10

# Medical and Bioethical Issues in Laboratory Animal

Matilde Jiménez-Coello, Karla Y. Acosta-Viana,
Antonio Ortega-Pacheco and Eugenia Guzmán-Marín
*Universidad Autónoma de Yucatán*
*México*

## 1. Introduction

Euthanasia in laboratory animals is a routinely procedure to properly complete the tests and experiments in which these models have a key role for the precise evaluation of various issues during the development of a scientific activity (Van Zutphen, 2001). The term euthanasia is derived from the Greek terms *eu* meaning good and *thanatos* meaning death (Webster, 1990). It is a necessary and accepted procedure in all aspects of veterinary medicine and many aspects of scientific procedures involving animals (Reilly, 2001). A "good death" would be one that occurs with minimal pain and distress.

It has been estimated that 75 to 100 million vertebrates are used per year worldwide in research, teaching and testing activities for a wide range of purposes. Only in Europe 10.7 million vertebrates are used annually for research purposes (Van Zutphen, 2001). Drug research, testing of vaccines and other biologicals, and cancer research account for about 70% of the animals used, while the remaining 30% are used for purposes such as fundamental research, for diagnostic purposes, for teaching, etc. (Fig. 1) (Baumans, 2005) therefore, animals need to be killed for various reasons, including the collection of blood and tissues, culling of breeding stock, disposal at the end of an experiment and in those circumstances where animals are experiencing pain and distress which cannot be alleviated (Reilly, 2001).

Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness (AVMA, 2007). For these reason, if an animal has to be killed, death must occur with the least fear, anxiety, pain and distress. The method used for euthanasia must either kill the animal very rapidly or instantaneously render the animal unconscious so that death ensues before consciousness is regained (Reilly, 2001).

Selection of the most appropriate method of euthanasia in any given situation depends on the species of animal involved, adequate methods of animal restraint, skill of personnel, number of animals, and other considerations. Available information focuses primarily on domestic animals, but the same general considerations should be applied to all species. For the best method of euthanasia, the following criteria must be considered: (1) ability to induce loss of consciousness and death without causing pain, distress, anxiety, or

apprehension; (2) time required to induce loss of consciousness; (3) reliability; (4) safety of personnel; (5) irreversibility; (6) compatibility with requirement and purpose; (7) emotional effect on observers or operators; (8) compatibility with subsequent evaluation, examination, or use of tissue; (9) drug availability and human abuse potential; (10) compatibility with species, age, and health status; (11) ability to maintain equipment in proper working order; and (12) safety for predators/scavengers should the carcass be consumed (AVMA, 2007).

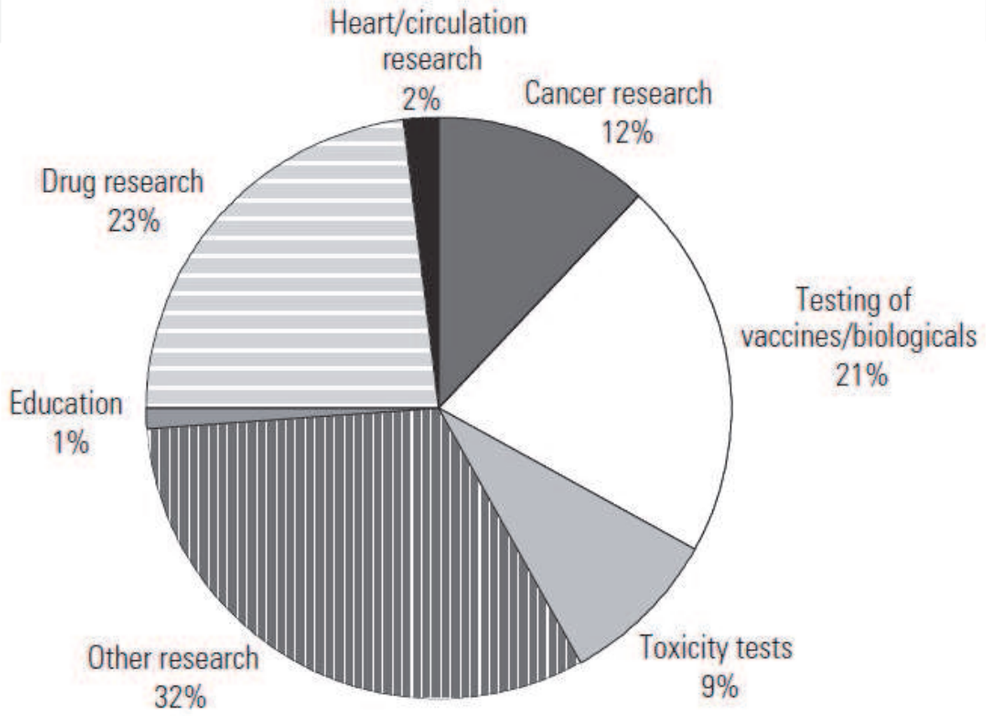



Fig. 1. Distribution of the purposes of animal use in research (Baumans, 2005)

In this chapter, attention is paid to provide investigators and technicians details of the current state of information relevant to the euthanasia of animals used for scientific purposes, so they can choose the better decision about the suitability of methods for euthanasia considering the welfare of animals.

## 2. Objectives of the euthanasia

The primary criteria for euthanasia in terms of animal welfare is that the method used should be painless, achieve rapid unconsciousness and death, require minimum restraint, avoid excitement, is appropriate for the age, species, and health of the animal, must minimize fear and psychological stress in the animal, be reliable, reproducible, irreversible, simple to administer (in small doses if possible) and safe for the operator, and, so far as possible, be aesthetically acceptable for the operator (Close et al., 1996).

| **Animals are killed in laboratories or breeding establishments for various reasons:** | **Statements to consider when is necessary to kill an animal,** |
|---|---|
| At the end of an experiment or when there might be continuing adverse effects; | Humane procedures must be used (avoiding distress, reliable, producing rapid loss of consciousness without pain until death occurs and procedures should also be compatible with the scientific or educational aims |
| To provide blood and ·other tissues for a scientific purpose; | Procedures must be performed only by persons competent in the method to be used, or under direct supervision of a competent person. |
| When levels of pain, distress and suffering are likely to exceed the designated level; | The appropriate means must be readily at hand |
| Where the health or welfare of the animals are grounds for concern; | Animals should be killed in a quiet, clean environment and normally away from other animals. There should be no disposal of the carcass until death is established. |
| When they are no longer suitable for breeding; | Dependent neonates of animals being killed must also be killed or provision made for their care |
| Unwanted stock or those with unsuitable characteristics, for example, type or sex, are not needed. | When fertilized eggs are used, the method of disposal must ensure the death of the embryo. |

Table 1. Reasons to explain why the laboratory animals must be euthanatized and statements to consider for do it.

### 2.1 Signs of pain or distress in animals

When animals are killed, both the method of euthanasia, particularly the time taken to produce unconciseness, and how the technique is performed can result in animals experiencing pain, distress, fear and anxiety. Furthermore, the animal's psychological response to the environment in which it is killed, including interactions with other animals and humans and how it is handled, can result in emotional distress.

*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" The use of the word pain implies a conscious awareness of the stimulus and not an unconscious reflex response (Close et al., 1996).

Physiologically, pain is that sensation (perception) that results from nerve impulses reaching the cerebral cortex via ascending neural pathways. Under normal circumstances, these pathways are relatively specific, but the nervous system is sufficiently plastic that activation of nociceptive pathways does not always result in pain and stimulation of other (non-nociceptive) peripheral and central neurons can give rise to pain. The term nociceptive is derived from the word *noci* meaning to injure and *ceptive* meaning to receive, and is used to describe neuronal input caused by noxious stimuli, which threaten to, or actually do, destroy tissue. These noxious stimuli initiate nerve impulses by acting at primary nociceptors and

other sensory nerve endings that respond to noxious and non-noxious stimuli from mechanical, thermal, or chemical activity. Endogenous chemical substances such as hydrogen ions, potassium ions, ATP, serotonin, histamine, bradykinin, and prostaglandins, as well as electrical currents, are capable of generating nerve impulses in nociceptor nerve fibers. Activity in nociceptive pathways can also be triggered in normally silent receptors that become sensitized by chronic pain condition (Vierck et al., 1989; Wall, 1992).

