986. Carbon dioxide narcosis: influence of short-term high concentration carbon dioxide inhalation on EEG and cortical evoked responses in the rat. *Acta Physiologica Scandinavica* **127:** 281-287.

Glen, J.B., Scott, W.N. 1973. Carbon dioxide euthanasia of cats. *British Veterinary Journal* **129:** 471-479.

Green, C.J. 1979. Euthanasia. Chapter 15. In: *Animal Anaesthesia.* Laboratory Animals Handbook, Laboratory Animals Ltd, London. pp 237-241.

Green, C.J. 1987. Euthanasia. Chapter 10. In: *Laboratory Animals: An Introduction for New Experimenters.* (ed A.A. Tuffery): 171-177. John Wiley & Sons Ltd, Chichester, UK.

Gregory, N.G., Wotton, S.B. 1986. Effect of slaughter on the spontaneous and evoked activity of the brain. *British Poultry Science* **27:** 195-205.

Gregory, N.G., Wotton, S.B. 1990. Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *The Veterinary Record* **126:** 570-572.
Gregory, N.G., Moss, B.W., Leeson, R.H. 1987. An assessment of carbon dioxide stunning in pigs. *The Veterinary Record* **121:** 517-518.

Hall, L.W. 1972. The anaesthesia and euthanasia of neonatal and juvenile dogs and cats. *The Veterinary Record* **90:** 303-306.

Hatch, R.C. 1982. Euthanatizing agents. In: *Veterinary Pharmacology and Therapeutics, 5th edition,* Ch 65. 1059-1064. (Eds Booth, N.H., McDonald, L.E.) Iowa State University Press, Ames.

Hellebrekers, L.J., Baumans, V., Bertens, A.P.M.G., Hartman, W. 1990. On the use of T61 for euthanasia of domestic and laboratory animals; an ethical evaluation. *Laboratory Animals* **24:** 200-204. In Dutch in: *Tijschrift voor Diergeneeskunde* **115(13):** 625-631.

Herin, R.A., Hall, P., Fitch, J.W. 1978. Nitrogen inhalation as a method of euthanasia in dogs. *American Journal of Veterinary Research* **39(6):** 989-991.

Herschler, R.C., Lawrence, J.R., Schiltz, R.A. 1981. Secobarbital/dibucaine combination as a euthanasia agent for dogs and cats. *Veterinary Medicine & Small Animal Clinician* **76(7):** 1009-1012.

Hewett, T.A., Kovacs, M.S., Artwohl, J.E., Bennett, B.T. 1993. A comparison of euthanasia methods in rats, using carbon dioxide in prefilled and fixed flow rate filled chambers. *Laboratory Animal Science* **43:** 579-582.

Hoenderken, R. 1983. Electrical and carbon dioxide stunning of pigs for slaughter. In: *Stunning of animals for slaughter.* (Ed G Eikelenboom): 59-63. Martinus Nijhoff Publishers, Boston.

Hoenderken, R., Lambooy, E., van Logtestijn, J.G., Sybesma, W. 1980. Dutch research on stunning of slaughter animals. *Proceedings of the 26th European meeting of Meat Research Workers.* Colorado Springs, USA.

Holson, R.R. 1992. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicology and Teratology* **14:** 253-257.

Holtzmann, M. 1991. Tierschutzgerechtes Töten von Versuchskaninchen mit Bolzenschußgeräten.(Killing of experimental rabbits with captive bolt guns according to animal welfare regulations). *Journal of Experimental Animal Science* **34:** 203-206.

Hornett, T.D., Haynes, A.P. 1984. Comparison of carbon dioxide/air mixture and nitrogen/air mixture for the euthanasia of rodents. Design of a system for inhalation euthanasia. *Animal Technology* **35(2):** 93-99.

Iwarsson, K, Rehbinder, C. 1993. A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and postmorten findings. *Scandinavian Journal of Laboratory Animal Science* **20:** 191-205.

Jaksch, W. 1981. Euthanasia of day-old male chicks in the poultry industry. *International Journal for the Study of Animal Problems* **2(4):** 203-213.

Kestin, S.C. 1993. *Pain and Stress in Fish.* A report prepared for the RSPCA, Horsham, UK.

Kestin, S.C., Wotton, S.B., Gregory, N.G. 1991. Effect of slaughter by removal from water on visual evoked activity in the brain and reflex movement of rainbow trout (*Oncorhynchus mykiss*). *The Veterinary Record* **128:** 443-446.

Klemm, W.R. 1964. Carbon dioxide anesthesia in cats. *American Journal of Veterinary Research* **25:** 1201-1205.

Lambooy, E., van Voorst, N. 1986. Electrocution of pigs infected with notifiable diseases. *The Veterinary Quarterly* **8(1):** 80-82.

Laursen, A.M. 1983. Choosing between CO_2 and electrical stunning of pigs. A preliminary examination of stress and ethics. In: *Stunning of animals for slaughter.* (Ed G Eikelenboom): 64-72. Martinus Nijhoff Publishers, Boston.

Longair, J., Finley, G.G., Laniel, M.A., McKay, C., Mould, K., Olfert, E.D., Rowsell, H., Preston, A. 1991. Guidelines for euthanasia of domestic animals by firearms. *Canadian Veterinary Journal* **32:** 724-726.

Lord, R. 1989. Use of ethanol for euthanasia of mice. *Australian Veterinary Journal* **66(8):** 268.

Lord, R. 1991. Humane Killing. *Nature* **350** (11 April): 456.

Lorden, J.F., Klemm, W.R. 1987. Letters on AVMA report about decapitation. *Laboratory Animal Science* **37(2):** 148-151.

Lucke, J.N. 1979. Euthanasia in small animals. *The Veterinary Record* **104(14):** 316-318.

Lumb, W.V. 1974. Euthanasia by noninhalant pharmacologic agents. *Journal of the American Veterinary Association* **165(9):** 851-852.

Lumb, W.V., Jones, E.W. 1984. *Veterinary Anesthesia. 2nd Edition.* Chapter 24: Euthanasia. Lea & Febiger, Philadelphia. pp 631-648.

Lumb, W.V., Doshi, K., Scott, R.J. 1978. A comparative study of T-61 and pentobarbital for euthanasia of dogs. *Journal of the American Veterinary Medical Association* **172(2):** 149-152.

Marshall, S., Milligan, A., Yates, R. 1994. Experimental techniques and anaesthesia in the rat and mouse. ANZCCART Facts sheet, *ANZCCART News* **7(1).** 4pp.

McFarland, W.N., Klontz, G.W. 1969. Anesthesia in fishes. *Federal Proceedings* **28(4):** 1535-1540.

Mikeska, J.A., Klemm, W.R. 1975. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Laboratory Animal Science* **25(2):** 175-179.

Ministry of Agriculture, Fisheries and Food, Welsh Office Agriculture Department. 1993. *Welfare of red meat animals at slaughter. Code of Practice.*

Moroji, T., Takahashi, K., Ogura, K., Toishi, T., Arai, S. 1977. Rapid microwave fixation of rat brain. *Journal of Microwave Power* **12(4):** 273-286.

Mullenax, C.H. Dougherty, R.W. 1963. Physiologic responses of swine to high concentrations of inhaled carbon dioxide. *American Journal of Veterinary Research* **24:** 329-333.

Noonan, D. 1994. The guinea pig (*Cavia porcellus*). ANZCCART Facts Sheet. *ANZCCART News* **7(3).** 8pp.

Olfert, E.D., Cross, B.M., McWilliam, A.A (Editors). 1993. *Guide to the Care and Use of Experimental Animals.* Volume 1. Canadian Council on Animal Care, Ontario, Canada.

Oliver, D.F. 1979. Euthanasia of horses. *The Veterinary Record* **105(10):** 224-225.

Phifer, C.B., Terry, L.M. 1986. Use of hypothermia for general anesthesia in preweanling rodents. *Physiology and Behaviour* **38:** 887-890.

Quin, A.H. 1963. Observations on a new euthanasia agent for small animals [T-61]. *Veterinary Medicine* **58:** 494-495.

Quine, J.P. 1980. Euthanasia by hypoxia using nitrogen. A review after 4 years of operation involving 20,500 animals. *Canadian Veterinary Journal* **21:** 320.

Quine, J.P., Buckingham, W., Strunin, L. 1988. Euthanasia of small animals with nitrogen; comparison with intravenous pentobarbital. *Canadian Veterinary Journal* **29:** 724-726.

Raj, M. Gregory, N.G. 1993. Time to loss of somatosensory evoked potentials and onset of changes in the spontaneous electroencephalogram of turkeys during gas stunning. *The Veterinary Record* **133:** 318-320.

Raj, M., Gregory, N.G. 1994. An evaluation of humane gas stunning methods for turkeys. *The Veterinary Record* **135:** 222-223.

Raj, M., Gregory, N.G., Wotton, S.B. 1990. Effect of carbon dioxide stunning on somatosensory evoked potentials in hens. *Research in Veterinary Science* **49:** 355-359.

Raj, M., Wotton, S.B., Gregory, N.G. 1992. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning with a carbon dioxide and argon mixture. *British Veterinary Journal* **148:** 147-156.

Reilly, J.S. (Editor). 1993. *Euthanasia of Animals Used for Scientific Purposes.* ANZCCART 1993. Glen Osmond. SA Australia.

Rowan, A.N. 1986. T-61 use in the euthanasia of domestic animals: a survey. In: *Advances in Animal Welfare Science.* 79-86. Martinus Nijhoff Publishers, Boston.

Rowsell, H.C. 1981. The present status of euthanasia by nonanaesthetic gases. *Canadian Veterinary Journal* **22(1):** 8.

Rowsell, H.C. 1990. In: *Experimental Animals in Biomedical Research*: Chapter 21. Euthanasia: acceptable and unacceptable methods of killing. Volume 1: 381-391.

Schatzmann, U, Leuenberger, T., Fuchs, P. 1991. Jet injection: the possibility of using a high pressure water jet for the stunning of slaughter pigs. *Fleischwirtschaft* **71:** 899-901.

Schatzmann, U, Howard, J., Pittino, J., Fuchs, P. 1994. Jet injection for the stunning of slaughter pigs. *Fleischwirtsch international* **1994(3)**: 18-19.

Smith, A.W., Houpt, K.A., Kitchell, R.L., Kohn, D.F., McDonald, L.E., Passaglia, M., Thurmon, J.C., Ames, E.R. 1986. 1986 Report of the AVMA Panel on Euthanasia. *Journal of the American Veterinary Medical Association* **188(3):** 252-268.

Summerfelt, R.C., Smith, L.S. 1990. Anesthesia, Surgery, and Related Techniques. In: C.B. Schreck & P.B. Moyle (eds) *Methods for Fish Biology.* American Fisheries Society, Bethesda, MD (USA). pp 213-272.

Tidswell, S.J., Blackmore, D.K., Newhook, J.C. 1987. Slaughter methods: electroencephalic (EEG) studies on spinal cord section, decapitation and gross trauma of the brain in lambs. *New Zealand Veterinary Journal* **35:** 46-49.

Universities Federation for Animal Welfare. 1989. *Guidelines on the care of laboratory animals and their use for scientific purposes. II. Pain, analgesia and anaesthesia.* UFAW, Herts, UK.

UFAW/WSPA. 1989. *Euthanasia of Amphibians and Reptiles.* Report of a Joint Universities Federation for Animal Welfare/World Society for the Protection of Animals Working Party. UFAW, Herts, UK.

Vanderwolf, C.H., Buzsaki, G., Cain, D.P., Cooley, R.K., Robertson, B. 1988. Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Research* **451:** 340-344.

Verheijen, F.J., Flight, W.F.G. 1995. Commercial slaughter of eel: experimental tests show methods are unacceptable. *Ecology of Freshwater Fish* (submitted).

Von Cranach, J., Gassmann-Langmoen, A.B., Schatzmann, U. 1991a. *Euthanasie bei Labornagetieren.* Anaesthesieabteilung der Kliniken, Veterinärmedizinische Fakultät der Universität Bern, Switzerland.

Von Cranach, J., Gassmann-Langmoen, A.B., Schatzmann, U. 1991b. *Euthanasie chez les rongeurs de laboratoires.* Resume. Source de référence pour toute l'étude: Office fédéral vétérinaire, Schwarzenburgstr 161, 3097 Liebefeld.

Wallach, M.B., Peterson, K.E., Richards, R.K. 1981. Electrophysiologic studies of a combination of secobarbital and dibucaine for euthanasia of dogs. *American Journal of Veterinary Research* **42(5):** 850-853.

Wallgren, H., Barry III, H. 1970. *Actions of Alcohol, Vol I, Biochemical, Physiological and Psychological Aspects.* Elsevier, Amsterdam.

Warrington, R. 1974. Electrical stunning, a review of the literature. *The Veterinary Bulletin.* **44(10):** 617-635.

Warwick, C. 1986. Euthanasia of reptiles - decapitation: an inhumane method of slaughter for the class "Reptilia". *Canadian Veterinary Journal* **27:** 34.

Warwick, C. 1990. Crocodilian slaughter methods, with special reference to spinal cord severance. *Texas Journal of Science* **42(2):**191-198.

Weisbrod, K.S., Dennis, M.D., Dong, W.K., Ramos, B., Russell, R.G., Bower, A. 1984. Physical methods of euthanasia for rabbits. *Laboratory Animal Science* **34:** 516.

Zeller, von W., Mettler, D., Schatzmann, U. 1989. Untersuchungen zzur tierschutzgerechten Betäubung des Schlachtgeflügels mit Mikrowellen (2550 MHz). *Deutsche Tierartzliche Wochenschrift* **96:** 285-332.

Zimmermann, M. 1986. Behavioural investigations in animals. In: *Assessing pain in farm animals.* Eds Duncan I.J.H., Moloney, V. Commission of the European Communities, Luxembourg. pp 16-27.

Zutphen, L.F.M. van, Baumans, V., Beynen, A.C. (Eds) 1993. *Principles of Laboratory Animal Science.* Elsevier, Amsterdam.

Zwart, P., Vries, H.R.de, Cooper, J.E. 1989. Humane methods of killing fish, amphibians and birds. *Tijdschrift voor Diergeneeskunde* 114(10):557-565.

5. FURTHER READING

GENERAL

Adolph, E.F. 1969. Regulations during survival without oxygen in infant mammals. *Respiration Physiology* **7:** 356-368.

Allert, J.A., Adams, H.R. 1987. Pharmacologic considerations in selection of tranquillizers, sedatives and muscle relaxant drugs used in inducing animal restraint. *Journal of the American Veterinary Medical Association* **191(10):** 1241-1244.

Anon. 1988. Reducing pain can magnify stress. *The Veterinary Record* November 26: 559-560.

Anon. 1990. Barbituraatvergiftiging bij dierentuindieren. *Tijdschrift voor Diergeneeskunde* **115(5):** 241-242.

Bancroft, R.W., Dunn, J.E.II. 1965. Experimental animal decompressions to a near vacuum environment. *Aerospace Medicine* **36:** 720-725.

Barr, F.M. 1987. Waste anaesthetic gas exposure in veterinary surgeries: a need for scavenging systems. *New Zealand Veterinary Journal* **35:** 68-71.

Battisti, G.A. 1984. CO2 euthanasia: Letter to the Editor/ Editor's note. *Laboratory Animal Science* **34(3):** 228.

Baumans, V., Herck, H. van,. Bartels, H., Bertens, A.P.M.G., Hoenderken, R., Schlingmann, F. 1991. Methods of euthanasia used for laboratory animals in the Netherlands. Abstract voordracht GV-SOLAS (Lubeck), 9-13 September 1991.

Benson, G.J., Thurmon, J.C. 1987. Species difference as a consideration in alleviation of animal pain and distress. *Journal of the American Veterinary Medical Association* **191(10):**1227-1230.

Billings, Ch. E. 1985. Effects of physical agents. In: *Sodeman's Pathologic Physiology,* (Ed. Sodeman, W.) 7th Edition. p 1059. W.B. Saunders Company.

Booth, N.H. 1982. Inhalant anesthetics. In: *Veterinary pharmacology and therapeutics. 5th edition.* Ch 12. (Booth, N.H., McDonald, L.E.eds): 175-202. Iowa State University Press, Ames, Iowa.

Borodkin, S., Macy, L., Thompson, G., Schmits, R. 1977. Stable nonaqueous pentobarbital sodium for use in laboratory animals. *Journal of Pharmaceutical Sciences* **66(5):** 693-695.

Brewer, N.R. 1982. The history of euthanasia. *Lab Animal* **11(4):** 17,19.

British Veterinary Assocation. 1988. Killing with Kindness. *Proceedings of the BVA Animal Welfare Foundations Sixth Symposium.*

Broom, D.M. 1988. The scientific assessment of animal welfare. *Applied Animal Behaviour Science* **20:** 5-19.

Broom, D.M. 1991. Animal welfare: concepts and measurement. *Journal of Animal Science* **69:** 4167-4175.

Bundesamt für Veterinärwesen. 1984. *Blutentnahme bei Labornagetieren, Kaninchen und Katzen.* Informationsblatt Tierschutz, CH-3097 Liebefeld-Bern 15.2.1984.

Bundesamt für Veterinärwesen. 1985. *Töten von Versuchstieren (Hund, Katze, Labornagetiere, Vögel, Amphibien und Reptilien, Fische).* Informationsblatt Tierschutz, CH-3097 Liebefeld-Bern 25.2.1985.

Bustad, L.K. 1982. An educator's approach to euthanasia. *Lab Animal* **11:** 37-41.

Butler, M.M., Griffey, S.M., Clubb, F.J., Gerrity, L.W., Campbell, W.B. 1990. The effect of euthanasia technique on vascular arachidonic acid metabolism and vascular and intestinal smooth muscle contractility. *Laboratory Animal Science* **40(3):**277-283.

Canadian Council on Animal Care. 1988. AVMA 1986 Report on Euthanasia. Euthanasia methods - cervical dislocation and decapitation with guillotine. Press release January 1988.

Carstensen, J. 1978. The need for better specification of the animal model in the test situation. In: *Papers and Abstracts/Symposium on Design of Experiments and Quality of Laboratory Animals, Kuopio 5-7 October 1978.* (ed T.Nevalainen, K Pelkonen).

Cavaliere, U., Andreano, C., Raducci, G., Andreoni, C., Iacovella, A. 1982. Intossicazione da T-61 (TanaxR). *Min. Anest.* **48:** 861-863.

Chatrian, G.E. 1980. Electrophysiologic evaluation of brain death: a critical appraisal. In: *Electrodiagnosis in Clinical Neurology.* 525-580. (Ed Aminoff, M.J.) Churchill Livingstone, New York, NY.

Conway, C.M. 1965. The anaesthetic ethers. *British Journal of Anaesthesia* **37:** 644-653.

Cuadros, G.R., Ocampo, C.L., Sumano, L.H. 1982. Estudio economico y comparativo de la eficacia del pentobarbital y T-61 como eutanasico en perros. (Comparative and economical study of pentobarbital and T-61 as an euthanasia agent). Short communication. *Veterinaria Mexico* **13(3):** 155-156.

Davison, M.H.A. 1965. Chloroform. *British Journal of Anaesthesia* **37:** 655-660.

Deutsche Tierärzteschaft e.V. 1987. Tötung von Tieren durch das Präparat T-61. *Deutsches Tierärzteblatt* **8:** 556.

Drawer, K., Ennulat, K.J. (Eds) 1977. *Tierschutzgerechtes Töten von Wirbeltieren Tierschutzpraxis,* Gustav Fischer Verlag Stuttgart, New York, 293-300.

Edgson, F.A., Payne, J.M. 1967. The dangers of poisoning domestic pets with meat from animals subjected to barbiturate euthanasia. *The Veterinary Record* **80:** 364.

Eikmeier, H. 1962. Experience with a new preparation for painless destruction of small animals (T-61). *Die Blauen Hefte Tieraerztl* **5:** 22-23.

Ewbank, R. 1983. Is CO_2 euthanasia humane? *Nature* **305:** 268.

Faupel, R.P., Seitz, H.J., Tarnowski, W., Thiemann, V., Weiss, C. 1972. The problem of tissue sampling from experimental animals with respect to freezing technique, anoxia, stress and narcosis. *Archiv. Biochem. Biophys.* **148:** 509-522.