Nerve impulse activity generated by nociceptors is conducted via nociceptor primary afferent fibers to the spinal cord or the brainstem where it is transmitted to two general sets of neural networks. One set is related to nociceptive reflexes (eg, withdrawal and flexion reflexes) that are mediated at the spinal level, and the second set consists of ascending pathways to the reticular formation, hypothalamus, thalamus, and cerebral cortex (somatosensory cortex and limbic system) for sensory processing. It is important to understand that ascending nociceptive pathways are numerous, often redundant, and are capable of considerable plasticity under chronic conditions (pathology or injury). Moreover, even the transmission of nociceptive neural activity in a given pathway is highly variable. Under certain conditions, both the nociceptive reflexes and the ascending pathways may be suppressed, as, for example, in epidural anesthesia. Under another set of conditions, nociceptive reflex actions may occur, but activity in the ascending pathways is suppressed; thus, noxious stimuli are not perceived as pain. It is incorrect to use the term pain for stimuli, receptors, reflexes, or pathways because the term implies perception, whereas all the above may be active without consequential pain perception (Breazile & Kitchel, 1969; Zinnerman, 1984).

Pain is divided into two broad categories: (1) sensory-discriminative, which indicates the site of origin and the stimulus giving rise to the pain; and (2) motivational-affective in which the severity of the stimulus is perceived and the animal's response is determined. Sensory-discriminative processing of nociceptive impulses is most likely to be accomplished by subcortical and cortical mechanisms similar to those used for processing other sensory-discriminative input that provides provides the individual with information about the intensity, duration, location, and quality of the stimulus. Motivational-affective processing involves the ascending reticular formation for behavioral and cortical arousal. It also involves thalamic input to the forebrain and the limbic system for perceptions such as discomfort, fear, anxiety, and depression. The motivational-affective neural networks also have strong inputs to the limbic system, hypothalamus and the autonomic nervous system for reflex activation of the cardiovascular, pulmonary, and pituitary-adrenal systems. Responses activated by these systems feed back to the forebrain and enhance perceptions derived via motivational-affective inputs. On the basis of neurosurgical experience in humans, it is possible to separate the sensory-discriminative components from the motivational-affective components of pain (Kitchel et al., 1993).

For pain to be experienced, the cerebral cortex and subcortical structures must be functional. If the cerebral cortex is nonfunctional because of hypoxia, depression by drugs, electric shock, or concussion, pain is not experienced. Therefore, the choice of the euthanasia agent or method is less critical if it is to be used on an animal that is anesthetized or unconscious, provided that the animal does not regain consciousness prior to death.

An understanding of the continuum that represents stress and distress is essential for evaluating techniques that minimize any distress experienced by an animal being euthanatized. Stress has been defined as the effect of physical, physiologic, or emotional factors (stressors) that induce an alteration in an animal's homeostasis or adaptive state

(Kitchen et al., 1989). The response of an animal to stress represents the adaptive process that is necessary to restore the baseline mental and physiologic state. These responses may involve changes in an animal's neuroendocrinologic system, autonomic nervous system, and mental status that may result in overt behavioral changes. An animal's response varies according to its experience, age, species, breed, and current physiologic and psychologic state (NRC, 1992).

### 2.2 Identification and recognition of death

It is essential that all personnel are trained to recognize and confirm death in the species they are working. The most important aspects in recognition of death include cessation of heartbeat and respiration, 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 (Close et al., 1996).

### 2.3 Personal 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 (Close et al., 1996). Personnel who perform euthanasia must have appropriate certification and training, experience with the techniques to be used, and experience in the humane restraint of the species of animal to be euthanatized, to ensure that animal pain and distress are minimized during euthanasia. Training and experience should include familiarity with the normal behaviour of the species being euthanatized, an appreciation of how handling and restraint affects that behaviour, and an understanding of the mechanism by which the selected technique induces loss of consciousness and death. Prior to being assigned full responsibility for performing euthanasia, all personnel must have demonstrated proficiency in the use of the technique in a closely supervised environment (AVMA, 2007). Training programmes should include courses on the biology of the species to be used, suitable methods of euthanasia for each species and national and animal welfare regulations. Training must include aspects such as recognition of pain, fear, distress, anxiety, insensibility and death for all species to be used.
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 (Close et al., 1996).

### 2.4 Equipment and Instrumentation required

Equipment, instruments 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 (Close et al., 1996).

### 2.5 Disposal of carcasses

After death has been verified, the carcase must be disposed of appropriately (Reilly, 2001). 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 (Close et al., 1996). This is particularly important for animal such as sheep, cattle, pigs and horses which may be used for human or pet food (Reilly, 2001). 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 (Close et al., 1996).

### 2.6 Modes of action of euthanatizing agents

Euthanatizing agents cause death by three basic mechanisms: (1) hypoxia, direct or indirect; (2) direct depression of neurons necessary for life function; and (3) physical disruption of brain activity and destruction of neurons necessary for life.

Agents that induce death by direct or indirect hypoxia can act at various sites and can cause loss of consciousness at different rates. For death to be painless and distress-free, loss of consciousness should precede loss of motor activity (muscle movement). Loss of motor activity, however, cannot be equated with loss of consciousness and absence of distress. Thus, agents that induce muscle paralysis without loss of consciousness are not acceptable as sole agents for euthanasia (eg, depolarizing and nondepolarizing muscle relaxants, strychnine, nicotine, and magnesium salts). With other techniques that induce hypoxia, some animals may have motor activity following loss of consciousness, but this is reflex activity and is not perceived by the animal.

A second group of euthanatizing agents depress nerve cells of the brain, inducing loss of consciousness followed by death. Some of these agents release inhibition of motor activity during the first stage of anesthesia, resulting in a so-called excitement or delirium phase, during which there may be vocalization and some muscle contraction. These responses do not appear to be purposeful. Death follows loss of consciousness, and is attributable to cardiac arrest and/or hypoxemia following direct depression of respiratory centers.

Physical disruption of brain activity, caused by concussion, direct destruction of the brain, or electrical depolarization of neurons, induces rapid loss of consciousness. Death occurs because of destruction of midbrain centers controlling cardiac and respiratory activity or as a result of adjunctive methods (eg, exsanguination) used to kill the animal. Exaggerated muscular activity can follow loss of consciousness and, although this may disturb some observers, the animal is not experiencing pain or distress (AVMA, 2007).

### 2.7 Acceptable methods of euthanasia in laboratory animals

#### 2.7.1 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 (Close et al., 1996).

#### 2.7.1.1 Shooting

Shooting in the head to ensure immediate destruction of the brain is an effective and humane way of killing large reptiles and mammals. This may be divided into two types: captive bolt or free bullet. The type of weapon used must be selected according to the species to be killed and the environment.

*Captive bolt:* A penetrating captive bolt is used for euthanasia of ruminants, horses, swine, laboratory rabbits, and dogs. Its mode of action is concussion and trauma to the cerebral hemisphere and brainstem (Blackmore, 1985; Dennis et al., 1988; Daly and Whittington, 1989). Captive bolt guns are powered by gunpowder or compressed air and must provide sufficient energy to penetrate the skull of the species on which they are being used (Blackmore, 1985). Adequate restraint is important to ensure proper placement of the captive bolt. A cerebral hemisphere and the brainstem must be sufficiently disrupted by the projectile to induce sudden loss of consciousness and subsequent death. Accurate placement of captive bolts for various species has been described (Clifford, 1984; Blackmore, 1985; Daly and Whittington, 1989). A multiple projectile has been suggested as a more effective technique, especially for large cattle (Blackmore, 1985). A nonpenetrating captive bolt only stuns animals and should not be used as a sole means of euthanasia (must be stunning under adjunctive methods). An advantage of the penetrating captive bolt is that could be an effective method of euthanasia for use in research facilities, in slaughterhouses, and on the farm when use of drugs is inappropriate or unavailable, but the disadvantages of this method is that is aesthetically displeasing and the death may not occur if equipment is not maintained and used properly. The use of the penetrating captive bolt is an acceptable and practical method of euthanasia in horses, ruminants, and swine, and it is conditionally acceptable in other appropriate species. However the non-penetrating captive bolt must not be used as a sole method of euthanasia.

*Free bullet*: Special care must be taken to avoid danger to the operator using this method. 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 (Oliver, 1979; Blackmore, 1985; Dodd, 1985).

#### 2.7.1.2 Concussion

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 specialized equipment such as the non-penetrating captive bolt must be used. **The use of the hammer or poleaxe is condemned as a method of stunning**. These methods must always be followed immediately by exsanguination, removal of the heart or destruction of the brain to ensure death. Training is essential for all operators. If not performed correctly, various degrees of consciousness with concomitant pain can ensue.

It is difficult to ensure consistency in performance by operators and therefore only a few animals should be killed using this method at any 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).

#### 2.7.1.3 Electrical stunning

Electrical current has been used for stunning species such as dogs, cattle, sheep, goats, hogs, fish and chickens (Warrington, 1974; Gregory & Wotton, 1986; Eikelenboom, 1986; Lambooy & Voorst, 1986; Anil & McKinstry, 1991).