Feldman, D.B., Gupta, B.N. 1976. Histopathologic changes in laboratory animals resulting from various methods of euthanasia. *Laboratory Animal Science* **26(2):** 218-221.

Flecknell, P.A., 1987. *Laboratory Animal Anaesthesia.* Academic Press, London.

Freed, D.L.J., 1983. CO_2 euthanasia. *Nature* **304(5926)**:482

Gärtner, K., Messow, C. 1975. Tierschutzgerechtes Töten von Versuchstieren, Töten von Wirbeltieren aus der Sicht des Tierschutzgesetzes vom 24 Juli 1972. *Archiv für tierärztliche Fortbildung* **3:** 85-88

Gärtner, K., Ruppert, E. 1973. Methoden zur tierschutzgerechten Tötung von Tieren. Uebersetzung von der 2. Aufl, von: UFAW: *Humane Killing of Animals* (1968), 8 Hamilton Close, South Mimms, Potters Bar, Herts, UK.

Gelfan. S., Nims, L.F., Livingston, R.B. 1950. Explosive decompression at high altitude. *American Journal of Physiology* **162:** 37-53.

Glass, H.G., Snyder, F.F., Webster, E. 1944, the rate of decline of resistance to anoxia of rabbits, dogs and guinea pigs to the onset of viability to adult life. *American Journal of Physiology* **140:** 609-615.

Goldbaum, L.R., Orellano, T., Dergal, E. 1976. Mechanisms of the toxic action of carbon monoxide. *Annals of Clinical Laboratory Science* **6:** 372-376.

Grätz, H. 1981. CO2-Betäubung und -Anlagen. *Fleischwirtschaft* **61(4):** 513-516.

Green, C.J., Knight. J., Precious, S., *et al.* 1981. Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Laboratory Animals* **15:** 163-170.

Gylstorff, I. 1976. Tierschutzgerechte Tötung von Wirbeltieren. *Archiv für tierärztliche Fortbildung* **3:** 52.

Hanson, M.A. 1987. Euthanasia of embryos and foetuses. In: *Euthanasia of Unwanted, Injured or Diseased Animals or for Educational or Scientific Purposes:* 15-18. UFAW, Herts.
Heinecke, H. 1989. *Angewandte Versuchstierkunde.* p254-257. Gustav Fischer Verlaag, Stuttgart.

Hilbrich, P. 1976. Tierschutzgerechte Tötung von Wirbeltieren. *Archiv für tierärztliche Fortbildung* **3:** 25.

Hughes, H.C. 1976. Euthanasia of laboratory animals. In: *Handbook of Laboratory Animal Science. Vol III.* (Eds Melby, Altman): 553-559. CRC Press, Cleveland.

Hughes, H.H., Warnick, C.C. 1986. Euthanasia. A comparison of the 1978 and 1986 AVMA Panel Reports. *Lab Animal* Nov/Dec: 30-32.

Iwarsson, K., Rehbinder, C., Warren, A., Weihe, W. 1985. Euthanasia in laboratory animals. *Zeitschrift für Versuchstierkunde* **27(2):** 60-61.

Keller, G.L. 1982. Physical euthanasia methods. *Lab Animal* **11(4):** 20-26.

Kingston, R.L., Saxena, K. 1979. Intentional poisoning by injection of veterinary euthanasia drug. *Clinical Toxicology* **15(4):** 492.

Kuepper, G. 1964. T-61 used in large animals. *Die Blauen Hefte Tieraerztl* **8:** 32-33.

Kurasawa, T., Tamura, H., Shikita, J., Maejima, J., Takagaki, Y., Nakagawa, M., Suziki, K. 1981. Use of CO2 euthanasia cabinet for experimental animals. *Jikken Dobutso* **30(3):** 317-321.

Lambooy, E. 1984. Bedwelmen en doden van dieren. Voordracht gehouden tijdens de 21e Biotechnische dag, 12 November 1983. Bilthoven: *Biotechniek* **23(6):** 81-83.

Lambooy, E. 1984. Euthanasie van huisdieren. *IVO rapport B-251:* (Zeist, Instituut voor Veeteeltkundig Onderzoek "Schoonoord"): 1-36, September 1984.

Lumb, W.V. 1985. *Veterinary anesthesia.* Lea & Febiger, Philadelphia.

Lumb, W.V., Jones, E.W. 1984. *Veterinary Anaesthesia:* Chapter 24, Euthanasia. Lea and Febiger, Philadelphia, 2nd Edition.

Lumb, W.V., Moreland, A.F. 1982. Chemical methods for euthanasia (veterinary medicine). *Lab Animal* **11(4):** 29,33,35.

MacDonald, L.E., Booth, N.H., Lumb, W.V., Redding, R.W., Sawyer, D.C., Stevenson, D.C., Wass, W.M. 1978. Report of the AVMA Panel on Euthanasia. *Journal of the American Veterinary Medical Association* **173(1):** 59-72.

Manser, C.E. 1992. *The Assessment of Stress in Laboratory Animals.* Published by the RSPCA, Horsham, UK.

Martinic, G. 1990. The animal technicians' role in the euthanasia of laboratory rodents. *Animal Technology* **41(2):** 145-150.

Medina, M.A., Deam, A.P., Stavinoha, W.B. 1980. Inactivation of brain tissue by microwave irradiation. In: *Cerebral Metabolism and Normal Function.* Chapter 8. (Eds Passoneau, R.A. *et al*). The Williams & Wilkins Co., Baltimore.

Messow, C. Tötung von Versuchstieren: Tierschutzrelevanz und die Bedautung des Tötungsvorganges für die postmortale morphologische und funktionelle Befunderhebung. In: K Gärtner: *Qualitätskriterien der Versuchstierforschung* pp 239-264. VHC - Verlag Weinheim.

Messow, C., Bungenstock, H., Korn, W.D., Hackbarth, H. 1987. Morphologische Tötungseffekte am Herzen. *Tierärztliche Umschau.* **42:** 803-896.

National Research Council. 1991. *Education and training in the care and use of laboratory animals. A guide to developing institutional programs.* National Academy Press, Washington, D.C.

Owens, C.E., Davis, R., Smith, B. 1981. The psychology of euthanizing animals - the emotional components. *International Journal of the Study of Animals Problems* **2(1):** 19-26.

Paton, W.D.M. 1983. Is CO_2 euthanasia humane? *Nature* **305:** 268.

Poole, T.B. (ed). 1989. *The UFAW handbook of the care and management of laboratory animals.* Sixth edition. Longman Scientific & Technical, Harlow, UK.

Port, C.D., Garvin, P.J., Garnote, Ch.E., Sawyer, D.A. 1978. Pathologic changes induced by euthanasia agent (T61). *Lab Animal Science* **28:** 448-450.

Rapport, M.B. 1967. On methods of killing laboratory animals. *Lab-Delo* **6:** 363-365.

Schatzmann, U., von Cranach, J, Gassmann, A.B. 1990. *Anästhesie, Analgesie und Euthanasie bei Labortieren.* Hauptreferat an der wissenschaftlichen Tagung der Schweizer Gesellschaft für Versuchtierkunde, Bern, 29.3.1990.

Schulze, W. 1987. Die Fähigkeit zur Euthanasie bei allen Haustierarten gehört zum Können des Tierarztes. *Tierärztliche Praxis* **15:** 123.

Schwink, K., Egger, E.L. 1980. Methods of euthanasia. *Iowa State Veterinarian* **42(2):** 78-81.

Seamer, J. 1992. Transport of live animals for slaughter. *Veterinary Record* **130(2):** 38.

Universities Federation for Animal Welfare. 1987. *Euthanasia of unwanted, injured or diseased animals or for educational or scientific purposes.*

Universities Federation for Animal Welfare. 1988. *Humane killing of animals.* Preprint of 4th Edition.

Veetch, R.L., Harris, R.L., Veloso, D., Veech, E.H. 1973. Freeze-blowing: a new technique for the study of brain *in vivo*. *Journal of Neurochemistry* **20:** 183.

Warren, R.G. 1983. *Small Animal Anaesthesia.* Mosby, St. Louis, Missouri.
World Society for the Protection of Animals (Scientific Advisory Panel). *Pain Assessment and Euthanasia in Ectotherms.*

Wright, M. 1982. Pharmacological effects of ketamine and its use in veterinary medicine. *Journal of the American Veterinary Medicine Association* **180:** 1462-1471.

FISH

Amend, D.F., Goven, B.A., Elliot, D.G. 1982. Etomidate: effective dosages for a new fish anaesthetic. *Transactions of the American Fish Society* **111:** 337-341.

Anders, J.J., Ostrow, M.E. 1986. Goldfish in research: use and maintenance. *Laboratory Animals:* 33-41.

Arena, P.C., Richardson, K.C. 1990. The relief of pain in cold-blooded vertebrates. *ACCART News* **3(1):** 1-4.

Azam, K., Strachan, N.J.C., Mackie, I.M., Smith, J., Nesvadba, P. 1990. Effect of slaughter method on the progress of rigor of rainbow trout (*Salmo gairdneri*) as measured by an image processing system. *International Journal of Food Science and Technology* **25:** 477-482.

Bell, G.R. A guide to the properties, characteristics and uses of some general anaesthetics for fish. *Fisheries Research Board of Canada Bulletin* **148:** (2nd edition revised)

Bernoth, E.M., Wormuth, H.J. 1990. Tierschutzaspekte bei der Tötung von Fischen. *Deutsche Tierärztliche Wochenschrift.* **97:** 154-157.

Blasiola, G.C. 1975. Quinaldine sulphate, a new anaesthetic formulation for tropical marine fishes. *Journal of Fish Biology* **10:** 113-119.

Boggers, T.S.Jr., Heaton, E.K., Shewfelt, A.L., Parvin, D.W. 1973. Technique for stunning channel catfish and their effects on product quality. *Journal of Food Science* **38:** 1190-1193.

Booke, H.E., Hollender, B., Lutterbie, G. 1978. Sodium bicarbonate, an inexpensive fish anaesthetic for field use. *Progressive Fish-Culturist* **40:** 11-13.

Bové, F.J. 1962. MS-222 Sandoz - the anaesthetic of choice for fish and other cold-blooded organisms. *Sandoz News* **3:** 12.

Bourne, P.K. 1984. The use of MS-222 (tricaine methanesulphonate) as an anaesthetic for routine blood sampling in three species of marine teleosts. *Aquaculture* **36:** 313-321.

Brambell, F.W.R. (Chairman) 1965. *Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems.* Cand 2836. London HMSO.

Curran, C.A., Poulter, R.G., Bruton, A., Jones, N.S.D. 1986. Cold shock reactions in iced tropical fish. *Journal of Food Technology* **21:** 289-299.

Dawson, V.K., Gilderhus, P.A. 1979. Ethyl-*p*-aminobenzoate (benzocaine): efficacy as an anaesthetic for five species of freshwater fish. *U.S. Fish and Wildlife Service Investigations in Fish Control 87.*

Escoubet, P. 1982. Utilisation et efficacite du metomidate comme anesthesiant sur dix especes de poissons mediterraneans. *Sci. Vet. Med. Comp.* **84(6):** 356-362.

Ferreira, J.T., Smit, G.L., Schoonbee, H.J., Holzapfel, C.W. 1979. Comparison of anaesthetic potency of benzocaine hydrochloride and MS-222 in two freshwater fish species. *Progressive Fish-Culturist* **41:**161-163.

Ferreira, J.T., Schoonbee, H.J., Smit, G.L. 1984. The anaesthetic potency of benzocaine hydrochloride in three freshwater fish species. *South African Journal of Zoology* **19:** 46-50.

Ferreira, J.T., Schoonbee, H.J., Smit, G.L. 1984. The uptake of the anaesthetic benzocaine hydrochloride by the gills and skin of three freshwater fish species. *Journal of Fish Biology* **25:** 35-41.

Gilderhus, P.A. 1990. Benzocaine as a fish anaesthetic: efficacy and safety for spawning-phase salmon. *The Progressive Fish Culturist* **52:** 189-191.

Gilderhus, P.A., Marking, L.L. 1987. Comparative efficacy of 16 anesthetic chemicals on rainbow trout. *North American Journal of Fisheries Management* **7:** 288-292.

Gilderhus, P.A., Berger, B.L., Sills, J.B., Harman, P.D. 1973. The efficacy of quinaldine sulfate as an anesthetic for freshwater fish. *U.S. Fish and Wildlife Service Investigations in Fish Control 49.*

Gilderhus, P.A. Berger, B.L., Sills, J.B., Harman, P.D. 1973. The efficacy of quinaldine sulfate:MS-222 mixtures for the anesthetization of freshwater fish. *U.S. Fish and Wildlife Service Investigations in Fish Control 54.*

Gooding, J.M., Corssen, M. 1976. Etomidate: an ultrashort-acting nonbarbiturate agent for anesthesia induction. *Anesthesia and Analgesia* **55:** 286-289.

Hartley, W.G. 1977. The use of electricity for anesthetizing fish. *Journal of Fish Biology* **11:** 377-378.

Hong, T.L., Wen, C.T. Studies on the packaging of freshwater aquarium fish. *Aquarama* **1:** 45-49.

Houston, A.H., Woods, R.J. 1976. Influence of temperature upon tricaine methanesulphonate uptake and induction of anesthesia in rainbow trout, *Salmo gairdneri. Comparative Biochemistry and Physiology C, Comparative Pharmacology* **54:** 1-6.

Johansson, N. 1978. Anesthetics of fish. *Salmon Research Institute, Report 5,* Sundsvall, Sweden.

Jolly, D.W., Mawdesley-Thomas, L.E., Bucke, D. 1972. Anesthesia of fish. *Veterinary Record* **91(18):** 424-426.

Klontz, G.W. 1964. Anesthesia of fishes. Pages 350-374 In: D.C.Sawyer (ed) *Proceedings of the symposium on experimental animal anesthesiology.* U.S. Air Force School of Aerospace Medicine, Aerospace Medical Division, Brooks Air Force Base, Texas.

Klontz, G.W., Smith, L.S. 1968. Methods of using fish as biological research subjects. *Methods of Animal Experimentation* **3:** 383-385.

Kreiberg, H., Powell, J. 1991. Metomidate sedation reduces handling stress in Chinook salmon. *World Aquaculture* **22:** 58-59.

Limsuwan, C.J., Grizzle, J.M., Plumb, J.A. 1983. Etomidate as an anesthetic for fish: its toxicity and efficacy. *Transactions of the American Fisheries Society* **112:** 544-550.

Locke, D.O. 1969. Quinaldine as an anesthetic for brook trout, lake trout, and Atlantic salmon. *U.S. Fish and Wildlife Service Investigations in Fish Control 24.*

Mischra, B.K., Kumar, D., Mischra, R. 1983. Observations on the use of carbonic acid anaesthesia in fish fry transport. *Aquaculture* **32:** 405-408.

Muench, B. 1958. Quinaldine, a new anaesthetic for fish. *Progressive Fish Culturist* **20:** 42.

Ohr, E.A. 1976. Tricaine methanesulphonate - I. pH and its effect on anaesthetic potency. *Comparative Biochemistry and Physiology C, Comparative Pharmacology* **54:** 13-17.

Orsi, J., Short, J.W. 1987. Modifications in electrical anesthesia for salmonids. *Progressive Fish-Culturist* **49:** 144-146.

Pickering, A.D. (ed). 1981. *Stress in Fish.* Academic Press.

Plumb, J.A., Schwedler, T.E., Limsuwan, C. 1983. Experimental anesthesia of 3 species of fish with etomidate. *The Progressive Fish-Culturist* **45(1):** 31-33.

Post, G. 1979. Carbonic acid anesthesia for aquatic organisms. *Progressive Fish-Culturist* **45:** 30-31.

Randall, D.J., Hoar, W.S. 1971. Special techniques. Pages 511-528 In: W.S. Hoar and D J Randall (eds) *Fish Physiology,* Vol 6. Academic Press, New York.

Ross, L.G., Ross, B. 1984. *Anaesthetic and sedative techniques for fish.* Institute of Aquaculture, University of Stirling, Stirling, Scotland.

Sado, E.K. 1985. Influence of the anesthetic quinaldine on some tilapia. *Aquaculture* **46:** 55-62.

Schaeffer, D.O., Kleinow, K.M., Krulisch, L. (eds). 1992. *The Care and Use of Amphibians, Reptiles and Fish in Research.* Proceedings from a SCAW/LSUSVM-sponsored conference, April 8-9 1991, Louisiana, Scientists Center for Animal Welfare 1992.

Schoettger, R.A., Steuke, E.W. 1972. *Anesthetization of fish.* U.S. Patent 3,644,625 (February 22, 1972)

Schulz, D. 1984. Forschungsvorhaben: Tierschutzgerechtes Töten von Fischen/Aalen (Abschlußbericht). *Der Fischwirt* **33:** 11-13, 17-19.

Scottish Salmon Growers Association: *Guidelines for Humane Slaughter of Farmed Atlantic Salmon.*

Smit, G.L., Schoonbee, H.J., Barham, W.T. 1977. Some effects of the anesthetic MS-222 on freshwater fish. *South African Journal of Science* **73:** 351-352.

Stuart, N.C. 1981. Anaesthetics in fish. *Journal of Small Animal Practice* **22:** 377-384.

Verbeek, F. 1984. Why anaesthetize fish? *The Catfish Association of Great Britain* **43(3):** 8-13.

Wood. E.M. 1956. Urethane as a carcinogen. *Progressive Fish Culturist* **18:** 135.

AMPHIBIANS

Arena, P.C., Richardson, K.C. 1990. The relief of pain in cold-blooded vertebrates. *ACCART News* **3(1):** 1-4.

Cooper, J.E. 1987. Euthanasia of captive reptiles and amphibians: report of UFAW/WSPA working party. 34-39.

Cooper, J.E., Ewbank, R., Platt, C., Warwick, C. 1986. Euthanasia of reptiles and amphibians (letter). *The Veterinary Record* November 8: 484.

Kaplan, H.M. 1969. Anesthesia in amphibians and reptiles. *Federation Proceedings* **28(4): 1541.**

Schaeffer, D.O., Kleinow, K.M., Krulisch, L. (eds). 1992. *The Care and Use of Amphibians, Reptiles and Fish in Research.* Proceedings from a SCAW/LSUSVM-sponsored conference, April 8-9 1991, Louisiana, Scientists Center for Animal Welfare 1992.

REPTILES

Arena, P.C., Richardson, K.C. 1990. The relief of pain in cold-blooded vertebrates. *ACCART News* **3(1):** 1-4.

Calderwood, H.W. 1971. Anesthesia for reptiles. *Journal of the American Veterinary Medical Association* **159:** 1618-1625.

Cooper, J.E. 1987. Euthanasia of captive reptiles and amphibians. In: *Euthanasia of Unwanted, Injured or Diseased Animals or for Educational or Scientific Purposes:* 34-39. UFAW, Herts.

Cooper, J.E., Ewbank, R. Rosenberg, M.E. 1984. Euthanasia of tortoises. *The Veterinary Record* **114(24):** 635.

Cooper, J.E., Ewbank, R., Platt, C., Warwick, C. 1986. Euthanasia of reptiles and amphibians (letter). *The Veterinary Record* **119:** 484.

Godfrey, C. 1985. Euthanasia of tortoises. *The Veterinary Record* **116(11):** 304.

Rosenberg, M.E. 1978. Thermal relations of nervous conduction in the tortoise. *Comparative Biochemistry and Physiology* **60A**: 57-63.

Schaeffer, D.O., Kleinow, K.M., Krulisch, L. (eds). 1992. *The Care and Use of Amphibians, Reptiles and Fish in Research.* Proceedings from a SCAW/LSUSVM-sponsored conference, April 8-9 1991, Louisiana, Scientists Center for Animal Welfare 1992.

Warwick, C. 1985. Euthanasia of reptiles (letter). *New Zealand Veterinary Journal* **34**:12.

Warwick, C. 1985. Euthanasia of reptiles. *Journal of the American Veterinary Medical Association* **187(11):** 1081.

Warwick, C. 1985. Euthanasia of tortoises. *The Veterinary Record* **116:** 82.

Warwick, C. (in press). Observations on collection, handling, storage and slaughter of western diamondback rattlesnakes *(Crotalus atrox). Herpetopathologia,* in press.