Experiments with dogs have identified a need to direct the electrical current through the brain to induce rapid loss of consciousness. In dogs, when electricity passes only between fore-and hind limbs or neck and feet, it causes the heart to fibrillate but does not induce sudden loss of consciousness (Roberts, 1954). For electrical stunning of any animal, an apparatus that applies electrodes to opposite sides of the head, or in another way directs electrical current immediately through the brain, is necessary to induce rapid loss of consciousness. Attachment of electrodes and animal restraint can pose problems with this form of stunning. Signs of effective electrical stunning are extension of the limbs, opisthotonos, downward rotation of the eyeballs, and tonic spasm changing to clonic spasm, with eventual muscle flaccidity.

Electrical stunning should be followed promptly by electrically induced cardiac fibrillation, exsanguination, or other appropriate methods to ensure death (AVMA, 2007).

#### 2.7.1.4 Cervical dislocation

Cervical dislocation is a technique that has been used for many years and, when performed by well-trained individuals, appears to be humane. However, there are few scientific studies to confirm this observation.

This technique is used to euthanatize poultry, other small birds, mice, and immature rats and rabbits. For 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. With the other hand, the base of the tail or the hind limbs is quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull (Clifford, 1984). For poultry, cervical dislocation by stretching is a common method for mass euthanasia, but loss of consciousness may not be instantaneous (Lambooy, 1986).

Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation (Vanderwolf, 1988), and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness (Derr, 1991; Holson, 1992).

#### 2.7.1.5 Decapitation

Decapitation can be used to euthanatize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study (Feldman & Gupta, 1976). Also, this procedure has been used for killing fish, amphibians and birds. 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 especially for that purpose to ensure rapid and quick severance in the correct position (Clifford, 1984). Guillotines that are designed to accomplish decapitation in adult

rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose.

Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation (Mikeska & Klemm, 1975), more recent studies and reports indicate that this activity does not infer the ability to perceive pain, and in fact conclude that loss of consciousness develops rapidly (Vanderwolf et al., 1988; Derr, 1991; Holson, 1992). The immediate lack of circulation of blood to the brain and subsequent anoxia is thought to render the head rapidly insensible (Derr, 1991) making prior stunning or sedation unnecessary. Use of the puntilla is not acceptable (Commission of the European Communities, 1993). Use of other methods is preferred where possible until further research can show rapid loss of consciousness (Close et al., 1996).

The advantages described for this procedure, is that decapitation is a technique that appears to induce rapid loss of consciousness (Vanderwolf et al., 1988; Derr, 1991; Holson, 1992). It does not chemically contaminate tissues and it is rapidly accomplished. On the other hand, the disadvantages are that is a technique requiring handling and restraint to perform it, and could be distressful to animals. Also, it has been widely discussed about the presence of electrical activity in the brain following decapitation, creating controversy and for that reason its importance may still be open to debate (Mikeska & Klemm, 1975; Vanderwolf et al., 1988; Derr, 1991; Holson, 1992). Decapitation must be done by trained personnel to perform this technique, which should recognize the inherent danger of the guillotine and take adequate precautions to prevent personal injury. And finally, decapitation may be aesthetically displeasing to personnel performing or observing the technique.

The recommendations when decapitation has been chosen, is to consider that this technique is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental design and approved by a Bioethical Committee. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

**2.7.1.6 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 (Close et al., 1996). The designed mechanical apparatus have rotating blades or projections, which causes immediate fragmentation and death of day-old poultry and embryonated eggs. A review (American Association of Avian Pathologists Animal Welfare and Management Practices Committee, 2005) about the use of commercially available macerators for euthanasia of chicks, poults, and pipped eggs indicates that death by maceration in day-old poultry occurs immediately with minimal pain and distress. Maceration is an alternative to the use of carbon dioxide for euthanasia of day-old poultry. Maceration is believed to be equivalent to cervical dislocation and cranial compression as to time element, and is considered to be an acceptable means of euthanasia for newly hatched poultry by the Federation of Agriculture Canada (1989), World Organization for Animal Health (OIE, 2006) and European Council (1993).

The advantage of this procedure is that death is almost instantaneous, the method is safe for workers and large numbers of animals can be killed quickly. But the disadvantages described for maceration are that special equipment is required and the macerated tissues may present bio-security risks. It is recommended to consider than maceration requires special equipment that must be kept in excellent working order. Chicks must be delivered to the macerator in a way and at a rate that prevents a backlog of chicks at the point of entry into the macerator and without causing injury, suffocation, or avoidable distress to the chicks before maceration.

#### 2.7.1.7 Microwave irradiation

Heating by microwave irradiation is used primarily by neurobiologists to fix brain metabolites in vivo while maintaining the anatomic integrity of the brain (Stavinoha, 1983). Only specialist equipment (this does not include domestic microwave ovens) designed for this purpose is to be used (Close et al., 1996). Microwave instruments have been specifically designed for use in euthanasia of laboratory mice and rats. The instruments differ in design from kitchen units and may vary in maximal power output from 1.3 to 10 kw. All units direct their microwave energy to the head of the animal. The power required to rapidly halt brain enzyme activity depends on the efficiency of the unit, the ability to tune the resonant cavity and the size of the rodent head (Stavinoha et al., 1978). There is considerable variation among instruments in the time required for loss of consciousness and euthanasia. A 10 kw, 2,450 MHz instrument operated at a power of 9 kw will increase the brain temperature of 18 to 28 g mice to 79$^{o}$ C in 330 ms, and the brain temperature of 250 to 420 g rats to 94 C in 800 ms. (lkarashi et al., 1984). It is only to be carried out on small animals such as amphibians, birds, mice, rats and small rabbits (less than 300 g) (Zeller et al., 1989). This method requires specialist expertise, but when carried out correctly is humane as death occurs in milliseconds (Andrews et al., 1993, Bermann et al., 1985, Olfert et al., 1993). Care must be taken to ensure correct positioning of the microwave beam but time taken to restrain the animal should be kept to a minimum to reduce stress prior to euthanasia.

The advantages for this procedure are that it cause loss of consciousness achieved in less than 100 ms, and death in less than 1 second. Besides, this is the most effective method to fix brain tissue *in vivo* for subsequent assay of enzymatically labile chemicals. The disadvantages mentioned for this technique are that the specific instruments are expensive and only small animals (with the size of mice and rats) can be euthanatized with commercial instruments that are currently available.

### 2.7.2 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 (Close et al., 1996).

### 2.7.3 Inhalational agents

Any gas that is inhaled must reach a certain concentration in the alveoli before it can be effective; therefore, euthanasia with any of these agents may take some time. The suitability of a particular agent depends on whether an animal experiences distress between the time it begins to inhale the agent and the time it loses consciousness. Some agents may induce

convulsions, but these generally follow loss of consciousness. Agents inducing convulsions prior to loss of consciousness are unacceptable for euthanasia. Certain considerations are common to all inhalant agents:

- In most cases, onset of loss of consciousness is more rapid, and euthanasia more humane, if the animal is rapidly exposed to a high concentration of the agent.
- The equipment used to deliver and maintain this high concentration must be in good working order and in compliance with state and federal regulations. Leaky or faulty equipment may lead to slow, distressful death and be hazardous to other animals and to personnel.
- Most of these agents are hazardous to personnel because of the risk of explosions (eg, ether), narcosis (eg, halothane), hypoxemia (eg, nitrogen and carbon monoxide), addiction (eg, nitrous oxide), or health effects resulting from chronic exposure (eg, nitrous oxide and carbon monoxide).
- Alveolar concentrations rise slowly in an animal with decreased ventilation, making agitation more likely during induction. Other noninhalant methods of euthanasia should be considered for such animals.
- Neonatal animals appear to be resistant to hypoxia, and because all inhalant agents ultimately cause hypoxia, neonatal animals take longer to die than adults. Glass et al, (1944) reported that newborn dogs, rabbits, and guinea pigs survived a nitrogen atmosphere much longer than did adults. Dogs, at 1 week old, survived for 14 minutes compared with a 3-minute survival time after a few weeks of age. Guinea pigs survived for 4.5 minutes at 1 day old, compared with 3 minutes at 8 days or older. Rabbits survived for 13 minutes at 6 days old, 4 minutes at 14 days, and 1.5 minutes at 19 days and older. The panel recommended that inhalant agents not be used alone in animals less than 16 weeks old except to induce loss of consciousness, followed by the use of some other method to kill the animal.
- Rapid gas flows can produce a noise that frightens animals. If high flows are required, the equipment should be designed to minimize noise.
- Animals placed together in chambers should be of the same species, and, if needed, should be restrained so that they will not hurt themselves or others. Chambers should not be overloaded and need to be kept clean to minimize odours that might distress animals subsequently euthanatized
- Reptiles, amphibians, and diving birds and mammals have a great capacity for holding their breath and anaerobic metabolism (AMMVA, 2007).

#### 2.7.3.1 Carbon dioxide

At concentrations above 60%, carbon dioxide acts as an anaesthetic agent and causes rapid loss of consciousness (Green, 1987). It is effective and humane for euthanasia of most small animals above 70%. Carbon dioxide stimulates the respiratory centre which may cause anxiety and stress in the animal as well as being aesthetically unpleasant for the observer. Carbon dioxide may form carbonic acid when in contact with the nasal mucous membranes which could produce a fizzy or tingling effect which may be mildly irritating to some species when applied at lower concentrations (Lucke, 1979). In most animals it is recommended to place them 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 (Forslid et a1., 1986; Blackshaw et a1., 1988). One hundred per cent $CO_2$ may cause severe dyspnoea and distress in conscious animals (van Zutphen et a1., 1993).

The advantages described for this procedure include: the rapid depressant, analgesic, and anesthetic effects of $CO_2$ are well established. Also, carbon dioxide is readily available and can be purchased in compressed gas cylinders. Carbon dioxide is inexpensive, nonflammable, nonexplosive, and poses minimal hazard to personnel when used with properly designed equipment. The euthanasia with carbon dioxide does not result in accumulation of tissue residues in food-producing animals and finally, carbon dioxide does not distort murine cholinergic markers (Bereger-Sweeney et al., 1994) or corticosterone concentrations (Urbanski & Kelly, 1991).

On the other hand, the disadvantages for using this euthanasia procedure are that $CO_2$ is heavier than air, and incomplete filling of a chamber may permit animals to climb or raise their heads above the higher concentrations and avoid exposure; some species, such as fish and burrowing and diving mammals, may have extraordinary tolerance for $CO_2$. Reptiles and amphibians may breathe too slowly for the use of CO2. Euthanasia by exposure to $CO_2$ may take longer than euthanasia by other means (Coenen et al., 1995). Also, induction of loss of consciousness at lower concentrations (< 80%) may produce pulmonary and upper respiratory tract lesions (Iwarsson & Rehbinder, 1993; Danneman et al., 1997) and must be considered that high concentrations of $CO_2$ may be distressful to some animals.

Room air contains 0.04% carbon dioxide ($CO_2$), which is heavier than air and nearly odourless. Inhalation of $CO_2$ at a concentration of 7.5% increases the pain threshold, and higher concentrations of $CO_2$ have a rapid anesthesic effect (Leake & Waters, 1929; Woodbury, 1958; Simonsen et al., 1981; Lecky, 1983; Matson et al., 1992; Klemm, 1994).

Several investigators have suggested that inhalation of high concentrations of $CO_2$ may be distressing to animals (Carding, 1968; Hoenderken, 1983; Gregory et al., 1987, Britt, 1987), because the gas dissolves in moisture on the nasal mucosa. The resulting product, carbonic acid, may stimulate nociceptors in the nasal mucosa. Some humans exposed to concentrations of around 50% $CO_2$ report that inhaling the gas is unpleasant and that higher concentrations are noxious (Anton et al., 1992; Danneman et al., 1997). Carbon dioxide has been used to euthanatize groups of small laboratory animals, including mice, rats, guinea pigs, chickens, and rabbits (Kline et al., 1963; Breazile & Kitchell, 1969; Kocula et al., 1971; Jaksch, 1981; Raj & Gregory, 1990), and to render swine unconscious before humane slaughter (Hoenderken, 1983; Gregory et al., 1987).

#### 2.7.3.2 Carbon monoxide

This causes rapid death as it combines with the red blood cells in preference to oxygen, thus causing hypoxia (Chalifoux & Dallaire 1983).There is little or no distress as there is no smell (Breazile & Kitchell, 1969; Smith et a1., 1986; Green, 1987; Blackmore, 1993). It is not acceptable for use in reptiles because of their low metabolic rate and hypoxic tolerance. It is acceptable for small animals, but in dogs and cats vocalizations and convulsions may occur after unconsciousness making it aesthetically unpleasant. Death should be confirmed by physical means.

In the past, mass euthanasia has been accomplished by use of 3 methods for generating CO: (1) chemical interaction of sodium formate and sulfuric acid, (2) exhaust fumes from idling gasoline internal combustion engines, and (3) commercially compressed CO in cylinders. The first 2 techniques are associated with problems such as production of other gases, achieving inadequate concentrations of carbon monoxide, inadequate cooling of the gas, and maintenance of equipment. Therefore, the only acceptable source is compressed CO in cylinders.

The advantages for this procedure are: carbon monoxide induces loss of consciousness without pain and with minimal discernible discomfort; hypoxemia induced by CO is insidious, so that the animal appears to be unaware and death occurs rapidly if concentrations of 4 to 6% are used.
The disadvantages are that safeguards must be taken to prevent exposure of personnel and any electrical equipment exposed to CO (eg, lights and fans) must be explosion proof.
As recommendations, must be considered that carbon monoxide used for individual animal or mass euthanasia is acceptable for dogs, cats, and other small mammals, provided that commercially compressed CO is used and the following precautions are taken: (1) personnel using CO must be instructed thoroughly in its use and must understand its hazards and limitations; (2) the CO chamber must be of the highest quality construction and should allow for separation of individual animals; (3) the CO source and chamber must be located in a well-ventilated environment, preferably out of doors; (4) the chamber must be well lit and have view ports that allow personnel direct observation of animals; (5) the CO flow rate should be adequate to rapidly achieve a uniform CO concentration of at least 6% after animals are placed in the chamber, although some species (eg, neonatal pigs) are less likely to become agitated with a gradual rise in CO concentration (Lambooy & Spanjaard, 1980); and (6) if the chamber is inside a room, CO monitors must be placed in the room to warn personnel of hazardous concentrations.

**2.7.3.3 Volatile inhalational anaesthetics**

When using any liquid anaesthetic care must be taken to ensure that it is not allowed to come in contact with the animal. Sufficient air or oxygen should be provided during the induction period to prevent hypoxia (Andrews et al., 1993). Exposure to trace concentrations of anaesthetic gases is a recognized human health hazard and requires gas scavenging apparatus to be used in the work environment. Volatile inhalational anaesthetics are neither flammable nor explosive.
Halothane 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 is a commonly used anaesthetic agent for small laboratory animals and is quick acting and stress free in overdose for euthanasia (Green, 1987). It has a depressant effect on the cardiovascular and respiratory systems. It may be preferred to halothane in situations where drug metabolism or toxicological work is being conducted as very little drug is metabolized in the liver.
Isoflurane 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.
Nitrous oxide ($N_2O$) may be used with other inhalants to speed the onset of anesthesia, but alone it does not induce anesthesia in animals, even at 100% concentration. When used by itself, $N_2O$ produces hypoxemia before respiratory or cardiac arrest. As a result, animals may become distressed prior to loss of consciousness.
The advantages of choose euthanasia with someone of these options are: Inhalant anesthetics are particularly valuable for euthanasia of smaller animals (< 7 kg) or for animals in which venipuncture may be difficult, and Halothane, enflurane, isoflurane, sevoflurane,

desflurane, methoxyflurane, and $N_2O$ are nonflammable and nonexplosive under ordinary environmental conditions.

As disadvantages, animals may struggle and become anxious during induction of anesthesia because anesthetic vapors may be irritating and can induce excitement. Ether is flammable and explosive. Explosions have occurred when animals, euthanatized with ether, were placed in an ordinary (not explosion proof) refrigerator or freezer and when bagged animals were placed in an incinerator. Another disadvantage to consider is that the induction with methoxyflurane is unacceptably slow in some species. Nitrous oxide will support combustion, and as well personnel as animals can be injured by exposure to these agents, with a potential for human abuse of some of these drugs, especially $N_2O$.