BIRDS

Cooper, D.M. 1967. Destruction of birds with carbon dioxide. *The Veterinary Record* **81:** 444-445.

Ewbank, R. 1987. Euthanasia of day-old chicks. In: *Euthanasia of Unwanted, Injured or Diseased Animals or for Educational or Scientific Purposes:* 11-14. UFAW, Herts.

Fedde, M.R. 1978. Drugs used for avian anesthesia: a review. *Poultry Science* **57:** 1376-1399.

Fiedler, H.H. 1976. Die Tötung aussortierter Eintagshähnchen - eine Literaturstudie unter tierschützerischem Aspekt. *Archiv für Geflügelkunde* **40:** 56-60.

Gerriets, E. 1969. Schlachten und Töten von Hühnern. *Berlin München Tierärztliche Wochenschrift.* **82:** 63-65.

Gregory. N.G., Wotton, S.B. 1991. Euthanasia of chickens (letter). *The Veterinary Record* **128:** 532.

Hilbrich, P., von Mickwitz, G. 1977. Tierschutzgerechte Töten aussortierter Eintagshähnchen und nicht schlupffähiger Küken im Brutei. *Berlin München Tierärztliche Wochenschrift.* **90:** 355-358.

Jaksch, W. 1980. Betrachtungen zur elektrischen Betäubung des Geflügels bei der Schlachtung. *Wiener Tierärztliche Monatsschrift* **67:** 77-86, 321-337.

Jaksch, W., Mitterlehner, A. 1979. Euthanasie von Eintagskucken in der Massentierhaltung. (Euthanasia of day-old (male) chicks in large-scale poultry production). *Wiener Tierärztliche Monatsschrift* **66(2):** 37-46, 145-149.

Kaltofen, G.S., Houben, G.J.T. 1973. Het doden van eendagskuikens. *De Pluimveehouderij* **3:** Spelderholt Mededeling 198.

Koktula. A.W., Dewniak, E.E., Davies. L.L. 1961. Experimentation with in-line carbon dioxide immobilization of chickens prior to slaughter. *Poultry Science* **40:** 213-216.

Ministry of Agriculture, Fisheries and Food, Welsh Office. 1987. *Disposal of unwanted day-old chicks, turkey poults and hatchery waste.* ADAS P568.

Trapp, A.L., Taylor, R.F. 1986. Methods of euthanasia in poultry and food-producing animals. *Veterinary Clinics of North America: Food Animal Practice* **2(1):** 31-41.

Woolley, S.C. Gentle, M.J. 1988. Physiological and behavioural responses of the domestic hen to hypoxia. *Research in Veterinary Science* **45:** 377-382.

RODENTS

Anderson L.C. 1987. Guinea pig husbandry and medicine. *Veterinary Clinics of North America: Small Animal Practice* **17(5):** 1045-1060.

Applebee, K.A., Cooper, J.E. An anaesthestic or euthanasia chamber for small animals. *Animal Technology* **40:(1)** 39-43.

Baldwin, D.M., Colombo, J.A., Sawyer, C.H. 1974. Plasma prolactin, LH and corticosterone in rats exposed to a novel environment. *American Journal of Physiology* **226:** 1366-1368.

Battisti, G.A. 1984. Euthanasia of small laboratory animals. *Laboratory Animal Science* **34(3):** 228.

Bauer, P., Forster, H., Fortmeyer, H.P. 1983. Einflusse von Exzitationszeit und Tötungsart auf die Stressreaktion von Ratten. *Zeitschrift für Versuchstierkunde* **25:** 149.

Bogaard, A.E.J.M.van den, Dam, E.van, Weekers, F.H. 1985. Het gebruik van een koolzuurgas euthanasie apparaat voor ratten. *Biotechniek* **24(3):** 34-38.

Britt, D.P. 1987. Humaneness of carbon dioxide as an agent of euthanasia for laboratory rodents. In: *Euthanasia of Unwanted, Injured or Diseased Animals or for Educational or Scientific Purposes:* 19-31. UFAW, Herts.

Carney, J.A., Walker, B.L. 1973. Mode of killing and plasma corticosterone concentrations in the rat. *Laboratory Animal Science* **23(3):**675-676.

Cate, C.C. 1969. A successful method for exsanguinating unanesthetised mice. *Laboratory Animal Care* **19(2):** 256-258.

Clifford, D.H., Cruse, E., Boatfield, M.P. 1985. Euthanasia by CO_2 and halothane alone and in combination in rats. *Laboratory Animal Science* **35(5):** 540.

Cooke, S.W. 1987. Anaesthesia of guinea pigs and reversing pneumothorax. *The Veterinary Record* March 28: 309.

Fawell, J.K., Thomson, C., Cooke, L. 1972. Respiratory artefact produced by carbon dioxide and pentobarbitone sodium euthanasia in rats. *Laboratory Animals* **6:** 321-326.

Geilen, H. 1984. Lichtmikroskopische Untersuchungen zum Einfluss von drei Tötungsarten (Dekapitation, Nembutal Überdosierung, Entbluten in Nembutal Narkose) auf die Morphologie der Nebenniere und der Schilddrüse bei Ratten. Inaugural Dissertation Tierärtzliche Hochschule Hannover.

Jaax, G.P. 1988. A mobile CO_2 inhalation chamber for small laboratory rodents. *Lab Animals* **17:** 26-27.

Johnson, I.T. 1976. Alternative methods of animal sacrifice: the effects on intestinal function *in vitro. Experentia* (Basel) **32:** 347-348.

Lord, R., Jones, G.L., Spencer, L. 1991. Ethanol euthanasia and its effect on the binding of antibody generated against an immunogenic peptide construct. *Research in Veterinary Science* **51:** 164-168.

Mayevsky, A. 1978. Ischaemia in the brain: the effects of carotid artery ligation and decapitation on the energy state of the awake and anesthetized rat. *Brain Research* **140:** 217-230.

Messow, C., Fiolna, A. Kaup, F,-J., Hackbarth, H. 1987. Morphologische Auswirkungen an der Lunge bei Ratten nach der Tötung. *Zeitschrift für Versuchstierkunde.* **29:** 219-227.

Modak, A.T., Weintraub, S.T., McCoy, T.H., Stavinoha, W.B. 1976. Use of 300 ms microwave irradiation for enzyme inactivation: a study of effects of sodium pentobarbital on acetylcholine concentration in mouse brain regions. *J. Pharmacol. Exp. Ther.* **197:** 245-252.

Mörch, E.T., Jobgen, E.A. 1959. Fluothane compared to chloroform and ether in mice. *Acta anaesth. Scandinav.* **3:** 173-179.

Scott, F.W., Trick, D. 1982. Variation of rat serum biochemical values following decapitation or anaesthesia with ether, halothane of Innovar-Vet: rapid Innovar-Vet-induced hyperuricemia and hyperglycaemia. *Clin. Exp* **31:** 514-519.

Schlingmann, H. 1988. Tekening van de Euthanasierotor. (DUPHAR - WEESP).

Swaab, D.F. 1971. Pitfalls in the use of rapid freezing for stopping brain and spinal cord metabolism in rat and mouse. *Journal of Neurochemistry* **18:** 2085-2092.

Thuring, J.A., v d Heuvel, A, Kamerman, J., Attia, M. 1983. CO_2/O_2 gas mixture as an anaesthetic agent in rats in terminal studies. Abstract. *Zeitschrift für Versuchstierkunde.* **25:** 179-180.

Venkataraman, B.V., Shetty, P.S., Joseph, T. 1981. Variations in brain and heart acetylcholine content in rat: cervical dislocation vs guillotine technique. *Indian Journal of Physiology and Pharmacology* **25(3):** 289-291.

RABBITS

Adki, T., Yoshiura, M., Iwamoto, T., Ozaki, Y., Iriyama, K. 1983. Changes in rabbit brain norepinenephrine and dopamine after decapitation. *Jikeilai Medical Journal* **29(4):** 385-392.

Chiboka, O. 1981. Stage dependency of the effect of fetal decapitation on gestation and parturition in rabbits. *Zentralbl. Veterinaermed. Reihe. A.* **28(4):** 338-344.

Dickel, H. 1975. Tierschutzgerechtes Töten von Kaninchen, Tierschutzgerechtes Töten von Wirbeltieren, *Schlütersche Verlagsanstalt,* Hannover, 1975.

Hattingh, J., Cornelius, S.T., Ganhoa, M.F., Fonseca, F. 1986. Arterial blood gas composition, consciousness and death in rabbits. *Journal of the South African Veterinary Association,* March 1986: 13-16.

Hunter, A.R., Pleuvry, B.J., Rees, J.M.H. 1968. The respiratory depressant effects of barbiturates and narcotic analgesics in the unanaesthetized rabbit. *British Journal of Anaesthesia* **40:** 927-935.

Veyssiere, G., Berger, M., Jean-Faucher, C., De Turckheim, M., Jean, C. 1982. Effects of decapitation on the androgen levels in plasma and testes of fetal rabbits. IRCS Medical Science Library Compend. **9(12):** 1089.

CARNIVORES

British Standards Institution. 1957. *Cabinets for the Electrical Euthanasia of Dogs.* British Standard 2909: 1957.

Carding, A.H. 1968. Mass euthanasia of dogs with carbon monoxide and/or carbon dioxide: preliminary trials. *Journal of Small Animal Practice* **9:** 245-254.

Carding, A.H. 1977. Euthanasia of dogs and cats: an analysis of experience and current knowledge with recommendations for research. *Animal Regulation Studies* **1:** 5-21.

Croft, P.G. 1972. the EEG as an aid to assessment of state of consciousness in the dog. *Journal of Physiology* **151:** 6p-8p.

Dallaire, A., Chalifoux, A. 1985. Premedication of dogs with acepromazine of pentazocine before euthanasia with carbon monoxide. *Canadian Journal of Comparative Medicine = Revue Canadienne de Medecine Compares* **49(2):** 171-178.

De Vries. H.W., Zimmermann, A.N.E., van Leeuwen, S.W. *et al.* 1977. An experimental study of acute carbon monoxide intoxication in dogs. *Acta. Pharmacol. Toxicol. Suppl.* **41:** 374-392.

Eisele, J.H., Eger, E.I., Muallem, M. 1967. Narcotic properties of carbon dioxide in the dog. *Anesthesiology* **28:** 856-865.