As final recommendations, in order of preference, halothane, enflurane, isoflurane, sevoflurane, methoxyflurane, and desflurane, with or without nitrous oxide, are acceptable for euthanasia of small animals (< 7 kg). Ether should only be used in carefully controlled situations in compliance with state and federal occupational health and safety regulations. It is conditionally acceptable. Nitrous oxide should not be used alone, pending further scientific studies on its suitability for animal euthanasia. Although acceptable, these agents are generally not used in larger animals because of their cost and difficulty of administration.

| **Agents** | **Classification** | **Mode of action** | **Rapidity** | **Ease of performance** | **Safety** | **Species suitability** | **Efficacy and comments** |
|---|---|---|---|---|---|---|---|
| *Blow to head* | Physical damage to brain | Direct concussion of brain tissue | Rapid | Requires skill, adequate restraint, and appropriate force | Safe | Young pigs < 3 weeks old | Must be properly applied to be humane and effective |
| *Carbon dioxide (bottled gas only)* | Hypoxia due to depression of vital centers | Direct depression of cerebral cortex, subcor-tical structures and vital centers; direct depression of heart muscle | Mode-rately rapid | Used in closed container | Minimal hazard | Non-human primates, free ranging wildlife | Effective, but time required may be prolonged in immature and neonatal animals |
| *Carbon monoxide* (bottled gas only) | Hypoxia | Combines with hemoglobin, preventing its combination with oxygen | Moderate onset time, but insidious so animal is unaware of onset | Requires appropriately maintained equipment | Extre-mely hazardous, toxic and difficult to detect | Non-human primates, free ranging wildlife | Effective, acceptable only when equipment is properly designed and operated |

| Agents | Classification | Mode of action | Rapidity | Ease of performance | Safety | Species suitability | Efficacy and comments |
|---|---|---|---|---|---|---|---|
| *Cervical dislocation* | Hypoxia due to disruption of vital centers | Direct depression of brain | Moderately rapid | Requires training and skill | Safe | Poultry, birds, lab mice, and rats (< 200 g) or rabbits (<1 kg) | Irreversible. Violent muscle contractions can occur after cervical dislocation |
| *Chloral hydrate* | Hypoxia from depression of respiratory center | Direct depre-ssion of brain | Rapid | Personnel must be skilled perform IV injection | Safe | Horses, ruminants and swine | Animals should be sedated prior to administration |
| *Decapitation* | Hypoxia due to disruption of vital centers | Direct depression of brain | Rapid | Requires training and skill | Guillotine pose potential employee injury hazard | Laboratory rodents, small rabbits, birds, some fish, amphibians, and reptiles (latter 3 with pithing) | Irreversible. Violent muscle contraction can occur after decapitation |
| *Electrocution* | Hypoxia | Direct depression of brain and cardiac fibrillation | Can be rapid | Not easily performed in all instances | Hazardous to personnel | Used primarily in foxes, sheep, swine, mink (with cervical dislocation), ruminants, animals <5 kg | Violent muscle contractions occur at same time as loss of unconsciousness |
| *Gunshot* | Hypoxia due to disruption of vital centers | Direct concussion of brain tissue | Rapid | Requires skill and appropriate firearm | May be dangerous | Large domestic and zoo animals, reptiles, amphibians, wildlife, cetaceans (<4 meters long) | Instant unconsciousness, but motor activity may continue |

| Agents | Classifi-cation | Mode of action | Rapidity | Ease of perfor-mance | Safety | Species suita-bility | Efficacy and comments |
|---|---|---|---|---|---|---|---|
| *Inhalant anesthetics* | Hypoxia due to depression of vital centers | Direct depression of cerebral cortex, subcortical structures, and vital centers | Moderately rapid onset of anesthesia; excitation may develop during induction | Easily performed with closed container; can be administered to large animals by means of a mask | Must be properly scavenged or vented to minimize exposure to personnel | Nonhuman primates, swine | Highly effective provided that subject is sufficiently exposed |
| *Nitrogen, Argon* | Hypoxia | Reduces partial pressure of oxygen available to blood | Rapid | Use closed chamber with rapid filling | Safe if used with ventilation | Cats, small dogs, birds, rodents, rabbits, other small species, mink, zoo animals, nonhuman primates, free ranging wildlife | Effective except in young and neonates; an effective agent, but other methods preferable |
| *Penetrating captive bolt* | Physical damage to brain | Direct concussion of brain tissue | Rapid | Requires skill, adequate restraint, and proper placement of captive bolt | Safe | Dogs, rabbits, zoo animals, reptiles, amphibians, free ranging wildlife | Instant loss of consciousness but motor activity may continue |
| *Pithing* | Hypoxia due to disruption of vital centers, physical damage to brain | Trauma of brain and spinal cord tissue | Rapid | Easily performed, but requires skill | Safe | Some ectotherms | Effective, but death not immediate unless brain and spinal cord are pithed |
| *Thoracic compression* | Hypoxia and cardiac arrest | Physical interference with cardiac and respiratory function | Moderately rapid | Requires training | Safe | Small to medium sized free ranging birds | Apparently effective |

Table 2. Summary of Conditionally Acceptable Agents and Methods of Euthanasia - Characteristics and Modes of Action

### 2.8 Recommended methods of euthanasia in each species

Below are described the main methods of euthanasia suggested for the various species that can be used in the laboratory. A summary of these recommendations indicating characteristics of the euthanasia methods is presented on table 2.

#### 2.8.1 Fish

There are over 20 000 species of fish with enormously varying lifestyles which makes it very difficult to generalize on methods of euthanasia. 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 anyone 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.

In general, larvae and adults can be euthanized by:

Physical methods: Concussion, Cervical dislocation (Clifford, 1984) and Maceration. Cervical dislocation 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 must be choose only for small fish of less than 2 cm 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.

The most common chemical agents used are: Tricaine methane sulphonate (buffered MS-222), Benzocaine (ethyl aminobenzoate), Etomidate, Metomidate, Quinaldine (2-methylquinoline), Halothane, injectable agents (barbiturates).

The methods considered as acceptable for unconscious fish are decapitation and exsanguinations. By other hand, the methods not acceptable for euthanasia of fish are : removal from water, whole body crushing, electrical stunning, hypothermia, hyperthermia, carbon dioxide, diethyl ether, Urethane, Chloral hydrate, tertiary amyl alcohol, tribromoethanol, chlorobutanol, Methyl pentynol, pyridines.

#### 2.8.2 Amphibians

Because amphibians are ectothermic and thus accustomed to fluctuations in body temperature, their central nervous system (CNS) is less sensitive to hypoxia and anoxia. Even when the cranial nerves and brain are deprived of 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 (AVMA, 2007).

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 (Close et al., 1996)

For those reasons, death may be recognized by cessation of heartbeat and respiration and in cases where this is not obvious; it may be confirmed by destruction of the brain. In 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 anesthesia, followed by death. For adults, 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. However, the physical methods for adult amphibians include: Concussion, microwave and electrical stunning. The procedures where chemical agents are considered, usually are used: Tricaine methane sulphonate (buffered MS-222), Benzocaine, sodium pentobarbitone, T-61. The methods considered as acceptable for unconscious amphibians are: Pithing and decapitation. Contrarily, the methods not acceptable for euthanasia of amphibians are: hypothermia, hyperthermia, exsanguinations, strangulation, carbon dioxide, ether, chloroform, volatile inhalational anesthetics, chloral hydrate, ketamine, hydrochloride, chlorbutanol, methylpentynol, 2-phenoxyethanol, tertiary amyl alcohol, tribromoethanol, and urethane (Close et al., 1996; Reilly, 2001).

### 2.8.3 Reptiles

Similarly to the amphibians, reptiles are also ectothermic and even when the cranial nerves and brain are deprived of blood supply following decapitation; they 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. For that reason, 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 types of snake, especially when they are not used to being handled (Close et al., 1996). 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. 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 (Reilly, 2001).

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. 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 possess 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 (Close et al., 1996).

For eggs of reptiles, are recommended methods would include disruption of the egg and killing of the embryos by injection of sodium pentobarbitone, anaesthetic overdose or an appropriate physical method to destroy the brain or whole egg or early life form. For all practical purposes, all newly hatched reptiles can be treated inthe same way as 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. Physical accepted methods include the captive bolt, concussion and shooting. The chemical method considered is to overdoses of pentobarbital by intraperitoneal route. The methods acceptable for unconscious reptiles are phithing and decapitation; contrarily between the methods not acceptable for euthanasia of reptiles are: spinal cord severance, hypothermia or hyperthermia, exsanguination, chloroform, and tricaine methane sulphonate (MS-222). Because reptiles are capable of holding their breath for a relatively long period of time and therefore inhalational methods cannot be considered as practicable or humane due to slow induction (Close et al., 1996; AVMA, 2007).

### 2.8.4 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. The dead of birds, Death may be recognized by the absence of signs of breathing, cardiac arrest and absence of reflexes in the head (Close et al., 1996). Reflexes to be checked would include pinching of wattles or blink reflexes. The most commonly used method of destroying eggs is cooling or freezing. The recommended temperature is<4°C for 4 h. In cases where the embryo has been exposed to experimental conditions for studies, decapitation is considered an acceptable method of euthanasia as is an overdose of anaesthetic (AVMA, 2007).

For adult birds, the physical methods considered are: cervical dislocation, maceration, concussion, microwave and electrical stunning (Reilly, 2001). The chemical methods for euthanasia in birds are inhalational agents (carbon dioxide, volatile inhalational anaesthetics, carbon monoxide) and injectable agents (Sodium pentobarbitone, T-61). The acceptable methods for unconscious in birds include decapitation, pithing and potassium chloride. And the methods not acceptable for euthanasia of birds are neck crushing, exsanguination, decompression creating a vacuum, nitrous oxide, ether/chloroform, cyclopropane, hydrogen cyanide gas and must be consideder that there are other agents which have not to be used (methoxyflurane, trichlorethylene, chloral hydrate, strychnine, nicotine, magnesiumsulphate, ketamine alone and neuromuscular blocking agents)(Close et al., 1996).

### 2.8.5 Rodents

Rodents are the most commonly used animals for experimental purposes and include mice, rats, hamsters, guinea pigs, gerbils, shrews, and dormice (Close et al., 1996; AVMA, 2007).

Physical methods for rodents include concussion, cervical dislocation (only animals under 150 g), decapitation, and rapid freezing (only for fetus and neonates) (Reilly, 2001). There are many chemical methods including: the use of Inhalational agents, as volatile inhalational anaesthetics (Halothane, enflurane, isoflurane), carbon dioxide. Also are used injectable agents like sodium pentobarbitone (diluted, because it can cause irritation of the peritoneum). The methods acceptable for unconscious rodents are: rapid freezing (only for rodents <4g), exsanguination, air embolism, potassium chloride and ethanol. The methods not acceptable for euthanasia of rodents are hypothermia, nitrogen, nitrous oxide, cyclopropane, ether, chloroform, decompression, asphyxia, drowning, trichlorethylene, methoxyflurane, hydrogen cyanide gas, carbon monoxide, strychnine, nicotine, chloral hydrate, magnesium sulphate, curariform drugs and other neuromuscular blocking agents (see table 3) (Close et al., 1996, Reilly, 2001).

| Techniques | Recommended | Acceptable with reservations | Not acceptable |
|---|---|---|---|
| **Chemical** | | | |
| **Inhalant** | Carbon dioxide 💣 | Halothane ☣ | Either ☣👎<br>Hydrogen cyanide ☣☹<br>Carbon monoxide ☣<br>Nitrogen ☣<br>Chloroform |
| **Injectable** | Pentobarbitone sodium Ip (diluted) | Ethanol Ip | |
| | | | |
| Physical | | Cervical dislocation | Microwave irradiation –not yet proven to be humane 💣 |
| | | Possibly👎 in animals heavier than 150 g (acceptable if stunned or anaesthesied first) | Decompression ☹💣 |
| | | Decapitation ☹📖 | Asphyxia ☹👎💣 |
| | | Stunning and exsanguinations ☹📖 | Rapid freezing (unconscious and <4 g) 👎💣 |
| 💣 Requires specialised equipment 📖 Training required<br>☣ Occupational health and Safety Issues $ Expensive<br>☹ Aesthetically unpleasant 👎 Inhumane | | | |

Table 3. Recommended, acceptable with reservation and not accepted methods for euthanasia in rodents (Reilly, 2001).

### 2.8.6 Rabbits

Death must be recognized and confirm with the absence of reflexes. Must be confirm with exsanguinations, evisceration or decapitation. To euthanize embryos, they must be removed for decapitation, with a previous administration of increased amount of anaesthetic to the dam for longer to ensure that the anesthetic has crossed placenta. The 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 (Reilly, 2001).

The physical methods of killing adult rabbits include: concussion, cervical dislocation, captive bolt, decapitation, electrical stunning, and microwave. The chemical methods include inhalational methods with volatile agents (Halothane, isoflurane, enflurane, carbon dioxide, and carbon monoxide) or injectable agents.

The methods considered as acceptable for unconscious rabbits are: Exsanguination, nitrogen, potassium chloride and air embolism (5-50 mL/Kg) (AVMA, 2007). The non acceptable methods for euthanasia of rabbits are: nitrous oxide, methoxyflurane, clyclopropane, ether and chloroform and ketamine hydrochloride. 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 (Close et al., 1996).

### 2.8.7 Carnivores (dogs, cats and ferrets)

Recognition and confirmation of death must be verified. Cessations of respiration and heartbeats, as well loss of reflexes are good indicators of death in carnivores. To euthanize embryos, must be considered similar indications as previously described for rabbits. Neonate carnivores should generally be treated as adults. Sodium pentobarbitone is the preferred method, but $CO_2$, cervical dislocation and concussion may be considered (Hall, 1972).

For adults, the physical procedures includes: Captive bolt, shooting and electrocution. The chemical methods used for carnivore adults are: Inhalational methods (with volatile inhalational anaesthetics), injectable agents (sodium pentobarbitone, secobarbital/dibucaine and T-61). The acceptable methods for unconscious carnivores includes: exsanguination, dislocation of neck, and potassium chloride; and the not acceptable methods for carnivores are stricking of chest of cats, decompression, carbon dioxide, carbon monoxide, nitrogen, ether and chloroform (AVMA, 2007). 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 (Close et al., 1996).

Personnel using and having to kill any large mammal must receive special 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. It may be necessary to take the animals to approved slaughterhouses where specialized 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 (Close et al., 1996).

Cessation of respiration and heartbeat, and loss of reflexes are good indicators of irreversible death in these species. Death should be confirmed by exsanguination. 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 (Reilly, 2001). Foetuses may also be large and in general any method used on an adult is considered acceptable. Because the neonates of large mammals are born in an advanced stage of development, they should be treated as adults.

Physical methods for adults must be considered: captive bolt, free bullet, shooting, concussion and electrical stunning (Blackmore, 1979). The chemical methods include: inhalational methods, with volatile inhalational anaesthesics (halothane, isoflurane, enflurane using a mask for kids and lambs) and carbon dioxide –only for pigs - (death must be confirmed by exsanguination) but other methods are considered preferable and carbon dioxide must not be used on any other large animal (AVMA, 2007). Other methods acceptable for unconscious large mammals are exsanguination, potassium chloride and the intravenously administration of chloral hydrate in conjunction with magnesium sulphate and sodium pentobarbitone. The methods not acceptable for euthanasia of large mammals are methoxyflurane, trichlorethylene, strychnine, nicotine, magnesium sulphate, thiopentone sodium, ketamine hydrochloride, curariform drugs and other neuromuscular blocking agents (Close et al., 1996).

### 2.8.8 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 (Reilly, 2001). Cessation of heartbeat and respiration, and absence of reflexes may be considered as indicators of death. The only recommended method for killing primates is by overdose of anaesthetic. Sodium pentobarbitone injected intravenously is the most acceptable agent. Exsanguination under inhalation anesthesia is also considered acceptable, but this must be followed by perfusion (Close et al., 1996; AVMA, 2007).

### 2.8.9 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 (Close et al., 1996).

## 3. Conclusion

The use of animals in the laboratory is very useful as these are excellence models for the evaluation and development of multiple scientific studies. It is for this reason that researchers need to consider that in the course of these animal experiments, assure that they have a good quality of life and likewise, must choose the right method to perform euthanasia in order to avoid stress, anxious or pain when it is done.

## 4. References

Agriculture Canada (1989) Recommended code of practice for the care and handling of poultry from hatchery to processing plant. Publication 1757/E. 1989. Ottawa: Agriculture Canada. 12.02.2011. Available from: http://www.agr.gc.ca/poultry/pub1757e.pdf

American Association of Avian Pathologists (2005) Animal Welfare and Management Practices Committee: Review of mechanical euthanasia of day-old poultry. Athens, Ga.