Fox, M.N., Carding, A. 1978. *Euthanasia of dogs and cats.* Washington DC. The Institute for the Study of Animal Problems. pp 23-39.

Griesemer, R.A et al. 1978. Laboratory Animal Management - Cats. *ILAR News* **21(3):** C1-C20.

Hicks, T., Bailey, E.M Jr. 1978. Succinylcholine chloride as a euthanatizing agent in dogs. *American Journal of Veterinary Research* **39:** 1195-1197.

Körner, E. 1984. Tötung von farmgehaltenen Peltztieren. *Tierärztl. Praxis* **12:** 527-530.

Lambooy, E., Roelofs, J.A., van Voorst, N. 1985. Euthanasia of mink with carbon monoxide. *The Veterinary Record,* April 13, 416.

Loftsgard, G., Braathen, S., Helgebostad, A. 1972. Electrical stunning of mink. *The Veterinary Record* **91:** 132-134.

Moreland, A.F. 1974. Carbon monoxide euthanasia of dogs: chamber concentrations and comparative effects of automobile engine exhaust and carbon monoxide from a cylinder. *Journal of the American Veterinary Medical Association* **165(9):** 853-855.

Prynn, R.B., Redding, R.W. 1968. Electroencephalographic continuum in dogs anesthetized with methoxyflurane and halothane. *American Journal of Veterinary Research* **29:** 1913-1928.

Sawyer, D.C. 1975. Comparative effects of halothane. *Gaines Dog Research Program.* 2-3.

Sevcikova, E., Reichel, F. 1982. Klinicke overovani pripravku Pentobarbital Spofa inj. ad usum veterinarium. (Clinical testing of the preparation Pentobarbital spofa inj, ad usum veterinarium). *Biologizace a Chemizace Zivocisne Vyroby-Veterinaria* **18(4):** 375-382.

Simonsen, H.B., Thordal-Christensen, A., Ockens, N. 1981. Carbon monoxide and carbon dioxide euthanasia of cats: duration and animal behaviour. *The British Veterinary Journal* **137(3):** 274-278.

Vinter, F.J. 1957. The humane killing of mink. *British Fur Farmers Gazette.* August 1957.

LARGE MAMMALS

Anon. 1982. Guidelines for recommending euthanasia (of the horse, approved by the American Association of Equine Practitioners). *Veterinary Professional Topics: Horse, Equine Professional Topics* **8(1):** 1.

Barford, K. 1990. Carbon dioxide anaesthetization of pigs [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 9-10. *IVO rapport B-354.*

Barton-Gade, P.A., Nielson, N.J., Klovborg, H. Practical experience with CO2 stunning [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 14-15. *IVO rapport B-354.*

Birchall, A. 1990. Kinder Ways to Kill. *New Scientist* **126(1717)** 19 May: 44-49.

Birchall, A. 1990. The rough road to slaughter. *New Scientist* 24 November: 33-38.

Blackmore, D.K., Newhook, J.C. 1981. Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* **29:** 219-222.

Blackmore, D.K., Newhook, J.C. 1983. The assessment of insensibility in sheep, calves and pigs during slaughter. In: *Stunning of animals for slaughter.* (Ed G Eikelenboom) 13-25. Martinus Nijhoff Publishers, Boston.

Blackmore, D.K., Newhook, J.C., Peterson, G.V. 1979. Electrical stunning and humane slaughter. *New Zealand Veterinary Journal* **27:** 224.

Brambell, F.W.R. 1965. *Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems.* Cand 2836. London HMSO.

Dougherty, R.W. 1981. Anesthesia and euthanasia. In: *Experimental Surgery in Farm Animals*, Ch 3. p8-13. Iowa State University Press, Ames.

Forslid, A. 1990. Preslaughter CO2-anaesthesia in swine. [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 12. *IVO rapport B-354.*

Gregory, N.G., Wotton, S.B. 1984. Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* **37:** 141-143.

Gregory, N.G., Mohan-Raj, A.B., Audsey, A.R.S, Daly, C.C. 1990. Effects of CO2 on man. [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 7-9. *IVO rapport B-354.*

Hertampf, B von, Mickwitz, G von. Betäubung von Schlachttieren; Teil 1: CO2-Betäubung. Übersichtsreferat. *Deutsche Tierärtzliche Wochenschrift* **86:** 333-376.

Hoenderken, R. 1979. Zur Betäubung von Schlachtschweinen. *Schlachten und Vermarkten* **79(8):** 235-237.

Hoenderken, R., Logtestijn, J.G.van, Sybesma, W., Spanjaard, W.J.M. 1979. Kohlendioxid-Betäubung von Schlachtschweinen. *Die Fleischwirtschaft* **11:** 1572-1578.

Honkavaara, M. 1990. Effect of stunning method on early postmortem biochemical changes in pork. [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 14. *IVO rapport B-354.*

Jones, R.S. 1993. Euthanasia in horses. *Royal College of Veterinary Surgeons Newsletter.* February 1993.

Jones, R.S., Knottenbelt, D.K., Mason, K., O'Donnell, E. 1992. Euthanasia of horses. *The Veterinary Record* **130(24):** 544.

Lagerwey, E. 1990. CO2 inhalation in the pig. [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts].(Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 10-11. *IVO rapport B-354.*

Lambooy, E. 1982. Electrical stunning of sheep. *Meat Science* **6:** 123-135.

Lambooy, E., Spanjaard, W. 1980. Euthanasia of young pigs with carbon monoxide. *The Veterinary Record* **107:** 59-61.

Lambooy, E., Spanjaard, W. 1982. Electrical stunning of veal calves. *Meat Science* **6:** 15-25.

Lieske, R. 1980. Die Euthanasie von Pferden mit Eutha 77 (Euthanasia of horses with Eutha 77). *Tierärztliche Unschau* **35(3):** 170, 175-177.

Lomholt, N. 1983. CO2-Betäubung von Schlachttieren, Behauptungen und Realitäten. *Deutsche Tierärztliche Wochenschrift* **2:** 66-68.

Mickwitz, G.van. 1990. The behaviour of pigs during application of different stunning methods. [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 16-17. *IVO rapport B-354.*

Ring, C., Erhardt, W. 1990. CO2 anaesthesia for slaughter pigs: Animal protection and meat quality. [The use of CO2 for stunning of slaughter pigs; report of a meeting of experts]. (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 13. *IVO rapport B-354.*

Rose, M.A., Daly, D.M., Shaw, F.D. 1991. Humane slaughter of farm animals. *ACCART News* **4(1):** 2-3.

Schatzmann, U., Zeller, W. 1990. The use of CO2 for stunning of slaughter pigs; report of a meeting of experts [Observation of CO2 stunning under practical conditions] (Lambooy E. Instituut voor Veeteeltkundig Onderzoek "Schoonoord"; Zeist); 15. *IVO rapport B-354.*

Schultz, N.E. 1969. Succinylcholine for euthansia of swine. *Journal of the American Veterinary Medical Association* **154:** 38-39.

Thurmon, J.C. 1986. Euthanasia of food animals. *Veterinary Clinics of North America: Food Animal Practice* **2(3):** 743-756.

Trapp, A.L., Taylor, R.F. 1986. Methods of euthanasia in poultry and food-producing animals. *Veterinary Clinics of North America: Food Animal Practice* **2(1):** 31-41.

Troeger, W., Woltersdorf, W. 1989. Die Elektrobetäubung von Schlachtschweinen. *Deutsche Tierärztliche Wochenschrift* 100-103.

PRIMATES

International Primatological Society. 1988. *IPS international guidelines for the acquisition, care and breeding of nonhuman primates.*

Mattsson, J.L., Stinson, J.M., Clark, C.S. 1972. Electroencephalographic power-spectral changes coincident with onset of carbon dioxide narcosis in rhesus monkey. *American Journal of Veterinary Research* **33(10):** 2043-2049.

EXOTICS

Anon. 1979. Avlivning med kolsyra (CO_2) - en introduktion. (An introduction to the use of carbon dioxide (CO_2) for killing mink for euthanasia). *Vara Palsdjur* **50(10):** 240-241.

Cooper, J.E. 1984. Anaesthesia of exotic animals. *Animal Technology* **35:** 13-20.

Haugen, A.O., Svendson, M.J., Shult, M. Petersburg, S.J. 1976. Immobilization of adult bison with etorphine. *Proceedings of the Iowa Academy of Sciences* **83(2):** 67-70.

Korner, E. 1984. Tötung von farmgehaltenen Peltztieren (Euthanasia of fur-animals in farms). *Tierärztliche Praxis* **12(4):** 527-530.

Lambooy, E. 1984. Electrocutie van vossen; een ethisch acceptabele methode? (Electrocution of foxes; an ethically acceptable method?) *Tijschrift voor Diergeneeskunde* **109(11):** 460-464.

Rowell, S.F. 1985. Stranded whales. *The Veterinary Record* **116(6):** 167.

6. EUTHANASIA TRAINING MATERIALS

INTER-ACT ANIMAL CARE TRAINING PROGRAMMES.
This is a specially designed series of programmes produced by the Association of the British Pharmaceutical Industry (ABPI) as a resource for the training of laboratory staff involved in the care and use of animal under the Animals (Scientific Procedures) Act 1986. Topic 7 covers euthanasia. Using inter-active video technology, the full series of eleven programmes (held on ten laser discs) is available from Mr M Connelly, Redway Interactive Video, 34 Redway, Kerridge, Macclesfield, Cheshire SK10 5BA, UK.

PRINCIPLES OF PROPER LABORATORY ANIMAL USE IN RESEARCH.
This program is a basic program for research investigators, technicians and support staff. This program establishes the appropriate foundation for use of animals in biomedical research. It is a modular, flexible and easy to use software template. The program consists of a template divided into a series of modules containing information on the following topics; regulations, ethics, euthanasia, anaesthesia and analgesia, pre- and post-operative care, safety, disease, nutrition, alternatives and models, species information, USDA animal welfare act. The program is designed with many applications in mind.
Runs on Macintosh and IBM PC computers and requires a hard disk and 640K RAM.
MTM Associates Inc., P O Box 1606 Manassas, Virginia 2110, USA. Item No 4211WP.

USING ANIMALS IN RESEARCH: GUIDELINES FOR INVESTIGATORS. 1986.
This film records in its entirety a course sponsored by the USDA/Agricultural Research Service and presented to investigators on March 25 1986. Speakers for the Animal and Plant Health Service, National Institutes of Health, Public Health, and Agricultural Research Service present information on laws, policies and practices that affect the use of research laboratory; and provide references for those who want to learn more about specific procedures. Tape 1 - regulatory issues from an aphis perspective. Tape 2 - film "Unnecessary Fuss", assembled by PETA from U of PA research documentation. Tape 3 - principles and policies for animal use in NIH extramural programmes. Tape 4 - principles and policies for animal use in the Beltsville area. Tape 5 - technical information and training opportunities for animal users. Tape 6 - panel discussion of audience questions.
Videocassette, U-matic, 3/4", NTSC, English. 240 minutes (6 tapes).
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette no 186.

EUTHANASIA INTERACTIVE VIDEO PROGRAM
To provide the trainee with an understanding of the legal requirements of Schedule 1 (UK) and the Alderley Park site regulations to be found in the "Humane Policy for Euthanasia of Protected Species". Demonstrate the correct procedure for euthanasia when using the following methods: overdose of anaesthetic, physical methods, carbon dioxide. It provides a means by which the trainees understanding of the above can be assessed.
Approximately 1.25 hours. A printout of the trainees responses to questions posed during the programme is available. It may be viewed without the interactive component - this lasts about 25 minutes. English language only. Contact Mr Bob Kemp, Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK. Tel +44 1625 512726, Fax +44 1625 583074/586278.

COMMON PROCEDURES AND TECHNIQUES AND SURVIVAL SURGERY: TAPE II. 1988.
Handling laboratory animals in blood collection, gavage, injection and euthanasia: emphasises minimization of animal discomfort.
Videocassette, NTSC/U-matic/VHS. English. 21 mins.
MDA-TV, University of Texas Cancer Centre, 1515 Holcombe Houston, Texas 77030, USA. Order No 861188.

PRACTICAL METHODOLOGY: REPTILES PART III SPECIAL LABORATORY. 1988.
The second of a two part series, this program covers laboratory techniques such as sexing, blood sampling, catheter placement, injection sites, oral gavaging, intubation, restraint, anaesthesia and euthanasia for reptiles. A manual accompanies the videocassette.
Videocassette, ½" VHS, NTSC. English. 18 mins.
National Agricultural Library, Beltsville, Maryland 20705, USA.
Videocassette No 414 part II.

BIOMETHODOLOGY OF THE MOUSE.
This video shows a close-up, detailed footage demonstrating proper techniques of handling and restraint, identification, injections and blood sampling. The techniques presented are the most common, reproducible, safe and least stressful to the animal.
Video, VHS (½") U-matic (3/4") standard tape formats.
MTM Associates Inc., P O Box 1606, Manassas, Virginia 2110, USA. Item no 3211V.

THE MOUSE: HANDLING, RESTRAINT, AND OTHER TECHNIQUES. 1975.
This programme demonstrates basic technical skills required for the proper care of mice and their use in biomedical research. It includes handling and restraint, injections and oral administration of medicine, blood and urine collection, sexing, identification, anaesthesia and euthanasia. A manual accompanies the slide set.
Slides, 48 slides, audiocassette. 12 minutes. English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no 227, part 2.

BIOMETHODOLOGY OF THE LABORATORY MOUSE. 1987.
A demonstration of basic techniques involving laboratory mice including identification, restraint, injection, blood withdrawal and euthanasia.
Videocassette ½" VHS (NTSC), colour, English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 200.

THE MOUSE, RAT AND HAMSTER. 1988.
This training film provides information on the humane care and use of laboratory rodents for scientists, laboratory technicians and students. The recommendations are consistent with the Public Health Services Guide for the Care and Use of Laboratory Animals and regulations set by the USDA. Topics include housing, nutrition, environment, record keeping, animal health care, occupational safety, handling and restraint, experimental techniques, and euthanasia. A manual that includes a script, test and answer key accompanies the videocassette.
Videocassette ½" VHS (NTSC), English. 34 minutes.
National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no 338 vol 2.

THE LABORATORY RAT, BIOLOGY, HUSBANDRY, AND RESEARCH METHODOLOGY. 1977.
This slide set describes and illustrates basic anatomy and physiology of laboratory rats, discusses standard procedures for housing rats in the laboratory, and familiarizes the viewer with basic methodology employed for manipulating rats in research. Research methodology includes handling, restraint, blood collection, anaesthesia and euthanasia. Biological values, listed in the accompanying guide.
Slides, 59 slides, audiocassette. English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no 221.

BIOMETHODOLOGY OF THE RAT. 1987.
Demonstration of basic techniques involving laboratory animals including identification, restraint, injection, blood withdrawal, and euthanasia.
Video ½" VHS. English. 16 mins.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 200.

BIOMETHODOLOGY OF THE GUINEA PIG. 1987.
Demonstration of basic techniques involving laboratory animals including identification, restraint, injection, blood withdrawal, and euthanasia.
Videocassette ½" VHS. English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 200.

BIOMETHODOLOGY OF THE RABBIT. 1987.
Demonstration of basic techniques involving laboratory animals including identification, restraint, injection, blood withdrawal, and euthanasia.
Video ½" VHS. English. 15 mins.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 200.

BIOMETHODOLOGY OF THE CAT. 1987.
This film demonstrates to animal technicians basic techniques for handling, restraining, and manipulating cats for research. Covers removal from caging, injection routes, blood collection, and euthanasia.
Videocassette ½" VHS (NTSC), colour, 15 mins. English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 337.

THE DOG AND CAT. 1988.
This programme provides information on the humane care and use of laboratory dogs and cats for scientists, laboratory technicians, and students. The recommendations are consistent with the Public Health Services "Guide for the Care and Use of Laboratory Animals" and regulations set by the USDA. Topics include housing, nutrition, environment, record keeping, animal health care, occupational safety, handling and restraint, experimental techniques and euthanasia. A scripty, test, and answer key accompany the videocassette.
Videocassette ½" VHS (NTSC), colour. English. 35 mins.
National Agricultural Library, Beltsville, Maryland 20705, USA.
Videocassette No 338 vol 4.

BIOMETHODOLOGY OF THE DOG. 1987.
This film demonstrates to animal technicians safe and humane techniques for manipulating dogs in research including removal from caging, injection routes, blood collection, and euthanasia.
Videocassette ½" VHS (NTSC), colour, 15 mins. English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 335.

BIOMETHODOLOGY OF THE PRIMATE. 1987.
This film demonstrates basic techniques for manipulating primates for research. It includes manual and chemical restraint, identification, injection routes, blood collection, and euthanasia.
Videocassette ½" VHS (NTSC), colour, English.
National Agricultural Library, Beltsville, Maryland 20705, USA. Videocassette No 336.

THE NONHUMAN PRIMATES. 1988.
This programme provides information on the humane care and use of laboratory primates for scientists, laboratory technicians, and students. The recommendations are consistent with the Public Health Services Guide for the Care and Use of Laboratory Animals and regulations set by the USDA. Topics include housing, nutrition, environment, record keeping, animal health care, occupational safety, handling and restraint, experimental techniques, and euthanasia. A manual that includes a script, test and answer key accompanies the videocassette.
Videocassette ½" VHS (NTSC), English. 29 minutes.
National Agricultural Library, Beltsville, Maryland 20705, USA. Slide no 338 vol 5.

FURTHER INFORMATION ON TRAINING MATERIALS:

Information on training materials may be obtained from:

1. Dept of Laboratory Animal Science (Dr Jan Nab, Ing T.P. Rooymans) Utrecht University, Postbus 80.166, 3508 TD Utrecht, The Netherlands.

2. NORINA database. English language database of audiovisuals for use in the biological sciences. Contact Karina and Adrian Smith, Laboratory Animal Unit, Norwegian College of Veterinary Medicine, P O Box 8146 Dep., 00033 Oslo 1, Norway. Fax +47 22 96 45 35. Telephone +47 22 96 45 74. email (Internet): adrian.smith@veths.no.

European Commission

Euthanasia of experimental animals

Document

Luxembourg: Office for Official Publications of the European Communities

1997 — 98 pp. — 21 x 29.7 cm

ISBN 92-827-9694-9

Price (excluding VAT) in Luxembourg: ECU 14.50

Venta • Salg • Verkauf • Πωλήσεις • Sales • Vente • Vendita • Verkoop • Venda • Myynti • Försäljning

BELGIQUE/BELGIË

Moniteur belge/Belgisch Staatsblad
Rue de Louvain 40-42/
Leuvenseweg 40-42
B-1000 Bruxelles/Brussel
Tél. (32-2) 552 22 11
Fax (32-2) 511 01 84

Jean De Lannoy
Avenue du Roi 202/
Koningslaan 202
B-1060 Bruxelles/Brussel
Tél. (32-2) 538 51 69
Fax (32-2) 538 08 41
E-mail: jean.de.lannoy@infoboard.be

Librairie européenne/Europese Boekhandel
Rue de la Loi 244/
Wetstraat 244
B-1040 Bruxelles/Brussel
Tél. (32-2) 295 26 39
Fax (32-2) 735 08 60

DANMARK

J. H. Schultz Information A/S
Herstedvang 10-12
DK-2620 Albertslund
Tlf. (45) 43 63 23 00
Fax (45) 43 63 19 69
E-mail: schultz@schultz.dk
URL: www.schultz.dk

DEUTSCHLAND

Bundesanzeiger Verlag
Breite Straße 78-80
Postfach 10 05 34
D-50667 Köln
Tel. (49-221) 20 29-0
Fax (49-221) 20 29 278

GREECE/ΕΛΛΑΔΑ

G.C. Eleftheroudakis SA
International Bookstore
Panepistimiou 17
GR-105 64 Athens
Tel. (30-1) 331 41 80/1/2/3
Fax (30-1) 323 98 21
E-mail: elebooks@netor.gr

ESPAÑA

Mundi Prensa Libros, SA
Castelló, 37
E-28001 Madrid
Tel. (34-1) 431 33 99/431 32 22
Fax (34-1) 575 39 98
E-mail: mundiprensa@tsai.es
URL: www.tsai.es/mprensa

Boletín Oficial del Estado
Trafalgar, 27-29
E-28071 Madrid
Tel. (34-1) 538 22 95 (Libros)/
384 17 15 (Suscripciones)
Fax (34-1) 538 23 49 (Libros)/
384 17 14 (Suscripciones)
URL: www.boe.es

Mundi Prensa Barcelona
Consell de Cent, 39
E-08009 Barcelona
Tel. (34-3) 488 34 92
Fax (34-3) 487 76 59

FRANCE

Journal officiel
Service des publications des CE
26, rue Desaix
F-75727 Paris Cedex 15
Tél. (33-1) 40 58 77 01/31
Fax (33-1) 40 58 77 00

IRELAND

Government Supplies Agency
Publications Section
4-5 Harcourt Road
Dublin 2
Tel. (353-1) 661 31 11
Fax (353-1) 475 27 60

ITALIA

Licosa SpA
Via Duca di Calabria, 1/1
Casella postale 552
I-50125 Firenze
Tel. (39-55) 64 54 15
Fax (39-55) 64 12 57
E-mail: licosa@ftbcc.it
URL: id382.cilea.it/Virtual_Library/bibliot/vetrina/licosa/tt1.htm

GRAND-DUCHÉ DE LUXEMBOURG

Messageries du livre Sarl
5, rue Raiffeisen
L-2411 Luxembourg
Tél. (352) 40 10 20
Fax (352) 490 661
E-mail: mdl@pt.lu

Abonnements:

Messageries Paul Kraus
11, rue Christophe Plantin
L-2339 Luxembourg
Tél. (352) 499 88 88
Fax (352) 499 888 444
E-mail: mpk@pt.lu
URL: www.mpk.lu

NEDERLAND

SDU Servicecentrum Uitgevers
Christoffel Plantijnstraat 2
Postbus 20014
2500 EA 's-Gravenhage
Tel. (31-70) 378 98 80
Fax (31-70) 378 97 83
E-mail: sdu@sdu.nl
URL: www.sdu.nl

ÖSTERREICH

Manz'sche Verlags- und Universitäts-buchhandlung Gmbh
Siebenbrunnengasse 21
Postfach 1
A-1050 Wien
Tel. (43-1) 53 161 334 / 340
Fax (43-1) 53 161 339
E-mail: auslieferung@manz.co.at
URL: www.austria.EU.net:81/manz

PORTUGAL

Imprensa Nacional-Casa da Moeda, EP
Rua Marquês de Sá da Bandeira, 16 A
P-1050 Lisboa Codex
Tel. (351-1) 353 03 99
Fax (351-1) 353 02 94/384 01 32

Distribuidora de Livros Bertrand Ld.ª
Rua das Terras dos Vales, 4 A
Apartado 60037
P-2701 Amadora Codex
Tel. (351-1) 495 90 50/495 87 87
Fax (351-1) 496 02 55

SUOMI/FINLAND

Akateeminen Kirjakauppa /
Akademiska Bokhandeln
Pohjoisesplanadi 39/
Norra esplanaden 39
PL/PB 128
FIN-00101 Helsinki/Helsingfors
Tel. (358-9) 121 41
Fax (358-9) 121 44 35
E-mail: akatilaus@stockmann.mailnet.fi
URL: booknet.cultnet.fi/aka/index.htm

SVERIGE

BTJ AB
Traktorvägen 11
PO Box 200
S-22100 Lund
Tel. (46-46) 18 00 00
Fax (46-46) 18 01 25
E-mail: btj_tc@mail.btj.se
URL: www.btj.se/media/eu

UNITED KINGDOM

The Stationery Office Ltd (Agency Section)
51, Nine Elms Lane
London SW8 5DR
Tel. (44-171) 873 9090
Fax (44-171) 873 8463
URL: www.the-stationery-office.co.uk

ICELAND

Bokabud Larusar Blöndal
Skólavördustig, 2
IS-101 Reykjavik
Tel. (354) 55 15 650
Fax (354) 55 25 560

NORGE

NIC Info A/S
Ostenjoveien 18
Boks 6512 Etterstad
N-0606 Oslo
Tel. (47-22) 97 45 00
Fax (47-22) 97 45 45

SCHWEIZ/SUISSE/SVIZZERA

OSEC
Stampfenbachstraße 85
CH-8035 Zürich
Tel. (41-1) 365 53 15
Fax (41-1) 365 54 11
E-mail: urs.leimbacher@ecs.osec.inet.ch
URL: www.osec.ch

ČESKÁ REPUBLIKA

NIS CR - prodejna
Konviktská 5
CZ-113 57 Praha 1
Tel. (42-2) 24 22 94 33
Fax (42-2) 24 22 94 33
E-mail: nkposp@dec.nis.cz
URL: www.nis.cz

CYPRUS

Cyprus Chamber Of Commerce & Industry
38, Grivas Digenis Ave
Mail orders:
PO Box 1455
CY-1509 Nicosia
Tel. (357-2) 44 95 00/46 23 12
Fax (357-2) 361 044
E-mail: cy1691_eic_cyprus@vans.infonet.com

MAGYARORSZÁG

Euro Info Service
Európa Ház
Margitsziget
PO Box 475
H-1396 Budapest 62
Tel. (36-1) 11 16 061/11 16 216
Fax (36-1) 302 50 35
E-mail: euroinfo@mail.matav.hu
URL: www.euroinfo.hu/index.htm

MALTA

Miller Distributors Ltd
Malta International Airport
PO Box 25
LQA 05 Malta
Tel. (356) 66 44 88
Fax (356) 67 67 99

POLSKA

Ars Polona
Krakowskie Przedmiescie 7
Skr. pocztowa 1001
PL-00-950 Warszawa
Tel. (48-2) 26 12 01
Fax (48-2) 26 62 40

TÜRKIYE

Dünya Infotel A.S.
Istiklâl Cad. No: 469
TR-80050 Tünel-Istanbul
Tel. (90-212) 251 91 96
(90-312) 427 02 10
Fax (90-212) 251 91 97

BÁLGARIJA

Europress-Euromedia Ltd
59, Bld Vitosha
BG-1000 Sofia
Tel. (359-2) 80 46 41
Fax (359-2) 80 45 41

HRVATSKA

Mediatrade Ltd
Pavla Hatza 1
HR-10000 Zagreb
Tel. (385-1) 43 03 92
Fax (385-1) 44 40 59

ROMÂNIA

Euromedia
Str. G-ral Berthelot Nr 41
RO-70749 Bucuresti
Tel. (40-1) 210 44 01/614 06 64
Fax (40-1) 210 44 01/312 96 46

SLOVAKIA

Slovenska Technicka Kniznica
Námestie slobody 19
SLO-81223 Bratislava 1
Tel. (42-7) 53 18 364
Fax (42-7) 53 18 364
E-mail: europ@tbb1.sltk.stuba.sk

SLOVENIA

Gospodarski Vestnik
Zalozniska skupina d.d.
Dunajska cesta 5
SI-1000 Ljubljana
Tel. (386) 61 133 03 54
Fax (386) 61 133 91 28
E-mail: belicd@gvestnik.si
URL: www.gvestnik.si

ISRAEL

R.O.Y. International
17, Shimon Hatarssi Street
PO Box 13056
61130 Tel Aviv
Tel. (972-3) 546 14 23
Fax (972-3) 546 14 42
E-mail: royil@netvision.net.il

Sub-agent for the Palestinian Authority:

Index Information Services
PO Box 19502
Jerusalem
Tel. (972-2) 27 16 34
Fax (972-2) 27 12 19

RUSSIA

CCEC
60-letiya Oktyabrya Av. 9
117312 Moscow
Tel. (095) 135 52 27
Fax (095) 135 52 27

AUSTRALIA

Hunter Publications
PO Box 404
3167 Abbotsford, Victoria
Tel. (61-3) 9417 53 61
Fax (61-3) 9419 71 54

CANADA

Uniquement abonnements/
Subscriptions only:

Renouf Publishing Co. Ltd
1294 Algoma Road
K1B 3W8 Ottawa, Ontario
Tel. (1-613) 741 73 33
Fax (1-613) 741 54 39
E-mail: renouf@fox.nstn.ca
URL: fox.NSTN.Ca/~renouf

EGYPT

The Middle East Observer
41, Sherif Street
Cairo
Tel. (20-2) 39 39 732
Fax (20-2) 39 39 732

JAPAN

PSI-Japan
Asahi Sanbancho Plaza #206
7-1 Sanbancho, Chiyoda-ku
Tokyo 102
Tel. (81-3) 3234 69 21
Fax (81-3) 3234 69 15
E-mail: psijapan@gol.com
URL: www.psi-japan.com

SOUTH AFRICA

Safto
5th Floor Export House,
CNR Maude & West Streets
PO Box 782 706
2146 Sandton
Tel. (27-11) 883 37 37
Fax (27-11) 883 65 69

UNITED STATES OF AMERICA

Bernan Associates
4611-F Assembly Drive
MD20706 Lanham
Tel. (301) 459 2255 (toll free telephone)
Fax (800) 865 3450 (toll free fax)
E-mail: query@bernan.com
URL: www.bernan.com

MÉXICO

Mundi-Prensa Mexico, SA de CV
Río Pánuco, 141
Delegación Cuauhtémoc
ME-06500 México DF
Tel. (52-5) 553 56 58/60
Fax (52-5) 514 67 99
E-mail: 104164.23compuserve.com

RÉPUBLIQUE DE CORÉE

Kyowa Book Company
1 F1. Phyung Hwa Bldg
411-2 Hap Jeong Dong, Mapo Ku
121-220 Seoul
Tél. (82-2) 322 6780/1
Fax (82-2) 322 6782
E-mail: kyowa2@ktnet.co.kr.

ANDERE LÄNDER/OTHER COUNTRIES/
AUTRES PAYS

Bitte wenden Sie sich an ein Büro Ihrer Wahl / Please contact the sales office of your choice / Veuillez vous adresser au bureau de vente de votre choix

CR-03-97-490-EN-C

Price (excluding VAT) in Luxembourg: ECU 14.50

ISBN 92-827-9694-9

OFFICE FOR OFFICIAL PUBLICATIONS
OF THE EUROPEAN COMMUNITIES

L-2985 Luxembourg