Andrews, E.J.; Bennett, T.; 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*, Vol.202, No.2, (January 1993), pp. 229-249, ISSN 0003-1488

Anil, M.H. & McKinstry, J.L. (1991) Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *Veterinary Record* Vol. 128, No. 5 (February, 1991) pp. 106–107. ISSN 0042-4900

Anton, F.; Euchner, I. & Handwerker, H.O. (1992) Psycophysical examination of pain induced by defined CO2 pulses applied to nasal mucosa. *Pain* Vol. 49, No. 1 (April, 1992) pp.53–60 ISSN 0304-3959

Baumans, V. (2005) Science-based assessment of animal welfare: laboratory animals. *Revue Scientifique et Technique* Vol. 24, No. 2 (August, 2005) pp. 503-514 ISSN 0253-1933

Bereger-Sweeney, J.; Berger, U.V.; Sharma, M. & Paul, C.A. (1994). Effects of carbon dioxide-induced anesthesia on cholinergic parameters in rat brain. *Laboratory Animal Science*, Vol. 44, No. 4 (August, 1994) pp. 369–371. ISSN 0023-6764

Berman, E.; King, J.B.; All, J.; Carter, H.B.; Rehnberg, B. & Stead, A.G. (1985) Lethality ill mice and rats exposed to 2450 MHz circulatory polarized microwaves as a function of exposure duration and environmental factors. *Journal of Applied Toxicology* Vol. 5, No 1 (February, 1985) pp.23-31. ISSN 0260-437X

Blackmore, D.K. (1993) Euthanasia; not always eu. *Australian Veterinary Journal* Vol. 70, No. 11 (November, 1993) pp. 409-13 ISSN 0005-0423

Blackmore, D.K. (1985) Energy requirements for the penetration of heads of domestic stock and the development of a multiple projectile. *Veterinary Record* Vol. 116, No. 2 (January, 1985) pp. 36–40. ISSN 0042-4900

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* Vol. 22, No. 1 (January, 1988) pp. 67-75 ISSN 0093-7355

Breazile, J.E. & Kitchell, R.L. (1969) Euthanasia for laboratory animals. Federal Proceedings Vol. 28, No. 4 (July, 1969) pp. 1577-1579 ISSN 0014-9446

Britt, D.P. (1987) The humaneness of carbon dioxide as an agent of euthanasia for laboratory rodents. In: Euthanasia of unwanted, injured or diseased animals for educational or scientific purposes. *University Federation for Animal Welfare* (UFAW) pp. 19–31 Potters Bar, UK

Carding, A.H. (1968) Mass euthanasia of dogs with carbon monoxide and/or carbon dioxide: preliminary trials. *Journal Small Animal Practice* Vol. 9, No. 5 (May, 1968) pp. 245–259. ISSN 0022-4510

Chalifoux, A. & Dallaire, A. (1983) Physiologic and behavioral evaluation of CO euthanasia of adult dogs. *American Journal of Veterinary Research*. Vol. 44, No. 12 (December, 1983) pp. 2412-2417. ISSN 0002-9645

Clifford, D.H. (1984) Preanesthesia, anaesthesia, analgesia, and euthanasia. In: Fox, J.G.; Cohen, B.J. & Loew, F.M. eds. *Laboratory Animal Medicine*. Academic Press Inc pp. 528–563. ISBN 0122639510 New York

Close, B.; Banister, K.; Baumans, V.; Bernoth, E.M.; Bromage, M.; Bunyan, J.; Erhardt, W.; Flecknell, P.; Gregory, N.; Hackbarth, H.; Morton, D. & Warwick, C. (1996). Recommendations for euthanasia of experimental animals: Part 1. DGXI of the European Commission. *Laboratory Animals* Vol. 30, No. 4 (October, 1996) pp. 293-316 ISSN 0093-7355

Coenen, A.M.; Drinkenburg, W.H.; Hoenderken, R. & van Luijtelaar, E.L. (1995) Carbon dioxide euthanasia in rats: oxygen supplementation minimizes signs of agitation and asphyxia. *Laboratory Animals* Vol. 29 No. 3 (July, 1995) pp. 262–268 ISSN 0093-7355

Commission of the European Communities (1993) *Council directive on the protection of animals at the time of slaughter or killing*. 93/119/EC. No L340/21. 03.05.2011. Available from: http://ec.europa.eu/food/animal/welfare/references_en.htm

Daly, C.C. & Whittington, P.E. (1989) Investigation into the principal determinants of effective captive bolt stunning of sheep. *Research Veterinary Science* Vol. 46, No. 3 (May, 1989) pp.406-408 ISSN 0034-5288

Danneman, P.J.; Stein, S. & Walshaw, S.O. (1997) Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Laboratory Animal Science* Vol. 47, No. 4 (August, 1997) pp. 376–385 ISSN 0023-6764

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* Vol. 38 No. 4 (August, 1988) pp.459–462 ISSN 0023-6764

Derr, R.F. (1991) Pain perception in decapitated rat brain. *Life Sciences* Vol. 49, No. 19 pp. 1399–1402. ISSN 0024-3205

Dodd, K. (1985) Humane euthanasia. I. Shooting a horse. *Irish Veterinary Journal* Vol. 39, No. 3 pp. 150-151. ISSN 0368-0762

Eikelenboom, G. (1983) *Stunning of Animals for Slaughter. Current Topics in Veterinary Medicine*. 1st ed. Martinus Nijhoff Publishers pp. 1–227. ISBN 0898385989. Boston Massachusetts.

European Council (1993) European Council Directive 93/119/EC of 22 December 1993 on the protection of animals at the time of slaughter or killing. Annex G: killing of surplus chicks and embryos in hatchery waste. Brussels: European Council. 05.03.2011, Available from: http://ec.europa.eu/food/fs/aw/aw_legislation/slaughter/93-119-ec_en.pdf

Feldman, D.B. & Gupta, B.N. (1976) Histopathologic changes in laboratory animals resulting from various methods of euthanasia. *Laboratory Animal Science* Vol. 26, No. 2 (April, 1976) pp. 218-221 ISSN 0023-6764

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* Vol. 127, No. 3 (July, 1986) pp. 281-287 ISSN 0001-6772

Green, C.J. (1987) Euthanasia. In: *Laboratory Animals: An Introduction for New Experiments*. (Tuffery, A.A. ed.). John Wiley &. Sons, pp. 171-177 ISNB 978-0471952572 Hoboken, NJ

Gregory, N.G.; Moss, B.W. & Leeson, R.H. (1987) An assessment of carbon dioxide stunning in pigs. *Veterinary Record* Vol. 121, No. 22 (November, 1987) pp.517–518 ISSN 0042-4900

Gregory, N.G. & Wotton, S.B. (1986) Effect of slaughter on spontaneous and evoked activity of the brain. *British Poultry Science* Vol. 27, No. 2 (June, 1986) pp.195–205. ISSN 0007-1668.

Ikarashi, Y.; Marvyama, Y. & Stavinoha, W.B. (1984) Study of the use of the microwave magnetic field for the rapid inactivation of brain enzymes. *Japanese Journal of Pharmacology* Vol. 35, No. 4 (August, 1984) pp. 371–387. ISSN 0021-5198.

Hoenderken, R. (1983) Electrical and carbondioxide stunning of pigs for slaughter. In: Eikelenboom, G. ed. *Stunning of Animals for Slaughter* Martinus Nijhoff Publishers, pp. 59-63 ISBN 978-0898385984, Boston Massachusetts.

Holson, R.R. (1992) Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicology and Teratology* Vol. 14, No. 4 (July, 1992) pp.253–257. ISSN 0892-0362

Iwarsson, K. & Rehbinder, C. (1993) A study of different euthanasia techniques in guinea pigs, rats, and mice. Animal response and postmortem findings. *Scandinavian Journal Laboratory Animal Science* Vol. 20 pp.191–205. ISSN 09013393

Jaksch, W. (1981) Euthanasia of day-old male chicks in the poultry industry. *International Journal for the Study of Animal Problems* Vol. 2 pp.203–213. ISSN: 0195-7554

Kitchell, R.L.; Erickson, H.H.; Carstens, E. & Davis L.E. (1983). *Animal pain: perception and alleviation*. Bethesda: American Physiological Society. pp 221. ISBN 068304625X Baltimore, USA

Kitchen, N.; Aronson, A.L.; Bittle, J.L.; McPherson, C.W.; Morton, D.B.Pakes, S.P.; Rollin, B.; Rowan, A.N.; Sechzer, J.A.;Vanderlip, J.E.; Will, J.A.; Clark, A.S. & Gloyd J.S. (1987) Panel report on the colloquium on recognition and alleviation of animal pain and distress. *Journal American Veterinary Medical Association* Vol. 191, No. 10 (November, 1987) pp.1186–1191. ISSN 0003-1488

Klemm, W.R. (1964) Carbon dioxide anesthesia in cats. *American Journal Veterinary Research* Vol. 25 (July, 1964) pp.1201–1205 ISSN 0002-9645.

Kline, B.E.; Peckham, V. & Heist, H.E. (1963) Some aids in handling large numbers of mice. *Laboratory Animal Care* Vol. 13 (April, 1963) pp. 84–90 ISSN 094-5331

Kocula, A.W.; Drewniak, E.E- & Davis, L.L. (1961).Experimentation with in-line carbon dioxide immobilization of chickens prior to slaughter. *Poultry Science* Vol. 40 pp. 213–216. ISSN 1537-0437

Lambooy, E. & Spanjaard, W. (1980) Euthanasia of young pigs with carbon monoxide. *Veterinary Record* Vol. 107, No. 3 (July, 1980) pp. 59–61. ISSN 0042-4900

Lambooy, E. & van Voorst, N. (1986) Electrocution of pigs with notifiable diseases. *Veterinary Quarterly* Vol. 8, No. 1 (January, 1986) pp. 80–82 ISSN 0165-2176

Leake, C.D. & Waters, R.M. (1929) The anesthetic properties of carbon dioxide. *Current Researches in Anaesthesia and Analgesia* Vol. 8, No. 1 (December, 1929) pp. 7–19 ISSN 0003-2999

Lecky, J.H. (1983) Waste anesthetic gases in operating room air: a suggested program to reduce personnel exposure. *The American Society of Anesthesiologists*, Park Ridge Illinois USA.

Longair, J.A.; Finley, G.G.; Laniel, M.A.; Mackay, C.; Mould, K.; Olfert, E.D.; Rowsell, H. & Preston A. (1991) Guidelines for euthanasia of domestic animals by firearms. *Canadian Veterinary Journal* Vol. 32, No.12 (December, 1991) pp. 724-726.ISSN 0008-5286

Lucke, I.N. (1979) Euthanasia in small animals. *Veterinary Record* Vol. 104, No. 14 (April, 1979) pp. 316-18 ISSN 0042-4900

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 Veterinary Research* Vol. 33, No. 10 (October, 1972) pp.2043–2049. ISSN 0002-9645.

Mikeska, J.A. & Klemm, W.R. (1975) EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. *Laboratory Animal Science* Vol. 25, No. 2 (April, 1975) pp.175–179. ISSN 0023-6764

National Research Council (1992) Recognition and alleviation of pain and distress in laboratory animals. *Committee on Pain and Distress in Laboratory Animals Institute of Laboratory Animal Resources Commission on Life Sciences*. National Academy Press. ISBN 0-309-04275-5 Washington, DC.

Olfert, E.D.; Cross, B.M., & McWilliam, A.A. (1993) Guide to the care and use of experimental animals, Volume 1. *Canadian Council on Animal Care*, Ottawa, Canada. ISBN: 0-919087-18-3

Oliver, D.F. (1979) Euthanasia of horses. *Veterinary Record* Vol. 105, No. 10 (September, 1979) pp. 224-245. ISSN 0042-4900

Raj, A.B.M. & Gregory, N.G. (1990) Investigation into the batch stunning/killing of chickens using carbon dioxide or argon-induced hypoxia. *Research Veterinary Science* Vol. 49, No. 3 (November, 1990) pp. 364–366. ISSN 0034-5288

Reilly, J.S. (2001) Euthanasia of Animals Used for Scientific Purposes. *Australian and New Zealand Council for the Care of Animals in Research and Teaching* 2nd edition. Adelaide University ISBN 0 9586821 4 3 Adelaide Australia.

Roberts, T.D.M. (1954) Cortical activity in electrocuted dogs. *Veterinary Record* Vol. 66, No.5 pp.561–567. ISSN 0042-4900

Schatzmann, U.; Howard, J.; Pittino, J. & Fuchs, P. (1993) Jet injection for the stunning of slaughter pigs. *Fleischwirtschafst* Vol 73, No. 9 pp. 1027-1028 ISSN 0015-363X

Schatzmann, V.; Leuenberger, T.; Fuchs, P.; Howald, M. & Howard, J. (1991) Jet injection: the possibility of using a high pressure water jet for the stunning of slaughter pigs. *Fleischwirtschaft* Vol. 71, No. 8 pp 899-901 ISSN 0015-363X

Simonsen, H.B.; Thordal-Christensen, A.A. & Ockens, N. (1981) Carbon monoxide and carbon dioxide euthanasia of cats: duration and animal behavior. *British Veterinary Journal* Vol. 137, No. 3 (May, 1981) pp. 274-278. ISSN 0007-1935

Stavinoha, W.B.; Frazer, J. & Modak, A.T. (1978) Microwave fixation for the study of acetylcholine metabolism. In: Jenden, D.J. ed. Cholinergic mechanisms and psychopharmacology. *Proceedings of a Symposium on Cholinergic Mechanisms and Psychopharmacology*, held in La Jolla, California, March 28-31, 1977 Plenum Publishing Corp, 169–179. ISBN 0306379244 New York

Stavinoha, W.R. (1983) Study of brain neurochemistry utilizing rapid inactivation of brain enzyme activity by heating and mirowave irradiation. In: Black, C.L.; Stavinoha, W.B. & Marvyama, Y. eds. *Microwave irradiation as a tool to study labile metabolites in tissue* Pergamon Press; 1–12 Elmsford, NY

Urbanski, H.F. & Kelly, S.F. (1991) Sedation by exposure to gaseous carbon dioxide-oxygen mixture: application to studies involving small laboratory animal species. *Laboratory Animal Science* Vol. 41, No. 1 (January, 1991) pp. 80–82. ISSN 0023-6764

Van Zutphen, L.F.M. (2001). History of animal use. In: *Principles of Laboratory Animal Science* Van Zutphen, L.F.M.; Baumans, V. & Beynen A.C. eds. Elsevier, 2-5. ISBN 0444506128, Amsterdam

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* Vol. 451, No 1-2 (June, 1988) pp. 340–344. ISSN 0006-8993

Vierck, C.J.; Cooper, B.Y.; Ritz, L.A. & Greenspan, J.D. (1989). Inference of pain sensitivity from complex behaviors of laboratory animals. In: Chapman, C.R. & Loeser, J.D. eds. *Issues in Pain Measurement: Advances in Pain research and Therapy*. Raven Press, 93–115. ISBN 10 088167530X New York.

Wall, P.D. (1992) Defining pain in animals. In: Short, C.E. & Poznak, A.V. eds. *Animal pain*. Churchill-Livingstone Inc. 63-79. ISBN 978-0721659374, New York

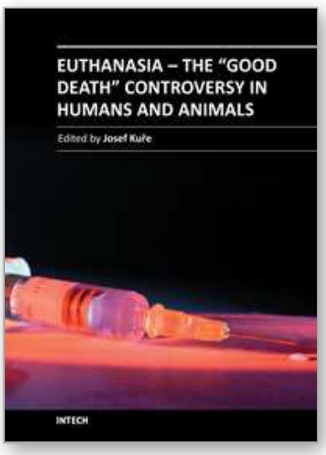
Warrington, R. (1974) Electrical stunning, a review of the literature. *Veterinary Bulletin* Vol. 44, No.10 pp. 617–635. ISSN 0042-4854

Woodbury, D.M.; Rollins, L.T.; Gardner, M.D.; Hirschi, W.L; Hogan, J.R.; Rallison, M.L.; Tanner, G.S. & Brodie, D.A (1958) Effects of carbon dioxide on brain excitability and electrolytes. *American Journal of Physiology* Vol. 192, No. 1 (January, 1958) pp.79–90. ISSN 0002-9513

World Organization for Animal Health (OIE). (2006) Terrestrial animal health code, appendix 3.7.6: *Guidelines for the killing of animals for disease control purposes*. 15.03.2011. Available from http://www.oie.int/print.php?p=http://www.oie.int/eng/normes/mcode/en_chapitre_3.7.6.htm

Zeller von, W.; Mettler, D. & Schatzmann, U. (1989) Untersuchungen zm tierschutzgerechten Betiiubung des Schlachtgeflugels mit Mikrowellen 12550 MHzl. *Deutsche Tierdrtzliche Wochenschrift* Vol.96, pp. 285-332. ISSN: 0341-6593.

Zimmerman, M. (1984). Neurobiological concepts of pain, its assessment and therapy. In: Bromm B., ed. *Pain measurement in man. Neurophysiological correlates of pain*. Elsevier Publishing Co. 15–35. ISBN-10: 0444805710, Amsterdam.




**Euthanasia - The "Good Death" Controversy in Humans and Animals**

Edited by Prof. Josef KuÅ™e

ISBN 978-953-307-260-9
Hard cover, 248 pages
**Publisher** InTech
**Published online** 15, September, 2011
**Published in print edition** September, 2011

No one really wants to die, or do they? From classical times to our post-modern era of medical high tech, societies have struggled with the thorny issue of euthanasia, and what it entails. Who shall be entitled to a "good death" and in what form shall it arrive? This book provides the reader with insight and enlightenment on the medical, philosophical, social, cultural and existential aspects of "good death" amid our digitized, individualized and ageing society, hampered by rising health care costs but unchained from one standardized level of care.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Matilde Jiménez-Coello, Karla Y. Acosta-Viana, Antonio Ortega-Pacheco and Eugenia Guzmán-Marín (2011). Medical and Bioethical Issues in Laboratory Animal, Euthanasia - The "Good Death" Controversy in Humans and Animals, Prof. Josef KuÅ™e (Ed.), ISBN: 978-953-307-260-9, InTech, Available from: http://www.intechopen.com/books/euthanasia-the-good-death-controversy-in-humans-and-animals/medical-and-bioethical-issues-in-laboratory-animal

INTECH
open science | open minds

**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

**InTech China**
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen