“Welfare Aspects of Animal Stunning and Killing methods”

Scientific Report of the Scientific Panel for Animal Health and Welfare on a request from the Commission related to welfare aspects of animal stunning and killing methods

(Question N° EFSA-Q-2003-093)

Accepted on the 15th of June 2004

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1. TERMS OF REFERENCE

1.1. BACKGROUND

The EFSA (European Food Safety Authority) Scientific Panel on Animal Health and Welfare (AHAW) was asked by the Commission to report on the welfare aspects of the main systems of stunning and killing the main commercial species of animals with consideration of Directive 93/119/EC.

The mandate was accepted by the AHAW Panel at the first Plenary meeting, on the 21st of May 2003. It was decided to establish a Working Group of AHAW experts (TWG) chaired by one Panel member. Therefore the Plenary entrusted a Scientific Report to a working group under the Chairmanship of Professor H. Blokhuis. The members of the working group are listed at the end of this report.

This Scientific Report is considered as basis for the discussion to establish the relevant conclusions and recommendations by the AHAW Panel.

1.2. MANDATE

The working group was requested by the EFSA AHAW Scientific Panel to draft a scientific report including the most recent and available scientific data on the welfare aspects of the main systems of stunning and killing the main commercial species of animals with consideration of Directive 93/119/EC.

The Directive applies inside and outside slaughterhouses, and when animals are killed for the purpose of disease control. Annex C to the Directive provides a specific list of permitted methods of stunning and killing animals (except for animals kept for fur production and surplus chicks in hatchery) with specific requirements for each method. Annex E concerns killing methods for disease control purposes and mainly refers to Annex C. Article 13 (2) provides that annexes shall be amended in order to adapt them to technological and scientific progress. In addition, it calls for the Commission to submit to the Council a report drawn up on the basis of an opinion from the competent Scientific Committee with possible appropriate proposals in relation to Annex C (free bullet pistols, gases, combined methods, other methods). Furthermore it requires that the Commission shall submit a report to the Standing Committee on the Food Chain and Animal Health (SCFCAH) drawn up on the basis of an opinion from the competent Scientific Committee, together with appropriate proposals with a view to laying down:

- the strength and duration of use of the current necessary to stun the various species concerned;
- the gas concentration and length of exposure necessary to stun the various species concerned;

Since the adoption of the Directive in 1993, the competent Scientific Committee has adopted three scientific opinions on these matters:

- Report on the Slaughter and Killing of Animals (ScVC, 1996).

- The Killing of Animals for Disease Control Purposes (ScVC, 1997).
- The use of Mixtures of the Gases carbon dioxide, oxygen and nitrogen for Stunning or Killing Poultry (SCAHAW, 1998).

However no amendment to the Directive has yet been proposed. Several reasons support reviewing the current Directive. In particular:

- Since 1993, there has been scientific and technological progress in this area.
- In light of the development of new methods of stunning and killing, a uniform approach within the Community is necessary.
- Concerns have been expressed in relation to the methods by which animals were stunned and killed in field conditions under disease control measures.
- The Scientific Steering Committee expressed, in September 2001, a preliminary opinion on Stunning Methods and BSE risks (SSC, 2001). Since captive bolt stunning is most commonly used for stunning adult bovine animals in the Community, there is an important need to examine the welfare aspects of alternative methods to the captive bolt.

Consequently, a comprehensive review is necessary in order to verify the extent to which previous scientific opinions are still valid.

Species referred in the present opinion are: cattle, sheep, pigs, poultry, horses and farmed fish.

1.3. SCOPE OF REPORT

The report presents the scientific basis of consciousness and stunning. It then presents available methods commonly used to stun/kill animals and, for each of them, the following three areas are developed:

- The minimal conditions by which the method is likely to be effective from the animal welfare point of view in field conditions;
- The criteria or procedures that could ensure that the stunning and the killing method is properly enforced;
- The advantages and disadvantages of the method used, taking into account the commercial/field conditions.

Two separate contexts are considered: stunning and killing methods used in slaughterhouses and those used for disease control measures.

Slaughter without stunning is also considered.

2. ABBREVIATIONS

A: ampere

AC: alternating current

AEPs: auditory evoked potentials

AHAW: Animal health and welfare

AP: action potential

AVMA: American Veterinary Medical Association

BSAEPs: brain stem auditory evoked potentials

BSE: bovine spongiform encephalopathy

cm: centimeter

CNS: central nervous system

CSF: cerebrospinal fluid

DC: direct current

EAA: excitatory amino acid

EFSA: European Food Safety Authority

EEG: electroencephalogram

EC: European Communities

ECG: electrocardiogram

ECoG: electrocorticogram

E.g.: example given

EU: European Union

EPSP: excitatory post synaptic potential

g: gram

GABA: gamma amino butyric acid

HALF: High Amplitude, Low Frequency waves

Hg: mercure

Hz: Hertz (frequency of electrical current or activity)

IAA: inhibitory amino acid

ICES: International Classification of Epileptic Seizures

IPSP: inhibitory post synaptic potential

ILAE: International League Against Epilepsy

J: Joules

l: litre

m: meter

mA: milliampere

min: minutes

mm: millimeter

mV: millivolts

NMDA: N-methyl-D-aspartate

Pers. comm.: personnal communication

PDS: paroxysmal depolarisation shift

RMP: resting membrane potential

RMS: root mean square current of an AC

ScVC : EU Scientific Veterinary Committee

SCAHAW : EU Scientific Committee on Animal Health and Animal Welfare

SD: standard deviation

SE: standard error

Sec: seconds

SEPs: somatosensory evoked potentials

V: volts

VEPs: visual evoked potentials

VOR: vestibule-ocular reflex

3. GLOSSARY

Anoxia: depletion of oxygen in atmosphere or blood.

Aversion: a tendency to show behaviour to avoid or withdraw from a situation which is associated with a noxious stimulus.

Chest sticking: severing major blood vessels in the thorax or chest by inserting a knife in front of the brisket or sternum (double cut: first the skin, then, with another knife, the vessels).

Blood splashing: Occurs before or during the stunning and subsequent slaughter process when blood leaves the blood vessels due to an excessive blood pressure or traumas of the vessels. It can be seen in small spots or bigger infiltration of whole muscles.

Clonic seizure: convulsions normally occurring as kicking or paddling movement of legs.

Corneal reflex: blinking response to touching the eyeball.

Coulomb: 1 Coulomb (C) = 1 Ampere (A) x second (s); C [A·s]; = electric charge quantity. To describe the total quantity of the electrical charge flowing through the animal during the stunning procedure, the current (A) during the stunning time (s) has to be registered. The electric charge quantity is expressed in the standard SI unit Coulomb (C).

Death: a physiological state of an animal, where respiration and blood circulation have ceased as the respiratory and circulatory centers in the Medulla Oblongata are irreversibly inactive. Due to the permanent absence of nutrients and oxygen in the brain, consciousness is irreversibly lost. In the context of application of stunning and stun/kill methods, the main clinical signs seen are absence of respiration (and no gagging), absence of pulse and absence of corneal and palpebral reflex and presence of pupillary dilation.

Electroencephalogram: electrical activity of the brain usually recorded from the surface of the skull using non-invasive techniques.

Electrocorticogram: electrical activity of the brain usually recorded on the surface of the brain or dura (a membrane covering the brain).

Evoked potentials: evoked activity in the brain using visual auditory or somatosensory stimuli.

Gagging or gasping: rudimentary respiratory activity occurring through mouth (oral breathing).

Generalised epilepsy: a pathological state of brain, involving both the cerebral hemispheres, occurring due to extreme neuronal synchrony leading to unconsciousness.

Grand mal epilepsy: Grand mal is a kind of generalized epilepsy that involves large groups of neurons and embraces widely dispersed neuronal networks in the brain and is associated with loss of consciousness and sensibility.

Hoisting carcass processing: lifting of unconscious animals and carcasses to an overhead rail, normally using shackles or chain attached to the legs, for the purpose of bleeding or processing.

Hypercapnea: increased blood carbon dioxide levels

Hypoxia: decrease in oxygen levels in atmosphere or blood.

Insensible: inability to perceive stimuli.

Neck cutting: severing major blood vessels in the neck (skin and vessels cut simultaneously).

Period: the period of a given electric current frequency (Hz) is expressed in milliseconds and is calculated using the formula 1000 (milliseconds) divided by the frequency (Hz) of current. For example, electric currents of 50, 400 and 1500 Hz sine wave have periods of 20 (1000/50), 2.5 (1000/400) and 0.67 (1000/1500) milliseconds.

Petit mal epilepsy: petit mal is a kind of epilepsy without convulsions, which is not always associated with loss of consciousness and sensibility.

Seizure: convulsions that may occur with or without loss of consciousness or pathological EEG.

Slaughter: in this report, slaughter means the process of bleeding to induce death, usually by severing major blood vessels supplying oxygenated blood to the brain.

Spiking: is a fish killing process whereby a spike or tube is driven into the brain through the top of the head, usually using a pneumatically operated pistol. It is similar to captive bolt stunning of red meat species.

Sticking or bleeding: act of severing major blood vessels (also see neck cutting, chest sticking).

Stun or stunning: stunning before slaughter is a technical process subjected to each single animal to induce immediate unconsciousness and insensibility in animals, so that slaughter can be performed without avoidable fear, anxiety, pain, suffering and distress.

Stun / kill or stunning / killing: process of rendering animals unconscious first and then inducing death or achieving these simultaneously.

Stun-to-stick interval: the time interval between the end of the stunning and the sticking.

Tetanus: rigidity of the whole body usually with legs extended.

Tonic seizure: a state of tetanus occurring during generalised epilepsy.

Unconsciousness: Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to respond to normal stimuli, including pain.

4. PREAMBLE

4.1. INTRODUCTION

The Treaty of Amsterdam, in force since 1st May 1999, lays out new ground rules for the actions of the European Union (EU) on animal welfare in a special "Protocol on the Protection and Welfare of Animals" (1997). It recognises that animals are sentient beings and obliges the European Institutions to pay full regard to the welfare requirements of animals when formulating and implementing Community legislation.

The primary purpose of this report is to describe the main stunning and stun / killing methods under commercial slaughterhouse or farm conditions in Europe, and to recommend procedures appropriate to the species and related minimum requirements such that unconsciousness and insensibility is induced without causing avoidable pain, suffering and distress. The report considers the main commercial species of farm animals: cattle, sheep, pigs, chickens and turkeys, fish and horses. The number of animals killed for disease control purposes is of course very variable but can be high when a disease occurs. For instance, during the classical swine fever epidemic in The Netherlands during 1997 / 1998, more than 4.5 million animals were killed in about one year (Pluimers *et al.*, 1999).

Although it is recognised that for instance transport to the slaughterhouse, lairage conditions, pre-slaughter handling and restraint prior to stunning may cause serious welfare problems, the present report concentrates on the point of application of the stunning and stun / killing techniques and will not consider in detail other preceding or subsequent procedures.

In drafting this Scientific Report, the working group did not consider ethical, socio-economic, cultural or religious aspects of the welfare during stunning and killing.

The basis of unconscious and insensibility and the measures to assess those are vital to evaluate the effectiveness of the different methods applied. Therefore, some basic neurophysiology and measures of unconsciousness are presented initially. As the underlying principles of functioning of different stunning and stun / killing methods, as well as many aspects of their proper use are often similar for all species, the initial chapter also gives a general overview of available stunning and stun / killing methods and their proper use. Separate chapters consider different scientific data and specific aspects of stunning and stun / killing methods for cattle, sheep, pigs, poultry (chickens and turkeys), fish and horses, respectively. Where specific scientific data are lacking, current practical applications are described in order to provide some relevant information. The stunning and stun / killing methods are described with certain requirements for effective use necessary to safeguard animal welfare. Each section also considers possible parameters that can be used as monitoring points to ensure good stunning practice under commercial conditions to safeguard animal welfare. Advantages and disadvantages from an animal welfare point of view are listed. Stun / killing methods for the purpose of disease control are considered in a separate chapter.

Since the mandate requested the AHAW Scientific Panel to consider welfare aspects of stunning and stun / killing methods, references to other issues such as food safety, BSE (bovine spongiform encephalopathy), human operator safety, economic impact are not included in the different chapters. For the information of the reader some points of consideration are briefly mentioned below.

4.2. FOOD SAFETY / BSE

Some stunning methods cause brain damage and this may cause brain particles to be disseminated in the blood. In these circumstances the tissues and organs are likely to be contaminated with CNS (central nervous system) material. The dissemination of brain particles and BSE risks continue to be a public heath concern.

This issue was addressed by the Scientific Steering Committee in its report 'Scientific Opinion on Stunning Methods and BSE Risks' (2002). The report addresses the risk of dissemination of brain particles into the blood and carcass when applying certain stunning methods. The risk of contamination of tissues and organs with BSE-infectivity from CNS material depends on the amount of BSE-infectivity in the brain of the slaughtered animal, the extent of brain damage and the dissemination of brain particles in the animal body.

Captive bolt stunners are cartridge-fired or pneumatic-powered. Pneumatic-powered stunners that inject compressed air through the captive bolt into the cranium disintegrate the brain tissue. Cartridge-fired guns cause least contamination, although levels might not be zero (Garland *et al.* 1996; Schmidt *et al.*, 1999; Anil *et al.*, 1999). Pithing involves insertion of a rod via the hole made by the captive bolt and mechanically destroying the brain and upper spinal cord. This procedure eliminates the chances of recovery of consciousness following captive bolt stunning and produces relaxed carcasses that are easy to shackle, hoist and bleed out. However, pithing has been known to contribute to dissemination of brain particles and hence is banned in Europe.

The Scientific Steering Committee gave the following ranking order of stunning methods in terms of decreasing risk for causing contamination:

- Pneumatic stunner that injects air;
- Pneumatic stunner that does not inject air;
- Captive bolt stunner with pithing;
- Captive bolt stunner without pithing.

Electrical stunning and stun / killing methods and gas mixtures being non-invasive are expected not to cause dissemination of brain particles into the carcass and meat. The risk associated with stunning of animals using non-penetrating captive bolt is considered to be low.

A potential conflict exists between the size of the sticking wound (neck cutting or chest sticking) and food safety. The sticking wound is a potential route of contamination when pig carcasses are immersed in the scald tank. Since pigs' lungs in some countries are used in meat products for human consumption, abattoirs are less inclined to make larger incisions at slaughter, especially chest sticking. In addition, any neck muscles exposed at the site of sticking appears cooked after scalding and is, therefore, normally trimmed off. Inevitably, the proportion of trimming increases with the size of the sticking wound.

There may also be risks to hygiene in slaughter without stunning if separate knives are not used for making incision of the skin and blood vessels, there is a risk of translocation of pathogens from the skin into the carcass and meat. On occasions, a slaughterman may manually hold the open wound, try to pull or massage blood vessels to prevent blood coagulation or to clear a blood clot. This can result in pathogens being introduced into the carcass and that is a food safety concern. These manual interventions and manipulations of cut wound would also inflict extra pain to animals and that is a welfare concern.

Electrical water bath stunning of poultry results in inhalation of water from the bath and this has been estimated to be 0.5 ml per chicken (Gregory and Whittington, 1992). Although the majority of birds defecate in the water bath stunner during the application of stunning and faeces are a potential source of pathogens in poultry, the public health significance of this is not known.

4.3. HUMAN OPERATOR SAFETY

Risks for human operators may be directly related to the stunning or stun killing method, e.g. contact-firing captive bolt guns, manual electrical stunning or stun / killing devices involving high voltages, or complex processes like water bath electrical stunning.

Other risks for operator safety relate to convulsions occurring after the application of certain stunning or stun / killing methods. For instance, electrical head-only stunning induces tonic and clonic seizures, which are the outward symptoms of generalised epilepsy. The clonic (kicking) phase follows immediately after the tonic phase. Thus, from a worker safety point of view, sticking should be performed ideally whilst the animal is still in the tonic phase. However, this may not always be possible to achieve in cattle because induced head-only stuns may not last long enough to allow humane slaughter.

Captive-bolt stunning of pigs also produces severe convulsions, which makes carcass handling difficult and hazardous to operators and also results in the development of PSE and blood splashing (captive-bolt stunning of pigs is mainly used in small abattoirs and in casualty slaughter situations or as a backup method in case of failure of another stunning method).

Ban on pithing without a potential alternative increases risk to the operator.

Shackling of live poultry prior to stunning or stun / killing using electrified water baths induces wing flapping and emission of dust and both of these have operators' safety implications.

Some methods of killing for disease control purposes, like a manually applied percussive blow on the head, are physically exhausting for personnel and therefore involve risks. Neck dislocation and decapitation of poultry result in severe wing flapping which can be hazardous, especially when dealing with turkeys. Also, gases like carbon monoxide and gaseous form of cyanide can create a hazard to personnel.

4.4. ECONOMIC IMPACT

The economic impact of different stunning and stun / killing methods may normally be directly related to investments for apparatus and running costs, however, there are also secondary consideration, such as environmental costs or product quality. For instance, with neck cutting, the skin and vessels are cut simultaneously; whereas with chest sticking, first the skin and then the blood vessels are cut and this takes more time.

4.5. PRODUCT QUALITY

Stunning methods may have a clear impact on carcass and meat quality. For instance, convulsions or wing flapping in poultry may result in blood spots and electrical stunning is known to cause blood splashing in sheep. Head-to-back method (one cycle method) is of current application in pigs and results in severe muscle contractions leading to broken vertebral bones and haemorrhages in muscles of some animals. In poultry, a commercial disadvantage of using 50 Hz AC in water bath stunners is that, at current levels greater than 105 mA per chicken and 150 mA per turkey, there are significant increases in the incidence of haemorrhaging in the breast and leg muscles, broken bones in the carcass and the appearance of carcass downgrading conditions (Gregory and Wilkins, 1989a and 1989b). Head-only stunning in chickens, unlike waterbath stunning, does not adversely affect carcass and meat quality provided the wing flapping is restricted. Similarly, anoxia stunning may lead to increased drip loss in pigs (Holst, pers. comm.; Anon, 1998). Blood splashing may occur in electrically stunned animals.

5. SCIENTIFIC BASIS OF CONSCIOUSNESS AND STUNNING

The European Union Treaty of Amsterdam explicitly acknowledges that farm animals are sentient beings, rather than agricultural products or commodities, and this is part of the response to society's concern for animal welfare.

One of the synonyms of the term 'sentient' is 'conscious' and various bases of consciousness exist, for example:

- Philosophical (Dennett, 1996);
- Neurophysiological (Zeman, 2001);
- Neurochemical (Perry *et al.*, 2002).

In general, conscious awareness may be described in terms of two components, one quantitative (‘level’), encompassing arousal, alertness, vigilance; the other qualitative (‘intensity’), encompassing selective attention and mental experience. From humane stunning and slaughter points of views, it is assumed that consciousness is a function of brain and therefore neurophysiological or neurochemical evidence of unconsciousness is sought to ascertain the impact of stunning, stun / killing and slaughtering methods.

Stunning before slaughter is a statutory requirement in Europe and is performed to induce unconsciousness and insensibility in animals so that shackling, hoisting (hanging upside down), and slaughter could be performed without causing the animals any avoidable anxiety, pain, suffering or distress. However, live and conscious poultry can be shackled prior to stunning. Religious slaughter is exempted from the statutory requirement of pre-slaughter stunning in some countries but some religious authorities accept pre-slaughter stunning.

The act of slaughter is also referred to as exsanguination, bleeding, sticking (neck or chest sticking in red meat species) or neck-cutting (in poultry species). The American Veterinary Medical Association (AVMA, 1993) Panel on euthanasia of animals describes 'good death' as the one 'that occurs without pain and distress' and 'the technique should minimise any stress and anxiety experienced by the animal prior to unconsciousness'.

5.1. PAIN CAUSED BY CUT

The animals that are slaughtered have pain systems that have evolved to protect animals from harm such as tissue damage (Broom, 2001a, b). The cuts which are used in order that rapid bleeding occurs involve substantial tissue damage in areas well supplied with nociceptors (Kavaliers, 1989). Whilst wounds which involve tearing of tissue or multiple cuts will affect a higher number of nociceptors than clean cuts, many nociceptors will still provide an input to pain centres in the brain when a long cut across the throat or a deep cut to sever blood vessels is made, however sharp the knife. Any cuts intended to kill the animal by rapid bleeding will greatly activate the protective nociceptive system for perceiving tissue damage and cause the animal to experience a feeling of pain.

Large wounds usually elicit major pain responses. In some situations responses to a wound may not occur because endogenous opioids which act as analgesics are released. These are usually situations in which the individual is involved in very vigorous activity of brain and body, frequently associated with emergency physiological responses, for example during fighting or other dangerous and demanding activities (Bodnar, 1984). The animal which is about to be slaughtered might be in such a state but would usually not be. Furthermore, only around 40% of humans experience such a syndrome of stress-induced analgesia in an emergency situation (e.g. Melzak *et al.*, 1982). Hence it is likely that endogenous-opioid-induced analgesia will often not occur during slaughter. As a consequence, there is a high risk that animals feel extreme pain during the cutting of the throat. The different degrees to which opioid analgesia occurs will lead to variation in the extent of pain perceived.

Grandin and Regenstein (1994) described little or no reaction by calves and cattle, restraint in low-stress state-of-the-art upright restraint systems to the throat cut, except for a slight flinch where the blade first touch the throat. But it may be difficult to observe reactions to the incisions in agitated or excitated animals. The animals made no attempt to pull away and there were almost no visible reactions of the animal's body or legs during the throat cut. Some observers feel that this is due to the mechanism of restraint although Grandin and Regenstein (1994) considered that this was not the cause. The cutting of the throat will have significant effects on the ability of the animal to show some of the responses which are used to evaluate the extent of pain. Whilst an animal which is in pain as a result of an injury will often vocalise, the ability to vocalise is often prevented by cutting the throat. Difficulty in movement may occur because of the restraint effects on muscles and blood pressure. Physiological shock may make movement difficult. Also, many animals which perceive that they are in extreme danger show a freezing response, common in animals that are preyed upon. Hence observations of low levels of behavioural responding following throat-cutting do not indicate that the individual was not feeling pain. While welfare might normally be assessed using adrenal cortex responses, these can not occur in animals whose throat has been cut, because ACTH is prevented from reaching the adrenal glands via the blood. In any event, glucocorticoid responses take more than 2 min to be evident. Hence the lack of an increase in blood cortisol reported in some studies (Tume and Shaw, 1992) is not surprising.

5.2. TIME TO LOSE CONSCIOUSNESS

After the blood vessels are cut, as a consequence of blood loss, there will be deficiencies of nutrients and oxygen in the brain and consciousness will be gradually lost. Further blood loss will damage brain function causing death. During the period when the animal, whose throat has been cut, is still conscious, serious welfare problems are highly likely to occur since the animal can feel anxiety, pain, distress and other suffering. The duration of this period is therefore of particular importance. Its duration depends on the method of restraint, the sticking method applied (how many of the major blood vessels supplying oxygenated blood to the brain are severed) as well as the animal species.

Among the main red meat species, adult cattle and calves appear to lose consciousness relatively slowly after throat cutting or sticking (see Table 7-1). After neck cutting, spontaneous brain activity was not lost until 19 to 113 sec later (mean 75). Somato-sensory and visual evoked potentials were lost only after 32-126 (mean 77) and 20-102 sec (mean 55), respectively (Daly *et al.*, 1988). Duration of spontaneous cerebral activity and evoked responses were strongly positively correlated (Daly *et al.*, 1988; Daly, pers. comm., 2003). Another study found that after severing the external jugular veins and the common carotid arteries, calves showed an isoelectric EEG after 35 to 50 sec in 3 animals, but after 680 sec in a fourth calf (Bager *et al.*, 1992). These results suggest that in some animals, unconsciousness may start 19 to 20 sec after the neck cut, but it may be much delayed in a significant proportion of animals.

In sheep, bleeding out by cutting the common carotid arteries and the external jugular veins is the quickest method of abolishing brain responsiveness. The effect of three different sticking methods and cardiac ventricular fibrillation on the time to loss of brain responsiveness (loss of visual evoked responses) according to Gregory and Wotton (1984b) is shown in Table 8-1.

Pigs are usually bled by chest sticking with incision of the major blood vessels, which arise from the heart. With an adequate incision, pigs lose between 40 and 60% of their total blood volume (Warriss and Wilkins, 1987), and within 30 sec the amount of blood lost is 70-80% of the total amount of blood which will be lost (Warriss and Wilkins, 1987). Experimental studies (see Table 9-1) indicate that, in pigs weighing between 8.8 and 12.2 kg that were not previously stunned, unconsciousness (based on reduction in the amplitude of EEG signals) occurred in 25 and 105 sec after bilateral severance of the common carotid arteries and external jugular veins and unilateral severance of one common carotid artery and one external jugular vein, respectively (Blackmore and Newhook, 1981). After chest sticking of pigs, the time between the first appearance of blood from the sticking wound and to loss of brain responsiveness (based on reduction in Visual Evoked Responses) was found to range between 14 and 23 sec (mean 18, SD: ±3) whereas time to loss of VER after cardiac arrest ranged from 17 to 22 sec (mean 19, SD: ±2) (Wotton and Gregory, 1986). The same authors found the development of an isoelectric electrocorticogram (ECoG) to range between 22 and 30 sec after the chest stick. Hoenderken (1978) has reported that chest stick by a skilled slaughterman induces an isoelectric ECoG in 12-20 sec.

Gregory and Wotton (1986), using anaesthetised and mechanically ventilated chickens (layer hens), investigated the time to loss of spontaneous EEG activity following decapitation, and various commercially-practised neck cutting procedures. In that study, the time to reach 5% of the pre-slaughter integrated EEG activity was used as one of the criteria to determine the state of brain function in chickens and the results are summarised in the Table 10-2. It is important to note that Gregory and Wotton (1986) ventilated the chickens (provided artificial respiration) following the slaughter procedures to simulate conditions where birds are able to maintain or resume normal breathing following neck cutting. However, these times were suggested to be overestimates because of the effects of anaesthetic used and mechanical ventilation provided to birds. Nevertheless, a minimum of 25 sec bleed-out time will be necessary to achieve brain ischemia through blood loss.

5.3. CONSEQUENCES OF THE CUT ON ABILITY TO RESPOND

After the throat has been cut there are several potential causes of pain and distress. The consequences of a cut across the neck include:

- Blood loss through severance of the common carotid arteries on both sides.
- Blood loss through severance of the external jugular veins on both sides.
- Severing the trachea.
- Severing the neck muscles
- Severing the vagus nerve and sympathetic trunk
- The sympathetic afferent input into the brain via the spinal cord is not affected. The sympathetic chain is unlikely to be cut as it lies near to the vertebral column.

As described in chapter 12, fish whose blood vessels to gills are cut may continue to respond to stimuli for 15 min (Morzel *et al.*, 2002).

5.3.1. Blood loss through severance of the common carotid arteries on both sides

The circulating blood volume in animals is estimated to be 8% of body weight and about 18% of total volume flows through the brain at any one time. This will affect the supply of blood to the brain and lead to a reduction in blood pressure. Blood pressure loss is very disturbing to humans (Hamlin and Stokhof, 2004) and probably to animals of other species. Severing the common carotid arteries would be expected to cause a constriction and narrowing of these arteries. This physiological response is frequently referred to as 'carotid occlusion' and has been known to retard bleeding and prolong the time to loss of consciousness in calves. In addition, the homeostatic mechanisms will come into play and partially compensate for this blood loss by the sympathetic efferent output to the heart to increase its rate and cardiac output. However, the vertebral arteries to the brain will still be intact and maintain a reduced blood supply to the brain until there is such blood loss that it will no longer be able to do so. At that point an animal will begin to lose consciousness (see Table 7-1, Table 8-1, Table 9-1and Table 10-2).

5.3.2. Blood loss through severance of the external jugular veins on both sides.

The external jugular veins are the major veins draining the head and brain and they have a wide bore and low pressure. On cutting, blood will escape profusely and result in less blood returning to the heart and hence a loss of cardiac output. This in turn will lead to a rapid reduction in blood supply to the brain as in point 5.3.1 above. This reduced venous return to the heart and the drop in overall circulating blood volume could induce distress prior to the onset of physiological shock.

5.3.3. Severing the trachea.

Cutting the neck can also cause poor welfare by suffocation. When the cut is performed properly, veins, arteries, muscles, trachea and oesophagus are opened so that blood and liquid from the stomach can be aspirated into the lung (Grandin and Regenstein, 1994). This could cause severe distress. The animals react strongly by movements that can be interpreted as signs of suffocation. The receptors in the airways and the lung detect these substances that have to be removed from the respiratory system. They try to cough to remove the liquid from the lungs. Animals may become distressed by the feeling that they cannot breathe and develop fear for as long as the brain is still functioning and the sympathetic afferent nerves are intact. The time span of brain function varies with animal species (see Table 7-1, Table 8-1, Table 9-1 and Table 10-2). This problem is exacerbated when animals are on their backs when the cut is performed. However, there is a lack of knowledge of how distressing these phenomena are.

Cutting across the trachea will also lead to an inability of the animal to vocalise. Vocalisation is often a way in which animals show signs of fear, pain and distress but its absence in animals which have had the throat cut could be because members of the species, e.g. sheep, do not usually vocalise when injured by a predator (Broom, 2001) or because the animals are incapable of vocalising.

5.3.4. Severing neck muscles

The position of the head is maintained by muscle tone of both extensor and flexor groups of muscles. Cutting the throat will remove the major flexor muscle group supporting the head so that the animal will try to compensate, ineffectually, for a sudden loss of posture. There will be a contraction of the cut ends of the muscle but the contractions are unlikely to be seen. The animal is likely to feel distress at this point.

5.3.5. Severing the vagus nerve

The vagus nerve helps to maintain cardiac tone and on cutting the throat, the heart will suddenly lose one of the neural inputs. This will lead to a dominant sympathetic tone, an increase in heart rate and possibly an unpleasant feeling known, in humans, as ‘palpitations’. The vagus nerve also plays an important role in the control of respiration and the parasympathetic system is activated in response to inhaled threats to respiratory function. Its severance, therefore, prevents an animal from expelling irritants from the upper respiratory tract and lungs and this would probably explain why animals subjected to slaughter without stunning are seldom reported to show any signs of distress due to inhalation or chocking with blood.

There may also be risks to hygiene in slaughter without stunning. If separate knives are not used for making incision of the skin and blood vessels, there is a risk of translocation of pathogens from the skin into the carcass and meat. On occasions, a slaughterman may manually hold open the wound in order to facilitate bleeding. This can also result in the introduction of pathogens into the carcass.

5.4. RESTRAINT OF ANIMALS DURING SLAUGHTERING WITHOUT STUNNING

Although this report does not consider pre-slaughter handling and restraint in detail (see chapter 4.1), it is clear that these may cause serious welfare problems. In the following chapters on different stunning methods for the various species, some of these problems are addressed. Here, the restraining methods used for slaughter without stunning are considered.

Cattle and calves are restrained either in an upright position in the so-called Cincinnati pen or ASPCA pen or turned on their side or back in rotary casting pens of the Weinberg, Dyne or North British type (Anil and Sheard, 1994; Dunn, 1990; and HSA, 1993). In some member states the use of the Weinberg pen (rotating cattle on their backs) is forbidden (Danish legislation). Dunn (1990) compared a rotating Weinberg pen and an ASPCA pen for slaughter of cattle in an upright position demonstrating the benefits of the ASPCA pen as shown in Table 5-1.

Table 5-1. Behaviour of cattle in two different restraining systems for slaughter without stunning (Dunn, 1990)

Action / Behaviour	Weinberg pen (n= 18)	ASPCA pen (n= 50)
Mean time from entering the pen until ready for cut	103,8 sec ± 18,4	11,1 sec ± 11,6
Total time of struggling before cut (means ± sd)	11,2 sec ± 7,0	1,2 sec ± 3,8
Number of vocalisations (means ± sd)	4,6 ± 6,1	0,3 ± 0,75

The mean time for which an animal was in the Weinberg pen, from when the rear gate was secure until its throat was cut was 103.8 sec. For about 70% of this time (73 sec) the animals were in an inverted position. By contrast in the ASPCA pen the mean time from entering the pen until the animal was ready for the cut was 11.1 sec. Cattle in the Weinberg pen struggled for a significantly longer period than those in the ASPCA pen. Inversion in the Weinberg pen initiated most of the struggling recorded and was in proportion to the time spent inverted. The total number of recorded vocalisations prior to neck cutting was significantly greater in the Weinberg pen than in the ASPCA pen and in the Weinberg pen a significantly higher proportion occurred when the animals were inverted (Dunn, 1990).

All restraining devices should use the concept of optimal pressure. The device must hold the animal firmly enough to facilitate slaughter without struggle but excessive pressure that would cause discomfort should be avoided. Struggling is often a sign of excessive pressure (Grandin and Regenstein, 1994).

A problem with all types of casting pens is that both cattle and calves will aspirate blood after the incision. This does not occur when the animal is held in an upright position (Grandin and Regenstein, 1994). Low stress restraint boxes for ritual slaughter of cattle are available on the market but several important modifications like improved lighting, pressure limitation of the chin lift or noise reduction of the pneumatic valves have to be made to reduce stress on the animal (Grandin and Regenstein, 1994.). The pre-slaughter handling and behaviour of the cattle has a negative impact on the cut and the bleed out because movements of the animals can lead to ineffective cuts or retraction of arteries and clotting of blood (Grandin and Regenstein, 1994).

If the belly lift of the ASPCA-pen is remained up during bleed out, bumping of the incision against the head opening when the animal collapses is prevented.

Sheep can be slaughtered in different ways. It can be done when sheep are standing on the floor and being manually restrained by lifting the head to stretch the neck with one hand and the other holds the knife and performs a transverse ventral cut. Sheep can also be placed on their back or the side in a cradle and held manually by one slaughterman while the other stretches the neck and performs the cut.

Poultry are restrained by hanging them on shackles or in a bleeding cone before their neck is cut. The welfare issues of shackling are further described in chapter 10. In Denmark, it is a mandatory requirement that for slaughter without stunning each chicken should be manually restrained for neck-cutting and bleeding.

Slaughter without stunning of pigs mainly takes place in small slaughterhouses or in private households. Pigs are restrained by fettering their legs and laying them on one side by tethering them with a rope around the upper mandibula or by shackling and hoisting them on one-hind leg before a chest-stick is performed. All these measures can lead to fear, stress and pain. Shackling and hoisting of slaughter pigs on one leg must be judged as a potentially painful procedure.

5.5. TRAINING OF STAFF IN RELATION TO WELFARE AT SLAUGHTER

As in many other areas of human work with animals, the training of personnel is necessary in order that animals are to be treated well, product quality is maximised and good food hygiene is ensured. This topic is discussed in the EFSA Report on the Welfare of Animals during Transport (EFSA, 2004). Studies show that the training of staff makes their attitude to animals more positive and improves the welfare of animals prior to and during the process of stunning (Grandin 2001 and 2003)

5.6. CRITERIA FOR STUNNING AND STUN / KILLING METHODS

Since the intention of humane slaughter regulations is to avoid as much as possible anxiety, pain, distress or suffering at slaughter, stunning and stun / killing methods should ideally fulfil the following criteria:

- induce immediate (e.g. <1sec) and unequivocal loss of consciousness and sensibility;

Or,

- When loss of unconsciousness is not immediate, the induction of unconsciousness should be non-aversive and should not cause anxiety, pain, distress, or suffering in conscious animals.

Humane slaughter regulations require that the duration of unconsciousness induced by a stunning method should be distinctively (appreciably and unequivocally) longer than the sum of the time interval between the end of stun and sticking and the time it takes for blood loss to cause death (see Figure 5-1). Sticking should therefore be performed quickly after the stun and, in this process, the major blood vessels supplying oxygenated blood to the brain must be severed to ensure rapid onset of death (see determination of death at slaughter).

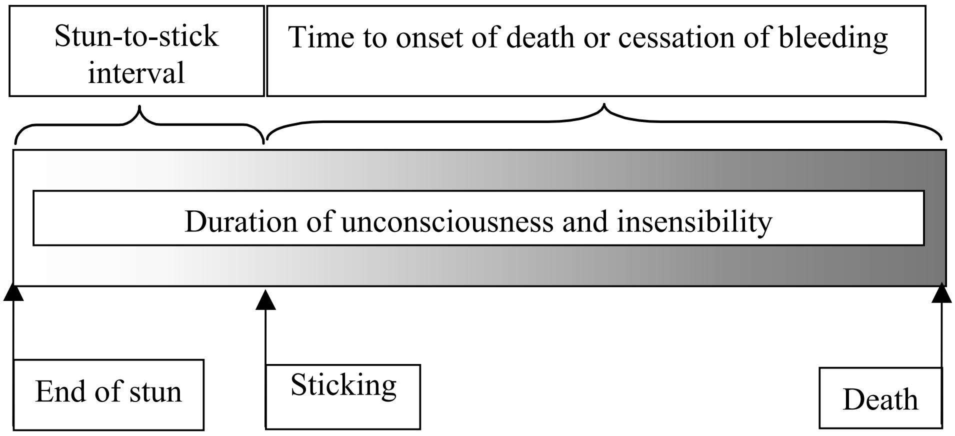


Figure 5-1. Illustration of time intervals required for humane slaughter

It is thought that a certain level of understanding of the neuronal basis of consciousness and neurophysiological bases of stunning procedures are vital to the successful implementation of good animal welfare standards during stunning and slaughter.

5.7. NEURONAL BASIS OF CONSCIOUSNESS

A neuron consists of a cell body and one or more cell-extensions, called axons and dendrites. Neurons receive all kinds of sensory and motor information from specialised peripheral receptor cells (e.g. chemoceptors, thermoreceptors, mechanoreceptors, nociceptors), and from other neurons. A neuron communicates with the other neurons by the release of chemical substances, known as neurotransmitters, into the gap between its axon and the axon, dendrite or cell body of the other neurons, called the synapse. There are two types of neurotransmitters, excitatory and inhibitory neurotransmitters, and when they are released at the synapses, they excite or inhibit the neurons that are synaptically connected.

In neurons, as in all cells of the body, there is a difference between the number of positive and negative ions between the inside and outside of the cell membrane.

Electrophysiological recording of the resting membrane potential (RMP) has shown that the inside of the cell has a negative charge of about 70 mV (Figure 5-2). When a neuron is stimulated, for example with a single electrical pulse, it undergoes a biphasic response. Firstly, its RMP will be disrupted. Sodium flows into the cell along its electrochemical gradient and causes a slight decrease in the RMP (e.g. from –70 to –65 mV), which is known as depolarisation or an excitatory postsynaptic potential (EPSP). The EPSP is followed by a longer latency inhibitory postsynaptic potential (IPSP) or hyperpolarisation, which occurs due to increased permeability of the membrane to chloride or potassium such that the RMP increases (e.g. –75 mV). If the depolarisation is slight, the RMP is quickly restored

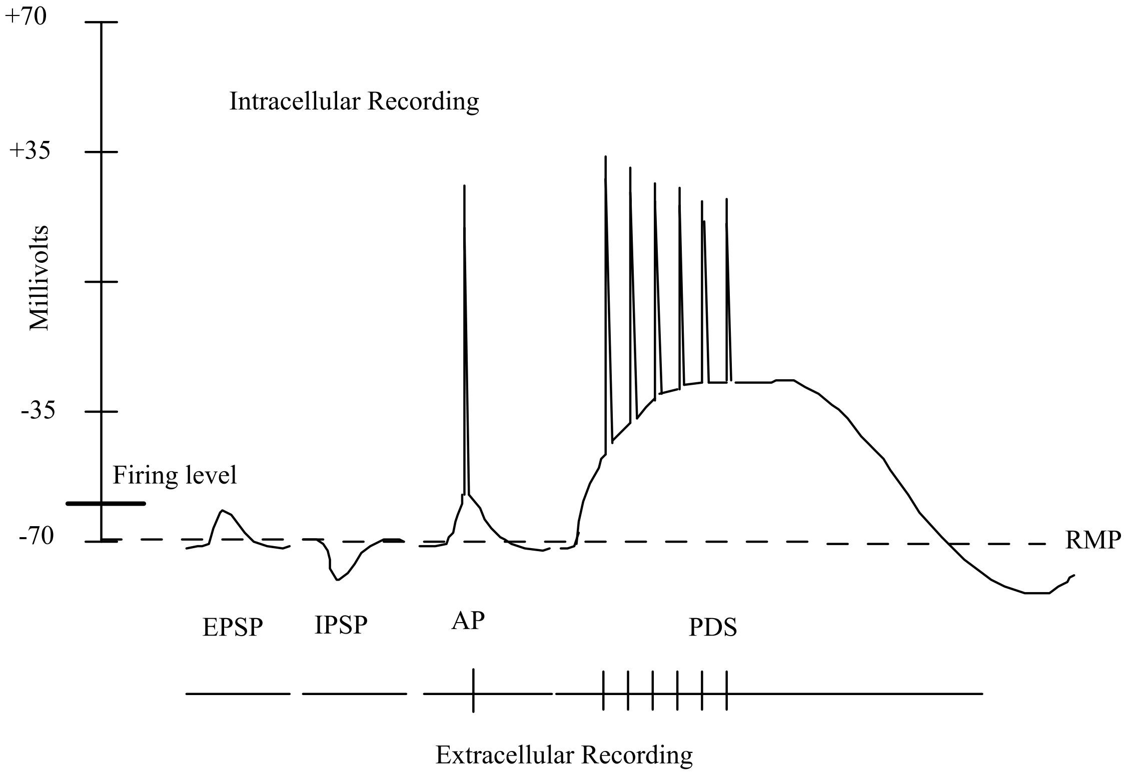
If the stimulus is strong or prolonged enough to decrease the RMP to about –60 mV (the firing level of neurons), a large depolarisation takes place and the membrane potential suddenly increases to +35 mV, which is known as an action potential. When the action potential travels along this neuron's axon (conducted action potential), it induces neurotransmitter release at its synapses, thus stimulating or inhibiting the other neurons that are synaptically connected. In normal neurons, an action potential lasts only a few milliseconds. When either the EPSP is exaggerated or the IPSP is inhibited, the depolarisation is prolonged and consecutive action potentials occur, known as paroxysmal depolarisation shift (PDS).

The existence of opposite functioning neurotransmitters is part of feedback mechanisms that help to maintain a proper balance and normal functioning of neurons in the brain. The level of excitability of a neuron at any particular moment is a function of competing influences of depolarisation and hyperpolarisation.

Electroencephalogram (EEG) or electrocorticogram (ECoG, referred to as EEG from now on) is widely used to record the brain electrical activity under different circumstances to determine the state of consciousness and brain disorders in humans and animals. Research on the origin of rhythmic brain electrical activity in various frequency (Hz) bands indicates that complex homeostatic systems involving large neuronal populations in the brain stem, thalamic and cortical processes regulate the EEG, which is the result of the summation of EPSPs and IPSPs on a large number of neurons that are situated beneath the recording electrodes.

The electrical activity recorded in the EEG can be classified into *delta* (< 4 Hz), *theta* (4 to 7 Hz), *alpha* (8 to 13 Hz) and *beta* (> 13 Hz) frequency bands. The amplitude and frequency of activity seen in the EEG is related to the degree of synchronisation of activity of neurons. For example, low-amplitude and high frequency (*beta*) waves occurring in the EEG during conscious state are generated by a large number of non-synchronised, independently firing neurons. It is assumed that the 'near-threshold' depolarised state of neurons in the thalamus and cerebral cortex of brain is a necessary condition for perceptual processes and consciousness.

With an increasing dose of most anaesthetic agents the spontaneous EEG changes progressively to higher amplitude, lower frequency (*delta*) pattern, referred to as slow waves. The increase in amplitude results from an increase in the degree of synchronisation in the electrical activity of neurons and the decrease in frequency of activity seems to correlate with a reduction in cortical metabolism. It is also known that quiescent or isoelectric EEGs occur in deep anaesthesia as well as brain death (Eger, 1981).



EPSP = excitatory post synaptic potential
IPSP = inhibitory post synaptic potential
AP = action potential
PDS = paroxismal depolarisation shift
RMP=resting membrane potential

Figure 5-2. Neurophysiology of depolarisation and hyperpolarisation.

Efficient stunning methods disrupt the neurons or neurotransmitter regulatory mechanisms in the brain causing a long-lasting depolarised neuronal state that render animals unconscious and insensible. Indeed, most of the known or established stunning methods also induce high degrees of synchronisation leading to a quiescent or isoelectric EEG.

5.8. NEUROPHYSIOLOGICAL BASES OF STUNNING AND STUN / KILLING METHODS

In conscious animals, EEG activity has amplitudes of 10 to 40 µV (Devine *et al.*, 1986), but higher levels may also be found in calves (Gregory and Wotton, 1984a). However, this may vary depending upon whether the EEG recording electrodes were inserted under the skin (subcutaneous needle electrodes) or placed intracranially, such that they rest on the dura or surface of the brain and according to the EEG signal amplification (e.g. sensivity of recording system, gain settings and time constants) used during recording. Scientific literature suggests that, in spite of the differences in the way stunning methods induce unconsciousness, an animal can be judged to be unconscious and insensible if one or more of the following is achieved:

(a) EEG shows changes that are incompatible with consciousness, e.g. grand mal epilepsy (>100 μV, predominantly 8-13 Hz), high amplitude low frequency activity (>100 μV at <4 Hz; slow waves), or a prolonged quiescent period with less than 10% of the pre-stun EEG power content.

(b) Abolition of evoked electrical activity in the brain (somatosensory evoked potentials: SEPs), brain stem auditory evoked potentials (BSAEPs) or flash visual evoked potentials (VEPs), which is indicative of the brain's incapacity to receive and process external stimuli.

The abolition of evoked potentials has been used as an objective and unequivocal indicator of loss of brain responsiveness and hence, loss of consciousness, in many species. The return of evoked activity seems to coincide with the return of behavioural signs of consciousness (Ommaya and Gennarelli, 1974). However, the presence of evoked potentials does not necessarily indicate consciousness, because visual evoked potentials can be present in anaesthetised animals and when the EEG is isoelectric, especially in poultry (Gregory, 1998; Gregory and Wotton, 1988). Similarly, their absence does not necessarily indicate unconsciousness. This is because somatosensory evoked potentials can also be experimentally abolished in conscious animals by the application of dopamine to the somatosensory cortex (Rolls *et al.*, 1984). Focal application of transcranial magnetic stimulation to the somatosensory or visual cortex of animals, which are conscious and able to process other sensory information reaching the brain, has been reported to result in abolition of these evoked potentials without causing any apparent changes in the EEG activity (Anil *et al.*, 2000; Masur *et al.*, 1993). Therefore, interpretations concerning the abolition of evoked potentials will have to be made in conjunction with the pathophysiology of the stunning method used and in association with the changes occurring in the EEG.

In spite of this cautious note, evoked potentials provide useful information about the site and nature of changes in cerebral activity not obtainable with EEG alone. In this regard, they are ideally suited for evaluating the extent of cerebral damage induced by stunning and slaughter methods. Complete bilateral (evoked in both the hemispheres) abolition of evoked potentials would indicate a state of profound brain failure, and therefore, frequently used in association with medical history to determine the prognosis of human patients under critical care (e.g. head trauma, coma, vegetative state and brain death) (Guerit, 1999). In general, the prognosis is bad (patients die) in most of the cases when the early latency evoked potentials are absent.

Some stunning methods (correctly applied captive bolt and electrical stunning, see below) can induce immediate loss of consciousness (e.g. in less than 1 sec) and others take a certain time (several sec) according to the method used (e.g. exposure to argon or nitrogen-induced anoxia or carbon dioxide mixtures).

Stunning methods induce temporary loss of consciousness and they rely solely on bleeding to cause death (e.g. head-only electrical stunning or exposure to gas mixtures for a brief period). If unbled, the adequately stunned animal has a potential to regain normal brain and bodily functions. It is a statutory requirement that animals showing signs of return of consciousness should be re-stunned immediately using an appropriate back-up method, mostly involving a captive bolt.

Stun / killing methods induce unconsciousness and death either simultaneously or sequentially. The actual moment of death can be immediate or delayed: e.g. head-to-body application (see electrical stun / killing) of an electric current would cause immediate death, while death due to exposure to gas mixtures would be delayed (of few sec or min).

One stunning method (penetrating captive bolt) however, being invasive, induces structural damage to the brain. If unbled, the extent of damage inflicted in the brain determines the outcome, *i.e.* whether the animal regains consciousness, goes from a stunned to a comatose state or dies immediately. The extent of the damage, thus determining whether the method stuns or kills, depends on the species (size and shape of the head, skull structure and thickness, density and porosity of the bone at the recommended shooting site), on the equipment used (bolt diameter, velocity and penetration depth), and on the place of impact.

Stunning versus stun / killing can be differentiated *post-hoc* on the basis of whether an animal that is not bled out after the application of a procedure resumes rhythmic breathing, which is a sign of potential return of consciousness following stunning.

Stunning methods used under commercial conditions are listed in Table 5-2.

Table 5-2. Stunning and stun / killing methods used at present

Species of animals	Stunning methods	Stun / kill methods
Cattle and calves	Mechanical Electrical	Electrical
Sheep	Mechanical Electrical	Electrical
Pigs	Mechanical Electrical Gas mixtures	Electrical Gas mixtures
Poultry	Electrical	Mechanical Electrical Gas mixtures
Horses		Free bullet
Fish	Electrical	Percussive Mechanically applied spiking Electrical Shooting / electric harpoon (large tuna)

5.8.1. Mechanical methods

Two kinds of mechanical methods are used at present:

- Non-penetrating captive bolts: involve firing of a mushroom-headed blunt bolt usually on the forehead of animals. In poultry however, such bolts cause severe skull fractures and therefore would not be considered as non-penetrating bolts.

- Penetrating captive bolts: involve firing of a sharp-rimmed bolt usually on the forehead of animals.

Captive bolts induce concussion of the brain on their impact with the skull of animals, provided the impact (kinetic) energy delivered by the bolt is appropriate to the species of animal. Cerebral concussion is usually a short-lasting disturbance of neural function, typically induced by the sudden acceleration of the head. It is characterised by a sudden brief impairment of consciousness, paralysis of reflex activity and loss of memory (Shaw, 2002). Research has shown that firing a captive bolt through a previously drilled hole in the skull does not induce concussion, at least in sheep (Daly, 1987).

Various researchers have studied the effect of acceleration of the brain in the context of stunning before slaughter. They have given different explanations for concussion induced unconsciousness:

- Brain haemorrhage often occurs at the place of impact (known as 'coup') and can further develop at the side opposite to the brain impacts ('contre-coup') (Ommaya *et al.*, 1971). Vacuolation may also occur within the brain as it is thrown forwards and backwards (Finnie, 1995).

- Acceleration of the head by the shot causes localised pressure at the midbrain as it jars against the rigid edge of the tentorium. This would disrupt nervous function associated with midbrain structures. Differences in pressure gradients caused disruption of synaptic transmission (Gregory, 1998).

- Following severe haemorrhage, intracerebral pressure may rise, blocking blood flow and causing ischemia (lack of blood supply to vital brain structures) (Ommaya *et al.*, 1964).

Others have experimentally studied the effects of brain trauma in rodents, cats and monkeys:

- The impact produces rotational forces in the brain, causing shearing and discrete brain lesions (Ommaya and Gennarelli, 1974).

- On the molecular level, loss of consciousness is related, at least partially, to changes in cholinergic and endorphinergic activity (Lestage *et al.*, 1998, Shah *et al.*, 1982; West *et al.*, 1981).

- Concussive injury produces changes in brain function that differ across various regions of the central nervous system, depending upon their distance from the site of impact, and may include both depression and focal activation of specific brain regions (Hayes *et al.*, 1988).

- The impact energy produces shock waves in the brain leading to depolarisation of neurons that are located away from the site of impact and disrupt normal functioning of ion channels. However, electrophysiological confirmation of this hypothesis is, as yet, lacking (Somjen, 2001).

During the last century, several theories of concussion have been prominent. These have been recently summarised and critically reviewed by Shaw (2002):

- Vascular hypothesis: loss of consciousness is due to a brief episode of cerebral ischemia.

- Reticular hypothesis: loss of consciousness is due to temporary paralyses, disturbances, or depression of the activity of the neural pathways within brain stem reticular formation / ascending reticular activating system (neuronal activating system).

- Centripetal hypothesis: loss of consciousness occurs due to sudden rotational forces that lead to shearing strains and stresses within the brain; these disengage or disconnect nerve fibres in a centripetal fashion.

- The thalamus and cortical regions of brain receives signals from two groups of neurons (primary projection neurons) in the brain stem, one of which is the reticular formation (reticular activating system; RAS) that includes pontine cholinergic system. This cholinergic system of projections appears critical to the overall modulation of cortical activity, e.g. arousal or unconsciousness (Pontine cholinergic system hypothesis).
- Convulsive hypothesis: in contrary to the vascular hypothesis, loss of consciousness occurs due to a direct mechanical insult to the neurons leading to initial convulsive phase (tonic-clonic seizures indicative of generalised epilepsy) followed by a quiescent phase.

The author suggests that the neurophysiological data on concussion is compatible with the convulsive theory. This theory suggests that the energy imparted to the brain by the sudden mechanical loading of the head generates turbulent rotatory and other movements of the cerebral hemispheres, increasing the chances of a tissue-deforming collision or impact between the cortex and the skull. Loss of consciousness would not be caused by disruption or interference with the function of the brainstem reticular activating system. Rather, it is due to functional differentiation of the cortex as a consequence of diffuse mechanically-induced depolarisation and synchronised discharge of cortical neurons (Shaw, 2002).

Penetrating bolts have additional effects:

- When the bolt penetrates the brain, it destroys and causes trauma to the cerebral hemisphere and brainstem. Effective captive bolt stunning produces a large, deep, and well-defined haemorrhagic track with severe destruction and loss of neural tissue in the cerebellum or midbrain (in the case of sheep, due to the occipital shooting position used), frequently involving the pons, medulla oblongata and caudal part of the cerebral hemispheres (Finnie, 1996).

- Cerebral damage is caused by negative pressure of the shock waves or by the collapse of the brain tissue at the moment of the obliteration of the temporary cavity induced by the retracting bolt (Lambooij and Spanjaard, 1981; Crockard *et al.*, 1977). However, Finnie (1996) suggested that the temporary cavity formed by a captive bolt, which acts as a low velocity missile (<165 m per sec), is restricted. These can have effects on distant neurons and in addition the penetrating bolt may cause further widespread subdural or subarachnoid haemorrhages (Palmer, 1982).

- The effectiveness of stunning animals with penetrating and non-penetrating captive bolts has been determined from the appearance of delta activity (high voltage, low frequency) in the EEG and abolition of evoked potentials

(Lambooij, 1981a; Daly, 1987). Effective concussion stunning leads to tonic-clonic seizures (see behaviour and physical reflexes for description) and therefore the occurrence of generalised seizures has been used as a sign of unconsciousness (Shaw, 2002).

- Use of the penetrating captive bolt has been found to have a double effect. The impact of the bolt on the skull and the resulting shockwave sent through the brain leads to instantaneous concussion-induced unconsciousness. Brain damage caused by subsequent penetration of the bolt causes irreversible physical damage. Brain penetration causes irreversible loss of consciousness provided the appropriate areas are destroyed. Concussion causes functional damage and may thus be reversible. Reversibility of concussion-induced unconsciousness depends on the severity and place of impact. Non-penetrating percussion stunning, using the Cash Magnum 9000, produced an isoelectric brain state in all cattle tested although it took slightly longer, on average, for the isoelectric state to develop compared to penetrating bolt stunning. This suggests that penetration is not essential for irreversibility. Therefore, some experts believe that a severe concussive blow produces global ischemia of the brain, responsible for the permanent loss of brain activity.

5.8.2. Electrical methods

Two kinds of electrical methods are used at present:

- Electrical stunning: involves transcranial application of an electric current (head-only stunning) in red meat species and poultry, in the latter however the current can also applied through the whole body.

- Electrical stun / killing: usually involves head-to-body application of an electrical current in red meat species and poultry.

In normal neurons, an action potential lasts only a few milliseconds (see Figure 5-2). As mentioned previously, when either the EPSP is exaggerated or the IPSP is inhibited, the depolarisation is prolonged and consecutive action potentials occur, known as paroxysmal depolarisation shift (PDS). PDS occurring in a group or small groups of neurons constitute focal epilepsy; whereas, when it occurs in large groups of neurons located in both cerebral hemispheres, it constitutes generalised epilepsy. The electrical stunning of animals, which involves stimulation of whole brain, with a current of sufficient magnitude above the threshold and duration (longer than 100 milliseconds) results in repeated firing of neurons, immediately followed by an exhausted state. Work on sheep (Cook *et al.*, 1995) revealed that an electrical stun lasting at least 200 milliseconds with a current of sufficient magnitude produces long-lasting strong depolarisation of the cell membrane leading to grand mal epilepsy. Predominance of 8 to 13 Hz high amplitude (>100 μV) EEG activity characterises the occurrence of grand mal epilepsy in mammals; however, poultry species seem to differ from this norm (Gregory 1986, see Chapter 10 for details). Grand mal epilepsy is a pathological extreme of neuronal synchrony and is considered to be incompatible with normal neuronal function and, hence, persistence of consciousness (Hoenderken, 1978; Cook *et al.*, 1992 and 1995).

Grand mal epilepsy is a kind of generalised epilepsy (see classification of epilepsies) that involves large groups of neurons and embraces widely dispersed neuronal networks, whereas focal epilepsy involves small groups of neurons and depends on excitatory networks within individual cortical structures. Experimental models of both primary generalised and focal epilepsy have revealed the importance of the intrinsic (membrane current) properties of neurons and the synaptic networks that connect them. In mammals, grand mal epilepsy is always followed by a period of quiescence in the EEG, which is referred to as spreading depression and occurs due to hyperpolarisation. When these two EEG manifestations occur after electrical stunning, the animals are considered to be unconsciousness and insensible. The power (V^2) content in the quiescent or isoelectric EEG is less than 10% of the pre-stun power content (Bager *et al.*, 1990; Lukatch *et al.*, 1997). By contrast, certain petit mal or partial epilepsy, which is not always associated with loss of consciousness, is not followed by the occurrence of a quiescent EEG; instead, a normal EEG resumes after the seizure activity (Gregory, 1986). The inference is that seizure is only a symptom and the site of origin of seizure in the central nervous system, as well as its extent of spread within the brain, determines the associated state of consciousness and insensibility.

Classification of epileptic seizures:

The International League Against Epilepsy (ILAE) has introduced the current International Classification of Epileptic Seizures (ICES), which is widely used in clinical practice (CCTILAE, 1981). Since electrical stunning induced epilepsy is based on human analogy, classification of epileptic seizures and associated state of consciousness in humans is vital to evaluating the impact of this stunning method.

A summary of this classification system is presented below (Dreifuss and Ogunyemi, 1992):

I. Partial (Focal, Local) seizures:

Partial seizures are those in which the first clinical and electroencephalographic changes indicate activation of a system of neurons limited to part of a cerebral hemisphere. A partial seizure is classified primarily on the basis of whether or not consciousness is impaired during the attack. When consciousness is not impaired, the seizure is classified as a simple partial seizure and it usually involves only one cerebral hemisphere. When consciousness is impaired, the seizure is classified as a complex partial seizure and it involves both cerebral hemispheres. Impairment of consciousness may be the first clinical sign, or simple partial seizures may evolve into complex partial seizure. A partial seizure may progress to a generalised seizure (known as partial seizure secondarily generalised).

II. Generalised seizures:

Generalised seizures are those in which the first clinical change indicates initial involvement of both cerebral hemispheres. Consciousness may be impaired and this impairment may be the initial manifestation. Various types of generalised epilepsies occur in humans and one group of them, known as generalised tonic-clonic epilepsies, is always associated with unconsciousness. Grand mal epilepsy is one type of the generalised tonic-clonic epilepsies.

Impairment of consciousness is defined as the inability to respond normally to exogenous stimuli by virtue of altered awareness or responsiveness.

The neurochemical basis of the occurrence of epilepsy in humans is well established. Under the conditions of normal neuronal function, the excitatory amino acid (EAA) neurotransmitters facilitate the excitatory bursts while inhibitory amino acid (IAA) neurotransmitters inhibit such actions. As these two neurotransmitter systems are often associated, it is believed that they jointly provide a controlled balance of neuronal activity under a variety of circumstances. It is known that low levels of deviations from the normal contents of EAA and IAA neurotransmitters can lead to an altered state of mind in humans (e.g. arousal and depression). The excessive release of two rapidly acting EAAs, which are glutamate and aspartate, into the extra cellular space is believed to play a crucial role in the initiation, spread and maintenance of epileptic activity in the brain (Meldrum, 1994). Gamma amino butyric acid (GABA) is the principal IAA neurotransmitter. When released into the extra cellular space, it inhibits neuronal activity and hence epilepsy (Meldrum, 1984). Therefore, spontaneous epilepsy can occur either due to excessive release of EAA or deficiency of IAA.

Nevertheless, Cook *et al.* (1992, 1995, 1996), using a combination of microdialysis and electrophysiological techniques, confirmed that the development of epilepsy following head-only electrical stunning of sheep is dependent on EAAs and revealed that glutamate, and possibly aspartate, induces or at least contributes to the epilepsy. The generalised epilepsy is accompanied by tonic (tetanus, rigid extension of legs) - clonic seizures (leg-kicking). The suppression of eye and pain reflexes (see behaviour and reflexes) is attributed to GABA release. Although GABA release can occur as a neuronal reflex mechanism in response to the release of EAA (to prevent neuronal exhaustion and loss), GABA release can occur independently of the EAAs. Both the neurotransmitter systems produce synergistically the state of unconsciousness and analgesia required to ensure animal welfare following electrical stunning.

Electrical methods are commonly used to stun or stun / kill sheep, pigs and poultry; however, their use in cattle is becoming popular. The effectiveness of electrical methods has been evaluated on the basis of occurrence of epilepsy in the brain (Hoenderken, 1978; Cook *et al.*, 1992 and 1995). Somatosensory or visual evoked potentials can be abolished following electrical stunning induced generalised epilepsy (Shaw, 1997; 1998). Since grand mal or generalised epilepsy in the brain is accompanied by tonic-clonic seizures (see behaviour and physical reflexes), the occurrence of these seizures has also been used to evaluate electrical stunning in animals (Anil, 1991; Anil and McKinstry, 1991 and 1992; Velarde *et al.*, 2002; Wotton *et al.*, 2000).

5.8.3. Gas mixtures

Gas mixtures are currently used for stunning or stun / killing poultry and pigs. Gas stunning or killing is performed by exposing animals, contained in cages, cradles, crates or conveyor, to a predetermined gas mixture contained within a well or tunnel. The composition of gas mixture and duration of exposure varies according to the species, manufacturer of the equipment and the requirement (stunning *vs*. killing). Since gas mixtures do not induce immediate loss of consciousness, the aversiveness of various gas mixtures and the respiratory distress occurring during the induction phase are important considerations with regard to the welfare of animals.

Table 5-3. Gas mixtures evaluated or used for stunning or stun / killing

Species	carbon dioxide in air	carbon dioxide plus oxygen	argon and / or nitrogen in air	argon and / or nitrogen plus carbon dioxide
Pigs	Used	Evaluated	Evaluated	Evaluated
Chickens	Used	Used	Used	Used
Turkeys	Used	Used	Used	Used
References	Holst, 2001. Barton-Gade *et al.*, 2001.	Mullenax and Dogherty, 1963. Barton-Gade *et al.*, 2001.	Raj, 1999. Meat Hygiene Service, 2002.	Raj, 1999. Raj *et al.*, 1997b. Meat Hygiene Service, 2002.

Used = commercially practised; evaluated = laboratory and pilot studies have been carried out but not implemented commercially.

Various physiological states and associated effects on the brain occur with gas stunning. Hypercapnia refers to the presence of an excessive amount of carbon dioxide in the blood. Hypoxia and anoxia refer to varying degrees of oxygen deficiency in the blood. Asphyxia refers to the physical separation of upper respiratory tract and the atmospheric air (e.g. drowning, choking and strangulation in terrestrial animals and removal of certain fish from water); it inevitably induces pain and distress and is not appropriate as a stunning or stun / killing procedure (Council Directive 93/119/EC).

5.8.3.1. Carbon dioxide mixtures (in air or oxygen)

Inhalation of carbon dioxide induces respiratory and metabolic acidosis and hence, reduces the pH of cerebrospinal fluid (CSF) and neurons thereby exerting its neuronal inhibitory and anaesthetic effects (Woodbury and Karler, 1960). In this regard, normal pH of CSF is 7.4 and a state of analgesia and anaesthesia are induced at 7.1 and 6.8, respectively. There is some evidence to suggest that carbon dioxide induced neuronal inhibition may be due to excessive release of GABA in the brain (Cook, 1999). On the other hand, brain GABA level also increases under distress. However, effective carbon dioxide stunning must accompany abolition of all the reflexes mediated by this neurotransmitter (see behaviour and reflexes). Research has shown that the time of induction of unconsciousness with carbon dioxide in pigs depends upon the concentration of this gas in the stunning unit (Troeger and Woltersdorf, 1991; Raj and Gregory, 1996). The duration of unconsciousness induced with a particular concentration of carbon dioxide depends upon the duration of exposure (magnitude of acidosis and neuronal inhibition induced) and prolonged exposure to high concentrations (>70%) causes death.

Owing to its inhibitory effects on neurons, carbon dioxide (anaesthetic concentration) has been reported to cause quiescent EEG and unconsciousness in rats (Woodbury and Karler, 1960). Mattsson *et al.* (1972) reported that, in rhesus monkeys, loss of power in the 10 to 14 Hz EEG signals is related to loss of consciousness during inhalation of carbon dioxide. Forslid (1987) reported that the onset of unconsciousness in pigs during exposure to carbon dioxide coincided with the increase in delta activity (1 to 4 Hz) in the EEG. Similarly, changes occurring in the EEG and abolition of somatosensory evoked potentials in the brain have been used as indicators of loss of consciousness and sensibility in pigs (Raj *et al.*, 1997a).

However, inhalation of carbon dioxide in high concentrations has been found to be aversive to pigs and poultry (chickens and turkeys) and induces severe respiratory distress prior to loss of consciousness, which is discussed in detail in later Chapters (gas mixtures for stunning or stun / killing pigs and poultry). It is worth mentioning here that carbon dioxide delivered to nasal mucous membrane is used as a painful stimulus to evoke electrical potentials in the brain, and therefore, induction of unconsciousness with this gas may not be free from pain or distress. The presence of intrapulmonary chemoceptors that are acutely sensitive to carbon dioxide but insensitive to hypoxia is well established in birds and mammals (Manning and Schwartzstein, 1995; Ludders, 2001). This is probably the reason why pigs and poultry withdraw immediately from this gaseous atmosphere. There are also irritant receptors in the lungs that acutely respond to inhalation of carbon dioxide. These could be the reason why pigs show sneezing, coughing and head shaking during exposure to carbon dioxide (Manning and Schwartzstein, 1995). The net result of stimulation of these receptors is avoidable breathlessness, pain, distress and suffering during the induction of unconsciousness. In addition, increased depth of breathing occurring during inhalation of carbon dioxide leads to stimulation of stretch receptors (mechanoceptors) which in turn triggers acute bradycardia, which is also distressing(Manning and Schwartzstein, 1995).

5.8.3.2. Hypoxia induced with argon or nitrogen

Hypoxia may be induced by the inhalation of inert gases. Xenon, krypton and argon are chemically inert under most circumstances, *yet all* have anaesthetic properties. Xenon is an anaesthetic gas under normal atmospheric pressure, whereas argon and krypton only have anaesthetic properties under hyperbaric conditions and they have been evaluated in humans and rats (Kennedy *et al.*, 92; Abraini *et al.*, 1998). However, owing to the prohibitive costs associated with the use of xenon and krypton, argon or nitrogen-induced hypoxia at normobaric conditions is commercially used to stun or stun / kill poultry. Hypoxia has also been evaluated to stun or stun / kill pigs; however, owing to the lack of purpose built equipment, it is not used commercially.

Exposure of animals to hypoxia induced with argon, nitrogen or other inert gases causes depolarisation and intracellular metabolic crisis leading to death in neurons (Rosen and Morris, 1991; Huang *et al.*, 1994). It is known that in humans, cerebral dysfunction - as indicated by the occurrence of highly synchronised electrical activity (slow waves) in the EEG - occurs when the partial pressure of oxygen in the cerebral venous blood falls below 19 mm Hg (Ernsting, 1963). Brain oxygen deprivation leads to accumulation of extra-cellular potassium and a metabolic crisis as indicated by the depletion of energy substrates and accumulation of lactic acid in the neurons. There is some evidence to suggest that the mechanism of induction of unconsciousness with xenon, argon, nitrogen and nitrous oxide (laughing gas) is due to the inhibition of N-methyl-D-aspartate (NMDA) receptor channels in the brain, which is essential for maintaining neuronal excitation during conscious state. It is worth noting that the effects of a number of modern analgesics, sedatives and anaesthetics are also mediated via NMDA receptor channels in the brain. Inert gas / oxygen mixtures have been found to be ideal for maintaining anaesthesia during laser surgery in the airway of horses (Driessen *et al.*, 2003).

In contrast to hypercapnia and asphyxia, anoxia or hypoxia induced by the inhalation of nitrogen is reported to be a pleasant or euphoric way of losing consciousness in humans and was recommended for euthanasia of animals (Ernsting, 1963, 1965 and Gregory, 1993a). Research has shown that hypoxia is not aversive to pigs and poultry and it doesn't induce any signs of respiratory distress prior to loss of consciousness, which is discussed in detail in later Chapters.

High amplitude low frequency EEG activity (delta activity or slow waves) occurring during cerebral anoxia indicates that large numbers of neurons are depolarised in unison and at a slow rate (Bager *et al.*, 1992; Raj *et al.*, 1997a). Evoked potentials are abolished during the occurrence of delta activity in the EEG (Raj *et al.*, 1997a).

5.8.3.3. Carbon dioxide and nitrogen or argon mixtures

Carbon dioxide may be mixed in various proportions with nitrogen or argon and inhalation of such mixtures leads to hypercapnic - hypoxia. Research has shown that pigs and poultry do not find 30% by volume of carbon dioxide aversive and therefore, a mixture of nitrogen and / or argon with up to 30% by volume of carbon dioxide has been used to stun / kill pigs and poultry (Raj and Gregory, 1995; Raj, 1996). The time to onset of EEG suppression and abolition of evoked potentials in the brain have been used to determine the time to onset of unconsciousness in pigs and poultry (Raj *et al.*, 1997a; Raj and Gregory, 1994; Raj, Gregory and Wotton, 1990 and 1991; Raj, Wotton and Gregory, 1992). In comparison with a high concentration of carbon dioxide in air or argon-induced hypoxia, exposure of poultry to a mixture of 30% carbon dioxide and 60% argon in air results in a quicker abolition of evoked potentials.

5.9. BEHAVIOUR AND PHYSICAL REFLEXES

During and immediately after stunning, depending on the method and species involved, animals show typical behaviour patterns and physical reflexes, which can help to monitor the effectiveness of stunning under commercial conditions. In general, vocalisation in animals during the induction of unconsciousness with any stunning method is indicative of pain or suffering. Absence of vocalisation does not, however, guarantee absence of pain or suffering.

Various physical reflexes can be measured, although their interpretation will vary depending on the species and the stunning / killing method used. The pathological EEG state induced by certain stunning methods (electrical and mechanical stunning methods) is associated with the absence of rhythmic breathing, which is also the first sign of return of consciousness in animals. Some general examples will be presented here although species-specific characteristics will be considered in later chapters.

Immediate collapsing of the body with spasms of the skeletal muscles occurs after captive bolt stunning and when an epileptic fit is elicited by passing a sufficient current through the brain. During exposure to gas mixtures, collapse may not be immediate and more progressive postural changes are seen.

Tonic and clonic seizures are physical signs of grand mal or generalised epilepsy that occur after head-only electrical stunning. During tonic phase, the animals show tetanus (rigidly extended legs), breathing is absent and the eyeballs may be obscured. The tonic phase is followed by two clonic phases, at least in sheep and pigs (Velarde *et al.*, 2002; Simmons, 1995), which can be either a galloping, cantering or erratic kicking action (Anil, 1991; Gregory, 1998).

The corneal reflex can be elicited by touching the cornea of the open eye with the fingertip or a pencil. If positive, the eyelid will close and a positive corneal reflex indicates that the brainstem is responsive. In electrical stunning the corneal reflex can occur shortly before or after the commencement of rhythmic breathing (*i.e.* end of tonic-clonic seizures). The presence of a corneal reflex does not distinguish accurately between consciousness and unconsciousness. But when it is absent, it is likely that the animal is unconscious (Anil, 1991; Gregory, 1998). The corneal reflex is generally the last reflex to disappear during loss of consciousness or onset of death (e.g. exposure to gas mixtures and after cardiac arrest) and the first one to reappear as consciousness returns in effectively stunned animals; although in electrical stunning, rhythmic breathing may reappear before return of the corneal reflex. In order to distinguish exactly between reactions on touching the palpebra or the cornea, small objects should be used for checking the corneal reflex. If the eyelids are shut it is not possible to test for corneal reflex.

When the closed eyelid is lifted (if needed, a torch light may be shone in the pupil), the pupil reduces its diameter. The reflex indicates that the animal is still alive.

Electrical stunning induces pupillary dilatation that leads to gradual constriction as the animal returns to consciousness.

Fixed eyes, that is eyes glassy with no pupillary or other eye reflexes, indicates that the animal is either unconscious or dead.

The presence of rhythmic breathing (full cycle of inspiration and expiration occurring usually through the nose), observed by regular flank movements or by condensation on a cold mirror placed in front of the mouth and nostrils, indicates that the brain stem has resumed some normal function and the animal is already or close to becoming conscious. In electrically stunned pigs, it has been reported to occur after the clonic phase (Anil, 1991). However, it is not certain whether rhythmic breathing occurs after the end of first or second clonic phase, as Simmons (1995) observed two clonic phases in electrically stunned pigs. More recently, Velarde *et al.* (2002) reported that two clonic phases occur in electrically stunned sheep and rhythmic breathing returns after the end of the first clonic phase. Ease of recognition of rhythmic breathing depends on the species, position and stunning method used.

Gagging and gasping may occur in animals following the application of certain stunning or stun / killing methods (e.g. carbon dioxide stunning and electrical stun / killing).

The response to painful stimuli like a repeated nose prick with a hypodermic needle can be useful to determine the perception of a noxious stimulus. The pain-perceiving animal will show withdrawal of the head, sometimes followed by the righting reflex (Anil, 1991; see below for righting reflex). If ear pinching induces an ear movement, or pinching the nose induces shaking of the head, or if pinching the skin between the toes of fore or hind limb induces front and back pedal reflexes, the animal is conscious. However, absence of response to a painful stimulus can occur in conscious animals due to the analgesia induced by certain stunning methods (carbon dioxide and electrical methods).

If an animal is attempting to recover a normal body position (righting reflex), it is likely that consciousness has fully returned.

Under practical conditions, eye reflexes and reactions to painful stimuli should always be investigated and evaluated in combination with the resumption of normal rhythmic breathing and righting reflexes to assess stunning effectiveness. Metabolic acidosis induced by exposures to carbon dioxide gas mixtures may result in recumbent and flaccid animals even after the recovery of consciousness.

The signs described under sections 5.9.1 to 5.9.5 are normally evaluated at the end of application of stun or stun / kill methods to determine their efficiency. Signs such as immediate collapse and apnoea occur from the start of application of certain stun or stun / kill methods and they indicate successful application. Some of the signs, such as return of rhythmic breathing and response to painful stimulus (e.g. nose prick or comb pinch), are normally evaluated from the end of application of stun or stun / kill methods and they should not occur at any time after the successful application of a method.

5.9.1. Signs of recognition of a successful mechanical stunning

The signs to recognize a successful mechanical stun are:

- Immediate collapse (it may not be applicable to poultry restrained in a cone or shackle in which severe wing flapping occurs due to the destruction brain).
- Apnoea (absence of breathing).
- Immediate onset of tonic seizure (tetanus) lasting several seconds.
- Loss of corneal reflex.
- Gradual pupillary dilation.
- Absence of response to a painful stimulus (to nose prick with a hypodermic needle for all red meat species and to comb pinch for poultry).

5.9.2. Signs of recognition of a successful electrical stunning

The signs to recognize a successful electrical stun are:

- Immediate collapse (it may not be applicable to poultry restrained in a cone or shackle).
- Immediate onset of tonic seizure (tetanus) lasting several seconds, followed by clonic seizure (un-coordinated kicking or paddling leg movements), applies to all red meat species and to water bath electrical stunning of poultry. Head-only electrical stunning of poultry leads to clonic-tonic convulsions (a reverse of sequence seen in red meat species).
- Apnoea (absence of breathing) lasting throughout tonic-clonic periods.
- Upward rotation of eyes (except for poultry).
- Dilated pupils due to prolonged apnoea.
- Absence of response to nose prick with a hypodermic needle for all red meat species.

5.9.3. Signs of recognition of a successful electrical stun / killing

The signs to recognize a successful electrical stun / kill are:

- Immediate collapse (it may not be applicable to poultry restrained in a cone or shackle).
- Immediate onset of tonic seizure (tetanus) lasting several seconds.
- Immediate onset of apnoea (absence of breathing).
- Dilated pupils.
- Clonic seizure (un-coordinated kicking or paddling leg movements) ensues the tonic seizure but is less pronounced than with an electrical stun.
- Corneal reflex may be briefly present but there is no response to nose prick with a hypodermic needle.
- Gagging or gasping may be present for a short period.
- Complete relaxation of carcass without a pulse.

5.9.4. Signs of recognition of a successful stunning or stun / killing with gas mixtures

All the signs are normally evaluated at the exit from the gas mixture:

- Dilated pupils apply to pigs and poultry.
- Absence of corneal reflex applies to pigs and poultry. After carbon dioxide stunning of pigs, a low percentage (< 5%) of the animals showing corneal reflex at the time of sticking is acceptable, but it should disappear shortly during bleeding.
- Absence of rhythmic breathing.
- Gagging or gasping may be present briefly in pigs but not in poultry.
- Absence of response to nose prick with a hypodermic needle in pigs and absence of response to comb pinch in poultry.
- Complete relaxation of carcass in stun without a pulse under stun / kill in both pigs and poultry.

5.9.5. Signs of ineffective stunning or stun / killing

Ineffective stunning or stun / killing can be recognised by the presence of one or more of the following signs. Those signs apply to all species with all stunning and stun / killing methods:

- Rhythmic breathing.
- Constricted pupil.
- Attempts to raise the head.
- Vocalisation during stunning and / or seizures.
- Corneal reflex (applies to mechanical stunning also).
- Response to a painful stimulus.
- Ears held stiff (not floppy) especially after captive bolt stunning.

5.9.6. Signs of recovery of consciousness

The signs of recovery of consciousness are:

- Rhythmic breathing.
- Corneal reflex.
- Constricted pupils.
- Righting reflex.
- Attempts to raise the head.
- Return of stiffness (muscle tone) in ears.

5.10. DETERMINATION OF DEATH

Humane slaughter regulations require that further operations (e.g. electrical stimulation, decapitation or carcass dressing procedures) shall not begin until the animal is dead. The Animal (Scientific Procedures) Act 1986 of the United Kingdom, which implements the requirements of the European Directive 86/609/EEC (EEC, 1986), stipulates that an animal "shall be regarded as continuing to live until the permanent cessation of circulation or the destruction of its brain". Therefore, from slaughter or killing point of view, death can be recognised from the absence of cardiac activity (e.g. pulse or heart beat) when bleeding has ceased or destruction of brain.

Brain death in animals can be recognised from the absence of brain stem reflexes such as pupillary light reflex, corneal reflex and gagging.

In laboratory situations, complete and irreversible abolition of evoked potentials, especially brain stem auditory and visual evoked potentials, can be used to confirm brain death in animals as used in humans (Guerit, 1999).

It is worth noting that spinal reflexes and automatisms associated with apparent brain death are frequently reported in humans. Brain dead patients may inconsistently extend their elbow and wrist after painful stimuli or touch (Christie *et al.*, 1996). Other reflexes include spontaneous head turning or shaking, neck-arm flexion, neck-hip flexion, neckabdominal flexion, arm extension, and elbow and finger flexion mimicking voluntary grasping or clasping. The Lazarus' sign, named after the biblical man who rose from dead, was coined to reflect some of these reflexes. Urasaki *et al.* (1992) demonstrated that somatosensory evoked potentials could be recorded from the dorsal horn of spinal cord but not the brain of a brain dead human showing respiration-like movements. This suggests that the complex movements shown by brain dead humans may either reflect partial function in spinal neurons or represent the physiologic potential of the intact isolated spinal cord. Spittler *et al.* (2000) found phenomenonological diversities of spinal reflexes and spinal automatisms in brain dead humans and described them according to the time of observation in relation to the development of brain death.

Such reflexes may also occur in animals subjected to a stun / kill method or after bleeding or destruction of brain in effectively stunned and slaughtered animals. Dying brain state could be recognised under field conditions from the absence of gagging or gasping, and papillary or corneal reflexes, which are brainstem reflexes. However, a disconcerting fact is that the occurrence of various kinds of spinal reflexes and spinal automatisms in brain dead animals under stun / kill or slaughter situations has not been clearly identified or elucidated, using neurophysiological tools.

6. AVAILABLE STUNNING AND STUN / KILLING METHODS AND THEIR USE

6.1. INTRODUCTION

All stunning and killing methods should only be used by properly trained, skilled, and licensed personnel (Grandin, 2003).

As a general rule, each method should be applied only once, *i.e.* animals must be rendered unconscious and insensible by a stunning or stun / killing method applied for the first time. In the event of a failure (unsuccessful stun), the personnel should employ appropriate backup stunning method. Two consecutive failures to stun an animal with any method / device must warrant immediate investigation and the fault must be rectified before staring again stunning and slaughteing.

In carbon dioxide stunned pigs however, the excessive GABA release that has occurred is thought to prevent manifestation of epilepsy and therefore, electrical stunning is not normally used. However, it is yet to be demonstrated experimentally. It is worth noting that electrical stunning also results in excessive release of GABA lasting for up to 20 min, at least in sheep (Cook *et al.*, 1992 and 1995); yet re-stunning of recovered pigs with an electric current is a common practice under commercial conditions.

Table 6-1. Back-up stunning methods

First method	Re-stun method
Electrical	Electrical or mechanical
Mechanical	Electrical or mechanical
Gas mixtures	Mechanical

In effectively stunned animals, sticking must be performed promptly by cutting the blood vessels supplying oxygenated blood to the brain. In most of the species, cutting both the common carotid arteries would be sufficient to induce a rapid onset of brain death. In cattle and calves however, the vertebral arteries continue to supply oxygenated blood to the brain after cutting the common carotid arteries at the apex of the neck (neck-sticking) and therefore, cutting the brachiocephalic trunk or artery at the chest (chest-sticking) is essential.

6.2. MECHANICAL DEVICES

The use of free bullets is described in detail in the chapter dealing with on-farm killing methods for disease control, as it is not an appropriate method for killing of animals in slaughterhouses.

Two types of captive bolt guns are commonly used: the penetrating and non-penetrating type. A captive bolt gun has a steel bolt that is powered by either compressed air or a blank cartridge. The tip of the penetrating steel bolt is concave and has a relatively sharp rim, while the non-penetrating has a mushroom headed large bolt (see Figure 6-1 to Figure 6-4). Both types are normally fired on the forehead (usually frontal bone) of an animal, but other sites may be selected due to the presence of horns or thick ridges on the skulls. Captive bolts must always be fired perpendicular (at right angle) to the skull bone surface (at the chosen site), otherwise bolts may skid and fail to fully impact the skull Captive bolts should be generally fired away from the sutures of the cranial bones because they are known to absorb impact energy (e.g. American long-horn rams, which butt each other with great force, have more sutures in their cranial bones than domesticated animals) and also prone to fracture on impact such that the energy is not transferred to the brain fully. Nevertheless, the characteristics of the chosen captive bolt gun (mass, velocity, diameter and length of the bolt) will vary depending on the type of animal it is used for and the expected outcome of their application (stunning or stun / killing).

Penetrating and non-penetrating captive bolt guns are generally designed to use .22 or .25 calibre cartridges (1.25 to 3.0 grains in some countries) or compressed air. The bolts have different shapes to facilitate their use in various species and circumstances. Bolt lengths vary between 70 and 121 mm, diameters between 12 and 14 mm. When properly maintained, the velocity of the commonly used bolts when shot into the air is about 60 m/sec and their kinetic energy is 400 to 420 J.

Although the impact of a blunt penetrating bolt with the skull may induce unconsciousness when the impact energy is sufficient (the gun is functioning properly and correct cartridge is used), the depth of penetration and hence the severity of structural damage induced by the bolt may be limited. This could occur due to lack of a bolt tip that is not sharp enough to shear through the cranial bone and brain tissue (e.g. axonal network) and the bone fragments may contribute to increased resistance against the bolt (limiting its travel distance). Owing to these effects, a blunt bolt may not travel deep enough into the brain to induce sufficient damage to the brain stem where the vital (e.g. respiratory) centres are located. Another factor could be that the impact energy is absorbed by the fractured skull (induced by a blunt bolt), instead of being transmitted to the brain beneath.

Captive bolts may be trigger-operated or contact firing and they have a bolt that is recessed within the muzzle or is level with the edge of the muzzle. Contact-firing guns should be struck against the animal's head to force the cartridge on to a non-moving firing pin. The advantage of these guns, when compared with trigger-operated guns, is the reduced likelihood of an inadequate stun due to holding the gun too far away from the head (Gregory, 1998). The contact-firing model is less suited from a human operator safety point of view and consequently, their use is prohibited in several countries. The guns with bolts that are in level with the muzzle should be held slightly away from the animal's head (e.g. up to 5 mm) to allow the bolt to accelerate before impacting the skull. In captive bolts guns with recessed bolt, the bolt accelerates within the barrel of the gun before impacting the skull.

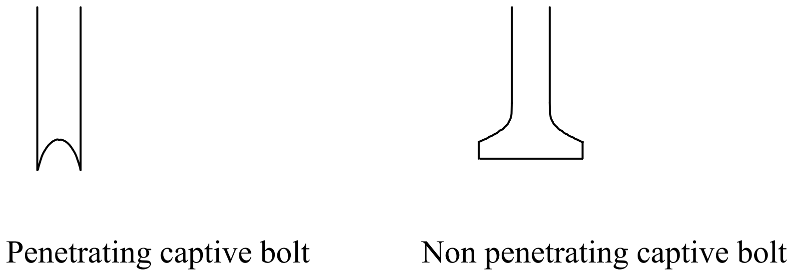


Figure 6-1. Typical bolt tip shapes

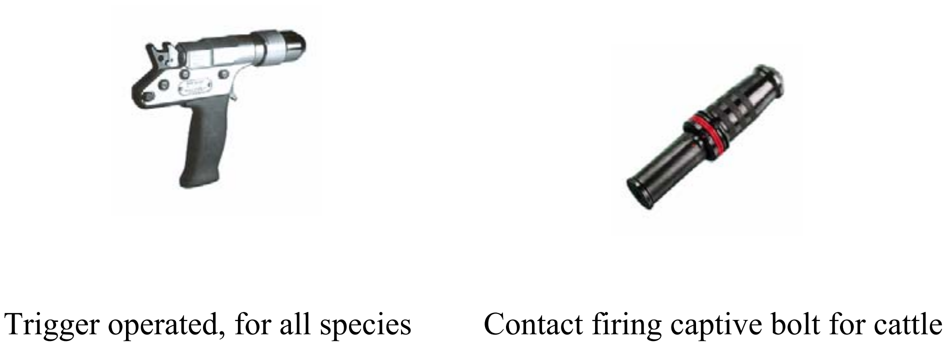


Figure 6-2. Examples of penetrating bolt captive bolts

Trigger operated, for cattle Contact firing, for cattle

Figure 6-3. Examples of non penetrating bolt captive bolts

Figure 6-4. Example of a non-penetrating captive bolt for poultry

The kinetic energy applied to the head is directly proportional to the mass of the bolt but proportional to the square of the bolt velocity (KE=1/2 mv^2). Consequently, increasing bolt velocity will have a relatively greater impact on stun efficiency than increasing the bolt mass. Independently from this observation, the diameter must be sufficiently large to deliver impact. A bolt with a very small mass and a very high velocity will result in an ultra-short time span during which the skull as a whole will not move during transfer of energy. Instead, during impact, there will be a high transfer of momentum and energy locally resulting in the perforation of the skull without transfer of the impact to the head and brain (Karger, 1995). Consequently, the penetration of a narrow bolt into the brain tissue may not always produce immediate loss of consciousness. For example, firing a penetrating captive bolt through a trephined skull failed to induce satisfactory stunning in sheep (Daly and Whittington, 1989). Similarly, firing a 3 mm diameter bolt (7 mm penetration depth) into the skulls of chickens penetrated the head without inducing unconsciousness (Raj and O' Callaghan, 2001).

The power of cartridges or compressed air line pressure needs to be sufficient to deliver the impact energy to achieve immediate unconsciousness. The guns should therefore be used and maintained properly. Stunner cartridges need to be stored in a dry and safe place and cartridges used are required to be appropriate for each species, based on manufacturer's recommendations. It is necessary to ensure that the bolt is fully returned or retracted into the barrel after each shot and to clean the chamber whenever necessary (Gregory, 1998). After the animal is shot, the bolt retracts and is reset for the next animal. If there is a build-up of carbon inside the gun, the bolt fails to return fully to the primed position, which reduces the power of the next shot and, hence, the effectiveness of the stun.

It is necessary that captive bolt guns are frequently cleaned and maintained in good working condition in line with the manufacturer's recommendations. The guns are fitted with several buffer rings, which regulate penetration depth and are also necessary to retract the bolt out of the head; otherwise the captive bolt would remain stuck in the skull of the animal and bend while the animal falls down. Care is required to ensure that the rubber rings are maintained in a good working state and bent bolts are replaced promptly.

When using a non-penetrating captive bolt, unconsciousness should be induced with a single blow at the frontal position of the head. Subsequent shots may not be effective due to the swelling of the skin occurring from the first shot, and therefore, should not be allowed. If exceptionally the first shot is unsuccessful, the animal should be stunned immediately using a penetrating captive bolt or electric current.

6.2.1. Description of effective use

Animal's head must be suitably presented to the operator to facilitate accurate shooting.

Bolt must be fired by a mechanical (compressed air) or explosive device (cartridge) and not by any other instrument.

Severe and irreversible damage to the brain should be induced.

Animal must be rendered unconscious using a single shot.

Bleeding is required and needs to be performed immediately after stunning. Both the common carotid arteries (or blood vessels from which they arise) must be severed to ensure rapid brain death following exsanguination.

Appropriate cartridge selection and storage and gun maintenance, should be done according to the manufacturer guidelines. Air line pressure should be appropriate to the species of animal, as recommended by the manufacturer.

Appropriate backup stunning system shall be readily available.

6.2.2. Monitoring Points

A successful use of the mechanical methods induces:

- Unconsciousness with a single shot at the indicated position of the head (if more than 1 shot is required, the welfare of the animal will be adversely affected).
- Immediate collapse and apnoea (absence of breathing).
- No animal show signs of recovery of consciousness during bleeding (e.g. rhythmic breathing). Species-specific signs will be described in later chapters.

- Ears should be floppy due to general loss of muscle tone.

6.2.3. Advantages

Immediate onset of a sustained period of unconsciousness can be achieved if effectively used.

6.2.4. Disadvantages

Missed firings are frequently caused by bad maintenance or improper use of the gun, and result in poor welfare of the animals.

Investigations of the effectiveness of non-penetrating captive bolts are lacking in some species.

6.3. ELECTRICAL METHODS

6.3.1. Head-only electrical stunning method

Electrical stunning involves transcranial application of an electric current of sufficient magnitude by using a pair of tongs (or electrodes) placed on either side of the head, or through the whole body using an electrified water bath in poultry species only. Electrical stunning may be achieved manually, by the application of electrified tongs on either side of the head, or automatically, by purpose-built devices. The amount of current (A: ampere) flowing through the brain is determined by the amount of voltage applied during the stun (Ohm's Law). At a constant voltage, the amount of current flowing through the brain is inversely proportional to the total electrical resistance in the pathway between the two electrodes or tongs. Therefore, the resistance in the pathway must be kept low by using clean electrodes. In addition, maintaining good electrical contact during stunning and supply of voltage high enough to deliver a recommended current are also essential.

Modern electrical devices involve varieties of waveforms and frequencies of currents. The generic waveforms of currents are pulsed direct currents (DC) and sine wave alternating currents (AC). Precise optimal current / frequency combinations are not known for all species.

The voltage used to deliver AC is expressed as root mean square (RMS) or peak-to-peak because it flows in both the positive and negative directions (bipolar). The amount of current delivered using a sine wave AC is also expressed as RMS current. Sine wave has been modified to produce clipped waveforms resembling a saw tooth and the proportion of edges clipped varies widely. Owing to the clippings, the peak or peak-to-peak voltage necessary to deliver a fixed amount of RMS current with these waveforms is greater than that required with a full sine wave (Gregory *et al.*, 1995).

There is some evidence to suggest that, at a given current level, the depth and duration of unconsciousness, as determined from the magnitude of neuronal inhibition (EEG suppression), induced by electrical stunning is determined by the duration for which the current stays at the maximum level within each cycle, otherwise known as the period (period = 1000 / frequency). For example, electric currents of 50, 400 and 1500 Hz sine wave AC have periods of 20, 2.5 and 0.67 milliseconds, respectively. It is therefore possible to suggest that the effectiveness of electrical stunning depends upon the period of current used and it decreases markedly when the period is insufficient to induce sustained neuronal inhibition following the epileptiform activity, at least in poultry (Raj and O'Callaghan, 2004a). The effect of stunning waveform / frequency has not been quantitatively evaluated in red meat species.

When using a pulsed direct current (DC), the voltage and current employed to stun is expressed as the peak or average since it flows from zero to a peak voltage (unipolar). The period of a pulsed DC consists of mark (current ON time), otherwise known as pulse width of a DC or duty cycle, and space (current OFF time). The mark : space ratio determines the relationship between the peak and average currents of a DC at any given frequency, according to the formula peak current = average current x period in milliseconds / mark in milliseconds. Therefore, the peak current used to deliver an average current of 130 mA of a 50 Hz pulsed DC, which has a period of 20 milliseconds, will be 520 mA at 1 : 3 (130 x 20 / 5), 260 mA at 1 : 1 (130 x 20 / 10), and 173 mA at 3 : 1 (130 x 20 / 15) mark : space ratios. Theoretically, the peak voltage necessary to deliver an average current of 130 mA will also decrease with increasing mark or pulse width for a DC. However, despite the decreasing peak current and peak voltage necessary to deliver the same average current, stunning should be more effective with a mark : space ratio of 3 : 1 than that achievable with a ratio of 1 : 3 for a pulsed DC. In other words, the current ON time within each cycle would determine the depth and duration of unconsciousness.

The head-only method induces tonic and clonic seizures, which are the outward symptoms of grand mal epilepsy. Following the stun, the hind legs are flexed under the abdomen and the forelegs fully extended. The body is tense and tonic (rigid), breathing is absent and the eyeballs may be rotated to a great extent that the pupils may not be visible. In some animals there will be running or paddling movements with the legs. A quiet phase can follow which is linked to exhaustion of the nervous system. The clonic (kicking) phase, which can be either a galloping, cantering or erratic kicking action, follows usually immediately after the tonic phase (Anil, 1991; Gregory, 1998). Electrically stunned pigs and sheep show two clonic phases and they show spontaneous breathing and signs of consciousness and sensibility at the end of the first clonic phase (Simmons, 1995 and Velarde *et al.*, 2002). Electrical stunning further induces pupillary dilatation, which gradually constricts as the animal returns to consciousness.

Application of a current lower than the threshold necessary to induce grand mal epilepsy (either due to low voltage, high resistance or misplaced electrodes) or when the current does not pass through the brain will induce a potentially painful arousal or seizures rather than unconscious state.

Electrodes need to be kept clean to reduce resistance to flow of current. If water or saline are not used, electrodes are required to be routinely cleaned with a powered wire brush (Gregory, 1998). Poor electrode maintenance or contact with the head can be recognised from the burning of the skin, hair or feathers due to the development of heat, which normally occurs due to increased electrical resistance.

Effective stunning will occur when a current of sufficient magnitude is passed through the brain. The total impedance or resistance in the pathway between the electrodes varies between animals depending upon the shape, size, material and cleanliness of the electrodes, tissue resistance, pressure applied during stunning and voltage used, at least, in pigs (Wotton and O'Callaghan, 2002). The time taken to breakdown this resistance seems to be shorter when high voltages (250V or more) are employed, with other conditions being ideal. Nevertheless, when a constant voltage stunner is used, the current starts to flow from zero to the maximum, which would take certain time depending upon the voltage. By contrast, constant current stunners are designed and constructed in such a way that they anticipate high resistance in the pathway and hence start with the maximum available voltage, which is usually in excess of 250V. Owing to this, the target current is reached within the first few current cycles (within milliseconds of the start of current application) and the applied voltage may also be modulated according to the changes in the resistance. Therefore, constant current stunners are preferred to constant voltage stunners.

In addition, constant-current electrical stunning devices could be fitted with an acoustic and / or optic signals, to indicate, (a) an interrupted stun, (b) excessively short stun duration and / or (c) increase in total electrical resistance in the pathway (due to dirt, fleece or carbonisation), which could lead to inadequate stunning. Such devices would facilitate effective monitoring of electrical stunning and stun / killing methods under commercial conditions (see Electrical stun / killing methods).

Electrical stunning devices should display visibly the delivered voltage and current during each stunning cycle, the voltage and current measuring devices should be appropriate to the waveform of the current used in the stunner. Furthermore, a calibrated volt and / or current meter appropriate to the waveform of the current should be used to verify the output of the stunner. The sampling rate of the meter needs to be fast enough and appropriate to the electrical parameters. Slow rate of sampling will distort the waveform of current. The effective use of such meters in conjunction with a dummy electrical load (electrical resistors) appropriate to the expected total electrical resistance in the pathway will also facilitate correct setting up of voltages in stunners or stun / kill devices to deliver sufficient current. This must be ideally performed at the beginning of each shift before the stunning or stun / kill devices are applied to animals and as required thereafter.

Electrical stunning and monitoring equipment need to be adequately protected from both physical and water damage. Access to the animals showing signs of recovery of consciousness on the bleeding rail is required to employ back-up procedures efficiently.

The equipment must be inspected at regular intervals, in order to ensure that it is operating correctly according to the specification and that it is in good state of repair.

The details of electrical parameters, such as waveform, frequency and the output voltage and current in appropriate units (average or RMS) need to be readily available for inspection to verify that correct parameters are applied, ensuring that a current of sufficient magnitude beyond that needed to induce generalised epilepsy is applied. This could be helped by evaluating the stunners in designated laboratories, using established neurophysiological criteria as mentioned above, and obtaining a certificate (kite mark) accordingly. This accountability on the part of equipment manufacturer could facilitate and improve monitoring of electrical stunning under commercial conditions.

6.3.2. Electrical stun / killing methods

Effective head-only electrical stunning produces a brief period of unconsciousness and is always accompanied by tonic-clonic seizures. The seizures are not conducive to prompt and accurate sticking of animals to prevent return of consciousness following stunning. Electrical stun / killing methods on the other hand involve induction of cardiac ventricular fibrillation (rapid and irregular beating of the heart), by passing an electric current across the heart in unconscious animals that have been stunned by head-only electrical stunning or simultaneous induction of unconsciousness and cardiac ventricular fibrillation.

Cardiac ventricular fibrillation threshold testing in experimental models suggests that cardiac tissue is most sensitive to stimulation between 30 and 60 Hz of sine wave alternating current and increased stimulus duration increases efficiency (Weirich *et al.*, 1983). However, successful induction of cardiac ventricular fibrillation would depend upon the delivery of sufficient electrical current to the myocardium. The amount of current delivered will depend upon the voltage and total impedance in the pathway (between the electrodes). Scientific literature concerning termination of ventricular fibrillation, in pig models, by electrical counter-shocks reveals that impedance is affected by size of the electrodes, applied pressure, the phase of respiration during which the shock is applied, use of coupling gel and its salt content and the distance between the electrodes, which is dependent upon the circumference of chest during transthoracic application (Niemann, Garner and Lewis, 2003). However, published scientific information regarding the effects of these variables during the induction of cardiac ventricular fibrillation in food animals is lacking.

When resuscitation is not attempted, cardiac ventricular fibrillation leads to cardiac arrest, sometimes within seconds, but often after about 5-10 min. In any case, cardiac ventricular fibrillation impairs cardiac output (reduced to less than 30% of normal) and normal blood circulation. Consequently, it induces hypoxia in the brain and myocardium, which either prolongs the period of unconsciousness and insensibility induced by the head-only electrical stun or leads to death under electrical stun / killing methods (Von Mickwitz *et al.*, 1989). Under these circumstances, at the least, the ability of an animal to regain consciousness and sensibility is seriously impaired, even if it is not bled out. In addition, the severity of clonic seizure is also reduced or eliminated in animals subjected to electrical stun / killing methods. In this regard, the tonic seizure leads to muscle relaxation in the carcass. Therefore, electrical stun / killing methods are preferable to electrical stunning methods on animal welfare grounds. Animals may show brain stem reflexes, such as gagging or gasping and corneal reflex, for a short time after the application of electrical stun / killing method and these brain stem reflexes indicate dying brain rather than presence of consciousness and sensibility. Problems may occur in automatic stun / killing devices due to variations in shape and size of animals and the way the carcasses are subsequently handled.

Electrical stun/kill is usually induced in automatic systems, whose settings should be adapted to the size of the animals.

Cardiac ventricular fibrillation can be effectively induced with a 50 Hz sine wave AC. Higher frequencies do not produce the cardiac ventricular fibrillation, but they may reduce muscle spasms and convulsions. Voltage should be at least 100 mV/cm at the level of the heart (Von Mickwitz *et al.*, 1989). In small animals (piglets, young lambs, rabbits), it may be difficult to induce cardiac ventricular fibrillation because, due to the small size of the heart, the current passes through tissues surrounding the heart, rather than through the heart. The electrical resistance of various other tissues in the pathway may also play roles in this.

Under commercial conditions, cardiac ventricular fibrillation may be induced using a *single cycle* or a *two-cycle* system. In a single cycle system, induction of cardiac ventricular fibrillation involves application of an electric current by using a pair of tongs (or electrodes) placed on the head (in front of the brain) and body (behind the apex of the heart) or an electrified water bath (in poultry species only), such that the electrical field spans the brain and heart. In a two-cycle system, two separate electric current cycles are used: a transcranial application immediately followed by a second application of an electric current from head-to-body (behind the position of heart) or across the chest (transthoracic).

6.3.3. Description of effective use for both electrical methods

Sufficient current should flow through the target organs (brain and heart) to achieve effective stun or stun / kill. Currents and voltages used should be (a) based on scientific evidence, (b) of sufficient magnitude, and (c) appropriate to the species.

Animals should be restrained suitably to facilitate uninterrupted application of the electrical current to stun and / or kill.

Electrodes should be placed so that target organ lies between them for effective stunning or killing, either head-only or head-to-body applications respectively.

Good electrical contact should be maintained between the tongs and the head (taking account of animal hair and wool), or between head and body, during the application for stunning and killing respectively.

Electrical current must be applied once only.

Bleeding should be ideally performed while the animal is in the tonic phase (under head-only electrical stunning).

No animal should show signs of recovery of consciousness after application of the stun or stun/kill methods.

In any case, a back-up stunning device must be readily available.

6.3.4. Description of effective use for electrical stun / killing

Cardiac ventricular fibrillation or body electrodes must be placed such that the electrodes span the heart and cardiac arrest is induced.

No animal shall survive the treatment.

6.3.5. Advantages

If sufficient current is used, electrical stunning or stun / killing are immediate in 100% of the animals.

6.3.6. Disadvantages

Duration of unconsciousness can be short after head-only stunning.

Particular restraint of animal is needed to facilitate proper application of the electrodes, which can be distressing.

Use of inadequate electrical parameters and / or inappropriate electrode placement would cause pain and distress.

6.4. GAS MIXTURES

Carbon dioxide is commonly used to stun or stun / kill pigs and poultry.

In humans, inhalation of high concentrations of carbon dioxide is described as pungent and causes breathlessness (Gregory *et al.*, 1990; Stark *et al.*, 1981). Therefore, it is not surprising to note that carbon dioxide has been used as a pain stimulus and acute stressor in some studies (Thurauf *et al.*, 1991; Anton *et al.*, 1992; Hummel *et al.*, 1994; Barbaccia *et al.*, 1996). Danneman *et al.* (1997) evaluated the human experience of inhalation of 50 to 100% carbon dioxide, the results indicated that the majority (14 out of 20) of human volunteers found inhalation of 50% or more carbon dioxide 'uncomfortable' and almost all of them (18 out of 20) found 'unable to take full breath' of 100% carbon dioxide. Inhalation of carbon dioxide has been reported to induce cardiac arrhythmias in humans (MacDonald and Simonson, 1953).

Studies involving laboratory rats and mice have also demonstrated that carbon dioxide, even at 20%, is aversive in these species (Leach *et al.*, 2001). It is worth noting that the carbon dioxide and oxygen mixture was also evaluated for sedating rodents, and research has shown that carbon dioxide and oxygen mixture is extremely aversive to them and, given a free choice, they too avoid it.

Research carried out under laboratory conditions has shown that the majority of pigs trained to obtain a reward (an apple) in an experimental set-up will avoid an atmosphere of high (80% or more) concentrations (Raj and Gregory, 1995). This aversion was found to be greater than the motivation to obtain a reward in the carbon dioxide atmosphere, even after 24 hours of fasting. It has also been reported that turkeys and chickens find 72% and 47% by volume of carbon dioxide in air aversive, respectively and given a free choice, they avoided a feeding chamber containing these levels of carbon dioxide (Raj, 1996).

Clearly, the aversive effects of initial exposure and subsequent inhalation of carbon dioxide has been demonstrated in many species of animals, including humans. The animals will have to tolerate these adverse effects until they become unconscious or the occurrence of analgesic stage just prior to unconscious stage and the time to loss of consciousness, determined on the basis of abolition of SEPs, can be up to 36 sec, in pigs exposed to 80 to 90% carbon dioxide in air (Raj *et al.*, 1997a). Forslid (1987) reported that exposure of pigs to 80% carbon dioxide for 1 min resulted in quiescent EEGs. In 90% carbon dioxide in air, the AEPs are abolished within 14 sec (Martoft *et al.*, 2001).

Owing to these concerns, it has been argued whether the cumulative distress occurring during the induction of unconsciousness with carbon dioxide is less than that occurring during decapitation and cervical dislocation of laboratory rodents (Humane Society of the United States (HSUS) draft communication on the use of carbon dioxide for euthanasia and anaesthesia). A similar argument can be raised with regard to killing of poultry using these methods for disease control purposes, and it has also been raised during discussion on slaughter of animals without stunning (Katme, 1987). Current knowledge does not allow calculating an 'overall suffering score' based on duration and degree of suffering. The usefulness of such arguments remains, therefore, disputable.

Carbon dioxide induces unconsciousness in pigs and poultry even at a concentration of 40% by volume in air (Raj and Gregory, 1996; Raj and Gregory, 1990a). However, the time to loss of consciousness will be prolonged with such a low concentration, especially in pigs (Raj and Gregory, 1996), which may not be feasible under commercial conditions where high throughput rates are required. In addition, research has shown that pigs show escape attempts when the rate of induction of unconsciousness is slow (e.g. during exposure to 40 to 60% by volume of carbon dioxide in air) (Raj and Gregory, 1996; Troeger and Woltersdorf, 1991). It has been suggested that a minimum of 55% by volume of carbon dioxide would be necessary to stun / kill chickens in a transport crate and a minimum of 70% by volume would be required to stun or stun / kill pigs (Raj and Gregory, 1990b; Hoslt, 1999).

As an alternative to using high concentrations of carbon dioxide, hypoxia induced with argon has been evaluated for pigs and poultry. The results of these studies have shown that pigs and poultry do not find hypoxia aversive and, given a free choice, they voluntarily enter hypoxic atmosphere and get stunned or killed (Raj, 1996; Raj and Gregory, 1995 and 1996). Woolley and Gentle (1988) evaluated the use of nitrogen-induced hypoxia for stunning / killing poultry and reported that the birds did not show any signs of distress during the process. Together, these studies indicated that hypoxia induced with argon, nitrogen, or mixtures of these two gases, is ideally suited to stun or stun / kill pigs and poultry.

As another alternative to using high concentrations of carbon dioxide, a mixture of 30% carbon dioxide and 60% argon in air has been evaluated. This is because, poultry do not avoid an atmosphere containing this gas mixture and pigs do not avoid an atmosphere containing 30% carbon dioxide in air (Raj, 1996; Raj and Gregory, 1995 and 1996).

Under commercial conditions, the choice of gas mixture to be used will determine the design of the stunning or stun / killing chamber. In this regard, the specific gravity of a gas or gas mixture is its relative density with respect to air, at the same pressure and temperature. The specific gravity of air is considered to be unity (one). Gases with a specific gravity less than one will be 'lighter than air', whilst those with a specific gravity of greater than one are 'heavier than air'(Table 6-2, Kettlewell, 1986).

Table 6-2. Specific gravity of gases used for stunning or stun / killing (Kettlewell, 1986)

Gas	Specific gravity at 300K (27°C) at 1atm.
Air	1.00
Argon	1.38
Carbon dioxide	1.50
Nitrogen	0.97

Heavier than air gas mixtures are normally preferred to stun or stun / kill animals. This is because it is relatively easy to contain them within a concrete pit or steel tunnel into which the animals could be conveyed. A mixture of predominantly (e.g. 80% by volume) nitrogen and low concentrations of heavier than air (e.g. 20% by volume of argon or carbon dioxide) would create an atmosphere that is slightly heavier than air and could be contained within a tunnel.

Practical experience has shown that it is difficult to stun 100% of the animals effectively without some being inadequately stunned (and regain consciousness before sticking or during bleeding) and some being killed (Zeller *et al.*, 1988). This is possibly due to either biological variation between animals or age, weight and metabolic state. Mixture of carbon dioxide and air or oxygen or argon appears to be aversive (pungency, hyperventilation, breathlessness, impression of suffocation). Inert gas mixtures that do not have less aversive effects during the induction phase are being used to stun / kill poultry under field conditions and have also been tested, using existing equipment, for pigs under field conditions. However, development of purpose built equipment necessary to implement inert gas mixtures for stun / killing pigs under field conditions is warranted.

6.4.1. Description of effective use

It is important that the stunning and monitoring equipment is inspected at regular intervals in order to ensure that it is operating correctly according to the manufacturer's specifications and that it is in a good state of repair.

The chamber in which animals are to be exposed to the gas mixtures and the equipment used for conveying animals through it need to be designed, constructed and maintained in such a way as to avoid injury, pain and suffering.

The gas concentrations must be continuously monitored and maintained at the prescribed levels such that animals continuously inhale the recommended gas mixture from the time of introduction until unconsciousness or death occurs.

A clearly audible and visible warning is required if the gas concentrations deviate from the recommended levels.

Animals should be able to stand in a normal position and breathe without restraint. They should inhale the maximum gas concentrations for the recommended exposure time.

The concentration of carbon dioxide and duration of exposure determines whether the animals are stunned or not, the duration of unconsciousness or the onset of death.

6.4.2. Monitoring points

No animal should show signs of recovery of consciousness (see Chapters relevant to individual species).

6.4.3. Advantages

In adequately designed systems, the method requires less handling of the animals and no restraint.

6.4.4. Disadvantages

The induction of unconsciousness with carbon dioxide mixtures appears to be aversive and distressing to animals (pungency, breathlessness, impression of suffocation).

7. STUNNING AND STUN / KILLING METHODS FOR CATTLE

Three main methods exist to stun adult cattle and calves:

- non-penetrating captive bolt stunning,
- penetrating captive bolt stunning,
- electrical stunning.

The penetrating captive bolt is the method far most used for adult cattle. Calves are usually stunned by a non-penetrating or penetrating captive bolt. Non-penetrating stunning is generally not used for adult cattle.

Among the main red meat species, cattle and calves appear to loose consciousness due to sticking relatively slowly (Table 7-1). After shechita slaughter (neck cutting without previous stunning), spontaneous brain activity was lost after 19 to 113 sec (means 75 sec). Somatosensory and visual evoked potentials were lost after 32-126 (means 77 sec) and 20-102 sec (means 55 sec), respectively (Daly *et al.*, 1988). Duration of spontaneous cerebral activity and evoked responses were strongly positively correlated (Daly *et al.*, 1988; Daly, pers. comm., 2003). Another study found that after severing the external jugular veins and the common carotids, calves showed an isoelectric EEG after 35 to 50 sec in 3 animals, but after 680 sec in a fourth calf (Bager *et al.*, 1992). These results suggest that in some animals, unconsciousness may start 19 to 20 sec after the neck cut, but in others it may be much delayed.

Table 7-1. Comparison between captive bolt stunning and slaughter without stunning in cattle (mean + SD) (Daly *et al.*, 1988)

	Captive bolt			Shechita (neck cut)		
	Mean time (s)	Range (s)	n	Mean time (s)	Range (s)	n
Onset of HALF*	10 ± 5	4-17	8	7.5 ± 2	5-13	8
Duration of HALF	44 ± 20	21-58	3	28 ± 28	9-85	8
Onset of <10 μV cortical activity	69 ± 1.5*	67-71	5	75 ± 48	19-113	8
Loss of SERs**	0	-	8	77 ± 32	32-126	7
Loss of VERs**	0	-	8	55 ± 32	20-102	8

* HALF: High Amplitude, Low Frequency waves.
** Somatosensory evoked response
*** Visual evoked response

Two phenomena have been invoked to explain the delayed loss of consciousness. First, in cattle, at neck cutting, the external jugulars and the common common carotids are severed. In contrast, vertebral arteries are not cut as they are protected within the foraminae of the cervical vertebrae. Therefore, part of the blood supply to the forebrain is maintained via the vertebral-occipital anastomosis, the vertebral-maxillary anastomosis and carotid rete (**Figure 7-1**). It was initially believed that the animal loses consciousness when blood loss via the carotid stumps reduces blood pressure sufficiently to reduce blood flow to the brain via the alternative anastomotic routes (Newhook and Blackmore, 1982). It was subsequently found, however, that previous surgical occlusion of the vertebral arteries of animals that had their common carotid arteries cut did not influence onset of unconsciousness. The results indicate that the blood flow through the vertebral arteries alone does not explain the prolonged time to loss of consciousness (Shaw *et al.*, 1990). In addition, it does not explain variability between animals. Other factors that are yet to be revealed may also play a role. Specifically, it was found that in some of the animals, blood clots appear at the caudal severed ends of the common carotid arteries, causing occlusions of the arteries (Anil *et al.*, 1995a, b). Anil *et al.* (1995a, b) suggested that several mechanisms could be responsible for this effect. First, the artery is elastic and may spring back into its connective tissue sheath. Second, platelets can aggregate at the cut end of the common carotid, leading to the rapid production of a clot. Third, after the cut, the artery may produce an annular spasm, facilitated by the release of vasoactive amines from platelets (Graham and Keatinge, 1975). In calves, mean arterial blood pressure progressively drops from about 110 mm Hg to close to 0 in about 35 to 40 sec after neck cutting. When carotid occlusions occur, levels of 0 may be obtained only after 200 sec because blood reaches the brain via the anastomic routes. Consequently, onset of unconsciousness was delayed in these animals (Anil *et al.*, 1995a, b; Bager *et al.*, 1992). When the chest-sticking technique was used (preceded by 7 sec head-only stunning, see below), by cutting the major blood vessels arising from the heart, no such occlusions occurred and mean arterial blood flow fell from about 70 mm Hg to near 0 within 8 sec (Anil *et al.*, 1995b). Summarising, the chest sticking technique was more efficient for two reasons: (1) blood loss is faster resulting in a rapid drop in blood pressure and (2) this result was reliably obtained in all animals. However, chest sticking takes longer than the neck cut as it is preceded by a skin cut (see point 4.4).

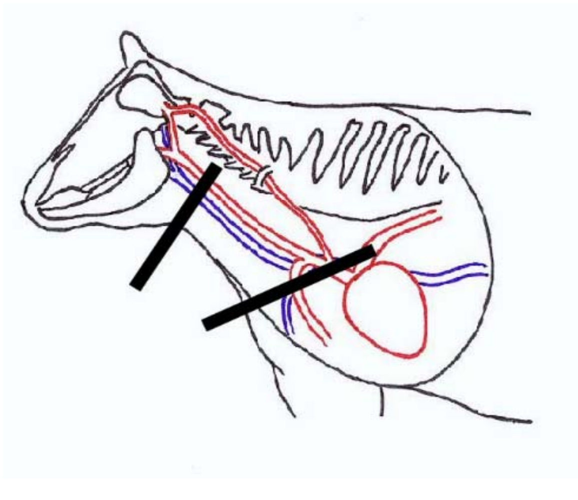


Figure 7-1. Sites of neck cutting and chest sticking in cattle

7.1. MECHANICAL METHODS

7.1.1. Penetrating captive bolt

To ensure efficient stunning in adult cattle, the captive bolt must be fired at the cross-over point of imaginary lines drawn between the base of the horns and the contralateral eyes (MIDAS, 1978), and certainly no further away than 2 cm radius from this point (Lambooij, 1981a and b). The muzzle of the captive bolt must be directed towards the centre of the brain and placed at right angles to the skull (Finnie, 1993). Adequate restraint of the animal and fixation of the head allows proper positioning of the instrument and shooting of the animal. For calves and adult cattle, the cartridge or air pressure chosen should produce sufficient penetration force to cause trauma to the cortex and deeper parts of the brain (Lambooij *et al.*, 1983). Recoil should be taken into account; it depends on the make of the captive bolt gun and on the weight and recoil properties of the operator. In one study, bolt velocities below 58 m/sec were found insufficient for 31% of the cattle (Daly *et al.*, 1987). This study used 16 Hereford x Friesian steers (291-403 kg) and 19 Friesian cows (455-735 kg), and reports that bolt velocity or the animal's body weight did not alter success rate. However, the results are difficult to interpret in terms of impact, as the study does not mention bolt diameters or gun characteristics. Another text recommends bolt velocities of 55 m/s for steers, heifers and cows, and 72 m/s for young bulls (Gregory, 1998), but again, no information was given on bolt diameters or gun characteristics.

The mean (± SD) and ranges of times to the onset of delta waves, their duration, and loss of somatosensory and visual evoked responses after captive bolt stunning and shechita slaughter are presented in Table 7-1 (Daly *et al.*, 1988). After shechita, cortical activity remained unchanged for a period after sticking followed by a period of high amplitude low frequency activity which eventually developed first into a period of very low amplitude (10 - 15 μV) high frequency activity and then into the isoelectric EEG (Daly *et al.*, 1988).

When adult cattle or calves are properly stunned, the muscles in the back and legs go into spasm. Forelegs and hindlegs are flexed and after 5 sec the forelegs will straighten and become extended. If the muscles are flaccid immediately after stunning, this is an indication that the state of unconsciousness is not profound and there is a risk that the animal will regain consciousness (Gregory, 1998). The eyes should not be rotated. Rotated eyeballs indicate that a deep stun is not present and there is a risk of return of consciousness (Gregory, 1998). There should be no reflex responses to a nose prick or ear pinch (Gregory, 1998). The chest-sticking technique, by cutting the main vessels arising from the heart, is more efficient (see above: Anil *et al.*, 1995b). If chest-sticking is done rapidly after the stun, the return of consciousness will be avoided.

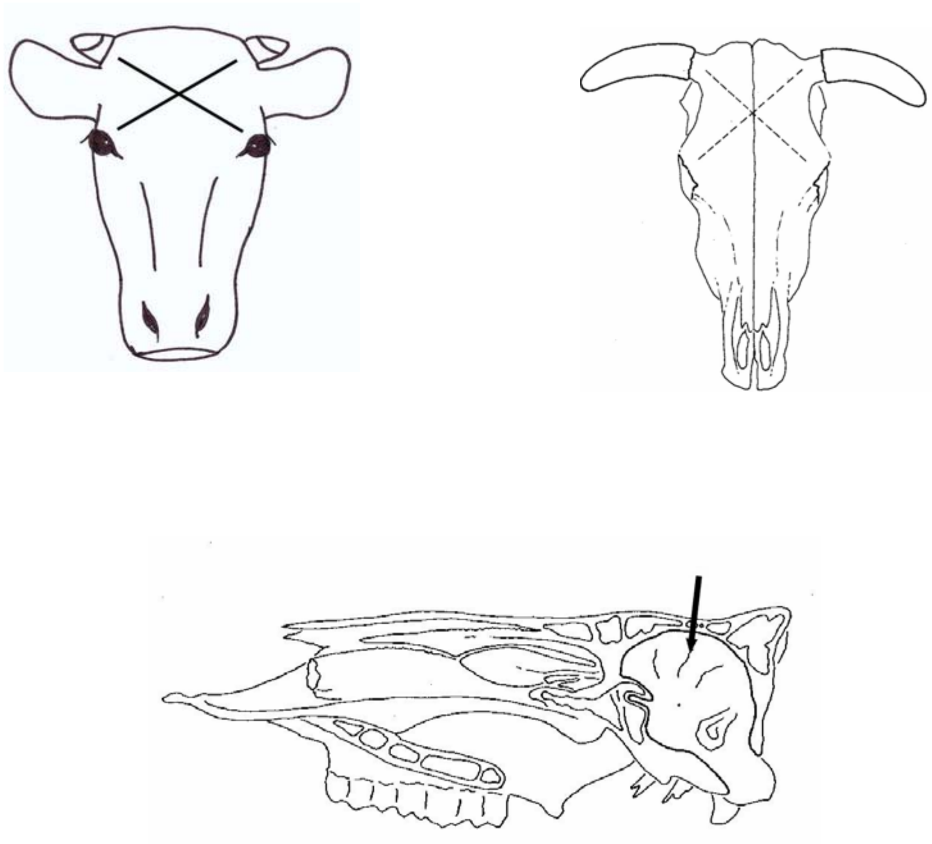


Figure 7-2. Recommended, frontal shooting site for penetrating and non-penetrating captive bolt stunning in adult cattle and calves

In adult cattle, when properly used, frontal captive bolt stunning (Figure 7-2) may achieve a 100% success rate (Daly *et al.*, 1988; Gregory and Shaw, 2000). Following frontal captive bolt stunning of cows (455 ± 24 kg), evoked responses were immediately abolished, while delta waves occurred 4 to 17 sec after the stun, lasting 21 to 58 sec (Daly *et al.*, 1988). The latter study established that isoelectric EEG developed before sticking (at 60 sec) in 3 animals, 21, 53 and 58 sec after the stun. In the remaining 5 animals, isoelectric EEG developed 9.5 ± 1.5 sec after sticking (Daly *et al.*, 1988). Respiratory activity usually ceases immediately after stunning (Vimini *et al.*, 1983).

In another experiment (Fricker and Riek, 1981), 12 cows were chest stuck on average 124 sec (minimum 78) after stunning: iso-electric EEG was observed at 40 to 115 sec after stunning for 11 cows. One cow had an iso-electric EEG only after more than 428 sec after the shot (30 sec after bleeding), undoubtedly due to bleeding rather than to the stun. This animal resumed breathing temporarily 2 min after the shot although EEG data indicated unconsciousness and no other signs of recovery (palpebral, pupillar, or corneal reflex) could be detected. The sticking procedure performed after 398 sec of stunning did not cause a change in the EEG. In this cow in contrast to the others the brain stem showed no macroscopic detectable lesions.

The iso-electro EEG state was accompanied with uncontrolled kicking movements (Fricker and Riek, 1981; Daly *et al*., 1988). Heart activity continued for about 4 min if the animal is bled immediately following stunning, but continued for 10 min if the animal is not bled. Respiratory activity had ceased in all animals immediately following the stun, suggesting that they were unconscious (Vimini *et al*., 1983).

Work on stunning calves (6 months, 200 kg) with a penetrating captive bolt found that frontal stunning and occipital stunning (into the direction of the brain; Figure 7-3) ensured immediate unconsciousness as shown by the appearance of delta (< 4 Hz) and *theta* waves (4-8 Hz), tending to an isoelectric EEG (Lambooij and Spanjaard, 1981; Blackmore and Newhook, 1982). Additionally, the corneal reflex was absent. Occipital shooting did not cause macroscopical damage to the cortex to the same extent as frontal stunning. Shooting in the nape of the neck (behind the horns, into the direction of the throat; Figure 7-3) is not satisfactory, since it only caused unconsciousness (measured by the appearance of delta and *theta* waves) 21 ± 6 sec after shooting (Lambooij and Spanjaard, 1981; Hofer, 1985).

Despite the potential efficiency of penetrating captive bolt stunning, practical information shows that mis-stuns occur relatively frequently. These often result in a faster recovery (within 1 or 2 min). In Germany, where the law prescribes to abattoirs a check of their guns every two years by an authorized instance (pneumatic or cartridge driven captive bolt guns must be inspected by the manufacturer at least every two years or earlier if severe functional defects appear), 4% of 1100 bulls (> 600 kg) needed a second stun (Von Wenzlawowitz, pers. comm., 2003). In other countries levels between 4 and 6.6% have been reported (Gregory, 1993b).

In summary, captive bolt stunning, when fired with appropriate cartridge, or air pressure in the case of pneumatic stunners, and applied frontally, induces reliably effective stunning in all adult cattle and calves. In calves, occipital shooting may be adequate if properly directed, although there is a risk that it may be mis-directed. This would result in shooting in the nape of the neck and this method gives unsatisfactory results.

Since the duration of unconsciousness may last up to 10 min or longer, stun-to-stick interval is not critical. However, when mis-stuns arise, immediate re-stunning and bleeding of the animal would prevent further suffering.

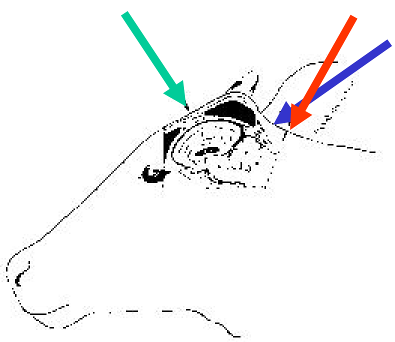


Figure 7-3. Different shooting positions (adapted from Lambooij and Spanjaard, 1981)

7.1.1.1. Description of effective use

The animal should be restrained and the head should be presented in such a way that the gun can be placed and fired correctly. The interval between restraining of the animal and the stun should be as short as possible.

The gun should be placed correctly.

After effective stunning, the stun to stick interval is not critical. If mis-stunned, the animal should be immediately re-stunned and bled. Chest sticking is recommended.

7.1.1.2. Monitoring points

Effective captive bolt stunning produces the following outwardly signs. Effects should last until the end of bleeding:

- Immediate collapse.
- Immediate and sustained absence of rhythmic breathing.
- Absence of righting reflex.
- The muscles in the back and legs go into spasm. Forelegs and hindlegs are flexed, and after 5 sec the forelegs will straighten and become extended.
- The eyes should not be rotated. Rotated eyeballs indicate that a deep stun is not present and there is a risk of return of consciousness.
- No reflex responses to a nose prick or ear pinch.
- Absence of vocalisation.

7.1.1.3. Advantages

If properly used, penetrating captive bolt stunning ensures very rapid and sustained loss of consciousness in 100 % of the animals.

7.1.1.4. Disadvantages

Animal restraint and proper presentation of the head is required, which can be stressful to the animal. It is difficult to achieve 100% effective stuns and a risk of mis-stunning exists, depending on the skill of the operator and the state of maintenance of the gun.

Investigations under practical conditions in 5 slaughterhouses done by Ilgert (1985) showed that 44 out of 1100 (4%) investigated cattle required a second shot. The mis-stunnings occurred due to insufficient head restraint and wrong position of the operator.

7.1.2. Non-penetrating captive-bolt-stunning

To ensure effective stunning in adult cattle, the captive bolt must be placed 2 cm above the cross-over point of imaginary lines drawn between the base of the horns and the contralateral eyes (HSA, 1998). Proper restraint of the animal and fixation of the head allows adequate positioning of the instrument and shooting of the animal.

Studies on 12 adult cattle found that frontal non-penetrating captive-bolt-stunning (Figure 7-2) resulted in immediate loss of consciousness in all animals, as indicated by immediate collapse and, after brief tetanic spasms, in slow uncoordinated hindlimb movements of increasing frequency (Finnie, 1995). Rhythmic breathing was absent. The animals presented a depressed fracture of the frontal bone and widespread subarachnoid haemorrhage, particularly beneath the impact site, in the temporal and frontal lobes, and around the brainstem. Petechial haemorrhage was observed in the basal ganglia and thalamus. Duration of unconsciousness has not been reported as animals were immediately bled.

A recent field study (Moje, 2003) on non penetrating captive bolt stunning on about 1200 cattle in 2 abattoirs found that 20 to 30% needed an immediate re-stun. Depending on the abattoir, more than 90% or 40% of the animals showed skull fractures. The author concludes that from the animal welfare point of view the method is unsatisfactory, due to the relative high failure rate. He suggests that improvement of the shape of the bolt, better fixation of the head, and standardisation of cartridge power, may improve the results. He further indicates the necessity of rapid sticking.

Electroencephalic studies found that frontal non-penetrating captive-bolt-stunning (Cash knocker, cartridges for light weight cattle) produced immediate unconsciousness in 80% of 31 calves (6 months, 200 kg; Lambooij *et al.*, 1981). Increasing cartridge force may help to improve efficiency, but may also cause skull bone fracture. In the latter case, efficient stunning may be more difficult to obtain as the skull absorbs part of the impact, the kinetic energy transferred to the brain is reduced proportionally to the extent of the fracture (Von Wenzlawowicz, pers. comm., 2003). Another study found that 80% of 90 calves (1 to 2 weeks of age, Hantover knocker, using air pressures of between 35 and 50 kg/cm^2) were effectively stunned as determined by behavioural observations (Blackmore, 1979).

In calves, outward signs of effective non-penetrating captive-bolt stuns were described as the appearance of 5-15 sec tonic convulsions and spasms prior to relaxation, or of extensor rigidity and some generalised muscular tremors, followed by slow hind leg movements, developing into vigorous hind leg kicking (clonic spasms or convulsions) (Blackmore, 1979; Lambooij *et al.*, 1981). Absence of the righting reflex lasted at least 60 sec. In effectively stunned calves, palpebral, corneal, labial, spinal and digital reflexes were absent, but the corneal reflex often returned within 20 sec, while respiration stopped for up to 35 sec (Blackmore, 1979). These observations suggest that duration of unconsciousness depends on the animal and on the impact energy, but could last 20 to 35 sec In addition, nystagmus (involuntary, rhythmical, repeated oscillations of one or both eyes), inward rotation of the eyes and a reflex vocal response were frequently observed immediately following the stun. Inward rotation of the eyes may indicate that the state of unconsciousness is not profound (Gregory, 1998).

Non-penetrating captive bolt stunning was accompanied by gross brain haemorrhage in 50 to 100% of the calves (Blackmore, 1979; Lambooij *et al.*, 1981). In calves, occipital non-penetrating captive bolt stunning (Figure 7-3) had a lower success rate (3 out of 8 calves) than frontal application (15 out of 19 calves) and therefore, occipital shooting is less effective and not advisable (Lambooij *et al.*, 1981).

The chest-sticking technique, by cutting the main vessels arising from the heart, is more efficient (Anil *et al.*, 1995b) and should be rapid to avoid the return of consciousness. As the stun may last as short as 20 sec (determined for calves; no data for cattle), and as chest sticking induces a blood pressure of near 0 after about 8 sec (Anil *et al.*, 1995b), animals should be chest stuck no later than 12 sec after the stun.

Summarising, limited data show that frontal non-penetrating captive bolt stunning induced unconsciousness in 100% of the adult cattle and in 80% of calves. Occipital non-penetrating captive bolt stunning in calves had an even lower success rate.

7.1.2.1. Description of effective use

Since the duration of insensibility induced by this method could be as short as 20 sec, animals should be bled as soon as possible after stunning.

The air has to be sufficiently compressed or the cartridge chosen has to produce sufficient velocity to stun the animal.

A penetrating captive bolt as a backup stunning device is essential.

7.1.2.2. Monitoring points

Effective non-penetrating captive bolt stunning produces the same outwardly signs as penetrating captive bolt stunning (see 7.1.1.2.).

7.1.2.3. Advantages

There are no animal welfare advantages compared to penetrating captive bolt stunning

7.1.2.4. Disadvantages

Non-penetrating captive bolt stunning is not always effective for all types of animals. When the skull is immature, bones (calves) may be crushed and the impact may be insufficient. When the skull is very thick (bulls), the power of the gun may be insufficient. Occipital and temporal non-penetrating captive bolt stunning should be avoided.

The duration of unconsciousness is relatively short. Animals should be chest stuck within 12 sec.

7.1.3. Free bullet

Free bullets are not used in slaughterhouses, except under exceptional conditions (their use under disease control situations will be described in chapter 13)

7.2. Electrical stunning and electrical stun / killing methods

Some of the major drawbacks of head-only electrical stunning of cattle and calves are the short duration of the epileptiform insult and the occurrence of clonic convulsions. Induction of cardiac ventricular fibrillation or rapid sticking resolves the problem of the short duration of unconsciousness. Chest sticking, by cutting the major blood vessels arising from the heart, should be preferred, to ensure efficient haemorrhaging and a rapid loss of brain activity (see above; Anil *et al.*, 1995b). In currently used systems, a low voltage spinal discharge (electro-immobilisation) is necessary to stop clonic convulsions and to make sticking possible. However, electro-immobilisation may mask signs of consciousness.

Studies have been carried out using experimental equipment and using two types of automatic cattle stunners, the first type is commercially used throughout the world (5 European abattoirs use the stunner at present), the second type is under development in one EU member state. The first type consists of a modified cattle restraint used at electrical stunning where the head of the animal is restrained by a pair of metal neck yokes, a chin lift, and a nose (muzzle) plate that can be electrified. A third and fourth electrode are placed on the brisket and on the tail base. It runs 3 programmes sequentially:

- First, a 3 sec head-only cycle (neck yoke / nose electrodes) induces loss of consciousness and sensibility.
- Second, a 15 sec cardiac cycle (brisket/nose electrodes) induces ventricular fibrillation.
- Third, a 4 sec spinal cycle (rear end/nose electrodes) reduces uncontrolled leg kicking after death. The stunner delivers current at 550 V, 50 Hz sinusoidal AC, via a choke, which limits the current to a maximum of about 3.5 A.

The second type of automatic electrical beef stunner (Troeger, 2002) delivers 300 V, constant current while frequency can be selected from a 10-990 Hz range. For the trials, a frequency of 50 Hz has been used. It has only two cycles: a head-only cycle followed by a head-body cycle.

7.2.1. Different methods

7.2.1.1. Electrical head-only stunning

Several studies describe procedures to induce unconsciousness through head-only stunning in adult cattle, manually or automatically. Type of animals is not always extensively described. Data have been obtained:

- on 6 steers and cows of various breeds (Jones *et al.*, 1988);
- on 7 steers and cows (536-670 kg), photographs suggesting that they were of the dairy type (Schatzmann and Jäggin-Schmucker, 2000);
- on 92, 2 years old cattle of mixed breeding (495 ± 5 kg; Wotton *et al.*, 2000); and
- on 12 cows, steers and bulls (380-450 kg) of various breeds (Friesian, Angus and Jersey; Devine *et al.*, 1986).

Information on presence of horns is mostly not given, but their presence in European dairy and beef breeds of cattle is generally not a problem for application of the electrodes.

In adult cattle, head-only electrical stunning has been induced using different types of electrodes. Thus, in a stunning box or crush, stunning was achieved by passing 4 sec current limited to 2.5 A (400 V, 50 Hz) through neck yoke electrodes placed behind the ears (metal vertical bars placed on both sides of the neck behind the ears and horns (buds) restraining the animal; Devine *et al.*, 1986). Another study used modified neck yoke electrodes that could be rotated forwards and upwards so that the neck was partially extended (constant current, 3 A, 50 Hz; Jones *et al.*, 1988). Manual application of enlarged tongs placed across the head, with an electrode behind one eye and another behind the contralateral eye or front of the ear were also efficient (Von Mickwitz *et al.*, 1989: 2.5-3 A, 250 V for > 20 sec; Schatzmann and Jäggin-Schmucker, 2000: 2-3 A, 250 V, for 10 sec). The tongs were enlarged pig tongs, with longer handles and a larger maximal space between the electrodes, in order to be able to span the head (or heart, see below). In the above mentioned experiments, unconsciousness lasted for 31 to 90 sec, as measured by resumption of normal breathing. As chest sticking induces a blood pressure near 0 within 8 sec (Anil *et al.*, 1995b), simple calculation of 31 minus 8 sec suggests that sticking should be carried out within 23 sec after the stun.

The automatic cattle stunners effectively use nose-to-neck yoke electrodes. Work on the first type of cattle stunner found that effective head-only stunning can be induced in adult cattle in less than 1 sec using a >1.28 A sinusoidal AC 50 Hz, 200 V current (Wotton *et al.*, 2000). Another paper reports that 1.1 A, using a 400 V open circuit, suffices (type of stunner not specified; Cook and Devine, in press).

Head-only stunning must result in tonic – clonic seizures (Devine *et al.*, 1986; Jones *et al.*, 1988; Schatzmann and Jäggin-Schmucker, 2000).

To stun 100% of calves (6 months, 200 kg), at least 1 sec of 1.25 A constant current (50Hz, 150 V) is needed when the current is applied on the temporal region of the skull (Lambooij, 1982; Lambooij and Spanjaard, 1982). Cook *et al.* (1991) found that placing electrodes on the first few cervical vertebrae and applying 1.5 A for 4 sec (50 Hz, 400 V) caused a seizure without stun, but only 1 calf (3-4 months) was tested in this trial. Effective electrical stunning induced by electrodes on the temporal region of the skull or on the first cervical vertebrae lasted 26 to 61 sec. Breathing was inhibited for at least 20 sec (Lambooij and Spanjaard, 1982; Bager *et al.*, 1990; 1992; Devine *et al.*, 1986; 1987; Blackmore and Newhook, 1982). Gregory *et al.* (1996) found that a 50 Hz current applied at 100 V across the head for 3 sec failed to induce insensibility in 1 out of 11 young calves. In the remaining 10 calves, the stun produced a tonic and clonic phase lasting 10 and 21 sec, respectively. Average times to return of breathing, of corneal reflex and head righting were 33, 75 and 78 sec. A voltage of 200 V (3 sec) produced insensibility in all of the 10 tested calves, inducing a tonic and clonic phase each lasting 11 sec. Average times to return of breathing, corneal reflex and head righting were 25, 67 and 67 sec. Increasing the duration of application (200 V, 7 sec) induced a tonic and clonic phase lasting 15 and 16 sec, respectively. Average times to return of breathing, of corneal reflex and of head righting were 31, 79 and 75 sec. Finally, comparison of electrocortical data of calves stunned with 150 or 250 V for 3 sec found a success rate of 100% in both cases, and a return of visual evoked potentials after 41 ± 7 sec (range 19-63) and 74 ± 7 (range 47-98) sec. Due to the one failed stun with 100 V for 3 sec, the authors recommend using at least 150 V (Gregory *et al.*, 1996), which is in accordance with other scientific data (Lambooij and Spanjaard, 1982).

Rapid sticking after stunning is one way to guarantee that animals do not recover before death ensues through blood loss. If the throat is cut (bilateral severance of the common carotid arteries and external jugular veins) 10 sec after the head-only stun, an isoelectric EEG is observed within 30 to 127 sec after stunning (Bager *et al.*, 1990; 1992; Devine *et al.*, 1986; 1987). Chest sticking, by cutting the major blood vessels arising from the heart, reduced variability in bleeding rate between animals: chest-sticking 30 sec after head-only stunning induced an isoelectric EEG within 36-60 sec following sticking (Anil *et al.*, 1995b). As chest sticking reduces blood pressure to near 0 within 8 sec (Anil *et al.*, 1995b), simple calculation of 20 minus 8 sec (shortest time to return of breathing 20 sec, see above: Lambooij and Spanjaard, 1982; Bager *et al.*, 1990; 1992; Devine *et al.*, 1986; 1987; Blackmore and Newhook, 1982) suggests that sticking should be carried out within 12 sec after the stun.

7.2.1.2. Electrical head-to-body stun / killing method

In practice, 60 sec is the time interval between end of stunning and sticking (stun to stick interval). Electrical stunning gives therefore satisfactory results only if head-only stunning is followed by the induction of cardiac ventricular fibrillation. Due to the cardiac ventricular fibrillation, cardiac output and hence blood circulation is impaired, adding an anoxic insult to the epileptic brain state induced by the stun. Consequently, unconsciousness will be more profound and lasts longer. In addition, most of the time, the cardiac ventricular fibrillation is followed by cardiac arrest, ensuring that the animal will not recover.

In adult cattle, cardiac ventricular fibrillation is achieved by a head-brisket discharge (>1.5 A sinusoidal AC at 50 Hz, 175 V, 5 sec; Wotton *et al.*, 2000), by placing electrodes across the chest, or by placing them along the ventral / dorsal axis (one ventrally on the chest and one on the dorsal surface of the mid thoracic region; Schatzmann and Jäggin-Schmucker, 2000; Von Mickwitz *et al.*, 1989). During manual electrical stunning of adult cattle or calves using enlarged pig tongs (see above), it is recommended to induce cardiac fibrillation following the head-only stun (Schatzmann and Jäggin-Schmucker, 2000; Von Mickwitz *et al.*, 1989). Von Mickwitz *et al* (1989) describe the following procedure to kill manually adult cattle (no bleeding): wetting of the skin with a long-handled brush on both sides of the head where the electrodes will be placed, stunning (placing electrodes bilaterally, eye / eye or eye / ear, at least 20 sec, 2.5 - 3.0 A), wetting chest for electrode placement, induction of cardiac ventricular fibrillation (preferably across the chest, otherwise along the ventral / dorsal axis, 25 sec, 1.8 - 2.8 A), and finally a second head-only stun (same procedure as the first, lasting >12 sec). After 10 min, it is necessary to check whether cardiac fibrillation has evolved into cardiac arrest. Schatzmann and Jäggin-Schmucker (2000) describe the following procedure to stun manually adult cattle before bleeding: head-only stun (eye / eye or eye / ear; 5 or 10 sec, 250 V), followed by induction of heart fibrillation (electrodes across the chest for 5 or 10 sec, 2.3-3.5 A, 50 Hz, 250 V). Recent trials found that cattle may be introduced in a cattle restrainer with open sides and front (or removable sides) and be manually stunned. One person applies the head-only stun. A second person applies a bent electrode over the heart on the left side of the animal. All three electrodes are applied during the total stunning duration. After a fixed time (head-only-stun) the left head electrode is switched off by the stunning device and the heart electrode is switched on. Consequently, the current passes from the heart region to the head and induces efficiently heart fibrillation. This system is believed to be usable by smaller abattoirs in future (Moje, 2003, pers. comm.).

The first type of automatic cattle stunner induced cardiac ventricular fibrillation with a 5 sec >1.5 A sinusoidal AC 50 Hz, 175 V, head-to-brisket current (Wotton *et al.*, 2000; Schatzmann and Jäggin-Schmucker, 2000). The voltage supplied to this cattle stunner is sufficient. Another paper however, reports that only 1.1 A, using a 400 V open circuit, suffices (type of stunner not specified; Cook and Devine, in press). It was earlier suggested that the brisket electrode of the first type of automatic cattle stunner should be improved to ensure better contact (Wotton *et al.*, 2000). Since, modifications involving articulation of the brisket electrode, allow reliable and adequate contact (Daly and Pleiter, 2003, pers. comm.).

It has been reported that handling of carcasses during shackling and hoisting may assist resuscitation and, hence, heart activity may resume despite previous successful induction of cardiac ventricular fibrillation. When there is a delay of 30-60 sec before hoisting, this phenomenon has not been observed (Daly, 2003, pers. comm.). Under practical conditions, when assessed by heart palpation, post pluck removal, to identify the presence of rigor which indicates ventricular fibrillation, induction of cardiac ventricular fibrillation shows a failure rate of 11 to 31% for this method, depending upon season. It may be related to the temperature of the animal or to fluidity of the blood: animals are probably thirstier in summer (having no access to water) and there is probably less blood volume for approximately equal amount of blood cells. These data would include any animals that were defibrillated due to physical handling post stun (Wotton, 2003, pers. comm.). This range of failure rate is too high and further investigations should clarify the problems. A high incidence of sustained heart function is probably not due to an intrinsic problem of the system, but the details of how the current is being applied (Daly, 2003, pers. comm.). In this regard, devices fitted with a ‘choke’, which restricts the maximum voltage / current applied, seem to fail more often than those without. The suggestion that handling of carcasses leads to resuscitation implies that cattle should be left in the restraining device for at least 60 sec following the induction of cardiac ventricular fibrillation to avoid this potential welfare problem. This indicates that during the first 5 min, it is important to watch carefully the animals for signs of recovery and to handle them very gently to avoid the heart to return to its normal function. The relative inefficiency of delayed heart massage to obtain resuscitation is also known from experience with humans, where it was found that heart resuscitation is much more effective when applied soon (within sec) after start of fibrillation.

Experimental use of the second type of automatic cattle stunner found that a head-only current lasting 4 sec (3-3.5 A, constant current, 50 Hz) followed by a head-body cycle of 14-16 sec (3-3.5 A, constant current, 50 Hz) gave satisfactory results, effectively inducing physical signs of loss of consciousness and cardiac ventricular fibrillation (Troeger, 2002).

For adult cattle, the induction of unconsciousness and cardiac ventricular fibrillation was reported to be associated with gasping and / or rhythmic breathing movements in most animals, and reflex rotation of the eyeballs in half of the animals (Wotton *et al.*, 2000). Such breathing movements have been described as regular, very slow and shallow flank movements, and have been observed on many occasions in the context of electrical head-to-body stunning (Gregory and Daly, pers. comm., 2003). On 220 adult cattle, the incidence of: (a) gasping following electrical stunning of cattle using all 3 cycles was found to be 37.6% in the summer and 38.5% in the winter and (b) apparent rhythmic breathing was 25.0% in the summer and 47.7% in the winter (Wotton *et al.*, 2000; Wotton, 2003, pers. comm.). It is well known that season has large effects on many physiological aspects around slaughter, including death of the animals and meat quality, even if the reason is not known. Breathing-like activity can be seen after head-only stunning and sticking, even after the bleeding process has been completed (Daly, 2003, pers. comm.). Wotton *et al.* (2000) report that following cardiac ventricular fibrillation, on some occasions, the corneal and then the palpebral reflexes returned temporarily before animals died. However, Wotton *et al.* (2000) suggest that the temporary return of eye reflexes may occur despite unconsciousness and that after cardiac arrest, the mid-brain and cortex succumb to anoxia first (within 40 sec), while the brain stem, controlling the eye reflexes, survives longer. It is known that corneal and spinal reflexes may be present for a short time after cardiac arrest (see point 5.10). It is not certain whether the breathing-like activity, eye movement and positive palpebral response could be considered as spinal reflexes or automatisms.

In studies on calves, following a head-only stun, cardiac ventricular fibrillation has been induced using different electrode placements. Lambooij and Spanjaard (1982) used electrodes to be placed on the back and on the withers to ensure better contact (600 V, 50 Hz, for 1 to 2 sec; see Table 7-2). Blackmore and Newhook (1982) have used head-to-back stunning where in a single cycle, two electrodes were placed behind the ears and one electrode to the dorsal surface of the mid thoracic region (0.9 A, 50 Hz, 300 V, for 5 sec). 4 out of 7 calves (1 week of age) showed immediate and permanent insensibility, associated with cardiac fibrillation. An iso-electric EEG was observed 40 to 135 sec after head-to-back stunning, without prior signs of recovery (Blackmore and Newhook, 1982). In the 3 remaining calves, heart fibrillation was not induced and permanent insensibility did not occur: animals were insensible for 36 - 61 sec and showed signs of recovery 60 - 85 sec after the stun. Blackmore and Petersen (1981) applied head-to-leg or head-to-back stunning in 1-week-old calves. For head-to-leg stunning, 2 electrodes were applied to the head (no further specification) while the lower portion of the legs were in contact with electrodes at the base of the crush conveyor (50 Hz, alternating current up to 400 V, 0.5-2.0 A). None of the 100 calves showed normal cardiac function after a current of 2 A for 5 sec. For the head-to-back stunning, 2 electrodes were applied to the head (no further specification) and a third curved one to the dorsal surface of the mid thoracic region. After a current of 1 A (50 Hz) for 5 sec, 2 out of 250 calves showed normal cardiac function. After application of 0.8 A (duration not specified), 9 out of 20 calves had normal cardiac function. Cook *et al.* (1991) placed 2 electrodes (acting as a single electrode) on either side of the neck (cervical vertebrae, C2 to C5 region) and 1 on the brisket of bull calves (3-4 months, 75-100 kg). Application of 1.5 A (50 Hz, 400 V) for 4 sec produced cardiac ventricular fibrillation in 2 out of 2 animals, without producing any epilepsy.

Summarising, cardiac ventricular fibrillation can be induced using different electrode placements. In adult cattle, electrodes may be placed across the chest, or along the ventral/dorsal axis. Manual application of 1.8 A for 25 sec induces cardiac ventricular fibrillation, as does automatic application of >1.5 (175 V, 50 Hz) A for 5 sec. In calves, application of 2 A (400 V, 50 Hz) for 5 sec, is sufficient for most - if not all – calves, when using head-to-leg electrodes. When head-to-back electrodes are used, 0.9 A, 300 V, 50 Hz for 5 sec is sufficient for most calves, but lower current strength is clearly insufficient. Current strengths above 1 A are likely to improve success rate. Further research is needed to confirm the possibility to induce cardiac fibrillation while placing electrodes on the neck and brisket.

Table 7-2. Summary of methods used in various studies (electrical parameters are reported to the extent they were described in the relevant paper)

Study	Application	Description of voltage/current	Duration of application	Number of animals
CATTLE				
Devine *et al.*, 1986	Head-only and electro-immobilisation	50 Hz, 400 V, current limited to 2.5 A (stunning) 80 V peak, 14.3 Hz, 5 ms square wave pulses, 300 mA (electro-immobilisation)	4 sec (stunning) 30-37 sec (electro-immobilisation)	12
Jones *et al.*, 1988	Head-only	50 Hz, constant current, 3 A	4 sec	6
Wotton *et al.*, 2000 (automatic cattle stunner)	Head-only and head-to-brisket	50 Hz sinusoidal AC, 160-275 V and 0.46-3.57 A (head-only), 100-350 V and 0.5-3 A (head-to-brisket)	1 sec (head-only) and 15 sec (head-to-brisket)	67 65
Schatzmann and Jäggin-Schmucker, 2000	Head-only and across chest	220-250 V, 2-3 A	5 or 10 sec	9
Von Mickwitz *et al.*, 1989	Head-only and across the chest	2.5-3.0 A 1.8-2.8 A	>20 sec (head-only) >25 sec (across chest)	340 in total
Troeger, 2002 (automatic cattle stunner)	Head-only and head-to-chest	Constant current 3.0 - 3.5 A, 50 Hz, (head-only and head-to-chest)	4 sec (head-only) 14-16 sec (head-to-chest)	-
CALVES				
Blackmore and Petersen, 1981	Head-to-back or head-to-leg	50 Hz, 400 V, alternated current between 0.5 and 2.0 A	5 sec	270 and 100, resp.
Blackmore and Newhook, 1982	Head-to-back	50 Hz, open circuit, 0.9 A, 300 V	5 sec	6
Lambooij and Spanjaard, 1982	Head-only/ withers-to-back (V-shaped electrodes)	50 Hz, constant current, open circuit 0.8- 1.1 A, 300-600 V / 600 V	1 - 4 sec/1-2 sec	62/16
Devine *et al.*, 1986	Head-only or head-to-back	50 Hz, 400 V open circuit, current limited to 1.0 A	4 sec	5 and 2, resp.
Devine *et al.*, 1987	Head-only, electroimmobilisat-ion	50 Hz, open circuit 400 V, 1.0 A (stunning); 14.3 Hz, 5ms square wave (electro-immobilisation)	4 sec (stunning) 5-60 sec (electro-immobilisation)	34
Cook *et al.*, 1991	Neck-to-neck and neck-to-brisket	50 Hz, open circuit, current limited to 1.5 A, 400 V	4 sec	3
Bager, *et al.*, 1990	Head-only	50 Hz, 400 V open circuit, current limited to 1.5 A	4 sec	12
Gregory *et al.*, 1996	Head-only	50 Hz sinusoidal AC, 100-250 V, 0.5-1.1 A	3 or 7 sec	61
Cook *et al.*, 1996	Head-only	50 Hz, 400 V open circuit, current limited to 1.5 A	4 sec	9

7.2.1.3. Post-stunning carcass movements: Use of electro-immobilisation technique

A major drawback with electrical stunning is that uncontrolled kicking movements (clonic seizure) of the animal start 2 to 21 sec after stunning, making prompt sticking difficult and dangerous. In adult cattle, reflex movements can be avoided by the application of a 4 to 15 sec low voltage front to rear-end current, nose to tail, muzzle to rump, spinal discharge (electro-immobilisation) (Devine *et al.*, 1986 and 1987; Wotton *et al.*, 2000). Observations of the first type of cattle stunner found that the spinal discharge is sometimes ineffective due to poor electrical contact. In calves, electro-immobilisation could be achieved using a 5-60 sec current (80 V peak, 14.3 Hz, 5 ms duration square wave pulses (current strength not indicated; Devine *et al.*, 1987). The authors further observed that the addition of at least 15 sec electro-immobilisation following a head-only stun accelerated the fall in the EEG amplitude towards iso-electricity (Devine *et al.*, 1987), but this effect may be secondary to the spinal discharge impeding transfer of impulses to the brain. Electro-immobilisation inhibits breathing, reducing the possibility of sustained oxygen supply to the brain (Devine *et al.*, 1986; Anil *et al.*, 1995a, b), which may also explain the accelerated fall in EEG activity. Electro-immobilisation facilitates carcass handling and bleeding, but on the negative side, in inadequately or poorly stunned animals, it masks signs of consciousness and is painful. This procedure was originally developed to induce carcass immobilisation, and thus prompt and accurate sticking, after head-only electrical stunning of cattle under religious slaughter situations. A general application under practical conditions is considered to have negative consequences for animal welfare.

The restrainer used in the second type of beef stunner provides a belly support. Consequently, the animal can be bled in the box, immediately following the stun. Electro-immobilisation is not necessary. In addition, convulsions were inhibited by the restraint system, and no damage was found on the carcass (Troeger, 2002). However, the blood collection procedure has to be improved to avoid soiling of the box.

7.2.2. Description of effective use

7.2.2.1. Cattle

For head-only electrical stunning, a minimum current of 1 sec, > 1.28 A (200 V, 50 Hz), can be used to effectively stun adult cattle. Ventricular fibrillation can be induced in an automatic stunning system by a head-brisket discharge (5 sec, 1.5 A (175 V, 50 Hz)) or by placing manually electrodes across the chest (25 sec, 1.8-2.8 A or 5-10 sec, 2.3-2.9 A (250 V, 50 Hz)).

7.2.2.2. Calves

For head-only electrical stunning, a minimum current of 1 sec, 1.25 A (150 V, 50 Hz) can be used to effectively stun calves (6 months) when applied on the temporal region of the skull. Ventricular fibrillation can be induced using withers-to-back (1-2 sec, current not reported, 600 V, 50 Hz), head-to-back (5 sec, 0.9 A, 300 V, 50 Hz) or head-to-leg (5 sec, 0.5-2.0 A, 400 V, 50 Hz) application of electrical current.

7.2.2.3. For both types of animals

Chest sticking by cutting the major vessels arising from the heart should be used. If head-only stunning is used, sticking should be carried out within 23 sec after the stun (cattle) or within 12 sec (calves). If the head-to-body stun / kill technique is used, sticking is not very urgent if it is certain that cardiac fibrillation has occurred.

Post-stun convulsions may be minimised or eliminated by 'spinal discharge' to facilitate prompt and accurate sticking.

7.2.3. Monitoring points

7.2.3.1. Effective electrical head-only stunning

Effective electrical head-only stunning produces the following signs:

- Immediate*, tonic phase:* flexion of the hindlegs and collapse of the animal. Subsequently, the gradual rigid extension of the forelegs and tense body (tetanus).

- Apnoea for at least 20 sec.

- About 10 sec later, *clonic phase*: uncontrolled paddling and kicking movements

- Finally, if bled rapidly, the animal may die due to blood loss. If bleeding is delayed, movements subside, and there is resumption of *rhythmic breathing* and return of *consciousness*.

7.2.3.2. Effective electrical head-only stunning followed by an electrical head-to-body current

This method produces the following signs:

- Immediate*, tonic phase:* flexion of the hindlegs and collapse of the animal. Subsequently, the gradual rigid extension of the forelegs and tense body (tetanus).

- Apnoea.

- About 10 sec later*, clonic phase*: uncontrolled paddling and kicking movements.

- Finally, if bled, *death* of the animal usually occurs due to blood loss, if bleeding is delayed, it occurs due to cardiac arrest.

7.2.3.3. Electro-immobilisation

Electro-immobilisation after head-to-body stunning results in tetanic contractions, abolished leg movements and inhibited breathing. Signs of resumption of consciousness may be obscured. However, immediate and efficient sticking after the procedure produces death before resumption of consciousness in effectively stunned animals.

7.2.4. Advantages

Head-only: Stunning is immediate

Head-to-body: Stun-to-stick interval is not critical so the chances of compromises in animal welfare is reduced

7.2.5. Disadvantages

The electrode placement in the automatic stunner is not consistent, due to variations in the size of cattle (breed and age effects) and the degree of soiling on the electrode position.

Most of the scientific publications relating to electrical stun / killing method have used dairy breeds and data concerning large European beef breeds of cattle are not available.

8. STUNNING AND STUN / KILLING METHODS FOR SHEEP

8.1. INTRODUCTION

The most common methods for stunning and killing of sheep in slaughterhouses are penetrating captive bolt and electrical stunning / killing.

In sheep, cutting both common carotid arteries and external jugular veins is the quickest method of abolishing brain responsiveness via bleeding out. The main sticking methods are:

- Stab sticking: The knife is stuck in the neck ventral to the vertebral column where it should sever both common carotid arteries, which can not be verified due to the small wound.
- Gash sticking: The knife is stuck in the neck ventral to the vertebral column and moved ventral in order to cut all soft tissues including both common carotid arteries.
- Full ventral cut: The knife cuts from the ventral part of the neck up to the vertebral column. This type of cut severs all blood vessels and other tissues lying ventral of the vertebral column.

The effect of three different sticking methods and cardiac ventricular fibrillation on the time to loss of brain responsiveness (loss of visual evoked responses) according to Gregory and Wotton (1984b) is shown in Table 8-1.

Table 8-1. Time to loss of evoked activity in the brain of sheep following slaughter or cardiac arrest

Sticking method	Number of sheep	Time to loss of brain responsiveness (sec)	SD
Both common carotid and both external jugular veins	20	14	1
One common carotid artery and both external jugular veins	8	70	7
No common carotid arteries and both external jugular veins	8	298	34
Electrically-induced cardiac fibrillation	8	28	2

Stunning methods should induce unconsciousness that lasts for at least 17 sec (calculated as follows: average time to loss of brain responsiveness after cutting both common carotid and external jugular veins + 3 SD = 17 sec) plus the interval between the end of stunning and sticking.

8.2. PENETRATING CAPTIVE BOLT

Ideal shooting position for polled sheep is the highest point of the head (front or crown position, Figure 8-1) in the mid-line, pointing straight down to the throat. The ideal shooting position for horned sheep is the position just behind the middle of the ridge that runs behind the horns (poll position, Figure 8-2). Then the captive bolt should be aimed towards the mouth (The Sheep Veterinary Society, 1994).

The head of the sheep must be restrained properly, which can be difficult in distressed or strong animals.

Figure 8-1. Shooting positions for hornless sheep (front or crown position)

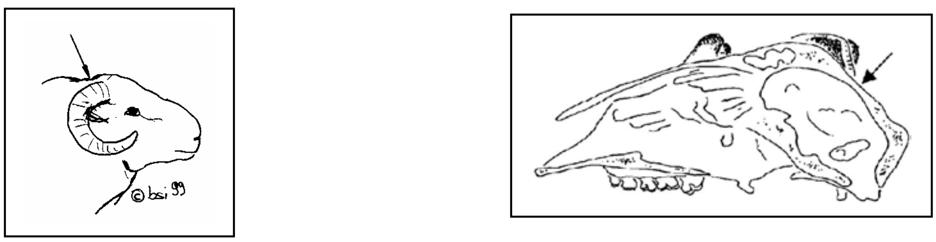


Figure 8-2. Shooting positions for horned sheep (poll position)

Captive bolt stunning in the frontal position results in immediate and irreversible loss of brain activity (visual evoked responses) (Daly *et al.* 1986, Daly and Whittington, 1989). Changing the shooting position to the poll (projected line running between the base of the ears and aiming towards the throat) during captive bolt stunning may alter the mechanics of the impact such that the diffuse damage to the brain is reduced, possibly owing to reduced acceleration of the head (Daly and Whittington, 1986). Captive bolt shooting in the poll position can be associated with rapid recovery of brain function (average: 49.6 sec, earliest: 32.9 sec) after the shot. Because such recovery could be associated with return of sensibility, shooting in the poll position should only be used when it is essential (*i.e.*, in horned animals) and then always followed promptly by sticking (Daly and Whittington, 1986). In such cases, bleeding must commence within 16 sec of shooting (calculated as follows: earliest recovery after shooting in the poll position = 33 sec minus time to loss of brain responsiveness = 17 sec).

Based on practical experience, behaviour post-stunning is very similar to that seen in cattle and already described in the previous chapter.

Both common carotid arteries should be severed to keep the time to loss of brain responsiveness as short as possible (Gregory and Wotton, 1984b).

When stunning free standing sheep in a pen, it is a typical behaviour that the animals crowd together and keep their heads low, which can make it difficult to reach the correct shooting position. Also the last sheep of a group may be agitated or showing flight reactions in order to run away from the operator or try to follow the stunned companions which can be already hanging on the bleeding rail.

8.2.1. Description of effective use

Ideal shooting position for polled sheep is the highest point of the head (front or crown position) in the mid-line, pointing straight down to the throat. The ideal shooting position for horned sheep is the position just behind the middle of the ridge that runs behind the horns (poll position). Then, the captive bolt should be aimed towards the mouth.

Bleeding should be performed immediately after the shot and when using the poll position within 16 sec at the latest. Both common carotid arteries should be severed to keep the time to loss of brain responsiveness as short as possible.

8.2.2. Monitoring points

Captive bolt stunning produces the following signs:

- Immediate collapse.
- Immediate onset of tonic seizure.
- Apnoea.
- The position of the eyeball is fixed (*i.e.* facing straight ahead).

8.2.3. Advantages

When performed correctly, captive-bolt stunning is an effective method of stunning sheep and loss of consciousness is immediate.

When properly used by skilled personnel with well-maintained equipment, captive-bolt stunning of sheep may result in less fear and anxiety and be more rapid.

8.2.4. Disadvantages

To apply this method properly, individual animals may have to be restrained.

8.3. NON-PENETRATING CAPTIVE BOLT

Captive bolt stunning with non-penetrating bolt has been reported to result in skull fracture in 5 out of 10 4 to 5-week-old Merino lambs. Based on gross pathology in all the lambs used in this study it was found that the structural brain damage, a mixture of focal and diffuse injury, produced by penetrating and non-penetrating captive bolts was similar and of sufficient severity to suggest that both types of bolts are acceptable to stun / kill lambs (Finnie *et al.*, 2000).

Data on effectiveness in heavier sheep and duration of unconsciousness in adult sheep are not available for this method.

Concussion of the brain and unconsciousness should be induced with a single blow at the frontal position of the head.

8.3.1. Description of effective use

Animal's head should be suitably presented to enable accurate stunning.

Bleeding should be performed as soon as possible after stunning.

In order to ensure rapid brain death following exsanguination both of the common carotid arteries should be severed.

8.3.2. Monitoring points

Non-penetrating captive bolt stunning produces the following signs:

- Immediate collapse.
- Immediate onset of tonic seizure.
- Apnoea.
- The position of the eyeball is fixed (*i.e.* facing straight ahead).

8.3.3. Advantages

There are no animal welfare advantages.

8.3.4. Disadvantages

The prevalence of mis-stunning under abattoir conditions is unknown, however it remains to be a major concern.

8.4. FREE BULLETS

Free bullets are mainly used under emergency situations (casualty slaughter or killing for disease control) and will be described in chapter 13. However, they could be used in slaughterhouse as a back-up killing method in case another method failed.

8.5. ELECTRICAL HEAD-ONLY STUNNING

Head-only stunning of sheep can be carried out on individual animals within a group in a pen, or on individual animals in a restrainer. Isolation of sheep from the group can be stressful and maintenance of visual contact between animals would be beneficial in terms of welfare. However, stunning of sheep kept groupwise in a pen has the following disadvantages:

- Sheep crowd together and often hide their heads (keep their heads low) under other animals which makes it difficult to reach and correctly place the electrodes on the head.

- Sheep in a group standing close to the electrodes or the head of a sheep being stunned can receive electric shocks when coming accidentally in contact with that part of the stunned animal that is under voltage.

- It is difficult to stun the last sheep of a group without causing distress.

To avoid painful electric shocks due to wrong placement of the electrodes, sheep should be individually restrained manually in a trap or in a restrainer. Unrestrained sheep can be incompletely stunned and subjected to a painful electric shock (Gregory and Wotton, 1984a; HSA, 2000).

The tongs should be positioned between the eyes and the base of the ears on both sides of the head preferably on wet skin. The presence of wool and a dry skin surface lead to a lower effectiveness of stunning when tongs are in caudal position (behind the ears on the occipital condyle on either side of the head) (Velarde *et al.*, 2000).

A poor initial contact or a slow rise in current levels may not stun the animal immediately, and instead it could experience an electric shock (Gregory, 2001). Therefore, good electrical contact is necessary to provide an effective stun. The contact places and the stunning electrodes should be wetted with water to improve the effectiveness of stunning.

Pointed electrodes (electrodes with pins) give good grip and electrical contact, because they penetrate the wool and make better contact with the skin when compared to electrodes without pins. Electrodes with serrated edges may work in shorn sheep and if area of application is wetted. Though the heads of the sheep are small and the skin is woolly, often burning of electrodes occurs which leads to intensive carbonising of the electrodes. This, in turn, leads to a poor electrical contact due to an increased electrical resistance in the pathway.

Under laboratory conditions, several variables were used to achieve a complete epileptic seizure and are listed in Table 8-2.

Stun durations as low as 0.2 sec and currents below 0.5 A can be used under laboratory conditions to elicit epileptic seizures in sheep.

Sheep can be stunned effectively at durations between 2.0 and 20 sec with a minimum current of 1.0 A (50 Hz) (Gregory and Wotton 1984b; Cook *et al.*, 1995; Velarde *et al.*, 2002).

The times to onset and end of seizures and physical reflexes following head-only electrical stunning (250 V, 50 Hz, 3,0 sec) in sheep (Velarde *et al.*, 2002) are listed in Table 8-3.

Table 8-2. Electrical stunning variables reported in the literature

Study	Application	Number of animals	Description of voltage/current	Duration of application
Cook *et al.*, 1992 and 1996	Head-only	10 / 6	50 Hz, 400 V, AC, current limited to 1,5 A	4.0 sec
Cook *et al.*, 1995	Head-only	17	50 Hz, 500 V, current limited to 1,0 A	0.2 – 20 sec
Gregory and Wotton, 1985	Head-only	22	50 Hz, 200 V, AC	3.0 sec
Gregory and Wotton, 1988	Head-only	21	50 Hz, 150 V, AC, average current 0,46 (+/- 0,12) A	3.5 sec
Velarde *et al.* 2000	Head-only	89	50 Hz, 250 V, AC, sinusoidal, 0,343-0,485 A	0.2 sec
Velarde *et al.* 2002	Head-only	24	50 Hz, 250 V, AC, sinusoidal, average current 2,14 +/- 0,47 A	3.0 sec

Table 8-3. Times to onset and end of seizures and physical reflexes in electrically stunned sheep

Reaction	n	Onset mean +/- SD sec	End mean +/- SD sec
Tonic phase	24	0	10,2 +/- 0,29
First clonic phase	24	10,2 +/- 0,29	36,4 +/- 1,23
Second clonic phase	24	36,4 +/- 1,32	70,4 +/- 1,79
Spontaneous breathing	24	29,5 +/- 1,55	
Corneal reflex	24	38,5 +/- 1,75	-
Sensibility to pain (ear prick)	24	240 +/- 1,34	-

In order to check for clinical signs of recovery in sheep, the return of normal rhythmic breathing is the safest indicator. Resumption of rhythmic breathing occurred earliest 24.85 sec (29.5 minus 3 x SD (4.65 sec) = 24.85 sec) after electrical stunning. Generalised epilepsy is accompanied by tonic and clonic seizures which can be used to evaluate unconsciousness and the effectiveness of stunning. In lambs the seizure activity after head-only stunning includes a tonic and two clonic phases. During the second clonic phase signs of return of consciousness, like normal rhythmic breathing, can be observed (Velarde *et al.*, 2002).

The maximum stun-to-stick interval can be calculated as follows: Resumption of rhythmic breathing after electrical stunning minus time to loss of brain responsiveness after cutting both common carotid arteries and external jugular veins: 24.85 - 17 = 7.85 sec.

8.5.1. Description of effective use

Sheep should be individually restrained.

The tongs should be positioned between the eyes and the base of the ears on both sides preferably on wet skin or using damp and pointed electrodes.

Effective head-only stunning in sheep should be induced using minimum currents of 1.0A (RMS or average). A minimum RMS voltage of 150 V, 50 Hz, AC) would be necessary to deliver the current. Stun duration should be a minimum of 2 sec.

The maximum stun-to-stick interval is 8 sec.

8.5.2. Monitoring points

Electrical stunning produces the following signs:

- Immediate onset of tonic and clonic seizures.
- Immediate onset of apnoea
- Severance of both common carotid arteries must be assured.

8.5.3. Advantages

When performed properly, electrical stunning induces an immediate loss of consciousness.

8.5.4. Disadvantages

Maintenance of good electrical contact is difficult in woolly sheep.

Electrical stunning of unrestrained sheep in a pen can cause incomplete stunning or electric shocks.

8.6. ELECTRICAL STUN / KILLING METHODS

8.6.1. Two methods

8.6.1.1. One cycle method (Head-to-back)

This method is carried out by passing a current simultaneously through the brain and through the heart of the animal. To ensure correct positioning of the electrodes and to maintain contact, it must only be carried out on animals held in a restrainer. In order to achieve this, head-to-back systems have the electrodes fixed to a handpiece, which is applied and operated manually by the slaughterman.

The two front (head) electrodes must be placed on the forehead above each eye (in front of the brain), and the rear electrode must be placed on the back behind the position of the heart.

Stunning sheep with 300-400 V, AC, 50 Hz, minimum current of 1.0 A, for 3 sec, leads to epileptiform activity and cardiac ventricular fibrillation (Anil and McKinstry, 1991, Gregory and Wotton, 1984c).

Even though, the corneal reflex and respiratory gasps were present in 10 out of 12 sheep there were no concomitant visual and somatosensory evoked responses following a stun / kill (Anil and McKinstry, 1991).

When it is assured that all sheep receive cardiac fibrillation, the stun-to-stick interval is not critical to prevent recovery after the stun / kill. In studies of Gregory and Wotton (1984c), a stun-to-stick interval of 43 sec after the start of the application of current (which was 3 sec) was used without any sheep regaining consciousness.

8.6.1.2. Two cycle method: Head-only followed by passing a current through the heart

This method is not used under slaughterhouse conditions but it can be used effectively for killing under disease control situation (see chapter 13).

This method is carried out by passing a current first through the brain immediately followed by the application of current across the chest in order to induce cardiac ventricular fibrillation.

For the first cycle, the tongs should be positioned between the eyes and the base of the ears on both sides of the head, preferably on wet skin. For the second cycle, the electrodes should be applied on both sides of the chest or between the ventral part of the chest and the back (Von Mickwitz *et al.*, 1989).

To ensure correct positioning of the electrodes and to maintain contact, it must only be carried out on individually restrained animals.

Head-only stunning can be performed with high frequency, but the cardiac fibrillation cycle must be applied using a 50 Hz sine wave AC.

For killing sheep, a minimum duration for the current flow during the first cycle should be 2 sec and 4 sec for the second cycle (Von Mickwitz *et al.*, 1989).

8.6.2. Description of effective use

8.6.2.1. One cycle method

Sheep should be individually restrained.

The head electrode must be placed on the forehead above each eye (before the brain) and the rear electrode must be placed on the back behind the position of the heart.

Wetting electrodes or wool allows to reduce electrical resistance in the pathway.

Effective head-to-back stunning in sheep and goats should be induced using minimum currents of 1.0 A. Therefore, minimum voltages of 300 V, 50 Hz, AC (rms) are necessary. The minimum stun duration should be 2 sec.

Good electrical contact is necessary to provide an effective stun. Therefore, wettening of the contact places or pointed electrodes are recommended.

8.6.2.2. Two cycle method

Sheep should be individually restrained.

The head electrode must be placed on both sides of the head between the eye and the ear. For the second cycle, the electrodes should be applied on both sides of the chest or between the ventral part of the chest and the back.

For the cardiac fibrillation cycle, a 50 Hz sine wave AC must be used.

The minimum stun duration should be 2 sec for the first cycle (head), and 4 sec for the second cycle (heart).

8.6.3. Monitoring points

Application of those methods produces the following signs.

8.6.3.1. One cycle method

- Immediate onset of tonic seizures.
- Immediate onset of apnoea.
- Relaxation of the carcass.

Even though it is a killing method for slaughter, the severance of both common carotid arteries is necessary.

8.6.3.2. Two cycle method

- Immediate onset of tonic seizures
- Immediate onset of apnoea
- Stretching of legs (mild tonic seizure) followed by relaxation

When used as a killing method, bleeding is not necessary but the carcass has to be inspected for signs of recovery in the case of a fail stun/kill.

8.6.4. Advantages

When performed properly, electrical stun / killing induces an immediate and permanent loss of consciousness.

8.6.5. Disadvantages

To apply the one cycle method properly, a restrainer is necessary.

Electrical stunning / killing of unrestrained sheep with the two cycle method in a pen can cause incomplete stunning or electric shocks.

The maintenance of good electrical contact is not always easy.

9. STUNNING AND STUN / KILLING METHODS FOR PIGS

9.1. INTRODUCTION

The most commonly used method for stunning pigs is electrical stunning. In some countries, carbon dioxide stunning is used almost exclusively while stunning with penetrating captive bolt is rarely used. Electrical stun / killing methods are used for killing pigs. Furthermore, alternative stunning / killing methods using anoxia alone or a combination of hypercapnia / anoxia respectively induced by argon or argon / carbon dioxide have been tested.

The objective of preslaughter stunning is to render pigs unconscious for sufficient time for the major blood vessels in the neck or thorax to be severed. The use of either a neck cut or a thoracic stick, severing the major vessels near the heart, should result in a swift and permanent fall in blood pressure and lead to rapid loss of blood supply to the brain leading to death.

Pigs are usually bled by chest sticking with incision of the major blood vessels, which arise from the heart. With adequate incision size, pigs lose between 40 and 60% of their total blood volume (Warriss and Wilkins, 1987). Within 30 sec after adequate incision, the amount of lost blood is 70-80% of the total loss of blood (Warriss and Wilkins, 1987).

When a chest stick is used, heart activity has no effect on rate of bleeding, which has been demonstrated in pigs by Warriss and Wotton (1981). In this experiment, pigs were head-only electrically stunned (90 V, 50 Hz) and exsanguinated normally or after the heart had been stopped by inducing ventricular fibrillation. Cardiac arrest did not affect the weight of blood lost, the rate at which it was lost or the amount apparently retained in the carcass. After high voltage electrical head-only stunning (300V, 50Hz) of pigs, Swatland (1982) recorded no electrocardiogram at sticking and concluded that the heart had stopped. During bleeding, heart activity resumed in most pigs after varying intervals up to 85 sec. Later work by Swatland (1983) showed that exsanguination in pigs after high voltage electrical stunning (565 V) was largely completed without any detectable ventricular contraction and it was concluded that, overall, the effective exsanguination of pigs was not prevented by stunning methods which stopped the heart.

Experimental studies concerning this issue indicated that in pigs weighing between 8.8 and 12.2 kg that were not previously stunned, unconsciousness (based on reduction in the amplitude of EEG signals) occurred in 25 and 105 sec after bilateral severance of common carotid arteries and external jugular veins and unilateral severance of a common carotid artery and an external jugular vein, respectively (Blackmore and Newhook, 1981). Evidently, severance of major blood vessels in the neck with a large sticking wound will facilitate rapid blood loss.

After chest sticking of pigs, the time between the first appearance of blood from the sticking wound and to loss of brain responsiveness (based on reduction in VEP) was found to range between 14 and 23 sec (18, SD: ±3 sec) whereas time to loss of VEP after cardiac arrest ranged from 17 to 22 sec (19, SD: ±2 sec) (Wotton and Gregory, 1986). The same authors found the development of an isoelectric electrocorticogram (ECoG) to range between 22 and 30 sec after the chest stick. Hoenderken (1978) has reported that chest stick by a skilled slaughterman induces an isoelectric ECoG in 12-20 sec.

Table 9-1. Effects of different slaughter method and cardiac arrest on brain activity in pigs

Treatment	N (pigs)	Time to loss of brain responsiveness (VER) in sec (± SD) (mini – maxi)	Time to loss of sensibility (ECoG) In sec	Time to isoelectric ECoG in sec (± SD) (mini – maxi)	Reference
Both common carotid and external jugular vessels severed	3		13 - 25	80 – 115	Blackmore and Newhook, 1981
Chest stick	26			12 – 20	Hoenderken, 1978
Chest stick	8	18 (± 3) (14 – 23)		26 (± 3) (22 – 30)	Wotton and Gregory, 1986
Cardiac arrest	20			23	Hoenderken, 1978
Cardiac arrest	8	19 (± 2) (17 - 22)		25 (± 3) (Wotton and Gregory (1986) did not report the mini / maxi)	Wotton and Gregory, 1986

Based on the above evidence, if the chest or thoracic sticking is used, unconsciousness must last longer than the time interval between the end of stunning and sticking, plus the 23 sec required to induce brain death. However, this duration may be shorter if the size of the wound is larger. The maximum time to loss of brain responsiveness after an efficient chest stick has been found to be 23 sec (Wotton and Gregory, 1986). These experiments were made under ideal and controlled experimental conditions and it must be concluded that the duration of unconsciousness in pigs must at least last longer than the time interval between the end of stunning and sticking plus the 23 sec required to induce loss of brain responsiveness. However, under practical conditions the sticking may not always be ideal (depending on how skilled the sticking operator are), and the rate of which blood is lost may be prolonged, thus the time required to induce loss of brain responsiveness may be prolonged as well. The length of the sticking wound determines the rate of bleeding and hence, the onset of brain death as determined from the time to loss of VEPs. It has been demonstrated that the rate of blood loss is significantly slower in pigs with short sticking wounds (< 5 cm) when compared with long sticking wounds (10 cm) and, as a result, the average time to onset of brain death, as determined from the abolition of VEPs, was also prolonged in pigs with short sticking wounds (on average 19 sec with sticking wound < 5cm *vs.* 11 sec with sticking wound of 10 cm) (Anil *et al.*, 1995c). Likewise, VER was lost sooner following long sticking wounds than with short incisions, 10.8 *vs.* 19 sec, respectively (Anil *et al.*, 1995c).

In a study including 570 slaughtered pigs, the influence of 9 different stunning methods (5 electrical, 2 carbon dioxide gas and 2 captive bolt) on the welfare of slaughtered pigs and the resulting meat quality were examined in two common commercial slaughterhouses in Germany under usual working conditions (Nowak, 2002).

The five electrical stunning methods were:

1. **EA3 (n=70 pigs):** a three point electrode procedure (head-to-body application) with constant electrical current at the head electrodes (HE: 1.3 A, 300 Hz, 2.2 sec) and at the chest electrode (CE: 0.9 A, 100 Hz, 3.2 sec); or

2. **EA5 (n=60 pigs)**: a three point electrode procedure (head-to-body application) with constant electrical current at the head electrodes (HE: 1.3 A, 500 Hz, 3.2 sec) and at the chest electrode (CE: 0.9 A, 100 Hz, 3.2 sec); or

3. **EV20 (n=73 pigs)**: two point electrode procedure (head-only stunning) with a constant voltage (250 V) and a pulsating direct current of 100 Hz for 20 sec; 100 Hz means that the frequency is always the same and consequently, the mark:space ratio is also the same; therefore, the amplitudes are always congruent (rectified AC); or

4. **ER8 (n=68 pigs)**: two point electrode procedure (head-only stunning) with a special device delivering a controlled and regulated current for 8 s with a quantity of electric charge (50 Hz) of 15 Coulomb over a given characteristic amperage curve; or

5. **ER14 (n=74 pigs)**: two point electrode procedure (head-only stunning) with a special device delivering a controlled and regulated current for 14 sec with a quantity of electric charge (50 Hz) of 25 Coulomb over a given characteristic amperage curve.

The two carbon dioxide stunning methods used concentrations of either :

1. **GK8:** Carbon dioxide (carbon dioxide) stunning method with concentration of 80% in air in a dip-lift system, using a duration of exposure of 73 sec (69 pigs tested); or

2. **GK9**: Carbon dioxide (carbon dioxide) stunning method with concentration of 90% carbon dioxide in air in a dip-lift system, using a duration of exposure of 73 sec (80 pigs tested).

The captive bolt was used as usual **(BSo, n=61 pigs)** and with the application of compressed air to destroy the medulla oblongata **(BSm, n = 15)**.

Clinical parameters such as positive corneal reflex, response to nose prick, gasping respiration, phonendoscopic count of heart beats and aligned movements of the stunned animal immediately after thrown out of the stunning system and during bleeding were recorded with each stunning method.

The results revealed that 38% of the pigs stunned with 80% carbon dioxide showed a positive corneal reflex when tested by finger tipping 25 to 30 sec after being released from the stunning chamber. When pigs were stunned with 90% carbon dioxide over the same period of time (73 sec), only 11% of the animals showed a positive corneal reflex.

For the application of the EA3 and EA5 electrical stunning methods, it is not known whether 1.3 A together with 300 Hz is able to cause full epileptic seizure under practical conditions.

The respective figures for the different electrical stunning methods tested were for insensitiveness and unconsciousness, which were respectively of 23% and 29% for EA3; 39% and 51% for EA5; 15% and 29% for EV20; 19% and 23% for ER8, and 21% and 18% for ER14. The reactions after captive bolt stunning (BSo) were 12% and7 %. When using captive bolt with compressed air to destroy the Medulla oblongata (BSm), none of the two reflexes were observed.

When testing the depth of unconsciousness and reaction to pain using nose pricking on the nasal septum by a hypodermic needle, positive reactions were seen in 9% of the pigs stunned in an atmosphere of 80% carbon dioxide and 0% of the animals stunned in 90% carbon dioxide. These two reflexes are good indicators of a successful stunning procedure (insensitiveness and unconsciousness).

In addition to these practical clinical tests, shortly after the stunning, electroencephalograms (EEG) of pigs were recorded during the whole stunning procedure from the walking in the stunning gondola, the dipping into the carbon dioxide atmosphere through to the end of bleeding. When entering the gondola before the dipping procedure, the animals show a brain activity in the EEG which is addressed in human medicine as *beta*-activity (>15 Hz). Immediately after and up to 60 sec following the ejection from the stunning chamber, *theta*-waves are seen in the EEG of animals comming from an 80% carbon dioxide atmosphere. *Theta*-waves are slow waves (3.5 to 7.5 Hz), and are interpreted as an indicator of a latent consciousness of the animal. EEGs of animals stunned in a 90% carbon dioxide atmosphere show mainly *delta*-waves after ejection with increasing parts of isoelectric zero lines. These are typical pattern indicating that brain death is happening or starts shortly (Hartung *et al.*, 2002).

In the first sticking blood, adrenaline, nor-adrenaline and lactate concentrations were measured. The relationship between stunning effectiveness and blood parameters is still subject to various scientific investigations. Catecholamines such as adrenalin and noradrenalin are short term stress indicators, the levels of these compounds in the sticking blood of the pigs vary significantly with the different stunning methods. For example, the basic average adrenalin value of slaughter pigs under usual housing conditions on the farm is 60 ng/l. After transport, the values rise by a factor of 5 to about 300 ng/l. In the sticking blood after electrical stunning, about 30000 ng/l are found, after carbon dioxide stunning more than 100000 ng/l are observed, after captive bolt stunning about 25000 ng/l are seen. This tends to show that the stunning method has a distinct influence on this stress parameter. The meat quality of the slaughtered animals was determined by the pH value (pH_1, pH_{24}), the temperature (T_1, T_{24}), the water holding capacity (Q_1, Q_{24}), together with the occurrence of a red rim zone in the muscles *semimembranosus* and *longissimus dorsi* 45 min and 24 hours post mortem, the impulse impedance (Py value) 60 min post mortem (Py_1) and 24 hours post mortem (Py_{24}). Carcass damages in the halfs of the slaughtered animals, such as separations in the spinal column, were recorded 80 min post mortem. Bleedings in the muscles of the shoulder and inside the muscle *longissimus dorsi* were recorded 24 hours post mortem. Meat quality parameters can be used as one of the indicators of the stress the animals suffer during pre-stunning handling and stunning. In a detailed study on 80 slaughtered pigs in a big German abattoir, 49% of the animals showed broken bones and 73% blood spots and blood splashes in the muscles of the shoulder (23% in the *longissimus dorsi* muscle alone) after electrical stunning (Nowak, 1998). Mostly the L4 to L6 vertebra were affected, displaying blood infiltrations which indicate that the process of breaking took place *intra vitam* (when the animals were still living, that means during stunning). In a subsequent study in another abattoir, the respective figures for bone breakages and blood spots were 27% and 80% (Nowak, 2002). No broken bones and low incidences of blood spots were observed when carbon dioxide and captive bolt stunning was properly applied. When comparing the parameters: pH-value 45 min, pH-value 24 hours post-mortem, conductivity, water holding capacity and temperature of the meat, there are little differences between electrical and gas stunning. The best results are usually obtained after captive bolt stunning. Because of the fact that many factors of pre-slaughter handling and application of the stunning method influence meat quality, it is difficult to differentiate between the effects of the different electrical stunning methods and the pre-handling stressors. However, it is clear that a badly performed carbon dioxide stunning with 80% of gas reveals lower meat quality than a properly carried out electrical stun by one of the ER methods.

The comparison of the stunning methods in respect to animal welfare and meat quality displayed a ranking: the electrical stunning methods using a regulated current (ER8) were more animal friendly than the other methods using electrical current (see frequency of the clinical recorded reflexes). This was also the case in respect to meat quality. The constant voltage method EV20 is the most unfavourable concerning animal welfare and meat quality, because of the amount of voltage and duration of current flow which induce the start of epileptic seizure starts in the restrainer. Carbon dioxide concentrations of 80% over 73 sec (GK8) are not suitable to ensure a successful stun with a deep and long lasting unconsciousness. This seems to have a negative influence on the meat quality too. Using 90% carbon dioxide over a period of 73 sec (GK9) gave a quicker death and better meat quality. A disadvantage is that a large part of the pigs is already dead at the point of bleeding, which may cause hygienic concerns, as there is a risk of translocation of bacteria from the gut into the blood vessels and the meat, if the time between the death of the animal and sticking is longer than 5 min.

The study therefore indicated that at least 85% carbon dioxide over 90 sec to stun pigs gave good meat quality and rapid death. The correctly executed captive bolt stunning with air pressure (BSm) seems to have little negative influence on animal welfare and meat quality. When looking at all the results, least reactions were seen after captive bolt stunning, particularly when air pressure was additionally used. This can be demonstrated by clinical reactions blood constituent parameters and meat quality. The best results of all stunning methods are displayed by captive bolt using compressed air to destroy the *Medulla oblongata* (BSm). The number of pigs showing no heart beats by auscultation was the same as after 90% carbon dioxide stunning. The results above indicate that captive bolt carried out by an experienced person in a proper way represents the lowest stress for the animals. However, captive bolt systems have the disadvantage that there is no automatic slaughter routine available until now where it is required to stun hundreds of pigs per hour.

After having compared those 9 different stunning methods, it is obvious that there is still a lot of detailed research necessary to insure animal welfare during the stunning procedures. It seems that captive bolt, if applied properly, causes least disturbances for the animal, followed by gas stunning and electrical stunning procedures. Captive bolt has the disadvantage that there is no automatic method for practical use available today. carbon dioxide-stunning can be improved by either increasing the carbon dioxide-concentration or prolonging the stunning time. Nevertheless this cannot prevent the very unpleasant situation the animals are exposed to in the first 10 to 12 sec after entering the controlled atmosphere compartment.

9.2. MECHANICAL METHODS

9.2.1. Free Bullets

Free bullets are only occasionally used in slaughterhouses (e.g. for casualty or emergency slaughter or as a back-up method in case of failure of another method). Therefore, the use of this method is described later in chapter 13.

9.2.2. Penetrating captive-bolt equipment

There are only limited scientific data available concerning the use of captive bolts in pigs.

For stunning of pigs only cartridge driven captive bolt guns are used.

Captive-bolt stunners can be used in most pigs, but it is recommended that the cartridge recommended for the equipment by the manufacturer is used, and that in all cases the animal is bled immediately to ensure rapid brain death (HSA, 2001).

Pigs are probably the most difficult farm animals to stun with penetrating captive bolts because (i) the target area is very small and this can be exacerbated by the dished forehead found in certain breeds and in aged pigs (especially sows), and (ii) in comparison with other species, the brain lies deep in the head with a mass of sinuses lying between the frontal bone and the brain cavity (HSA, 1998).

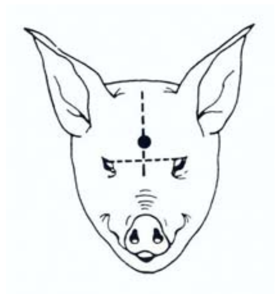
Proper restraint of the animal and of its head is needed (due to the poor welfare implications of inadequate stunning and consideration that the target area for shooting is very small). Furthermore, pigs are very difficult species to approach and it is difficult to achieve proper placement of the gun.

In slaughter pigs, the ideal shooting position is in the mid-line of the forehead, 1-2 cm above eye level, and the muzzle of the captive bolt should be placed against the head (in the case of guns with recessed bolts) and directed towards the tail which mean that the angle to the forehead may vary depending on the breed and the shape of the head (Pig Veterinary Society, 1996) (see Figure 9-1).

Boars and large sows may have a ridge of bone running down the centre of the forehead. This may interfere or prevent the bolt penetrating the brain cavity and the pig will not be stunned effectively. In such cases the recommended shooting position is 3-4 cm above the eye level and the muzzle of the captive-bolt should be placed slightly to one side of the ridge, aiming into the centre of the head (HSA, 2001). The captive bolt should be directed towards the tail, which means that the angle to the forehead may vary depending on the breed and the shape of the head.

These shooting positions ensure that maximum damage is caused to the cerebral hemispheres and deeper parts of the brain.

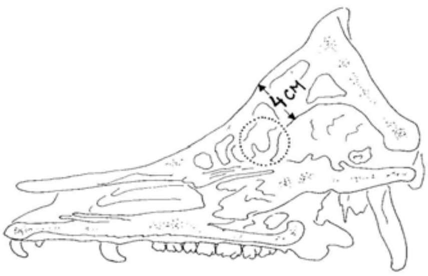
Large boars are more difficult or impossible to stun using this method as the sinuses in the forehead are well developed and the brain is laying deeper in the head than in other pigs and are preferably stunned using carbon dioxide or electrically, or killed by use of a free-bullet firearm (HSA, 1998; Blackmore *et al.*, 1995) (see Figure 9-2).



The ideal shooting position is in the mid line of the forehead 1-2 cm above the eye level

The muzzle of the captive bolt should be directed towards the tail which mean that the angle to the forehead may vary depending on the breed and the shape of the head

Figure 9-1. Ideal position for shooting pigs with penetrating captive bolt guns



Large boars are difficult or impossible to shoot efficiently with captive bolt as the sinuses in the forehead are well developed, thus the brain is lying deeper in the head than in other pigs

Figure 9-2. Large frontal sinuses may impede stunning with penetrating captive bolts in large boars.

Animals should be rendered unconscious in a single shot. This is mainly because the structural damage to the skull induced by an unsuccessful or inaccurate first shot reduces the chance of a successful stun through the subsequent shots.

The physical response to an effective captive bolt stunning is the immediate onset of tonic-clonic seizures (Van der Wal, 1971). The animal will immediately collapse and there is a period of tonic spasm, which in pigs is particularly violent (Van der Wal, 1971) but the duration may be for only 3-5 sec (Overstreet *et al.*, 1975). Correct use of a captive bolt pistol leads to abolition of corneal reflexes and apnoea (respiration ceases).

The tonic seizure is followed by clonic seizure, which may increase in intensity and persist for several min. Pupillary dilatation occurs during seizures. If an animal fails to show tonic seizure and instead immediately shows paddling or kicking movements on collapse, it is almost certain that it has not been effectively stunned and it should be re-stunned immediately (Van der Wal, 1971).

To prevent the risk of recovery, pigs should be bled as soon as possible after stunning, and a maximum stun-to-stick interval of 15 sec is recommended (HSA, 2001). When properly used by skilled personnel with well-maintained equipment, captive bolt stunning of single pigs is an effective method of stunning and loss of consciousness is immediate.

This method is not very effective in large boars.

9.2.2.1. Description of effective use

The head of the animal should be suitably presented to the operator to enable accurate stunning.

The ideal shooting position in pigs is in the midline of the forehead, 1-2 cm above the eye level. In large sows, the ideal position is 3-4 cm above the eye level.

Chest stick should be performed immediately after stunning, ideally during the tonic phase before the start of the clonic phase.

Both common carotid arteries or the major blood vessels near the heart from which they arise should be severed to reduce the time to loss of brain responsiveness as much as possible.

9.2.2.2. Monitoring points

Following a successful stun by use of a penetrating captive bolt, the pig should show the following reactions:

- Immediate collapse.
- Immediate onset of tonic seizure followed by clonic seizure within 3-5 sec.
- Apnoea.
- Immediate loss of corneal reflex.

9.2.2.3. Advantages

Immediate unconsciousness is induced.

Penetrating captive bolt can be used on farms.

9.2.2.4. Disadvantages

Penetrating captive bolt are difficult to shoot accurately and are not 100% effective in mature sows and boars of breeds.

Severe clonic seizures impede prompt and accurate sticking.

9.2.3. Non-penetrating captive-bolts

Concussion stunning by non-penetrating captive-bolts is not applied to pigs neither for slaughter nor for killing for disease control purposes.

9.3. ELECTRICAL STUNNING AND ELECTRICAL STUN / KILLING

Electrical stunning of pigs is performed either by head-only stunning or by head-to-body stun/kill involving cardiac arrest for killing. In the first method, an electrical current is applied across the head to span the brain for stunning. In the latter method, an electrical current is first applied across the brain to stun the animal combined with a current through the heart to induce cardiac arrest or fibrillation.

Handling of single pigs, or pigs in small groups, can be performed on-floor in a pen for head-only stunning with a manual tong, or using a two cycle stun / killing procedure where the manual tong is first applied to the head to stun the animal followed by application across the chest to induce cardiac arrest (Troeger, 1999).

Pigs can be restrained in a V-shaped restrainer, which can be stressful for pigs, with manual or automatic positioning of the stunning tongs for head-only stunning. The stunners may be provided with a third electrode for head-and-body application of a cardiac arrest current. However, automatic placement of electrodes is not always adequate because animals differ in size, consequently inefficient stunning occurs. (Troeger, 1999; Lambooij *et al.*, 1992; Von Wenzlawowicz *et al.*, 1999). In such systems, different size of the pigs can give problems with proper placement of the electrodes in either very small or large animals. Preselection of animals based on size and modifying electrode placement may overcome the problem.

An automatic electrical stunning device have been developed where the pigs are moved forward on a conveyor belt system (Belt restrainer) riding on the chest with the legs hanging down on both sides of the belt (Lambooij *et al.*, 1992; Troeger, 1999; Von Wenzlawowicz *et al.*, 1999). The head and heart electrodes are positioned automatically from a certain head size and form when passing a photo sensor, and the stunning process starts. The head electrodes consist of three pointed metal-electrodes, which pierce the skin. The heart-electrode consists of two blunt metal bolts, which are pressed onto the left side of the chest. This band restrainer appeared to be very useful to restrain and to stun pigs (Lambooij *et al.*, 1992; Von Wenzlawowicz *et al.*, 1999).

Table 9-2. Restraining and electrical stunning methods used for pigs

Restraining device	Approx. pigs/hour	Stunning method
Pen, group wise	30-60	Head-only or head-only followed by cardiac arrest across the chest (two step method)
Trap for single pigs	150	Head-only or head and body (heart) with 3rd electrode
V-shaped restrainer	120-240	Head-only or head and body (heart) with 3rd electrode
V-shaped restrainer	240-600	AAutomatically head-only high voltage (> 450 V)
Belt restrainer	150-600	Automatically head and body (heart)

Source: Von Wenzlawowicz, 2003, pers. comm.

9.3.1. Electrical Head-only stunning

The voltage and thus the current flowing through the brain determine how quickly the unconsciousness sets in (Troeger, 1991). The voltage must be high enough to overcome the total electrical resistance in the pathway between the electrodes (*i.e.* electrode material, fleece, skin, thickness and porosity of skull, brain tissue and distance between the electrodes) such that the required amount of current can flow within the shortest possible time, e.g. 200 milliseconds, from the beginning of the stun application (Troeger, 1991).

Good electrical contact must be maintained between the electrodes and the head during the stunning. The design and construction of the electrodes and the pressure applied during the initiation of stun are important to delivering the current (Sparrey and Wotton, 1997; Wotton and O' Callaghan, 2002). Poor electrode maintenance and/or contact with the head can be recognised from the burning of the skin due to the development of heat, which normally occur due to increased electrical impedance.

The best design of the electrode face for manual application is a rectangular shape, which gives the highest current flow (Troeger, 1991). In modern high voltage automated stunning systems, the head electrodes are positioned automatically and consists of three pointed metal electrodes (of approximatively 25 mm), which pierce the skin to facilitate the electrical conduction (Von Wenzlawowicz *et al.*, 1999). These have been shown to be adequate to achieve an effective stun or kill when using high (> 240 V) voltages (Wotton and O' Callaghan, 2002). Electrodes must be kept clean to reduce resistance to flow of current between the tongs and the head during the stunning.

Stunning tong electrodes should be placed in such a manner that they span the brain, enabling the current to pass through it (Sparrey and Wotton, 1997; Wotton and O'Callaghan, 2002).

Five different stunning tong placements have been tested under manual stunning situations by Anil and McKinstry (1998):

- Position 1: between the eyes and base of the ears on either side of the head;
- Position 2: below the base of the ears on either side of the head;
- Position 3: on either side of upper neck behind the ears;
- Position 4: one electrode on the forehead and the other under the head and behind the mandibles;
- Position 5: on the snout or jaws.

The results of this study showed that the ideal position of electrodes is between the eye and the ear on either side of the head (position 1). However, since this tong position is difficult to achieve consistently in all the pigs, positions 2 to 4 are commonly used under field conditions. Anil and McKinstry (1998) concluded that tong positions 2 to 4, which also span the brain, are effective in stunning pigs, as determined from the occurrence of tonic-clonic seizures, provided a minimum of 250 V was applied, and that position 5 is ineffective in stunning pigs. Therefore, Anil and McKinstry (1998) also concluded that tong position 5 should not be used.

Hoenderken (1978), whilst placing the electrodes at the upper neck (position 3), behind the ears, showed that generalised epilepsy could be induced in the brain within 1 sec of application of a minimum of 1.25 A. Research has shown that the current strength of 1.25 A or more could be reached within 1 sec by using a voltage of at least 250 V (Troeger and Woltersdorf, 1990). Together, these studies indicate that a minimum of 250 V applied using tong positions 1 to 4 will induce effective stunning in pigs. This is reiterated by a recent finding that stunning equipment delivering voltages lower than 240 V was not always able to produce the required minimum stunning current of 1.3 A within 1 sec (Von Wenzlawowicz, 2003, pers. comm.). In such low-voltage systems, the stunning is not always efficient and pigs may experience a painful electrical shock before onset of unconsciousness.

Berghaus and Troeger (1998) have found that an effective stun (epileptic seizure) of pigs can be induced within a minimum current flow time of 0.3 sec using a constant stunning current of 1.3 A with frequencies of both 50, 500 and 800 Hz and electrodes placed on either sides at the base of the ear (Position 2). However using current flow time of only 0.2 sec duration was not sufficient to stun all pigs (Berghaus and Troeger, 1998). Duration of unconsciousness was not measured in this experiment. Stunning pigs with 800 Hz (240 V) for 3 sec produced an effective stun in all tested pigs when using position 2 (Lambooij *et al.*, 1997).

It is also possible to produce an effective stun with higher current frequencies. However, when compared with 50 Hz sine wave AC, the time to onset of rhythmic breathing and return of reflexes are shorter with high frequencies. For example, Anil and McKinstry (1992) compared the effectiveness and duration of stunning, as judged from seizure activity and time to return of reflexes, pigs with 50 Hz sine wave AC (150 V), 1592 Hz sine wave AC (146 V) as well as 1642 Hz square wave DC current (162 V) And the results are presented in Table 9-3.

Table 9-3. The effects of certain electrical stunning frequency, waveform and stun duration on return of reflexes in pigs

Frequency and waveform	Stun duration	Average time (sec) to return of			
		Rhythmic breathing	Corneal reflex	Response to nose prick	Head righting reflex
50 Hz sine wave AC	3	41	47	57	65
1642 Hz square wave	3	39	38	47	52
1592 Hz sine wave	3	36	37	46	50
50 Hz sine wave AC	7	44	52	62	67
1642 Hz square wave	7	42	41	50	55
1592 Hz sine wave	7	37	38	46	51
S.D. of difference		1.05	1.12	1.29	1.44

Electrical stunning should only be applied once. Repeat stunning should only be applied if the first application fails to stun the animal (Anil *et al.*, 1997a). Inadequately stunned animals and those showing signs of recovery during bleeding should be immediately re-stunned using a captive bolt (Anil *et al.*, 1997a).

Ideally, the equipment should have a current sensor indicating the minimum current under load as a fail-safe device to safeguard welfare (Anil *et al.*, 1997a; Wotton and Whittington, 1994). Such sensors require high voltages since impedances measured using low voltages are not accurate and the measured impedance seems to be inversely proportional to the voltage (Wotton and O'Callaghan, 2002). However, the voltages required for the measurement of impedance are higher than normal stunning voltages used.

Therefore stunning equipment with pre-set current flow (variable voltage / constant-current stunner using electronic voltage adoption) or high voltage equipment (minimum 240 V) ensuring minimum of 1.3 A should be used.

When electrical stunning is carried out effectively, it produces grand mal epilepsy and the accompanying tonic-clonic seizures. During the current application the whole body of the animal will become rigid, breathing will stop and the position of the eye will become fixed. When current flow ceases the tonic seizure continues during which the head is raised and the hind legs are flexed under the abdomen. The forelegs may initially be flexed, but they usually straighten out (Anil, 1991; Anil *et al.*, 1997a). The tonic phase should last for 10 to 20 sec.

The clonic seizures begin immediately after the tonic seizure and they are manifested as involuntary kicking of both the fore and hind legs of which the kicking of the hind legs are more pronounced, whereas the foreleg movements are more like paddling. There appears to be two clonic seizures (Simmons, 1995) and they last for 15 to 45 sec. As the clonic seizures subside and finally end, rhythmic breathing returns (Anil, 1991; Anil *et al.*, 1997a).

The return of rhythmic breathing is the most practicable and useful indicator of recovery of sensibility (Anil *et al.*, 1997a). The corneal reflex is difficult to elicit in an electrically stunned animal and also highly variable. Therefore this reflex is less reliable to use in electrically stunned animals. The next sign of recovery is the head-righting reflex, which indicates full recovery of consciousness and sensibility (Anil *et al.* 1997a).

A chest stick resulted in abolition of VEPs on average within 18 sec in pigs (range 14-23 sec) (Wotton and Gregory, 1986). Anil (1991) demonstrated that, when sufficient current is applied to stun pigs, the minimum time to return of rhythmic breathing movements was 38 sec. By subtracting the longest time to profound brain failure (23 sec) following an accurate chest stick, from the minimum duration of unconsciousness produced by an accurate stun, the maximum recommended calculated stun-to-stick interval can be 15 sec (Anil, 1991). However, in practice sticking is often performed earlier than this during the tonic phase (within 10 sec) to ensure worker safety and accurate sticking.

Anil and McKinstry (1992) concluded that the duration of unconsciousness induced with the high frequencies are significantly shorter than that was achieved with 50 Hz AC and therefore, sticking will have to be performed as soon as possible after stunning. The recommended interval for high frequency low-voltage stunning may need to be as short as 6 sec, instead of the 15 sec under low frequency stunning, post-stun, as the shortest time to return of response to a painful stimulus was 28 sec for the 3 sec square wave stunning. However, stun-to-stick intervals appropriate to all the frequency and waveforms of currents used under slaughterhouse conditions are not known.

A slaughterhouse survey in the late 1980s has shown that, under commercial conditions, the tong positions used in manual on floor stunning systems falls outside the ideal site (position 1) in a high percent of applications and, while using low voltages (150 V) necessitating a repeat application (cited by Anil and McKinstry, 1998). Thus a high proportion of these pigs were subject to low stunning current and inefficient stunning. The situation has improved probably due to increased automation of stunning, awareness and training of abattoir staff (Meat Hygiene Service, 2002).

The stun-to-stick interval can be long with traditional on-floor stunning situations when compared with the modern automated stunning systems. For example, under on-floor stunning systems, convulsing unconscious pigs will have to be manually restrained, shackled, hoisted and moved away for bleeding (MAFF, 1995). Whereas, in automated systems, unconscious pigs under tonic convulsions are ideally ejected on to a conveyor such that the operator can swiftly perform bleeding, either prior to or soon after shackling.

In some Member States, it was a statutory requirement in the past that animals shall not be bled out within the sight of other animals of the same species (e.g. WASK Regulations, 1995, UK). Therefore, pigs stunned within a pen had to be shackled, hoisted and moved away to a bleeding area, which prolonged stun-to-stick intervals. Under this situation, it has been reported that a significant proportion of pigs showed signs of recovery of consciousness either at sticking or during bleeding (Anil *et al.*, 1997a). However, further research has shown that witnessing slaughter does not induce physiological stress responses in pigs (Anil *et al.*, 1997b), and therefore, the ban on bleeding of animals in the sight of other animals of the same species has ended (WASK Regulations (Amendment) 2003, UK). The animal welfare implication of this change statutory requirement is that short stun-to-stick intervals are practiced without infringing the law.

It is essential that all staff involved in stunning and slaughtering should be able to recognise, and differentiate between, effective and ineffective electrical stunning and slaughtering, which can only be achieved through proper training and education. A back-up, electrical or captive bolt, stunner must always be available.

9.3.1.1. Description of effective use

For automatic stunning, pigs should be restrained and the head suitably presented to facilitate accurate application of electrodes and maintenance of the current flow. Good electrical contact between the electrodes and the head should be maintained during the stunning.

Accurate position of the electrodes, such that they span the brain, should be appropriate to the variations in pigs (size, weight, variation in skull porosity, density, thickness, fatness and fleece density).

Minimum current of 1.3 A (RMS or average) must be applied for at least 1 sec to induce immediate loss of consciousness. A minimum of 240 V would be required to achieve this current in the shortest time and lower voltages are not sufficient to achieve the required current within an appropriate time

Electrodes must be kept clean to ensure maximum current flow.

Sticking should be performed within 15 sec after the stun. Chest sticking is appropriate.

Proper maintenance of the equipment should be insured.

Appropriate and calibrated volt / current measuring devices should be used to set stunners.

9.3.1.2. Monitoring points

Following a successful electrical stun, pigs will show the following signs:

- Immediate collapse.
- Immediate onset of Tonic seizure.
- Immediate onset of apnoea
- Clonic seizures follow tonic seizure:
- Gradual relaxation of the body.

Indicators of inadequate stunning are:

- Absence of tonic or tonic-clonic seizures
- Presence of rhythmic breathing.
- Focused eye movements.
- Constricted pupils.
- Vocalisation during stunning.
- Return of the head righting reflex.

9.3.1.3. Advantages

When performed correctly, unconsciousness is immediate.

9.3.1.4. Disadvantages

Under commercial conditions manual stunning may not be consistently good under high throughput conditions.

Pigs have to be restrained one-by-one to facilitate correct placement of the electrodes.

The duration of unconsciousness induced by an electrical head-only stun can be short.

Low and high voltage stunning are efficient and not painful if correctly applied; if inadequately applied, it is painful.

9.3.2. Electrical head-to-body or head-to-back stun / killing

9.3.2.1. One cycle and two cycle methods

One Cycle Method (Head-to-back): This method involves passing a current simultaneously through the brain and the heart. The front (head) electrode must be placed on the forehead (in front of the brain) and the rear electrode must be placed on the body behind the heart. To ensure correct positioning of the electrodes and to maintain contact, this method must be carried out on pigs held in a restrainer. A current frequency of 50 Hz sine wave AC needs to be used to ensure induction of cardiac arrest ventricular fibrillation (Wotton *et al.*, 1992).

Two Cycle Method: In this method, a current is first applied to the head (similar to head-only stunning), immediately followed by the application of current either head-to-body or across the chest to induce cardiac ventricular fibrillation. The head-only stunning can be performed with either a low or high frequency AC or DC with the minimum required current of 1.3 A. The cardiac ventricular fibrillation current must be applied immediately after stunning (the 1st cycle) using a 50 Hz sine wave AC.

Cardiac ventricular fibrillation can be induced by 50 Hz sine wave AC (1.3 A) applied for 3.5 sec during the head-to-back stunning procedure (Wotton *et al.*, 1992). Troeger (1999) recommends a cardiac ventricular fibrillation / arrest current of 1 A, 50-60 Hz sine wave AC. It is recommended that the electrical current should be applied for 3 sec when using this one-cycle method (HSA, 2000). Practical experience has shown that 1.0-1.7 A, 50 Hz sine wave applied for 2.1-2.4 sec can produce cardiac ventricular fibrillation in automated stun / kill systems (Von Wenzlawowicz *et al.*, 1999)

A minimum of 125 V and 50 Hz sine wave AC applied for 3 sec is an effective method of inducing cardiac ventricular fibrillation (under 2-cycle method Lambooij *et al.*, 1996). Warriss and Wotton (1981) successfully induced cardiac ventricular fibrillation in pigs with a transthoracic application of 100 V, 50 Hz sine wave AC for 5 sec. When using two-cycle method for manual stunning and killing, it is recommended that each cycle of current should be applied for 3 sec (HSA, 2000).

It has been shown that a current flow time of 0.2 sec (high voltage, 1.3 A, constant current) was not sufficient to induce an effective stun in all pigs (Berghaus and Troeger, 1998). With a minimum current flow time of 0.3 sec (high voltage, 1.3 A, constant current), all pigs showed epileptic seizures as an indication of an effective stun using frequencies of 50, 500 and 800 Hz sine wave.

In modern automated electrical stunning systems, method 2 is used where the application time for the 1st stunning cycle across the head is 2.1 to 2.4 sec (2.3-2.6 A) and for the heart current 1.4 to 1.7 sec (1.0-1.7 A). Pre-setting holds the current flow as the system operates using an electronic voltage adoption. Furthermore, the systems have a high efficiency in correct electrode placement to the benefit of animal welfare (Von Wenzlawowicz *et al.*, 1999).

Experiments have shown that the position of the rear electrode in head-to-back stunning between the 4th and the 7th cervical vertebra (C4-C7) did not fibrillate the heart in 100% of the pigs and therefore could not meet the requirements of the system on welfare grounds (Wotton *et al.*, 1992). Positioning the rear electrode between the 1st thoracic and the 1st lumbar vertebra (T1-L1) produced heart fibrillation in 100% of pigs.

In electrical stun / killing methods, the animals show only little or no clonic seizures (Troeger, 1999). High throughput / manual stunning are not conducive to maintenance of electrode positions. Automatic restraint is less susceptible to human operator variation in tongs application, although machines need to be adjusted in line with the variation in size of pigs slaughtered.

In fully automated 2-cycle electrics stun / killing systems, the incidence of successful cardiac fibrillation was 99% when evaluated in 6,056 pigs (60-135 kg live weight) in 3 European plants with line speed of 156, 358 and 365 pigs per hour (Von Wenzlawowicz *et al.*, 1999).

9.3.2.2. Description of effective use

One-cycle method:

- Pigs should be restrained and presented to facilitate accurate application of electrodes and maintenance of the current flow.
- The front (head) electrode must be placed on the forehead, and the rear (body) electrode should be placed on the body behind the anatomical position of the heart, such that they span the brain and the heart.
- Minimum current of 1.3 A (RMS or average) must be applied for at least 1 sec to induce immediate loss of unconsciousness. A minimum of 240 V would be required to achieve this current in the shortest time.
- Good electrical contact between the electrodes and the head and body should be maintained.
- Electrodes must be kept clean to ensure maximum current flow.
- Proper maintenance of the equipment should be insured.
- Appropriate and calibrated volt / current measuring devices should be used to set stunners.

Two-cycle method:

- Pigs should be restrained and presented to facilitate accurate application of electrodes and maintenance of the current flow.
- The head electrodes must be placed such that they span the brain (as in head-only stunning), and the cardiac fibrillation electrodes must span the heart.
- Minimum current of 1.3 A must be applied for at least 1 sec to induce immediate loss of consciousness. A minimum of 240 V would be required to achieve this current in the shortest time.
- Minimum current of 1.0 A, 50 Hz sine wave AC is required to induce cardiac ventricular fibrillation.
- Minimum current application for 3 sec is preferred.

- Good electrical contact between the electrodes and the head and/or body should be maintained.
- Appropriate post-stun handling is required to ensure that pigs are not subjected to forceful impacts that could resuscitate the heart.
- Second cycle should be applied as soon as possible, latest within 15 sec of head-only electrical stunning (within 5-10 sec, most animals are in the clonic phase, and it is unwise to wait unnecessary long).
- Electrical stunning and stun / killing devices should display voltage and current delivered to each animal and in each current cycle.
- Appropriate and calibrated volt / current measuring devices should be used to set these devices.

For both methods:

No animals shall recover after the application of stun / killing method.

9.3.2.3. Monitoring points

One cycle method:

Following an effective electrical stun / kill:

- Pigs will show similar signs of behaviour as after head-only stunning with immediate collapse followed by a tonic and clonic phase, the clonic phase may be weak or even absent.
- Gagging and corneal reflex may be present briefly after the induction of cardiac ventricular fibrillation but they should disappear soon.
- Dilated pupils.
- Relaxed carcass.

Two cycle method:

Adequate electrical stun/kill is indicated by:

- Immediate collapse.
- Immediate onset of tonic seizure.
- Immediate onset of apnoea.
- Clonic seizures follow tonic seizure but may be absent in some cases.
- Gradual appearance of relaxation of the body.
- Dilated pupils.

Inadequate stun/kill is indicated by:

- Recovery of rhythmic breathing.
- Carcass convulsions during bleeding.
- Other signs are similar to those seen with inadequante head-only stun.

9.3.2.4. Advantages

When performed correctly unconsciousness and/or death is immediate.

9.3.2.5. Disadvantages

In high capacity automated stun/killing systems (V- and belt-restrainer), pigs will have to be restrained one-by-one to facilitate correct placement of the electrodes.

9.4. GAS MIXTURES FOR STUNNING AND STUN /KILLING

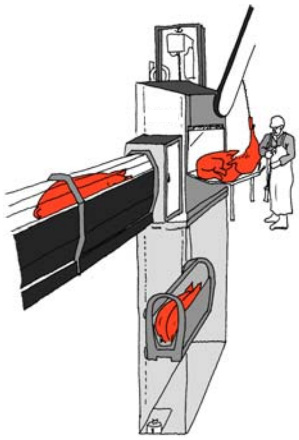
9.4.1. Carbon Dioxide / Air Mixtures

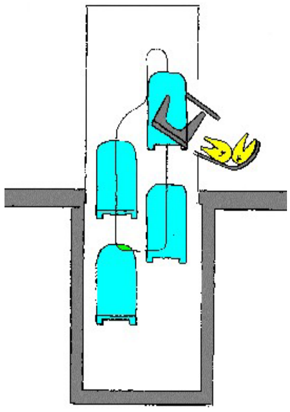
Carbon dioxide is heavier than atmospheric air and, under practical conditions, pigs are lowered into a pit with high concentration of carbon dioxide. There are two main types of carbon dioxide stunning systems for pigs: a dip-lift system and a paternoster system (Figure 9-3).

The dip-lift system works discontinuously. Small groups (4 to 6) of pigs in a box are lowered directly into the maximum carbon dioxide concentration at the bottom of the pit. After spending a pre-determined time at the bottom (depending on gradient of gas concentrations and appropriate exposure times) the box is again brought up and the unconscious pigs are tipped out for shackling, hoisting and bleeding.

The paternoster system works continuously with gondolas (cradles) like a Ferris wheel where pigs are lowered successively into the maximum carbon dioxide concentration at the bottom of the pit with stops during the procedure through an increasing carbon dioxide gradient as live pigs enter or unconscious pigs leave the gondolas for shackling. The paternoster system is the most commonly used. The number of pigs contained within each gondola or cradle varies according to the model and age of the system; older models have space to accommodate 1 to 3 pigs, whereas newer ones could take up to 6-8 pigs. The size of the chamber, size of individual cradle, and number of pigs per cradle could be varied according to the throughput rates.

The gradient of carbon dioxide contained within a chamber (pit or well) varies under commercial conditions (Figure 9-4). According to Directive 93/119/EC, pigs must be lowered into a minimum of 70% by volume of carbon dioxide in air, which is normally maintained at the top of the pit. The duration of exposure to carbon dioxide also varies depending upon whether stunning or stun / killing is desired.

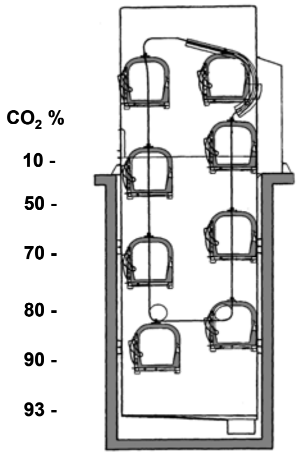




In dip-lift stunning systems pigs are lowered directly down into the bottom of the pit

In paternoster systems pigs are lowered successively into the bottom of the pit as live pigs during a stop enter an empty gondola and stunned pigs are tipped out of gondola (like principles in a “Paris Wheel”)

Figure 9-3. Principles in main types of carbon dioxide stunning systems



Principle in the carbon dioxide gradient in a stunning pit. carbon dioxide is heavier than atmospheric air and is admitted from the bottom. The concentration is highest at the bottom of the pit as air is mixed up with the gas in the upper part during operation of the system. The actual figures may however depend on the equipment used as well as the predetermined maximum concentration at the bottom position. The principles are equal for both dip-lift and paternoster systems.

Figure 9-4. Principles in the carbon dioxide gradient in a paternoster carbon dioxide stunning system

It has been shown in dogs that inhalation of high concentration of carbon dioxide reduces pH in the blood and hence in the CSF causing intracellular pH decreases in the neurons and thereby exerting its neuronal inhibitory and anaesthetic effect (Eisle *et al.* 1967). In this regard, normal pH of CSF is 7.4 and a state of analgesia and anaesthesia are induced at pH 7.1 and 6.8, respectively.

Aversive reactions in pigs during initial exposure to carbon dioxide:

Inhalation of carbon dioxide in concentrations of 50% or more are known to be pungent and cause breathlessness in most human subjects (Gregory *et al.* cited in Lambooij, 1990). Under laboratory experiments, it has been shown that while breathlessness on exercise in man was a common and known experience to all, exposure to elevated levels of 10% carbon dioxide by rebreathing was less pleasant and unfamiliar and was accompanied by an increased sense of breathlessness (Stark *et al.*, 1981).

Studies have shown that the majority of pigs (75%) avoided an atmosphere of 70% concentration of carbon dioxide (Cantieni, 1976), and Raj and Gregory (1995) found that pigs withdrew from an atmosphere of 90% in less than 5 sec. This aversion to carbon dioxide atmosphere was found to be greater than the motivation to obtain a reward (apples), even after 24 hours of fasting (Raj and Gregory, 1995). Moreover, 87.5% of pigs preferred to go without water for 72 hours rather than eudure exposure to carbon dioxide again (Cantieni, 1976).

Troeger and Woltersdorf (1991) reported that immediately after immersing pigs into the carbon dioxide gas mixture (60 to 90 %) under laboratory experiments there was no marked reaction, even at the highest concentrations, and overt panic or flight reactions did not occur. However, there was a slight drawing back and sniffing at the floor or at a normal head level. The first largely immobile phase - which could be a phase of tonic immobility - lasted for about 10 sec, was followed by a period in which the animals began to sway about and often leaned against the cage. Troeger and Woltersdorf (1991) concluded that the first contact with the stunning gas produces no definite sensation of pain or unease in the pigs.

In a full-scale research set-up behaviour studies in pigs during immersion into atmospheric air or high concentration of carbon dioxide has been carried out using a paternoster simulation with two stop positions between top and bottom of the pit (Holst, 2002). The pigs were lowered into either 70% carbon dioxide at the 1st stop position (reached within 10 sec) and 90% carbon dioxide at the bottom position or into atmospheric air in the same paternoster simulation. In these experiments, the carbon dioxide concentration was monitored 0.6 m above floor level of the stunning gondola at the 1st stop position, as pigs generally are standing upright in this position. In the bottom position the concentration was monitored 0.2 m above floor level of the stunning gondola, as by this time, pigs are recumbent.

The result of this study showed that there were few differences in behavioural reaction between pigs descended into atmospheric air compared with carbon dioxide. When immersed into atmospheric air, a majority of the pigs (77%) stood motionless (freezing) in the descending gondola. When in the stop position, the majority of pigs again began to explore the stunning box. When carbon dioxide (70%) was used, the majority (66%) of the pigs stood motionless during the descending movement and when they were in contact with the carbon dioxide there were no marked reactions (apart from normal exploratory behaviour). Some pigs again explored the stunning box while in the stop position. Based on these findings, it may be concluded, that the vertical movement of the stunning gondola by itself induces fear in the pigs expressed as tonic immobility.

The differences in reactions between immersion into atmospheric air and carbon dioxide were limited to sniffing and 'backing away' of the pigs. No pigs showed sniffing in atmospheric air while 53% showed sniffing at the floor or at normal head level during immersion into carbon dioxide (Holst, 2002). No sneezing or coughing during immersion into carbon dioxide was observed. It was also found, that 21% of the pigs slightly backed away during descent into atmospheric air and 49% backed away when exposed to carbon dioxide (Holst, 2002). A majority of the pigs did not show that reaction immediately after the contact with carbon dioxide and the first period of immobility lasted approximately 10 sec.

It is known that, when pigs are presented to an unpleasant situation, their first reaction is to back away (Dodman, 1977). Dodman (1977) reported that 60% of all the pigs exposed to 50 to 80% carbon dioxide in his study backed away; however, the incidence of this reaction varied according to the concentration of carbon dioxide. In this regard, 100% of pigs backed away in 50-55% carbon dioxide and 37% of the pigs showed this reaction in 76-80% carbon dioxide. Carbon dioxide is also known to have an analgesic effect (Mischler *et al*., 1994; Mischler *et al*., 1996) and so, another reason for a lower frequency of pigs backing in the higher concentrations of carbon dioxide may be because the analgesic effect of carbon dioxide is more potent at the higher concentrations. Thus, the aversiveness of carbon dioxide, if any, is less in high concentration compared with lower concentrations. Dodman (1977) also found that pigs remained motionless when they first came into contact with carbon dioxide. Remaining motionless could be interpreted as either a fear response (like tonic immobility or freezing behaviour to a potential threat) or that the pigs were unaffected by their environment.

Holst (2002) found that the number of pigs backing away decreased as the concentration of carbon dioxide was increased. Thus, 83, 79, 52 and 60% of pigs backed away during contact with 55, 65, 75 and 85% carbon dioxide, respectively. However, there were no significant alterations in other behavioural patterns such as sniffing, head shaking or vocalising.

Holst (2002) also found that when pigs were lowered into 90% carbon dioxide (with 70% in the 1st stop position reached within 10 sec), head shaking occurred in 4% of the pigs but no pigs vocalised or attempted to escape.

Under laboratory conditions, Raj and Gregory (1996) reported that some pigs showed escape attempts during exposure to carbon dioxide in concentrations of 70% or less in air, but no pigs showed escape attempts at 80-90% carbon dioxide. This lack of escape attempts in pigs that were exposed to 80-90% carbon dioxide could be interpreted as remaining motionless, freezing or a fear-induced inhibition of spontaneous behaviour. It could also be due to a more potent analgesic effect of carbon dioxide. In this study only one pig at a time were descended into the different gas mixtures and it was observed that 3 out of 36 pigs showed escape attempts when descended into atmospheric air. The results showed that isolation of an animal and caging might act as an aversive stimulus in themselves. In the study by Holst (2002), 3 pigs at a time were lowered into either atmospheric air or 70% carbon dioxide to avoid possible diverging behavioural reactions from isolated pigs, and at the highest concentration of 85% carbon dioxide, escape attempts were seen in only 5% of pigs.

Their reaction was expressed as fast running movements across the stunning box but without the pigs raising their forelegs on the side of the wall of the box. Dodman (1977) reported similar behaviour reactions.

Hartung *et al.* (2002) carried out EEG measurements, analysis of catecholamines and clinical investigations on slaughter pigs stunned with either 80 or 90% carbon dioxide. The stunning time for 90% carbon dioxide was 73 sec, but at 80% carbon dioxide, it was over 70 sec and was not sufficient to stun pigs properly.

Overall, the results above indicate that pigs adversely react to exposure to carbon dioxide, and they also react to the descending movement of the stunning box itself. Data indicate that they perceive the gas (increased sniffing) and also show signs of aversion: backing away, head shaking and escape attempts. Moreover, pigs that have been removed from an atmosphere of carbon dioxide and then allowed to recover refuse to return into that area, when given the choice. It is also possible that there is an interaction between the gas concentration and aversive reactions, as suggested by the lower aversive responses in the higher concentrations of carbon dioxide. The reason is probably due to a more potent analgesic effect of carbon dioxide in high concentrations compared with lower concentrations.

Physiological effects during exposure:

The severity of respiratory distress occurring prior to loss of posture (behavioural indicator of onset of unconsciousness) during exposure to concentrations of 20, 30, 40, 50, 60, 70, 80 or 90% carbon dioxide in air, was determined (Raj and Gregory, 1996). The audio tapes containing respiratory sounds occurring during exposure to various concentrations of carbon dioxide was played to experienced animal physiologists and they were asked to categorise the distress sounds as minimum, moderate or severe. The results showed that exposure to all the concentrations of carbon dioxide in air induced severe respiratory distress. Dodman (1977) also reported that 60% of pigs showed some degree of ‘excitement’ at 50 to 80% carbon dioxide prior to loss of posture, and that this behaviour was found to be severe in 20% of the total number of pigs used in his study. It is very likely that the ‘excitement’ reported by Dodman (1977) is similar to the vigorous shaking of head observed in pigs during the induction of unconsciousness with a high concentration of carbon dioxide in air. It is not certain whether this behaviour is suggestive of breathlessness in pigs.

The exposure to carbon dioxide stimulates respiration and pigs start to hyperventilate which causes respiratory discomfort (Raj and Gregory, 1996), and in humans this is interpreted as dyspnoea or breathlessness (Gregory *et al.*, 1990; Stark *et al.*, 1981). The severity of breathlessness in humans is known to increase with the rate of increase in blood carbon dioxide levels (Stark *et al.*, 1981).

The faster and deeper respiration during hyperventilation in high concentration carbon dioxide atmosphere will result in increased gas intake and thereby increased efficiency of the stunning method, which may shorten the induction period and time to loss of consciousness (Forslid, 1992). From an animal welfare point of view this may be an advantage. However, the time to onset of breathlessness is quicker with high than low concentrations of carbon dioxide (Stark *et al.*, 1981).

Time to onset of unconsciousness during exposure:

Dodman (1977) found that during exposure of pigs in a dip-lift equipment to 61-65, to 66-70, to 71-75 and to 76-80% carbon dioxide in air, the time to lateral recumbency (which was considered as a behavioural indicator of unconsciousness) decreased gradually from 38 to 34, 29 and 26 sec respectively.

Gregory *et al.* (1987) observed pigs in a compact stunning equipment with 86% carbon dioxide at the bottom of the well. The effectiveness of stunning was based on subjective changes in vocalisation patterns from a normal tone to slurred and muffled vocalisation (note that Holst (2002) found no effect on vocalisation). The results of Gregory *et al.* (1987), suggested that anaesthesia began 30 to 39 sec after the start of immersion procedure. However, pigs used in this were stunned in a Compact carbon dioxide stunning unit in which the floor of the gondolas moved away while descending the pigs into carbon dioxide and the pigs' chests were also restrained in the V-shaped gondolas. It is therefore possible to speculate that these could have resulted in compression of thorax and, hence, interfered with the full inhalation and uptake of carbon dioxide culminating in delayed loss of consciousness. Restraint is a known stressor to pigs and they may struggle and squeal as also noted by Gregory *et al.* (1987). It cannot be excluded, however, that they could have been squealing because of the carbon dioxide. With the Council Directive 93/119/EC on the protection of animals at the time of slaughter, those carbon dioxide stunning systems with drop floor where the floor fell away were banned on animal welfare grounds.

During stunning of pigs in a commercial dip-lift system with different carbon dioxide concentrations ranging from 50-80%, no vocalisation from the pigs was heard during the induction phase, whereas expiration was frequently accompanied by a muffled sounds during the excitation phase (Dodman, 1977). In an experiment with exposure of pigs to 80% carbon dioxide in an experimental stunning chamber, Ring *et al.* (1988) did not heard vocalisation during the induction phase. In a recent study of behaviour in slaughter pigs when stunned in 90% carbon dioxide in a full-scale research set-up, no vocalisation were recorded during the induction period (Holst, 2002).

Raj and Gregory (1996) have reported time to loss of consciousness in pigs, indicated by loss of posture, at 25, 17, 22 and 15 sec after immersion into 60, 70, 80 and 90% carbon dioxide, respectively. Preliminary investigations have shown that faster immersion of pigs into high concentrations of carbon dioxide reduces the time to unconsciousness, indicated by loss of posture, as compared with slower immersion (Barton-Gade, 1999).

When lowering pigs into increasing concentrations of carbon dioxide in the 1st stop position in a paternoster simulation under practical commercial conditions the time to loss of posture has been found to decrease (Holst, 2002). With increasing concentrations of carbon dioxide from 55 to 65, 75 and 85% in 1st stop position the time to loss of posture on average decreased from 32 sec to 27, 25 and 22 sec, respectively.

Several studies (Forslid, 1987; Ring *et al.*, 1988; Raj *et al.*, 1997a; Martoft *et al.*, 2001) have used EEG and shown fast changes towards depression of the CNS during induction of carbon dioxide anaesthesia in pigs.

As pigs convulse during exposure to carbon dioxide, it become important to note the time at which they appear to lose consciousness. At 80%, Forslid (1987) found that delta activity (<4 Hz) in the EEG became dominant at 21-30 sec and isoelectric EEG at 52.5±2.3 sec; whereas at 90% carbon dioxide, similar changes in the EEG were found after 11.8±0.3 sec and the EEG was isoelectric after 34±2 sec (Forslid, 1992). As convulsions started at 28±1 sec and 15.3±1.4 sec after exposure to 80% and 95% carbon dioxide respectively, Forslid (1987, 1992) concluded that the pigs would be unconscious by this time.

Ring *et al*. (1988) reported that, after immersion of pigs into 80% carbon dioxide in an experimental stunning chamber, they became unconscious in 15 to 20 sec and concluded that the procedure is rapid and humane. However, Ring *et al*. (1988) also found that Beta activity (13 to 30 Hz) in the EEG increased during this period. Since increases in Beta activity would be normally interpreted as arousal or distress, the finding is disconcerting on animal welfare grounds.

The results of another study, in which evoked potentials (somatosensory evoked potentials, SEPs) in the brain were used to determine the loss of consciousness during exposure to 80% carbon dioxide, showed that the average time to loss of SEPs was 21.2±6.5 (SD) sec but 1 pig took 36 sec to lose SEP (Raj *et al.* 1997a). Hoenderken *et al.* (1979) also reported that the time to onset of unconsciousness, as determined from changes occurring in the EEGs, during exposure of pigs to 80% carbon dioxide could be as long as 35 sec. However, the reason(s) for the wide variation between pigs in terms of onset of unconsciousness during exposure to carbon dioxide has not been clearly established and raises issues about pigs being completely unconscious at the time of convulsions.

Martoft *et al.* (2001) exposed pigs to 90% carbon dioxide in an experimental stunning chamber and showed a clear decline in CNS activity towards unconsciousness measured by Auditory Evoked Potentials (AEPs) at approximatively 14 sec after start of immersion.

Table 9-4. Time (in sec) to loss of consciousness measured by different methods after exposure to different concentrations of carbon dioxide

Carbon dioxide concentration (%)	Time to loss of sensibility (EEG) Sec	Time to loss of brain responsiveness (AEP/SEP) Sec	Average time to loss of posture Sec	Number of animals tested	Reference
95	11.8±0.3			4 (x2)[1]	Forslid, 1992
90		14		6 (x2)[1]	Martoft *et al*. 2001
90			15±3	5	Raj and Gregory, 1996
85			22±2	42	Holst, 2002
80	21-30			6 (x2)[1]	Forslid, 1987
80	15-20			44	Ring *et al*. 1988
80		21.2±6.5		12	Raj *et al*. 1997a
80			22±6	5	Raj and Gregory, 1996
76-80			26±6	16[2]	Dodman, 1977
75			25±3	42	Holst, 2002
71-75			29	16[2]	Dodman, 1977
70			17±4	5	Raj and Gregory, 1996
66-70			34±9	16[2]	Dodman, 1977
65			27±3	42	Holst, 2002
61-65			38	16[2]	Dodman, 1977
60			25±2	5	Raj and Gregory, 1996
55			32±4	36	Holst, 2002
50			34±8	5	Raj and Gregory, 1996

Notes: 1) Each pig tested twice.
2) Each of the 16 pigs in total tested several times in different concentrations.

The above studies have together shown that exposure of pigs to increasing carbon dioxide concentrations decreases the time to loss of consciousness. It is then reasonable to suggest that immersion of pigs into as high carbon dioxide concentration as possible (e.g. 80% according to Table 9-4) from leaving atmospheric air shortens the time to loss of consciousness and helps to reduce the duration of hyperventilation and potential distress.

Investigations have been carried out using carbon dioxide under commercial conditions (Raj, 1999) where it was found that, although vocalisation is normally considered to be a conscious response, there was reason to suggest that it may not have been a conscious reaction. First, the average time to loss of SEP's during exposure of pigs to the gas mixtures was shorter than the average time to the onset of vocalisation recorded. And secondly, the pigs vocalised when they were gagging, which appears to be a rudimentary brain stem response occurring in association with the process of exhalation.

Studies have shown that exposure of pigs to a minimum of 80% carbon dioxide is better than exposing them to lower concentrations and this should be achieved within 10 sec after that pigs have left the atmospheric air. This will keep the duration of potential distress and suffering short. The carbon dioxide concentration at the bottom of the pit in both the existing stunning systems should be 90% or more.

Stun stick interval and recovery from carbon dioxide exposure:

When pigs are returned to atmospheric air after exposure to carbon dioxide, they begin to regain consciousness (Blomquist, 1957; Dodman, 1977; Forslid, 1987; Holst, 2001; Martoft *et al.*, 2001 and Ring *et al.*, 1988). The reversibility of stunning of pigs for slaughter in high concentration of carbon dioxide depends on the gas concentrations used and the duration of exposure. In humans suffering from hypercapnea, rapid decline in blood carbon dioxide levels could induce post-hypercapnic ventricular fibrillation. However, the incidence of this occurring in pigs has not been established and so, other methods must be used to ensure death.

1 min of exposure to 80% carbon dioxide induced a flat (isoelectric) ECoG (Forslid, 1987), which lasted for about 1 min after the end of exposure and during the second post-stun min, the ECoG gradually attained a pattern characteristic of surgical anaesthesia (slow wave (delta) ECoG). Martoft *et al.* (2001) found that the period of marked depression of the CNS after emerging from 1 min of exposure to 90% carbon dioxide was approximately 60 to 90 sec.

Evaluation of insensibility after an effective carbon dioxide stunning of pigs can be performed by the clinical methods (corneal reflex, respiration, etc) normally employed to judge the effect of a chemical anaesthetic used for surgical procedures (Blackmore and Newhook, 1983). Gregory *et al.* (1987) and Raj (1999) have used brain stem reflexes such as absence of gagging or gasping and absence of corneal reflex to assess the effectiveness of carbon dioxide stunning of pigs. Gregory *et al.* (1987) found that 16% of the pigs showed a corneal reflex after stunning in carbon dioxide (total exposure 66 sec in a maximum concentration of 92%) as they were conveyed from the shackling table to the sticking point. Based on the absence of rhythmic breathing or voluntary movements, Gregory *et al.* (1987) concluded that all the pigs were effectively stunned by carbon dioxide, even though 5% of the pigs showed pedal reflex in response to pinching the cleat of a hind foot.

Experiments in a full-scale research set-up at a commercial abattoir have shown that, absence or presence of different distinct and easy reliable clinical brainstem reflexes (corneal reflex, gagging, convulsions), can be used for assessment of the efficiency of carbon dioxide stunning during sticking to ensure that no pigs regain consciousness during bleeding (Holst, 2001).

In this experiment, a total of 210 pigs were stunned one by one in a mixture of 90% carbon dioxide in air where the carbon dioxide concentration at the first stop was >70% and the total stunning time was 132 sec. After stunning, the pigs were tipped out on a receiving table and remained undisturbed, and each pig was continuously examined for behavioural and different physiological reflexes to evaluate the rate of regaining consciousness. The time from end of carbon dioxide exposure to reappearance of reflexes was registered for each pig.

No pigs had any reflexes just after the end of carbon dioxide exposure. The corneal reflex was the first reflex to reappear on average after 42 sec, and regular respiration reappeared after 68 sec and was used as the first sign of return to consciousness. Convulsions was noted on average after 76 sec and occurred in only 77% of the pigs. Spontaneous blinking of the eye reappeared on average after 93 sec and was used to indicate an imminent return of consciousness. Conscious movements of head or legs were observed after 171 sec and were evaluated as a complete return of consciousness. It is worth noting that the severe acidosis induced with inhalation of a high concentration of carbon dioxide may result in recumbent conscious animals, as seen in dairy cows with acidosis, without movement or response to stimulation.

Based on the findings of Holst (2001), a set of guidelines for monitoring the efficiency of carbon dioxide stunning of pigs has been suggested. At the time of sticking the following signs can be used as criteria for a successful stun:

- Absence of rhythmic breathing.
- Gagging or gasping may be present briefly.
- No convulsions may be present.
- Absence of spontaneous blinking of the eye.
- Corneal reflex may be present briefly in a low frequency (<5%) of the total number of pigs, provided that other reflexes are absent.

In order to monitor the stunning and bleeding efficacy, access to the slaughtered pigs must be possible from sticking to the beginning of the next slaughter steps (scalding) (Holleben *et al.*, 2002).

Under practical conditions, it is becoming more common to stun 2-3 pigs in each gondola, and the new group-wise stunning system operates with up to 7-8 pigs in each gondola. This means that the time required for shackling, hoisting and exsanguinatiing the last pig in a group increases with the group size and thus, the stun-to-stick interval also increases.

During anaesthesia, the corneal reflex is the last reflex to disappear when animals are very deeply anaesthetized and in a stage just prior to vascular collapse and death. Conversely, the corneal reflex is the first reflex to reappear during recovery from the very deep anaesthetic level. This has been used in a recent research study in pigs showing that, as exposure time to carbon dioxide increases, the time to regain consciousness is delayed as indicated by increasing time to first reappearance of the corneal reflex after end of exposure (Holst, 2001). Pigs were stunned in groups in a paternoster simulation with > 70% carbon dioxide at the 1st stop position (reached within 10 sec) and 90% carbon dioxide at the bottom position. The bottom position was reached within 40 sec and increasing the holding time at the bottom position increased the total exposure time. The whole experiment was performed in 9 separate sub-sections with different but increasing total exposure times to carbon dioxide ranging from 112 to 192 sec in intervals of 10 sec.

It was found that, as stunning time increase from 112 to 192 sec, the time to the earliest reappearance of the corneal reflex increased from 20 to 102 sec respectively (Holst, 2001). This indicates a deeper and longer lasting stunning or onset of death as a result of the increased exposure time to carbon dioxide (see Figure 9-5).

At the same time, the 5% limit (5% quantile) of pigs showing a corneal reflex was increased from 24 to 142 sec as time of exposure increased from 112 to 192 sec (see Figure 9-5).

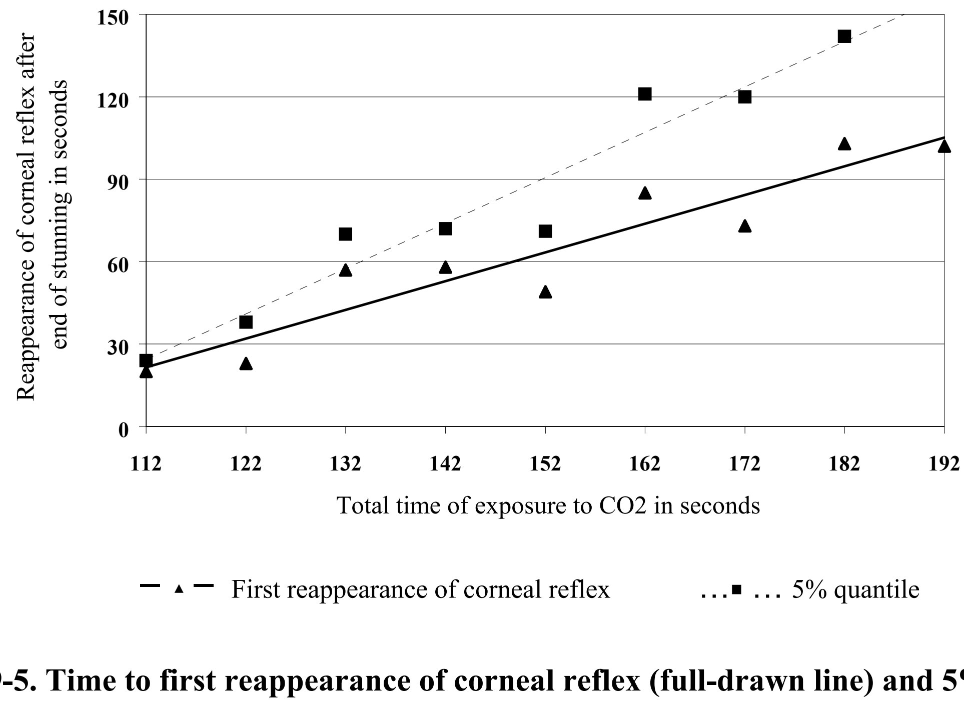


Figure 9-5. Time to first reappearance of corneal reflex (full-drawn line) and 5% quantile of pigs showing corneal reflex (dotted line) after end of increasing time of exposure to 90% carbon dioxide

Furthermore, it was found that, with increasing time of exposure to carbon dioxide, the number of pigs showing a corneal reflex within 150 sec after the end of exposure decreased from 91% to 3% when the exposure time increased from 112 to 192 sec respectively (Holst, 2001).

In conclusion, the results of these studies have shown that, with increasing exposure times to carbon dioxide, the duration and maintenance of unconsciousness increases and the stun-to-stick interval can be increased without jeopardising animal welfare. It was found that a stun-to-stick interval of up to 90 sec or even longer under practical conditions can be established without compromising animal welfare (Holst, 2001).

Based on these findings, recommendations for minimum stun-to-stick intervals in relation to total exposure times can be established for the paternoster system under the assumption that pigs are immersed in a minimum 70% carbon dioxide in the 1st stop position within 10 sec and that the minimum carbon dioxide concentration is 90% at the bottom position.

Using these criteria the following guidelines may be set up:

Table 9-5. Guidelines for stun-to-stick intervals in relation to total stunning time in the new group-wise carbon dioxide stunning equipments with a minimum of 70-80% carbon dioxide in air at the first stop position of the gondolas and 90% carbon dioxide at the bottom position.

Total time of exposure (sec)	Sticking within (sec)
120	30
130	45
140	60
150	75
160	90

It must however be stressed that Table 9-5 should only be used as a guideline as commercial carbon dioxide-equipment varies widely with respect to the position of gondolas in the equipment, carbon dioxide-gradients (even within the constraints minimum 70% carbon dioxide in the 1st stop position), as well as dwell time. If the equipment is such that the above times of exposure cannot be met, then good stunning practice with no pigs regaining consciousness before or during bleeding may be obtained **either** by increasing the carbon dioxide-concentration **or** by increasing dwell times. That is by slowing the rate at which pigs go through the system, the time of exposure to carbon dioxide can be increased. Under all circumstances, the stunning efficiency should always be monitored regularly to ensure that good stunning practice is obtained and no pigs regain consciousness before or during bleeding.

In a study under practical slaughterhouse conditions, Holleben *et al.* 2002 have found the following relationship between carbon dioxide concentration, time of exposure and recommended stun-to-stick interval.

Table 9-6. Recommended carbon dioxide-concentration, exposure time and stun-to-stick interval (Holleben et al. 2002)

Exposition in +/- 84 %	100 sec	Maximum stun-to-stick time 35 sec[1].
Exposition in >84 %	100 sec	Maximum stun-to-stick time 45 sec
Exposition in >84 %	150 sec	Maximum stun-to-stick time 60 sec
[1] applies to all animals in a chamber		

Meat quality and gas stunning:

Troeger *et al.* (2003) compared the meat quality of pigs stunned with argon alone and with mixtures of argon and nitrogen compared with carbon dioxide. Pigs stunned with hypoxic gas mixtures showed bloodspots in the muscles, especially in the muscle semimembranous, caused by hypoxia or convulsions during the excitation phase and was judged unacceptable for practical use by the authors.

Under practical conditions, Holleben *et al.* (2002) have found maximum stun-to-stick interval of 45 and 60 sec after exposure to >84% carbon dioxide for 100 and 150 sec, respectively. These figures are based on studies in older carbon dioxide stunning systems and only 2 of the new group-wise stunning units were included in the study (Holleben, 2003). This means that the recommended exposure times and stun-to-stick intervals in Holleben *et al.* (2002) can only be related to the older carbon dioxide stunning systems and not to the new group-wise stunning units. Insufficient data from the new group-wise stunning units are available at the time the study was conducted (Holleben, 2003). Based on experiments in a full-scale research set-up, Holst (2001) found that stun-to-stick intervals of up to 75 and 90 sec can be obtained by exposure to carbon dioxide for 150 and 160 sec, respectively. This is also in accordance with practical experience in the new group-wise carbon dioxide stunning units, where stun-to-stick intervals of 70-80 sec for the last pig in groups of up to 7-8 pigs have been established without compromising animal welfare (Christensen, 2003).

Based on these findings, it can be concluded that it is not possible to make the same recommendation for maximum stun-to-stick interval for all the exposure times, all group sizes, and all technical lay-outs of carbon dioxide stunning systems that are used in slaughterhouse conditions. Under all circumstances, stunning should be good enough to ensure that no pigs regain consciousness before death supervenes through bleeding.

9.4.1.1. Description of effective use

In dip-lift and pater-noster systems, pigs shall be exposed to a minimum 80% carbon dioxide within 10 sec from leaving atmospheric air. The carbon dioxide concentration at the bottom of the pit shall be a minimum 90%.

Exposure time to carbon dioxide shall be sufficient to ensure that no pigs regain consciousness before death supervenes through bleeding.

A minimum exposure time, which cannot be shortened by the personel, appropriate to the concentration gradient in the pit must be set.

In order to make sure that all pigs will be stunned properly, a minimum exposure time of 100 sec should be mandatory for all systems.

Practical experience has shown that pigs appear less calm during the induction phase when stunned singly and it is recommended that at least 2 pigs are stunned together, provided sufficient floor space is available in the gondola.

Bleeding must be done as soon as possible after the end of stunning and be carried out in such a way as to bring about rapid, profuse and complete bleeding. In any event, the bleeding must be carried out before an animal regains consciousness.

9.4.1.2. Monitoring points

Carbon dioxide concentrations should be monitored continuously, above the pig's head while standing, at both 1st stop position and bottom position in paternoster systems and at the position the gondola reaches after 10 sec in dip-lift systems. These concentrations shall be displayed.

In paternoster systems, monitoring of gas concentration in the bottom position should be done 0.2 m above floor level of the gondola.

In dip-lift systems monitoring of gas concentration should be done 0.6 m above floor level of the gondola

A clearly audible or visible warning must be given if the carbon dioxide concentration falls below the required levels.

The stunning efficiency shall be monitored regularly to ensure that no pigs regain consciousness during sticking or bleeding. This can be verified by:

- An absence of rhythmic breathing, although gagging or gasping may be present briefly (refer to section 5.3.4);
- An absence of carcass convulsions;
- An absence of spontaneous blinking of the eye but a corneal reflex may be present briefly in low frequency (<5%) at the moment when pigs are stuck.

9.4.1.3. Advantages

Carbon dioxide stunning of pigs has the advantage that restraint during stunning is not necessary other than confinement in a cradle or gondola and that more than one animal can be stunned at any one time.

In new Group-wise stunning systems, pigs do not need to be lined up in a raceway. Thus the use of force, such as electrical goads, is not necessary while guiding pigs up to and through the stunning equipment. Furthermore, the pulsatile start-stop nature of movement into the system has been eliminated.

It is possible to have variable stun-to-stick intervals as the increasing duration of exposure to carbon dioxide increases the duration of unconsciousness and onset of death in some pigs.

9.4.1.4. Disadvantages

Under the paternoster system of carbon dioxide stunning or killing, the loading of pigs into the older units is pulsatile; *i.e.* a pig can only be loaded when there is an empty gondola available in front of it. This stop-start nature of movement into the system is not conductive for handling pigs.

Carbon dioxide is an aversive gas. It induces breathlessness and some pigs may show attempts to escape, back away or head shake.

9.4.2. Other Gas Mixtures

At present, alternative gas mixtures are not used under commercial conditions for stun / killing of pigs.

Reactions occurring in animals during the initial exposure to other gas mixtures:

Aversion to the initial inhalation of argon or carbon dioxide was determined using passive avoidance tests in the presence of a reward (an apple) (Raj and Gregory, 1995). When 90% argon in air (with 2% residual oxygen) was presented in a feeding chamber, all the test pigs spent most of their allocated time (3 min) feeding on apples, and 6 out of 6 pigs in trial 1 and 3 out of 6 pigs in trial 2 either became unsteady or lost their posture while they were still feeding on apples presented inside the chamber. None of the pigs showed signs of hyperventilation whilst inhaling argon presented inside the chamber. By contrast, when 90% carbon dioxide was present in the feeding chamber, the time to withdrawal of their heads was immediate and the test pigs also spent significantly less time feeding in the box. When 30% carbon dioxide was presented in the feeding chamber, the test pigs withdrew their heads either immediately upon smelling the gas or when they became hyperventilated.

Physiological effects during exposure (e.g. respiratory distress):

The severity of respiratory distress occurring prior to loss of posture (behavioural indicator of onset of unconsciousness) during exposure to 90% argon in air, during exposure to concentrations of 20, 30, 40, 50, 60, 70, 80 or 90% carbon dioxide in air, or a mixture of 30% carbon dioxide and 60% argon in air was determined (Raj and Gregory, 1996). The respiratory sound was used to categorise the distress as minimum, moderate or severe. The results showed that exposure to 90% argon induced minimal respiratory distress prior to loss of consciousness, whereas, exposure to all the concentrations of carbon dioxide in air induced severe respiratory distress. Exposure to the carbon dioxide-argon mixture induced a moderate distress.

Dodman (1977) also reported that 60% of pigs that were exposed to high concentrations of carbon dioxide (50-80%) showed some degree of ‘excitement’ prior to loss of posture and, this behaviour was found to be severe in 20% of the total number of pigs used in his study. It is very likely that the ‘excitement’ reported by Dodman (1977) is similar to the vigorous shaking of head observed in pigs during the induction of unconsciousness with a high concentration of carbon dioxide in air. On the other hand it may as well be likely, that the ‘excitement’ just prior to loss of posture as also reported by Troeger and Woltersdorf (1991) as a dazed phase may be the result of the progressive affect on the brain during induction of unconsciousness.

Duration of exposure necessary to induce unconsciousness and insensibility:

Raj and Gregory (1995) reported that the time to loss of unconsciousness as determined from the loss of posture (which is considered as a behavioural indicator of the onset of unconsciousness) was on average 35, 24 and 15sec in 30 kg piglets after exposure to 90% argon, carbon dioxide-argon mixture and 80-90% carbon dioxide, respectively.

The time to loss of somatosensory evoked potentials (SEPs), and thus unequivocal loss of consciousness, was determined (Raj *et al.*, 1997a). In that study, the ranges of times to loss of SEPs were found to be 9-21, 11-20 and 16-36 sec after exposure to 90% argon in air, 30% carbon dioxide and 60% argon in air and 80 to 90% carbon dioxide in air, respectively. These results showed that the time to loss of brain responsiveness is rapid with argon and the carbon dioxide-argon mixture. By contrast, the time to loss of brain responsiveness during exposure to a high concentration of carbon dioxide in air is relatively long and is also highly variable. However, the longest times recorded for the onset of an isoelectric electrocorticogram (ECoG) were 86, 47 and 44 sec after exposure to 90% argon in air, a mixture of 30% carbon dioxide and 60% argon in air, and 80 to 90% carbon dioxide in air. This suggests that the exposure time required to kill pigs with anoxia would be longer than that required under hypercapnic conditions.

Under commercial conditions, investigations have been carried out to determine the feasibility of using alternative gas mixtures to stun / kill pigs in the existing Combi system (Raj, 1999). In this study, 2 or 3 pigs were loaded per cradle and immersed in either 90% argon in air or a mixture of 30% carbon dioxide and 60% argon in air. The pigs were exposed to gas mixtures for 3, 5 or 7 min to determine the proportion of pigs killed within the gas mixtures and to determine the ideal stun-to-stick intervals in pigs which survived after the exposure.

The approximate time interval between the pigs exiting the gas and sticking (bleeding out) was 25, 35 and 45 sec, respectively, for the first, second and third pig hoisted from a single batch of three (Raj, 1999). At the exit, animals were examined for the presence of gagging and corneal reflex. Animals were also examined about 5 sec after sticking (50 sec after exiting the gas) for the presence of gasping, corneal reflex and response to a nose prick performed with a hypodermic needle. The occurrence of carcass convulsions in pigs during bleeding was also noted.

The results of this study indicated that:

1. After exposure of pigs to either argon-induced anoxia or the carbon dioxide-argon mixture for 3 min, recovery can be avoided in pigs by sticking (bleeding) them within 25 sec from exiting the alternative gas mixtures, however, the carcass convulsions occur during bleeding.

2. Exposure of pigs to either argon-induced anoxia or the carbon dioxide-argon mixture for 5 min and bleeding within 45 sec prevented resumption of consciousness in pigs and carcass convulsions during bleeding.

3. Exposure of pigs to argon-induced anoxia for 7 min resulted in death in the majority of pigs. By contrast, exposure of pigs to the carbon dioxide-argon mixture for 7 min resulted in death in all the pigs.

The convulsions occurring during bleeding, after exposure of pigs for 3 min, might certainly cause concern that the animals might be recovering consciousness during bleeding. Because of this problem, 3 min of exposure to these novel gas mixtures may not be ideal under practical slaughterhouse conditions. Pigs could be exposed to either argon or a carbon dioxide-argon mixture for 3 min to stun them and then immediately kill them, when they are returned to atmospheric air, by inducing cardiac arrest through the application of an electric current across the chest. This could be achieved, for example, by the application of 90 V, 50 Hz sine wave AC across the chest for 5 sec (Warriss and Wotton, 1981), within 25 sec of pigs exiting the gas mixture. Cardiac arrest could also be induced when the pigs are still within the gas mixtures (*i.e.* before leaving the stunning unit) and this would eliminate the chances of recovery due to any inadvertent delay between gas stunning and the induction of cardiac arrest outside the stunning unit.

In summary, it is stated that the available scientific evidence overwhelmingly suggests that stunning of pigs with anoxia induced with inert gas mixtures (*i.e.* nitrogen and / or argon) is the best option on animal welfare grounds. However, due to the lack of purpose built equipment, inert gases are not implemented under field conditions and the FAWC (2003) recommended that the government and industry should fund research and development to achieve this.

Ideally, such a system should incorporate some general animal welfare principles that were also proposed by the FAWC (2003) to maximise pig welfare at slaughter as follows:

- Pigs should be maintained in a stable social group with the minimum of restraint;
- Pre-slaughter handling facilities should be designed to minimise stress;
- The gas used to induce unconsciousness should be non-aversive;
- All pigs should be rendered rapidly unconscious in the gas;
- An irreversible state of unconsciousness must be reached in all pigs prior to sticking; and,
- There should be adequate monitoring of the system and efficient evacuation in the event of any system failure.

9.4.2.1. Description of effective use

In order to achieve effective stunning, pigs must be exposed to a maximum of 2% by volume of oxygen in argon, nitrogen or other inert gases or to a maximum of 30% by volume of carbon dioxide and a maximum of 2% by volume of oxygen in mixtures with carbon dioxide and argon, nitrogen or other inert gases.

Exposure time to the gas mixtures shall be sufficient to ensure that no pigs regain consciousness before death supervenes through bleeding.

Bleeding must be done as soon as possible after end of exposure to gas mixtures and be carried out in such a way as to bring about rapid, profuse and complete bleeding. In any event, the bleeding must be carried out before the animal regains consciousness.

Table 9-7. Exposure times (min) and stun-to-stick intervals (sec) for non-aversive alternative gas mixtures (Raj, 1999)

Exposure time (min)	**Stun-to-stick interval** or maximum time to induce electrical cardiac ventricular fibrillation **(sec)**
3	< 25
5	< 45
7 (90% argon)	< 45
7 (mixture of argon/carbon dioxide)	Not critical, death occurs in majority of the pigs
>7 (90% argon until all pigs are dead)	Not critical

Both the common carotid arteries (or blood vessels from which they arise) should be severed to prevent return of consciousness in adequately stunned pigs. Alternatively, cardiac ventricular fibrillation shall be induced in unconscious pigs.

9.4.2.2. Monitoring points

Concentrations of oxygen and / or carbon dioxide shall be continuously monitored above the level of the pig's head both at the first position in the gas in paternoster systems, alternatively after 10 sec of descent in dip-lift systems, and at the bottom of the pit. These concentrations shall be displayed. A clearly audible or visible warning must be given if the carbon dioxide or oxygen concentration rises above the recommended levels.

The stunning efficiency shall be monitored regularly to ensure that no pigs regain consciousness during sticking or bleeding. Chest sticking must be performed as fast as possible to facilitate rapid bleed-out.

Based on the limited scientific data available, the following signs may be suggested as criteria for a successful stun:

- Absence of rhythmic breathing;
- No carcass convulsions;
- Corneal reflex may be present in some pigs (less than 5%) at the time of sticking as under carbon dioxide/air mixtures to be consistent.

9.4.2.3. Advantages

The use of argon to induce hypoxia is non-aversive and does not induce sense of breathlessness before loss of consciousness occurs.

9.4.2.4. Disadvantages

The duration of unconsciousness induced by a given exposure time of minimum 3 min to alternative gas mixtures is shorter than that achieved by stunning with high concentration of carbon dioxide.

As the duration of unconsciousness is very short, pigs have to be either killed in the gas mixtures or subsequent killed by inducing electrical cardiac fibrillation.

9.5. OTHER METHODS

The waterjet stunning method was developed and discontinued in Switzerland to stun / kill pigs. It works with high pressure water droplets which are shot in the head and brain of pigs by a watergun aplied on the frontal bone (Schatzmann *et al.*, 1993). It ensures immediate death of the animal but causes frequently heavy destruction of the rear parts of the head, resulting in very severe carcass convulsions, which lead to safety problems during carcass handling. Although electro immobilization would reduce carcass movements (lambooij and schatzmann, 1994), its disadvantages in terms of animal welfare have been exposed ealier in this document.

High energy microwave irradiation has been tested in pigs. The only article published in a trade journal (which was not peer reviewed) by Lambooij *et al.* (1990) involved irradiation of pigs' heads (obtained post mortem) with a power output of 6 kW delivered using 2450 MHz for 1.5 to 2.5 sec. The results of this study, although they showed a maximum temperature increase of 22°C, replicated the results of a previous report on rats regarding the uneven distribution of temperature within the brain. Nevertheless, Lambooij *et al*. (1990) extrapolated the results of experiments with rats and concluded that an output of 45 to 70 kW would be necessary to kill pigs. The animal welfare consequences of uneven heat distribution occurring in the brain during microwave irradiation could be reduced or alleviated by a simultaneous application of high energy electromagnetic fields (known as transcranial magnetic stimulation, TMS) as reported in rats Presumably owing to the lack of such a high energy device, no further experimental evidence on farm animals has been reported to substantiate this recommendation.

10. STUNNING AND STUN / KILLING METHODS FOR POULTRY (CHICKENS AND TURKEYS)

10.1. INTRODUCTION

Stunning methods used for poultry include head-only electrical stunning (50 to 1500 Hz AC or pulsed DC) and electrical water bath stunning with high frequency currents (> 50 Hz) that do not induce cardiac arrest.

Stun / killing methods used under slaughterhouse conditions are electrical water bath supplied with 50 Hz sine wave AC, and gas mixtures (either carbon dioxide and oxygen followed by high concentration of carbon dioxide in air, carbon dioxide and argon carbon dioxide and nitrogen or argon and nitrogen).

Captive bolts and neck dislocation are used as back-up methods.

In general, the depth and duration of unconsciousness depends upon the amount and frequency of currents applied during the head-only and water bath electrical stunning. During gas stunning, they depend upon the gas composition and the duration of exposure to the intended gas mixture.

In effectively stunned poultry, the onus of preventing return of consciousness during bleeding relies on the efficiency of the slaughter procedure. Cutting both the common carotid arteries in the neck, in comparison with cutting one common carotid artery and /

or one external jugular vein, induce rapid death in chickens and turkeys (Gregory and Wotton, 1986; Gregory and Wotton, 1988).

The time to onset of death at slaughter would depend upon the rate of bleed out which, in turn, depends upon the circulating blood volume and the blood vessels cut at slaughter. Research carried out during the 1960s indicated that blood volume in chickens has a curvilinear relationship with body weight. For example, blood volumes of chickens weighing 1.0, 1.5, 2.0, 2.5 and 3.0 kg were estimated to be 11.6, 8.9, 7.3, 7.3 and 7.4% of body weight, respectively (Kotula and Helbacka, 1966a). Kotula and Helbacka (1966a) also found that about 50% of total blood volume is retained in the carcasses (mainly capillary bed) and not bled out at slaughter. The entire circulating blood volume could not be drained at slaughter because various stunning methods contribute to retention of different proportion of total blood volume in the vital organs (e.g. liver). For example, captive bolt stunned chickens retained the least amount (9% of total blood volume) and carbon dioxide stunned chickens retained the highest amount (13% of total blood volume) of blood in the organs (heart, lungs, spleen, liver and kidneys; Kotula and Helbacka, 1966b). It has also been reported that slaughter without prior stunning results in maximum blood loss (45% of total blood volume) when compared with destruction of brain with a knife (43%) or decapitation (39%) (Newell and Shaffner, 1950). Since 1966, it is known that the intensive selection pressures applied during the past three decades in breeding broiler chickens and turkeys has significantly improved feed conversion ratio, increased muscle mass and reduced the age at slaughter. Consequently, modern broilers and turkeys are known to have serious cardiovascular deficiencies. However, from stunning and slaughtering point of view, the blood volume per kg body weight of today's commercial poultry is unlikely to have changed drastically.

A number of other studies have also shown that, regardless of the stunning or stun / killing method used, the rate of bleed out is faster when the common carotid arteries and external jugular veins, instead of one common carotid and one external jugular vein, in the neck are severed at slaughter (Gregory and Wilkins, 1989a; Raj and Gregory, 1991a; Raj, Gregory and Wotton, 1994 and Raj and Johnson, 1997). In addition, electrical stunning resulted in a relatively faster rate of bleed-out than electrical stun / killing method, although the total blood loss was very similar (stunning = 3.6% *vs* stun / killing = 3.4%) (Raj and Johnson, 1997). Nevertheless, the minimum amount of blood drained at slaughter constituted about 2.5% of body weight (Table 10-1).

Gregory and Wilkins (1989a) clearly demonstrated that cutting all the major blood vessels in the necks of electrically stunned chickens resulted in loss of blood amounting to more than 2% of body weight in less than 25 sec after neck cutting. Although direct scientific evidence is lacking, it can be speculated that this amount of blood loss in poultry may induce brain ischemia following slaughter and, hence, prevent return of consciousness.

Under commercial conditions, the interval between the end of stunning and neck cutting can be up to 20 sec. Under this situation, it can be estimated that the duration of unconsciousness induced by a stunning method should last longer than 45 sec (=20 sec stun-to-neck cut plus 25 sec to achieve brain ischemia through blood loss) to avoid return of consciousness following stunning. Cutting of vertebral arteries alone at slaughter will take a longer time to achieve the bleed out necessary to cause brain ischemia. Therefore, cutting all the major blood vessels in the necks of electrically stunned poultry is necessary.

Table 10-1. Examples of blood loss at slaughter in chickens and turkeys.

Reference	Stunning or stun / kill method used	Blood vessels cut	Blood loss in Chickens as a % of live weight (live weight kg)	Blood loss in turkeys (live weight kg)
Raj and Gregory, (1991a)	Electrical (105mA, 50Hz AC)	1 common carotid and 1 external jugular	3.3% (3.0)	_
	45% carbon dioxide in air	1 common carotid and 1 external jugular	3.1% (3.0)	_
	90% argon in air	1 common carotid and 1 external jugular	3.1% (3.1)	_
Raj, Gregory and Wotton, (1994)	Electrical (250mA, 50Hz AC)	1 common carotid and 1 external jugular	_	2.5% (7.6)
	90% argon in air	1 common carotid and 1 external jugular	_	2.5% (7.5)
	30% carbon dioxide and 60% argon in air	1 common carotid and 1 external jugular	_	2.5% (7.8)

Gregory and Wotton (1986), using anaesthetised and mechanically ventilated chickens (layer hens), investigated the time to loss of spontaneous EEG activity following decapitation, induction of cardiac arrest and various commercially practised neck cutting procedures. In that study, the time to reach 5% of the pre-slaughter integrated EEG activity was used as one of the criteria to determine the state of brain function in chickens and the results are summarised in Table 10-2. It is important to note that Gregory and Wotton (1986) have ventilated the chickens (provided artificial respiration) following the slaughter procedures to simulate conditions where birds are able to maintain or resume normal breathing following neck cutting. However, these times were suggested to be overestimates because of the effects of anaesthetic used and mechanical ventilation provided to birds. Nevertheless, a minimum of 25 sec bleed-out time will be necessary to achieve brain ischemia through blood loss and avoid return of consciousness following stunning.

Table 10-2. Effects of slaughter methods on the time to reach 5% of the pre-slaughter integrated spontaneous EEG activity in chickens (n = 8 birds per treatment)

Treatment	Average time (sec)	SD
Cardiac arrest	23	2
Decapitation	32	2
2 common carotid arteries cut	60	8
1 common carotid artery and 1 external jugular vein cut	122	22
2 external jugular veins cut	185	25
1 external jugular vein cut	233	58

Source: Gregory and Wotton, 1986.

Evidently, decapitation and induction of cardiac arrest were the most rapid slaughtering methods in terms of the time to loss of spontaneous EEG activity. All the slaughter methods tested in that study required significantly longer times to reach a similar end point. The average time to reach this criterion would be considerably longer when only the vertebral arteries at the back of the neck are severed at slaughter, which is a common practice in Europe.

There are no peer reviewed published scientific evidence concerning either the duration of unconsciousness or impact of neck cutting methods in terms of avoiding return of consciousness in electrically stunned turkeys and gas stunned poultry (see gas stunning for details).

Criteria used to determine unconsciousness after the application of stunning or stun / killing methods in poultry:

In contrast with the red meat species, electrical stunning (head-only or water bath) of poultry seldom produces grand mal epilepsy in the brain. Instead, only a small proportion of them develop "epileptiform" activity in the EEG following electrical stunning (Gregory and Wotton, 1987) and about 90% of the birds that develop "epileptiform" activity show low frequency (< 3Hz) polyspike or spike and wave activity. These kinds of low frequency polyspike activities in the EEG are not indicative of grand mal epilepsy and hence not always associated with unconsciousness in humans. Research so far indicates that electrical stunning-indicative of unconsciousness in chickens should lead to a period of epileptiform activity and a period – at least 30 sec - of profoundly suppressed or quiescent EEG immediately after epileptiform activity (Schutt Abraham *et al.*, 1983). For example, an electrically stunned chicken showing 15 sec of epileptiform activity and 30 sec of quiescent EEG can be assumed to be unconscious and insensible for 45 sec following stunning. It is important to note that the grand mal epilepsy in red meat species and epileptiform activity in poultry must always be followed by a profoundly suppressed EEG indicative of spreading depression or neuronal fatigue in the brain. The somatosensory evoked potentials (SEPs) in the brain are also abolished during the occurrence of a profoundly suppressed EEG in chickens and turkeys (Gregory and Wotton, 1986, 1989, 1990a, 1990b, 1991a and 1991b; Raj and O'Callaghan, 2004a and 2004b). The epileptiform activity, which normally last for about 15 sec, followed by the occurrence of a suppressed or quiescent EEG for 30 sec after electrical stunning would provide a period of unconsciousness and insensibility lasting about 45 sec. Alternatively, induction of cardiac arrest at stunning (see electrical stun / killing method) in poultry is the best option on bird welfare grounds.

The changes occurring in the EEG and the time to abolition of SEPs during exposure to gas mixtures have been used to determine the time to loss of consciousness. These seem to vary according to the oxygen and carbon dioxide levels in the mixture (other gases such as nitrogen and argon are used to displace atmospheric air and therefore, they determine the residual oxygen levels). Since the changes occurring in the EEG during exposure of poultry to gas mixtures and their interpretation vary depending upon the gas mixture used, abolition of SEPs has been used as an unequivocal indicator of loss of consciousness during exposure of chickens and turkeys to various gas mixtures (Wooley and Gentle, 1988; Raj, Gregory and Wotton, 1990 and 1991; Raj, Wotton and Gregory, 1992; Raj, Wotton and Whittington, 1992; Raj and Gregory, 1994 and Raj *et al.*, 1998).

Mechanical methods induce immediate and severe structural damage to the brain. The impact has been determined on the basis of induction of slow waves (high amplitude, low frequency activity) followed by a profoundly suppressed EEG. The visual evoked potentials (VEPs) are abolished during the occurrence of suppressed EEG (Raj and O'Callaghan, 2001).

10.2. HEAD-ONLY ELECTRICAL STUNNING

This method is used commonly to stun poultry on the farm and as a back-up method in commercial slaughterhouses using water bath electrical stunning systems. The method involves application of an electric current across the head. Head-only electrical stunning is normally performed on poultry that are restrained in a cone or shackle and both can be distressing to birds due to inversion (see water bath electrical stunning for details of welfare concern associated with inversion and shackling). However, poultry can also be restrained, in a sitting posture, between a pair of boards fitted with sponge cushion very similar to 'crushes' used for restraining pigs prior to electrical stunning (Raj *et al.*, 2001).

Head-only electrical stunning induces flexion of legs and wing flapping from the moment the current starts to flow across the head (initiation of proper stun). The duration of leg flexion was reported to be about 5 sec and is immediately followed by leg extension (Vernadakis and Burkhalter, 1965). The wing flapping leads to a distinct period of tonic seizure as indicated by stiffening and arching of the neck, rigidly extended legs, wings folded tightly around the breast and constant body movements. During tonic seizure, eyes will be wide open (no blinking when touched) and rhythmic breathing will be absent. As in other stunning methods, return of eye reflexes and normal breathing precedes a return of consciousness. Gregory (1989) suggested that this is probably the best single test that could be used to determine the return of consciousness and sensibility is the response to comb pinching and it returns at about 2 min after stunning chickens with low voltages (e.g. 140 V). On the other hand, research has shown that response to comb pinching is not a reliable indicator of state of consciousness following water bath electrical stunning (Schutt-Abraham *et al.*, 1983). Armington *et al.* (1957) found that the average time to return of breathing in chickens was 21 sec post-stun. The times to return of these responses in head-only electrically stunned turkeys are not known.

As reported in pigs, the effectiveness of stunning is affected by the size of the electrode surface area that is in contact with the head, the electrical properties (e.g. impedance) of the electrode material, the peak or peak-to-peak voltage available to the stunner, the amount and frequency of the current, and the pressure applied during stunning especially with low voltages.

Evaluation of electrical stunning of poultry (head-only or water bath) in laboratories seems to have involved stunners with different properties. For example, a constant voltage stunner, a constant voltage / constant current stunner, a variable voltage / constant current stunner that start to deliver a pre-set current with an unpredictable voltage spike of up to 620 V peak, and, a variable voltage / constant current stunner that always started at 0 V at the beginning of stun have been reported in the literature (Raj, 2003). Owing to the differences in the way these stunners function, the peak-to-peak voltage employed to deliver the stunning current and the time to reach (from 0) the maximum pre-set current during stunning would be expected to vary widely. Therefore, it is possible to suggest that the differences between the stunners could have partly contributed to contradicting reports regarding the minimum effective currents. Electrical stunning induced generalised epilepsy will only occur if the applied voltage is in excess of the threshold necessary to stimulate large groups of neurons in the brain. The voltage necessary to deliver a fixed amount of current at a given resistance seems to be higher with a pulsed DC than sine wave AC and it also increases with increasing frequency

(Bilgili, 1992). In addition, the rate of break down of electrical resistance in the pathway, which determines the rate of induction of electronarcosis, is dependent to a certain extent on the applied voltage. Therefore, low voltages may not induce immediate loss of consciousness instead it will result in distress. Gallup *et al.* (1970) reported that an electric shock applied at 1.25-5.25 mA between a chicken's feet increased the duration of tonic immobility reaction. Schutt-Abraham *et al.* (1983) reported that a current of less than 20 mA (in a water bath stunner) resulted in birds leaving the stunner squawking loudly and flapping their wings.

Electrical devices used for stunning or killing poultry have changed significantly since the publication of the Directive 93/119/EC and previous reports of the Scientific Committees (ScVC, 1996 and 1997). The frequency of currents used to stun poultry nowadays ranges from 50 to 1500 Hz. The generic waveforms of currents are pulsed direct currents (DC) and sine wave alternating currents (AC). The pulse width of a DC varies widely and half or fully rectified sine waves are also used as pulsed DC to stun poultry but their effectiveness is not known (Bilgili, 1992).

The variations in the waveform and frequency of currents used for stunning poultry make the measurement of voltage or current applied during stunning using a standard volt or current meter extremely difficult. The current meters used to set or monitor the output of a stunner must be calibrated prior to use and be appropriate to the waveform of the current (Ingling and Kuenzel, 1978).

The depth and duration of unconsciousness induced with all the waveform frequency combinations have not been determined to recommend one unanimous stunning current that will be adequate to achieve humane slaughter under all the conditions and further research is needed. This problem is further conpounded by the fact that the combination of, and number of, blood vessels severed in the neck at the time of slaughter vary widely (Gregory and Wotton, 1986). The cumulative effects of electrical stunning parameters (waveforms, frequency, and amount of current and duration of application) and blood vessels cut at slaughter have not been clearly established.

The minimum root mean square (RMS) currents of sine wave AC necessary to stun chickens and turkeys effectively were found to be 240 and 400 mA, respectively, whilst using conventional stunning electrodes made of three pins (Gregory and Wotton, 1990a and 1991b). These studies, however, involved prolonged administration of currents (minimum of 5 sec) using a constant voltage stunner. Nevertheless, when neck cutting was performed by severing all the major blood vessels in the neck within 10 to 15 sec from the end of stun, it prevented the return of consciousness in these birds. This may also apply to the use of a variable voltage / constant current stunner because, although the source of current would affect the rate of induction of stun, it is unlikely to alter the duration of unconsciousness and insensibility in adequately stunned poultry. However, there is no published scientific literature concerning this.

When a constant voltage stunner is used, the current starts to rise from zero to the maximum depending on the available supply voltage and the time it takes for the voltage to breakdown the total electrical impedance in the pathway (Sparrey *et al.*, 1993). Owing to this, there will be a delay between the start of the application of stun and the passage of recommended current through the brain, *i.e.* the latency to deliver the recommended current and induction of unconsciousness. On the other hand, a variable voltage / constant current stunner would expect infinite impedance in the pathway and therefore start with the maximum available voltage. It will also modulate the voltage according to the changes in the impedance during the stun. Under this situation, the recommended current would flow through the birds within 0.25 sec from the start of the stun (Sparrey *et. al*., 1993). Some authorities, responsible for providing guidelines to protect operators' safety, restrict the voltage supplied to hand-held stunners (e.g. 24 peak V or 110 RMS V in AC or 60 V DC). Therefore, high voltage electrical stunning is not always used under field conditions. From a bird welfare point of view, low voltages may not be adequate to stun poultry immediately if the stunning electrodes are not constructed with materials that have low electrical impedance. Under this situation, stunning current should be applied for a minimum of 7 sec or until the wing flapping stops (Gregory and Wotton, 1990a and 1991b). However, the induction of unconsciousness with low currents could be extremely painful to birds. Lee-Teng and Giaquinto (1969) reported that chicks receiving low stunning currents showed no convulsions and had low-frequency polyspike activity in their EEG, but were considered to be in a state of 'struck'. The authors' description of this state is that "these chicks squatted and raised their wings during the passage of the current, sometimes accompanied by shaking of the head. These motor responses appeared to be involuntary and normal posture was resumed right after the current, and there seemed to be no loss of consciousness for any period of time at all".

The minimum effective current increases with the frequency, possibly since the magnitude of neuronal inhibition induced by electrical stunning in chickens is determined by the duration for which the current stays at the maximum within each cycle, otherwise known as period (period = 1000 / frequency) and also due to the electrical frequency dependent nature of neurotransmitter release responses occurring in the brain (Wang and Kaczmarek, 1998). Recent research indicated that a RMS current of 100 mA of a 50 Hz sine wave AC would be sufficient to stun chickens whilst using a pair of tongs fitted with low impedance electrodes (300 Ohms) and stunning is delivered using a variable voltage / constant current stunner. By contrast, stunning broilers with 400 Hz and 1500 Hz AC would require minimum currents of 150 and 200 mA to achieve satisfactory depth and duration of unconsciousness (Raj and O'Callaghan, 2004a). In this study the current was applied for 1 sec and induction of epileptiform activity followed by a quiescent EEG (less than 10% of pre-stun EEG power content) were used as criteria. The minimum currents were suggested on the basis of inducing a minimum of 30 sec of quiescent EEG following a 15 sec period of epileptiform activity that is 45 sec of unconsciousness. As it takes 25 sec to produce brain ischemia, using these parameters, neck cutting should be performed within 20 sec to prevent return of consciousness.

Another recent study indicated that, at 130 mA average current of a 50 Hz DC, increasing pulse width from 5 to 10 or 15 milliseconds improved the effect of head-only stunning in chickens. The increase in the depth and duration of unconsciousness, as determined from the magnitude and duration of suppression in the EEG, seen with the wider pulse widths is attributed to the longer periods of the current. However, at this average current level, all the three pulse widths failed to induce unequivocal changes in the EEG that are normally associated with unconsciousness and insensibility following electrical stunning (Raj, O'Callaghan, Xavier, and Byessen, 2003). The effects of stunning poultry with high frequencies of pulsed DC are very likely to be similar to those found with sine wave AC. Further research should be aimed at determining the minimum current necessary to achieve effective stunning with pulsed DC. Richards and Sykes (1967) and Kuenzel and Wathers (1978) reported that satisfactory head-only electrical stunning resulted in a quiescent EEG in chickens. Based on the existing knowledge, it is suggested that, whilst using a pulsed DC, the mark: space ratio must be restricted to 1:1.

The effectiveness of stunning turkeys with various frequencies and waveforms of currents needs further investigation to prescribe appropriate minimum currents.

The minimum conditions recommended are based on the limited scientific information on the head-only electrical stunning of poultry.

10.2.1. Description of effective use

Birds should be restrained suitably to facilitate uninterrupted application of the stun. Metal shackles and bleeding cones are commonly used at present.

A minimum RMS or average currents of 240 and 400 mA should be applied for a minimum of 7 sec to chickens and turkeys, respectively, when using a constant voltage stunner (110 V RMS) supplied with 50 Hz AC. Neck cutting must be performed within 15 sec from the end of stun.

When a variable voltage / constant current stunners delivering sine wave AC and low impedance electrodes are used, the following minimum RMS currents are recommended (Table 10-3).

Table 10-3. Minimum currents for head-only electrical stunning of chickens with a variable voltage / constant current stunner delivering sine wave AC.

Sine wave AC frequency (Hz)	Minimum RMS current (mA)
50	100
400	150
1500	200

The current must be applied for at least 1 sec.

Neck cutting must be performed within 20 sec of the end of the stunning using the above-mentioned currents.

Both the common carotid arteries in the neck must be severed.

Birds should be dead when entering scald tanks.

10.2.2. Monitoring points

The following signs indicate a successful stun:

- Immediate onset of clonic – tonic seizure.
- A distinct period of tonic seizure.
- During tonic seizure, eyes will be wide open (no blinking when touched).

- Apnoea during tonic seizure.
- Adequately stunned and properly neck cut birds do not show wing flapping during bleeding.
- Eye reflexes must be absent when entering scald tank.

Poor electrode maintenance and / or contact with the head can be recognised from the singeing of feathers due to the development of heat, which normally occur due to increased electrical impedance. Response to comb pinching is not a reliable indicator of state of consciousness following electrical stunning. Return of eye reflexes and normal breathing precedes return of consciousness.

10.2.3. Advantages

Head-only electrical stunning involves the application of a current focally to the head which improves the effectiveness of stunning.

10.2.4. Disadvantages

This method may not be suitable for large-scale operations where high throughput rates are required.

Induction of unconsciousness using low currents can be painful.

10.3. WATER BATH ELECTRICAL STUNNING

Water bath stunning is commonly used under commercial conditions where large throughput rates (up to 220 birds per min) are required. Under this stunning system, conscious birds are hung upside down on a moving metal shackle line and passed through an electrified water bath, such that the current flows through the whole body towards the shackle. In general, the depth and duration of unconsciousness depends upon the amount and frequency of currents applied during water bath electrical stunning.

The duration between shackling and stunning varies according to the live bird transport system used and the layout of the processing plant. At present, shackling duration in conscious birds can be up to 3 min in chickens and up to 6 min in turkeys. However, under modern transport and processing conditions the shackling duration is less than 1 min. In the past, turkeys were transported in crates that were fixed on the back of lorries, and the birds were uncrated and shackled directly from a lorry parked in a ‘shackling area’. Under this scenario, it took a maximum of 6 min for turkeys on the far side of the vehicle to travel from the point of shackling to a water bath stunner. Nowadays, turkeys are transported in modules that allow shackling of birds, as close as desired by the processor, to the water bath stunners.

Investigations have revealed that up to 90% of birds hung on moving shackles flapped their wings (Kannan *et al.*, 1997; Parker *et al.*, 1997). While most birds (99.7%) cease flapping within 12 sec of shackling, many subsequently resume wing flapping if they are suddenly exposed to sunlight, jolting or pre-stun electric shocks at the entrance to the water bath stunner (Gregory and Bell, 1987). Wing flapping was found to be violent and prolonged if the shackles were tight fitting (Parker *et al.*, 1997). In the case of turkeys, no published scientific information is available regarding the incidence and duration of wing flapping. However, one unpublished survey (conducted by MAFF, UK) suggested that turkeys settle down within 20 sec of shackling (cited by Hewson and Russell, 1991).

Observations under commercial conditions have shown that provision of plastic or rubber curtains running along the line (known as breast comforting plate) had a quietening effect in chickens (Gregory and Bell, 1987). In addition, the latency to onset of wing flapping from the moment of shackling, duration of wing flapping and number of bouts of wing flapping were found to be significantly lower when the intensity of light in the shackling area was 5 lux or less when compared with 50 or 200 lux (Jones *et al.*, 1998). Some processing plants also use blue or violet light, which tend to have a calming effect on birds.

The fear responses of chickens to catching and handling by humans are well documented (Duncan *et al.*, 1986). Therefore, absence of wing flapping in poultry should not be considered as a sign of absence of pain or suffering.

Hanging upside down on shackles is a physiologically abnormal posture for poultry and compression of metatarsal bones by the metal shackle is extremely painful, and hence, induces wing flapping (Gentle and Tilston, 2000). Metal shackles have parallel slots for the insertion of each leg and the slot size determines the degree of compression on the legs. Broilers and turkeys show variable leg sizes, with males having consistently larger leg sizes than females. It has been calculated that the force on each leg of broilers could be 180 N applied over an area of $1cm^2$. A 14.5 mm leg will have to be compressed by 10% to fit into a 13 mm slot and by 20% to fit into a 11.5 mm slot and the latter requires four times more pressure (than the former) (Sparrey, 1994). The pressure applied during shackling increases exponentially with deformation of legs (Gentle and Tilston, 2000). Nevertheless, some modern shackle lines are designed to accommodate birds of different sizes but these are not commonly used under the existing processing conditions.

Inevitably, the pain and distress induced by shackling causes severe wing flapping which, in turn, increases the prevalence of dislocated joints and broken bones (Gregory and Wilkins, 1990a; Gregory *et al.*, 1989).

It has been reported that, in end of lay hens (N=375), shackling alone induces 8% broken bones (Gregory and Wilkins, 1989d). In another survey in which broilers were sampled just prior to entering water bath stunners (N=132) revealed that 3% had broken bones but it is not certain how much of this damage was induced by shackling per se (Gregory and Wilkins, 1990). In the 1980s, Kestin and Gregory (pers. comm.) noted that of 891 broilers slaughtered in an experimental processing plant, it was found that 1% had broken femurs and 54% and 45% of the broilers had ruptured hip ligaments and articular cartilage, respectively (Kestin and Gregory, pers. comm.). But again, it is not certain how much of this damage was actually induced by shackling. Nevertheless, the potential is there in a significant number of animals for dislocation and fractures to occur.

The pain and suffering during shackling is likely to be worse in birds suffering from painful lameness due to diseases or abnormalities of leg joint / bone (Butterworth, 1999; Danbury *et al.*, 2000). In this regard, the prevalence of extreme lameness in broiler chickens has been reported to be up to 15% (Berg and Sanotra, 2001; Sanotra *et al.*, 2001; Butterworth, 1999). This pain is also likely to be significant in birds suffering from dislocation of joints and / or fracture of bones induced by rough handling during catching, crating and uncrating. The pain and suffering associated with wing flapping can be worse in the case of turkeys due to their size / weight. Under commercial conditions, turkeys are processed at different ages (up to 22 weeks) and weights (e.g. 2 to 25 kg) according to market demands. The pain and suffering during shackling is likely to be greater in heavy turkeys due to the reason that their legs will have to be compressed more to fit into the slots of a shackle, the force applied by the operatives to push the legs into the slots is likely to increase with the size of the bird and, while on shackles, the pressure applied on the leg bones would increase with the weight of the bird. In addition, hanging heavy turkeys can be tiring to operatives and hence prone to compromises in bird welfare at hanging.

Potentially painful pre-stun electric shocks occur during the water bath stunning of poultry (Hewson and Russell, 1991; Wotton and Gregory, 1991). Pre-stun shocks usually occur when the birds' leading wings make contact with the water bath before their heads are fully immersed. Wing flapping invariably predisposes poultry to receiving pre-stun electric shocks at the entrance to the water bath stunners. Pre-stun shocks can induce wing flapping (Gregory and Bell, 1987) and, consequently, the birds may miss the electrified water bath completely or partially leading to total failure or inadequate stunning (Hewson and Russell, 1991). Turkeys' wings, owing to the wingspan, always hang lower than their heads, and therefore, one survey of turkey processing plants revealed that more than 80% of the turkeys receive pre-stun electric shocks under the commercial conditions (Wotton and Gregory, 1991). The situation is worse when the processing line speed is slow and shackle line slopes into the water bath. The incidence of pre-stun shocks in chickens has not been quantified or reported. The complexity of commercial water bath stunning systems and the physical contact between birds on the shackle line make it difficult to control the current pathway and eliminate this potential problem in chickens and turkeys.

The incidence of pre-stun shocks can be reduced by implementing certain measures: (1) water bath stunners should not overflow at the entrance, and (2) water bath stunners should be fitted with an electrically isolated 'entry ramp' that slopes upwards toward the bath. These entry ramps are fitted so as to facilitate swinging the birds' heads into the water bath stunner, especially in turkeys. In some instances, shackle lines are constructed such that they dip the heads into the water bath. For example, shackle lines are dipped (about 19°) at the entrance and rise again at the exit of the water bath stunners (Wotton and Gregory, 1991).

The water bath stunners used under commercial conditions are all supplied with constant voltages. The application time of the stun depends upon the processing line speed, in relation to the length of the water bath and the amount of current delivered to birds. Water bath stunning is normally carried out using frequencies well above 50 Hz, usually between 400 and 1500 Hz of sine wave AC and pulsed DC. When using low voltage stunners longer application times (and consequently longer water baths/slower processing line speed) are required.

The commercial electrical water bath stunner may contain up to 20 chickens (or up to 5 turkeys) at any moment and, as birds enter and leave a stunner supplied with a constant voltage, they form a continuously changing parallel electrical circuit (Sparrey *et al.*, 1993).

The voltage necessary to deliver a pre-set current seems to vary according to the depth of immersion of birds in the water bath. When this was tested using a variable voltage / constant current stunner, it was found that shallow immersion needed higher voltage than deeper immersion to deliver a pre-set constant current. Schutt-Abraham, Knauer-Kraetzel and Wormuth (1992a) suggested that, in geese, such variations are due to (1) distance between bird and the live electrode in the bath, (2) increase in contact area between bird and electrified water and (3) reduction in body mass between the live and earth electrodes (shackle). These could have been the reasons for recommending immersion of birds in water bath stunners up to the base of their wings (Schutt-Abraham *et al.*, 1983).

Most of the electrical impedance in the pathway between the electrified water bath and the earth is therefore attributed to the poor contact between the legs and metal shackle. The electrical impedance could be reduced significantly by wetting the leg-shackle contact area with a water spray (Griffiths and Purcell, 1984).

Although tighter fitting shackles provide good electrical contact, the pain and suffering associated with this is likely to be severe, which can be worsened by the wing flapping. Since the size of shanks of poultry processed commercially vary widely, it is doubtful whether this conflict can be resolved without compromising the efficiency of stunning. Modern shackle lines are designed to accommodate varying shank sizes or have two sets of slots with different widths, such that birds of varying sizes could be shackled without excessive compression of shanks, and they should be implemented. According to Ohm's law, each bird in a multiple bird water bath stunner will receive a current inversely proportional to the electrical resistance or impedance in the pathway (Sparrey *et al.*, 1992). The effective electrical impedance can vary between birds, usually 1000 to 2600 Ohms in broilers and 1900 and 7000 Ohms in layer hens (Schutt-Abraham *et al.*, 1987; Schutt-Abraham and Wormuth, 1991). The average electrical impedance in turkeys is reported to be 1200 Ohms for toms weighing 14 to 25 kg and 2300 Ohms for hens weighing 6 to 10 kg (Schutt-Abraham and Wormuth, 1988). Thinner metatarsal bones fitting loosely on wider metal shackles and dry scales on the legs could be attributed to relatively higher impedance in females than in males.

In addition, the electrical conductivity of water used in the stunner bath may vary depending upon its content of naturally occurring minerals. The conductivity improves with the time the water bath stunner has been in use. This is because minerals may be inadvertently added to the water bath either through the accumulation of dirt or faecal materials, as poultry are known to defecate during water bath stunning. However, addition of cooking salt even at 0.1% level, particularly at the beginning of the day when the water is fresh at the beginning of the operation, helps to overcome any deficit in the conductivity of the water (Bilgili, 1992).

In general, it is recommended that the electrode in the water bath stunner must extend to the full length of water bath. This is particularly important because the amount of current and voltage decreased as the measuring device was moved 5 cm or more away from the live electrode, the source of current in a water bath. The decrease has been reported to be considerable when fresh tap water is used without any added salt and the stunner is supplied with 200 V or more (Schutt-Abraham *et al.*, 1991). These findings support the recommendation that the electrode should extend to the full length of the water bath stunner and that the birds must be immersed up to the base of their wings such that the heads are always held close to the electrodes in the bath, where the current density is high.

The variation in electrical impedance in the pathway and, hence, the variation in the amount of current delivered to each bird in a water bath stunner can be overcome by the installation of constant current stunners that would ensure delivery of a pre-set current to each of the birds in a water bath (Sparrey *et al.*, 1993). During stunning with a variable voltage / constant current stunner, each bird is electrically isolated and the stunner modulates the voltage required to deliver a pre-set current by continuously monitoring the impedance in the pathway. A basic requirement to implementing constant current stunning is that each metal shackle carrying birds into a water bath stunner must be electrically isolated.

Electrically isolating individual poultry on a shackle line would require modification to the existing shackle line and a substantial capital investment. In addition, considering that the birds are suspended on shackles 15 cm apart and the processing line is operating at a speed of up to 220 chickens per min, it has been argued whether it will be possible to electrically isolate each bird for long enough to measure its resistance or impedance in the pathway and deliver the pre-set current (Bilgili, 1999). In spite of the additional costs and complexity associated with the implementation, there are no apparent improvements in carcass and meat quality of chickens that were stunned with a constant current stunner (Wilkins, Gregory and Wotton, 1999). It is worth noting that these are some of the concerns associated with the constant current stunners known to exist at present. Nevertheless, since the implementation of constant current stunning equipment will immensely improve bird welfare at stunning and slaughter, equipment manufacturers should develop systems that are cost effective and commercially viable.

Schutt-Abraham *et al.* (1983) evaluated electrical stunning of chickens and turkeys using the criteria listed below:

- Adequately stunned: the EEG showed a pattern of polyspike burst followed by a flat or "isoelectric" line as characteristic for a complete epileptic fit. This pattern had to last for at least 30 sec after the onset of current flow to ensure that the birds do not regain consciousness during bleeding.

- Inadequately stunned: the EEG pattern was similar to above but either lasted for less than 30 sec or lacked the flat or "isoelectric" line, the latter pattern being looked upon as incomplete epileptic fit.

- Not stunned at all: the EEG pattern remained similar to the pre-stunning one, no epilepsy occurred.

Based on these, Schutt-Abraham *et al.* (1983) concluded that a minimum of 120 mA per chicken in a water bath will be necessary to achieve humane stunning whilst using a 50 Hz sine wave alternating current (AC). A minimum RMS current of 150 mA per turkey in a water bath, delivered using a 50 Hz AC, would be necessary to achieve effective stunning.

Subsequent research showed that a minimum current of 120 mA per chicken in a water bath, delivered using either a 50 Hz sine wave AC or 350 Hz a pulsed direct current (DC), will be necessary to abolish SEPs following stunning (Gregory and Wotton, 1989;

1991a). Based on these reports, it may be safe to assume that a current (RMS or average) of 120 mA delivered for a minimum of 3 sec using a sine wave or a pulsed DC of up to 350 Hz would be adequate to stun chickens. With regard to turkeys, Gregory and Wotton (1991b) found that more than 250 mA per turkey would be necessary to abolish SEPs following stunning with a 50 Hz AC.

However, there are some potential problems associated with the use of a 50 Hz sine wave AC to deliver these currents in water bath stunners since sine wave AC is efficient in inducing cardiac arrest and therefore its use in water bath stunners will render it to be a killing, rather than a stunning, method. In this regard, the percentage of birds having cardiac arrest increases with the amount of current delivered to the birds. RMS currents of 105 and 148 mA per chicken will induce cardiac arrest in 90 and 99% of chickens (Gregory and Wilkins, 1989b; Gregory and Wotton, 1987). In the case of turkeys, using a multiple-bird water bath stunner, Schutt-Abraham *et al.* (1987) reported a 90% incidence of cardiac arrest with 175 mA for toms and 157 mA for hens. However, Gregory and Wotton (1991b), using a constant current single-bird stunner, found that 198 and 250 mA per turkey was needed to achieve cardiac arrest in 90 and 100% of birds, respectively. In another study, stunning turkeys with 150 mA per bird induced cardiac arrest in about 97% of birds (Gregory and Wilkins, 1989c). The welfare concern is that, in the presence of brain responsiveness in a turkey stunned with 150 mA current, the bird could experience a potentially painful cardiac arrest. Gregory and Wotton (1991b) reported that all the turkeys stunned with 250 mA suffered cardiac arrest but some of them retained brain responsiveness for a min following stunning.

This concern is supported by the findings of a recent study in which the effectiveness of water bath electrical stunning of chickens with a constant RMS current of 100 mA delivered for 3 sec using 100, 200, 400, 800 and 1500 Hz sine wave alternating current (AC) was investigated. The changes occurring in the spontaneous electroencephalogram (EEG) and somatosensory evoked potentials (SEPs) were used to determine the effectiveness of stunning. Birds were stunned individually (single bird stunner) using a variable voltage / constant current stunner. The results indicated that stunning of chickens with a constant RMS current of 100 mA delivered for 3 sec using 100 or 200 Hz induced epileptiform activity immediately followed by a profoundly suppressed EEG. It was therefore suggested that electrical water bath stunning of chickens with a RMS current of 100 mA delivered using 100 or 200 Hz induced satisfactory depth and duration of unconsciousness. However, both the common carotid arteries in the neck must be severed at slaughter to prevent return of consciousness. By contrast, water bath electrical stunning of chickens with a RMS current of 100 mA delivered for 3 sec using 400, 800 and 1500 Hz failed to induce epileptiform activity and EEG suppression and the SEPs were also retained in the majority of chickens. It was therefore suggested that stunning chickens with a RMS current of 100 mA delivered using these high frequencies do not fulfil the criteria set to protect the welfare of birds at slaughter (Raj and O'Callaghan, 2004a).

Therefore, on bird welfare grounds, a RMS current of greater than 100 mA should be applied whilst using frequencies of 400 Hz or more of sine wave AC for water bath electrical stunning of chickens.

Mouchonière *et al.* (2000) reported that electrical water bath stunning of turkeys for 4 sec with a RMS current of 150 mA delivered using 50, 300 and 600 Hz AC resulted in abolition of SEPs in turkeys. This finding is in contrast with those of Gregory and

Wotton (1991b) who reported that 4 out of 11 turkeys stunned with a RMS current of 250 mA delivered using 50 Hz AC retained their SEPs for up to 1 min. Mouchonière *et al.* (2000) also found that, when turkeys were allowed to recover from the stun, the average times to return of SEPs were 69 and 34 sec after stunning with 300 and 600 Hz AC, respectively. Therefore, the authors concluded that, at 150 mA stunning current, the time to recovery of consciousness decreased as the current frequency was increased. In addition, they found that stunning of turkeys with a RMS current of 75 mA per bird failed to abolish SEPs in the majority (71%) of birds and the average time to return of SEPs in other birds was 66 sec. It was concluded that 75 mA is not adequate to stun turkeys.

Mouchonière *et al.* (1999) evaluated the time to onset of physical reflexes (corneal reflex, neck muscle tension and wing flapping) following electrical water bath stunning of turkeys with a RMS current of 150 mA applied for 4 sec using 50, 300, 480, 550 and 600 Hz AC. The results of this study showed that the time to return of physical reflexes was significantly longer in birds stunned with 50 Hz than those stunned with other frequencies, and regardless of the sex of the bird, increasing stun frequency decreased the time to onset of physical reflexes. Wilkins *et al.* (1999) evaluated the time to recovery of breathing and neck tension in turkeys stunned with 150 mA per bird for 3 sec using 100 Hz DC produced by full rectification of sine wave AC and 500 or 1500 Hz pulsed DC and the results are presented in Table 10-4. The authors stated that the effectiveness of stunning, as judged from the time to return of reflex, was similar regardless of bird weight or stunning waveform / frequency.

Table 10-4. The minimum time (sec) to recovery of breathing and neck tension in turkeys following water bath electrical stunning with 150mA per bird applied for 3 sec

	Recovery of breathing			Recovery of neck tension		
Weight range (kg)	100 Hz	500 Hz	1500 Hz	100 Hz	500 Hz	1500 Hz
4.2 to 6.8	8	20	12	8	26	9
10.0 to 12.7	9	15	11	9	15	11
16.0 to 18.6	7	20	7	10	15	12

Therefore, a minimum RMS current of 400 mA, which was found to be satisfactory for head-only electrical stunning of turkeys, could also be recommended for water bath stunning of turkeys with high frequencies of AC or pulsed DC.

The recommendation that the current levels found to be satisfactory for head-only electrical stunning could also be applied in water bath is based on the fact that, in all the red meat species covered in this report, the minimum recommended currents are the same for stunning or killing with an electric current.

Gregory and Wotton (1990b) evaluated the time to return of breathing and neck tension following electrical water bath stunning of broilers with 5 different RMS current levels of a 50 Hz AC and the results are presented in Table 10-5.

Table 10-5. The minimum time (sec) to recovery of breathing and neck tension in broilers following water bath electrical stunning with different RMS currents per bird applied for 4 to 5 sec

Reflex	45 mA	60 mA	75 mA	90 mA	105 mA
Breathing	5	5	13	13	14
Neck tension	5	5	19	25	52

The times to return of spontaneous breathing in these studies do not concur with the duration of tonic seizures reported in the neurophysiological studies. In this regard, birds are in apnea during the tonic seizure and therefore, interpretation concerning the use of return of breathing in poultry has to be made cautiously.

It is very likely that consciousness and sensibility would return prior to the return of neck muscle tension. For example, Gregory and Wotton (1990b) found that some broilers showed escape behaviour soon after they had resumed breathing and some others showed escape behaviour during stunning. However, since stunning of chickens with 105 mA per bird induced cardiac fibrillation in 90% of chickens, the authors concluded that a minimum RMS current of 105 mA is adequate to stun chickens. The results of these studies concerning physical reflexes suggests that satisfactory electrical water bath stunning should lead to absence of breathing and neck muscle tension, and return of consciousness in poultry is indicated by occurrence of wing flapping (birds are not accessible under commercial conditions to test other reflexes) either prior to or during bleeding.

Some studies to evaluate the effectiveness of electrical water bath stunning entirely relied on the induction of seizures or loss of muscle tone as criteria for determining the effectiveness of water bath stunning and the time to recovery of neck muscle tension as an indicator of recovery of consciousness following electrical water bath stunning (Rawles *et al.*, 1995; Wilkins *et al.*, 1998; Wotton and Wilkins, 1999). In the absence of convincing neurophysiological evidence, it will be unwise to argue that poultry can be stunned with low currents (e.g. 10 mA), in particular, using high frequencies of a pulsed DC with a very short pulse width. Therefore the low currents (e.g. 10 mA) recommended by these investigations may not be appropriate in the absence of additional convincing evidence. Further investigation is needed on whether epileptiform activity followed by a profoundly suppressed EEG (< 10% of pre-stun level) is indeed induced by the application of such a low current and within 1 sec current application to avoid pain and suffering during the induction of electronarcosis.

In this regard, Gallup *et al.* (1970) reported that an electric shock applied at 1.25 to 5.25 mA between a chicken's feet was distressing as it increased the duration of tonic immobility. A pulsed DC (120 Hz) was found to be as aversive as a 60 Hz AC (Nash and Gallop, 1976). Schutt-Abraham *et al.* (1983) reported that a current of less than 20 mA is aversive because the birds leaving the water bath stunner were squawking loudly and flapping their wings.

Evidence to support this concern emanates from the literature concerning electroconvulsive therapy (ECT), which is widely used to treat drug resistant psychiatric human patients. The frequency most effective for seizure induction has been reported to be between 100 and 300 Hz (Robin and DeTissera, 1982; Hyrman *et al.*, 1985). Ultra brief pulse DC devices are also reported to be more effective than the conventional DC

(Sackeim *et al.*, 1994). Therefore, modern ECT devices deliver DC with a pulse width of 0.25 to 1 millisec and frequency of up to 120 Hz. Under these conditions the peak average current required to induce adequate seizure in humans is reported to be 800 mA, which is slightly lower than the 900 mA required under the conventional waveform (Hyrman, 1999). When a pulsed DC is employed in ECT, the electrode position appears to be very critical in inducing seizures and achieving desired effects (Bean *et al.*, 1991). However, irrespective of the electrode position and electrical parameters used in ECT, the criteria widely used in predicting the therapeutic response are the occurrence of high amplitude, low frequency electrical activity (slow waves indicative of synchronisation of neuronal activity) immediately after the stimulation followed by a profoundly suppressed EEG occurring due to spreading depression (Krystal and Weiner, 1999). These criteria (epilepsy followed by spreading depression) would also apply to electrical stunning situation as proposed by Schutt-Abraham *et al.* (1983).

Owing to the differences in the electrical resistance of various tissues in the pathway, it has been estimated that only a small proportion of current (10 to 28 per cent) applied in a water bath may flow through the brain and the majority may flow through the carcass (Wooley *et al.*, 1986a and 1986b). The amount of current flowing through the body is probably contributing to the carcass and meat quality defects seen under the water bath stunning systems. In this regard, it has been suggested that the amount of current flowing through the carcass increases with the live weight of poultry (Mouchonière *et al.,* 1999 and 2000). Physical contact between adjacent birds, variation in electrical resistance from bird to bird, variation in the depth of immersion due to different bird's size, and only small amount of applied current flowing through the brain are not conducive to maintaining good welfare standards. It has been known that the time to onset of brain death in chicken is quicker with the induction of cardiac arrest at stunning, decapitation and severance of two common carotid arteries supplying oxygenated blood to the brain than the other neck cutting procedures (Gregory and Wotton, 1986). In spite of this, the poultry industry practices continue to be to sever one external jugular vein or small vertebral arteries at the back of the neck of poultry. These inappropriate neck cutting procedures, if implemented following stunning with high frequency or low currents, could lead to recovery of consciousness during bleeding and, inevitably, live birds entering scald tanks. Live birds can enter scald tanks under two scenarios. Firstly, inadequately stunned live and conscious birds and those that have missed the stunner, due to wing flapping or being runts, miss the neck cutter by holding their heads up. Occasionally, effectively stunned birds also miss the neck cutting blades due to the fact that they miss the rails that guide the neck towards the blade(s). Hence, if these birds were not slaughtered manually, they will enter the scald tank live and conscious. Secondly, adequately stunned live but unconscious birds could have a poor neck cut and hence, enter the scald tank alive but unconscious. For example, neck-cutting machines have to be setup correctly so that they perform well (Gregory, 1989). However, the size of broilers in a flock may vary and, under this situation, not all the birds will have their necks cut at the same anatomical position. Although a manual back up should be present to cut necks of birds that missed the neck cutter, owing to fast throughput rates, manual back up alone is not sufficient to prevent this potential welfare problem.

Based on the existing scientific knowledge, it can be suggested that the minimum RMS current necessary to stun chickens would be 100, 150 and 200 mA per bird in a water bath supplied with up to 200, above 200 and up to 400, and above 400 and up to 1500 Hz AC, respectively. When currents of lower than this are applied, the depth and duration of unconsciousness induced by the stun may not be adequate to prevent resumption of consciousness before neck cutting or during bleeding.

On similar grounds, it is suggested that the use of pulsed DC for stunning poultry must be limited to wave forms with mark:space ratio of 1:1 only. It is also worth noting that the voltage necessary to deliver a pre-set current is higher with a 100 Hz DC (with 1:1 mark:space ratio) than that is required with a 60 Hz AC (Bilgili, 1992). The high voltages necessary to deliver a pre-set current that is required to stun poultry effectively with a pulsed DC could be detrimental to carcass and meat quality.

The effect current waveform and frequencies on the depth and duration of unconsciousness induced at water bath stunning needs to be investigated further to determine the minimum current. At present, inducing cardiac arrest at stunning (refer to electrocution under killing methods) would appear to be the preferred method. Alternatively, they could be stunned with a minimum RMS current of 400 mA delivered using 300 to 1500 Hz.

Satisfactory electrical water bath stunning of poultry results in tonic–clonic seizures followed by a period of complete muscle relaxation (Schutt-Abraham *et al.*, 83). The tonic seizure can be recognised from an arched neck, extended legs, a constant body tremor, and wings held closely to the body (Hewson and Russell, 1991). Unlike red meat species, the clonic seizure in poultry is mild and is manifested as leg kicking and wing movement (not wing flapping). During the period of seizures the eyes are wide open and apnoea occurs, and therefore, return of breathing is the earliest sign of resumption of consciousness.

'Electrical stunning monitors' or 'dummy chickens' have been developed in the UK. However, it is not known whether these devices are capable of registering all the waveforms and frequencies of currents used under commercial conditions. In particular, the electrical signal sampling rates will have to be very high to register waveforms and current details without distortion. Devices registering current details of one circuit (*i.e.* one shackle) of a multiple bird water bath stunner with constantly changing impedance can be misleading.

10.3.1. Description of effective use

The size and shape of the metal shackles should be appropriate to the size of legs of poultry such that secure electrical contact is provided without causing unnecessary pain.

Poultry must be hung on the shackle line by both legs.

There should be a sufficient delay between shackling and stunning to provide time for the birds to stop wing flapping. The minimum shackle duration should be 12 and 20 sec. in chickens and turkeys, respectively. The maximum shackle duration must be limited to 1 min.

Shackle line should not have bends and dips that induce wing flapping.

Breast comforting plates that help to calm the birds must be used from the point of shackling until the birds enter the water bath stunner.

Runts (smaller than average birds), which are likely to miss the water bath stunner, and injured birds that are in pain must not be shackled. Instead, they should be killed using emergency slaughter procedures.

Lighting conditions during shackling of live poultry should be controlled to reduce wing flapping.

There must be secure and uninterrupted contact between the shackle and the earth (rubbing) bar.

Water spray at the shackle / leg contact point should be provided to reduce electrical resistance and improve the efficiency of stunning.

The height of the water bath must be adjusted according to the size of poultry.

Food-grade salt, at least 0.1% weight / volume, should be added to the fresh water bath to improve electrical conductivity, where appropriate.

The electrodes in water bath stunners must extend to the full length of the water bath.

Birds must not receive pre-stun shocks, there must be provisions such as electrically isolated entry ramps at the entrance to the water bath to prevent pre-stun electric shocks.

Birds' heads must be completely immersed in the water bath, preferably up to the base of their wings.

Table 10-610. Minimum recommended RMS currents (mA per bird) for water bath stunning

Frequency (Hz)	Chickens	Turkeys
Up to 200 Hz	100	250
200 to 400 Hz	150	400
400 to 1500 Hz	200	400

The voltage supplied to the water bath must be sufficient to deliver these currents to each of the birds in the water bath.

The minimum average currents required to stun poultry with pulsed DC are not known. However, only pulsed DC 1:1 mark:space should be used.

Electrical devices must display visibly the total voltage and current delivered to the water bath and these should be appropriate to the waveform of the current used.

Neck cutting must be performed e.g. within 20 sec from the end of stunning.

Both the carotid arteries in the neck must be cut.

Birds should be dead when entering scald tanks.

In the event of line breakdown or a delay in stunning the birds, access must be available to unshackle the birds that have not reached the water-bath and have not been stunned, and bleed those birds that have been stunned and remain in the water-bath.

10.3.2. Monitoring points

The following signs indicate a successful stun:

- Immediate onset of tonic seizure.
- Eyes wide open during tonic seizure.
- Apnoea during tonic seizure.
- Clonic seizures occur as jerky movements of wings and legs (not as wing flapping).
- No wing flapping during bleeding.
- Eye reflexes must be absent when entering scald tank.

The output current from the stunner under load must be equal to, or greater, than the minimum recommended current multiplied by the number of birds in the water bath at any time (120 mA x 10 chickens = 1.2 A). This thumb-rule can be used to set up the stunner.

10.3.3. Advantages

If properly performed, it can be an efficient method of stunning.

10.3.4. Disadvantages

Catching, restraint applied by humans during shackling, and hanging inverted on shackles are distressing and painful to birds. The legs of birds are inevitably compressed during shackling and the degree of compression could be as high as 20%, which is extremely painful.

The pain associated with pre-stun electric shock is severe.

High proportion of current applied in water bath stunners flowing through the carcass, rather than the brain, does not ensure bird welfare.

Physical contact between adjacent birds, variation in electrical resistance from bird to bird, variation in the depth of immersion (due to different bird size) and only small amount of applied current flowing through the brain do not allow to maintaining good welfare conditions.

Certain commercial neck cutting practices (e.g. cutting vertebral artery at the back of the head) do not achieve rapid bleed out and death.

The possibility of live birds entering scald tanks can not be excuded.

Fast throughput rates do not facilitate execution of effective backup stunning procedures. Birds showing signs of consciousness during bleeding are not easily accessible to stun with the backup stunner.

10.4. ELECTRICAL STUN / KILLING METHODS

10.4.1. Electrical stun / killing in water bath

The only difference between the electrical water bath stunning and electrical stun / killing in water bath is the frequency of the electric current employed.

A 50 Hz sine wave (full or clipped) AC has been proven to be effective in inducing cardiac ventricular fibrillation at stunning in a water bath (Gregory *et al.*, 1995). A DC is less likely to induce cardiac ventricular fibrillation than an AC. Inducing cardiac arrest at the point of electrical stunning has welfare advantages since a delay between the end of stunning and neck cutting, and the efficiency of neck cutting become less important (Schutt-Abraham and Wormuth, 1988).

When a 50 Hz AC is used, the current necessary to induce cardiac ventricular fibrillation in 99% of chickens is 148 mA per chicken in a water bath (Gregory and Wotton, 1987). Since this amount of current is in excess of 120 mA required to induce immediate unconsciousness, as determined using EEG or SEPs, there are no perceived as welfare concerns.

In the case of turkeys, Schutt-Abraham, Wormuth and Fessel (1987) and Schutt-Abraham and Wormuth (1988), using a multiple bird water bath stunner, reported a 90% incidence of cardiac ventricular fibrillation with 175 mA for toms and 157 mA for hens. Gregory and Wotton (1991b), using a constant current single-bird stunner, found that 198 and 250 mA per turkey was needed to achieve cardiac ventricular fibrillation in 90 and 100% of birds, respectively. Unlike chickens, a major welfare concern with the induction of cardiac ventricular fibrillation in turkeys is that the current required to induce cardiac arrest in the majority of birds is less than the current required to disrupt the brain responsiveness. For example, Gregory and Wotton (1991b) found that 4 out of 11 turkeys though suffered cardiac ventricular fibrillation at stunning with 250 mA current, retained SEPs for a considerable time (min) following electrical stunning. Current levels higher than 250 mA that would abolish SEPs following electrical stunning need to be established for turkeys.

10.4.1.1. Description of effective use

Points 1 to 14 listed under Description of effective use of water bath electrical stunning apply to this method also.

A minimum RMS current of 150 and 250 mA delivered with a 50 Hz AC should be applied for a minimum of 1 sec to chickens and turkeys, respectively.

10.4.1.2. Monitoring points

Electrocution must induce cardiac ventricular fibrillation in the water bath, and hence, the usual tetanus seen at the exit of a water bath stunner will soon disappear and a total relaxation in the carcass will ensue. This can be recognised by the drooping of the wings at the time of neck cutting. Under no circumstances, birds shall show spontaneous or reflex movements during bleeding.

The details of electrical parameters, such as waveform, frequency and the output voltage and current in appropriate units (RMS) must be readily available to the inspection authorities.

A calibrated volt and / or current meter appropriate to the waveform of the current used should be made available to the inspection authorities to verify the output of the stunner, if needed.

The output current from the stunner under load must be equal to, or greater, than the minimum recommended current multiplied by the number of birds in the water bath at any one time (150 mA x 10 chickens = 1.5 A). This thumb-rule can be used to set up the stunner.

10.4.1.3. Advantages

Inducing cardiac ventricular fibrillation is the quickest method of inducing brain death in chickens; however, this does not apply to turkeys in which brain death occurs quicker after cutting common carotid arteries.

The bird welfare concerns associated with recovery of consciousness under water bath electrical stunning systems due to inadequate stunning and / or poor neck cutting will be eliminated.

10.4.1.4. Disadvantages

Some disadvantages of the electrical water bath stunning method (such as the welfare implications of shackling) also apply to water bath electrical stunning systems.

The amount of current necessary to induce cardiac ventricular fibrillation in turkeys is less than that required to abolish brain responsiveness.

As in water bath electrical stunning, physical contact between adjacent birds, variation in electrical resistance from bird to bird, variation in the depth of immersion due to different bird size and only small amount of applied current flowing through the brain do not allow to maintaining good welfare conditions.

10.4.2. Electrical stun / killing using dry electrodes

This method is not in use under commercial conditions and is being developed for chickens.

Electrical stun / killing can be applied to red meat species using two methods: head-to-body using a single current cycle, and head-only followed by head-to-body or across the chest using two separate current cycles. Since satisfactory head-only electrical stunning induced unconsciousness is accompanied with tonic seizure (tetanus), the two current cycles employed under the second method can be applied separately (interrupted) without compromising animal welfare or operator's safety. However, the problem with poultry is that satisfactory head-only electrical stunning induces severe wing flapping before tonic seizure begins. Under this situation, it will be very difficult to apply the second cycle effectively without compromising operator's safety. Therefore, electrical stun / killing method involving two separate current cycles should be applied uninterruptedly on adequately restrained poultry. Head-only electrical stunning immediately followed by head-to-vent application of the second current cycle has been successfully achieved uninterruptedly by using a relay switch in the current circuit to deliver these currents to previously positioned electrodes in broilers restrained in a wooden crush (Raj *et al.*, 2001).

Such an electrical stun / killing method appears to be more humane than the induction of cardiac ventricular fibrillation in a water bath stunner. Firstly, the stunning current is applied focally to the head in order to span the brain, before the induction of cardiac ventricular fibrillation. Secondly, it is envisaged that this method will be applied to birds which are restrained in a sitting position, using conveyors, thereby enabling shackling to be performed, either manually or automatically, on freshly killed carcasses.

A prototype electrical stun / killing system has been developed recently for chickens using a conveyor (without shackling of live birds). It involves head-only electrical stunning for 1 sec with 150 mA of 50 Hz AC, immediately followed by head-to-body (vent) application of the same current for 1 sec. The results of a study involving this prototype indicated that electrical stun / killing technique can be better than the water bath system on carcass and meat quality grounds (Raj *et al.*, 2001). Practical experience with this stun / killing system revealed that the tonic phase with fully extended legs last for about 20 sec, which might be conducive to automatic shackling. The electrical stun / killing method is still under research and development and is not used commercially.

This method should also be developed for stunning / killing turkeys and waterfowl (ducks and geese) under commercial conditions particularly because heads of waterfowl are not always adequately immersed in water bath stunners (Gregory and Wotton, 1992).

As mentioned under the head-only electrical stunning, the minimum current necessary to induce effective stunning in chickens was found to be 100, 150 and 200 mA delivered 50, 400 and 1500 Hz AC, respectively, while using a variable voltage / constant current stunner and a pair of electrodes that had low electrical impedance and conformed to the shape of the head of chickens. This data can be extrapolated to stun / kill methods. However, when constant voltage stunners supplied with 50 Hz AC are used, higher current (240 mA) applied for longer duration (>5 sec) would be necessary to achieve effective head-only stunning (Gregory and Wotton, 1990a). Since clipped sine waves AC were found to be effective in inducing cardiac ventricular fibrillation at water bath stunning, they can also be used to perform electrical stun / kill.

The minimum currents necessary to stun / kill turkeys are not known.

10.4.2.1. Description of effective use:

Birds must be stunned head-only first before killing them with a head-to-body current application.

Birds should be restrained suitably to facilitate uninterrupted application of the stunning and killing current cycles.

Good electrical contact must be maintained during stunning and killing.

When using a constant voltage stunner, a minimum RMS current of 240 mA of 50 Hz AC should be applied for at least 5 sec across the head to stun and another 5 sec across the body to kill chickens uninterruptedly.

When using a variable voltage / constant current stunner, a minimum RMS current of 150 mA of 50 Hz AC should be applied for at least 1 sec across the head to stun and 1 additional sec across the body to kill chickens uninterruptedly.

The minimum currents necessary to effectively stun / kill turkeys are not known.

The stunner must display visibly the voltage and current delivered during stunning and these should be appropriate to the waveform of the current used

No bird shall survive the treatment or show signs of recovery of consciousness during bleeding

10.4.2.2. Monitoring points

Birds killed by using electrical stun / killing method must show extension of legs, lasting for up to 20 sec, before relaxation occur in the carcass, which can be recognised from the drooping wings.

Under no circumstances, birds shall show spontaneous or reflex movements during bleeding.

Like in all dead poultry, pupils will be dilated and breathing absent.

Poor electrode maintenance and / or contact with the head can be recognised from the singeing of feathers due to the development of heat, which normally occur due to increased electrical impedance.

Stunners could be fitted with an alarm system to warn interrupted stun / kill current cycles.

10.4.2.3. Advantages

To deliver an effective stun, it involves application of a current focally to the head before killing and therefore it ensure good bird welfare.

10.4.2.4. Disadvantages

The electrical stun / killing method is still under research and development and is not used commercially. Therefore, it is not known how effectively this technique can be implemented without compromising bird welfare under commercial conditions, where high throughput rates are required.

10.5. GAS STUNNING

The main objective of gas stunning is to avoid the pain and suffering associated with shackling conscious poultry under water bath stunning and killing systems. Therefore, gas stunning should be limited to birds contained in crates or on conveyors only.

Gas stunning of poultry in their transport containers will eliminate the need for live bird handling at the processing plant and all the problems associated with the electrical stunning. Gas stunning poultry on a conveyor eliminates the problems associated with the electrical water bath stunning. Since birds can be gas stunned in large numbers and, if existing systems are still to be used, all birds will have to be shackled and neck cut. Therefore, the interval between the end of exposure to gas mixture and neck cutting is likely to be long, at least, in some birds. The duration of unconsciousness induced with gas stunning will have to be longer than required under electrical stunning situations to prevent a return of consciousness either prior to neck cutting or during bleeding. A variety of gas mixtures have been evaluated for stunning poultry:

- argon, nitrogen and their mixtures with up to 2% by volume of residual oxygen in the atmosphere;

- 30 to 80% by volume of carbon dioxide in air (leaving different concentrations of residual oxygen). This method is used in 4 plants in EU (one for broiler chicken and one for turkeys in Germany and two in Italy);

- a mixture of argon, nitrogen and their mixtures with up to 5% by volume of oxygen and up to 30% by volume of carbon dioxide. This method is mostly used (3-5 plants) in the UK and one in Belgium;
- a mixture of 40% by volume of carbon dioxide, 30% by volume of oxygen and 30% by volume of nitrogen. This method is used in 6 chicken processing plants (Finland, Belgium, Germany, France, UK and Sweden) in combination with a subsequent killing atmosphere containing 80% carbon dioxide for 2 min (it is called the Controlled Atmosphere Stunning system CAS which is the most commonly used system). This system is also used in 3 turkey processing plants (Italy, France and Germany, one system in each country). This method is also used for turkeys.

For welfare reasons, since the induction of unconsciousness with gas mixtures is a gradual process, the gas mixture should be non-aversive and the induction of unconsciousness should not be distressing to the birds.

Scientific investigations so far have addressed this concern by evaluating:

- Aversive reaction occurring during initial exposure.

- Respiratory discomfort prior to loss of consciousness.

- Time to loss of consciousness.

- Duration of unconsciousness.

Raj (1996) found that 3 out of 8 hens and 6 out of 12 turkeys avoided a feeding chamber to obtain food and water when it contained 47 and 72% carbon dioxide, respectively, in the atmosphere. By contrast, 6 out of 6 hens and 12 out of 12 turkeys spontaneously entered the feeding chamber containing 90% argon in air and were killed with the gas. Raj (1996) also found that 10 out of 12 turkeys entered the feeding chamber when it contained a mixture of 30% carbon dioxide and 60% argon in air and were killed with this gas mixture. The conclusion was that hypoxia is not aversive to poultry and that the carbon dioxide-argon mixture is better than using high concentrations of carbon dioxide on welfare grounds. The results also suggest that 30% by volume of carbon dioxide may not be aversive to poultry.

Woolley and Gentle (1988) exposed chickens to decreasing concentrations of oxygen created by using nitrogen and reported that at no time during the anoxic killing did the birds show any marked respiratory distress nor did they exhibit any of the behaviour to suggest distress. These reports suggest that hypoxia is the best option for stunning or killing poultry, which concurs with the opinion expressed in a review of stunning methods (Raj and Tserveni-Gousi, 2000).

Gerritzen *et al.* (2000) reported that broiler chickens did not avoid a gas tunnel containing either > 90% argon in air, 60% carbon dioxide in air, a mixture of 40% carbon dioxide and 30% oxygen in air, or a mixture of 70% argon and 30% carbon dioxide in air. However, behavioural observation of the birds revealed that they showed enforced respiration and head shaking before they lost posture and were unable to right themselves suggesting that they had detected and were reacting to the gas mixture.

In these studies, different methods were used to ascertain aversion and therefore different outcomes were reported. However, future studies should consider the possibility that fear responses, especially during aversion or passive avoidance testing, could suppress the behaviour (especially motivational state) of birds and, in the absence of acclimatisation, could change the outcome of the study (Gallup *et al.*, 1972; Gallup, 1977).

As discussed previously (Section 6.4. gas mixtures for stunning and stun / killing), a concentration of 50% or more of carbon dioxide is said to be unpleasant to inhale in humans, and laboratory animals avoid an atmosphere containing even lower concentrations of this gas. The concentration of carbon dioxide that becomes aversive to poultry is likely to be considerably lower because, unlike mammals, the lungs of birds have intrapulmonary chemoreceptors that are acutely sensitive to carbon dioxide and insensitive to hypoxia (Ludders, 2001). This is probably the reason why chickens and turkeys avoid carbon dioxide but not hypoxic atmospheres (discussed later in this section). In addition to these chemoreceptors, humans have other receptors (e.g. irritant) in their lungs that acutely respond to inhalation of carbon dioxide and contribute to a sense of breathlessness (Manning and Schartzstein, 1995), and these receptors are common to all animals.

McKeegan (2003) studied the impact of gas mixtures containing different percentages of carbon dioxide (10%, 25%, 40% and 55%) on the behaviour of broiler chicken during the first 10 sec of exposure. In 25% carbon dioxide, the number of chickens showing ‘gasping’ (4 out of 10 chickens) was higher than in 40% and 55% carbon dioxide (3 out of 10). In 40% carbon dioxide, the chickens began to withdraw from the gas, and in 55% carbon dioxide the reaction was described as 'marked withdrawal'. These results indicate that most broiler chickens seem to tolerate concentrations up to 40% carbon dioxide. Concentrations higher than 40 or 55% seem to cause pain or a higher unpleasantness as they caused an increase in withdrawal or marked withdrawal.

In addition to gasping and head shaking prior to loss of consciousness, carbon dioxide gas mixtures induce convulsions in unconscious poultry. Although addition of 40% oxygen to carbon dioxide seems to reduce or eliminate these convulsions, this mixture, may not be beneficial to bird welfare (Raj *et al.*, 1998). This suggestion is based on the observation that, during the induction phase, birds that were exposed to a mixture of 50% carbon dioxide and 50% oxygen showed signs of respiratory distress similar to those exposed to 50% by volume of carbon dioxide in air (Zeller *et al.*, 1988).

Lambooij *et al.*, 1999a (see also Barton-Gade *et al.*, 2001) compared the reactions occurring in chickens during exposure to 90% argon in air, 70% argon and 30% carbon dioxide, 60% carbon dioxide in air, and 40% carbon dioxide and 30% oxygen in air. The results indicate that the number of birds showing gasping, head shaking and convulsions are less using 90% argon in air than in the other gas mixtures tested.

The time to loss of consciousness (time to loss of SEPs) during exposure of poultry has been investigated in detail.

Table 10-7. The time (sec) to loss of SEPs in hens during exposure to gas mixtures (mean $\pm$ SD)

90% argon in air	49% carbon dioxide in air	31% carbon dioxide and 60% argon in air	30% carbon dioxide and 45% argon in air
29 $\pm$ 2	26 $\pm$ 3	19 $\pm$ 2	17 $\pm$ 3

Source: Raj, Gregory and Wotton, 1992; Raj, Wotton and Whittington, 1992.

Table 10-8. The time (sec) to loss of SEPs in broilers during exposure to gas mixtures (mean $\pm$ SD)

90% argon in air	31% carbon dioxide and 60% argon in air	A mixture of 40% carbon dioxide, 30% oxygen and 30% nitrogen
32 $\pm$ 2	24 $\pm$ 2	47 $\pm$ 4 *

*2 out of 12 broilers exposed to this gas mixture retained their SEPs for the entire 2 min exposure time.
Source: Raj *et al.* (1998).

Table 10-9. The time (sec) to loss of SEPs in turkeys during exposure to gas mixtures (range (mean))

90% argon in air	30% carbon dioxide and 60% argon in air	49% carbon dioxide in air	65% carbon dioxide in air	86% carbon dioxide in air
25-61 (44)	16-34 (22)	14-32 (20)	11-24 (15)	12-34 (21)

Source: Raj and Gregory (1994).

It is worth noting that increasing the concentration of carbon dioxide in the stunning atmosphere did not significantly reduce the time to abolition of SEPs in turkeys.

The presence of oxygen in a carbon dioxide atmosphere can prolong the time to loss of brain responsiveness, and thus, unequivocal loss of consciousness. The average time taken for broilers to lose SEPs was found to be longer than 2 min when exposed to a mixture of 40% carbon dioxide, 30% nitrogen and 30% oxygen in air (Raj *et al.*, 1998). By contrast, exposure of chickens to 45% carbon dioxide in air results in the loss of SEPs, on average, after 30 sec (Raj, Gregory and Wotton, 1990). However time to induction of unconsciousness is less important provided that the gas mixtures used in the induction phase are non-aversive.

The feasibility of using various gas mixtures for batch stunning of poultry has been evaluated to a certain extent. When using argon as a hypoxic agent, 5% by volume of residual oxygen and 2 min exposure time failed to stun chickens (Raj and Gregory 1990b). Raj and Gregory (1990b) found that chickens could be stunned with an exposure time of 2 min to 2% by volume of residual oxygen however they responded to comb pinching at 15 sec after returning to atmospheric air. Evidently, 15 sec of unconsciousness would not be sufficient to avoid return of consciousness and sensibility either before neck cutting or during bleeding. Therefore, Raj and Gregory (1990b) recommended that chickens should be killed, rather than stunned, by exposing them to 2% by volume of residual oxygen as a maximum. There are no scientific publications regarding the use of anoxia for stunning turkeys, but 3 min exposure to anoxia has been shown to kill them (Raj 1994a and b).

Kotula *et al.* (1957) recommended that chickens should be exposed for 75 sec to 33-36% by volume of carbon dioxide in air to stun them. The time interval between the end of exposure to carbon dioxide and neck cutting was not reported in this study. However, it is stated that cutting one common carotid artery and one external jugular vein at slaughter resulted in wing flapping during bleeding in most of the birds. Although the state of consciousness in these birds was not measured, the authors stated that the birds were unconscious when they flapped their wings.

Another study indicated that during exposure to various concentrations of carbon dioxide it would be difficult to effectively stun all the poultry without inducing death in some birds and even the duration of unconsciousness induced with a gas stun can be very short in some birds (Zeller *et al.*, 1988). Exposure of chickens to 45% by volume of carbon dioxide in air for 2 min has been reported to have resulted in death in the majority of the birds, and the survivors showed a positive response to comb pinching as early as 26 sec after returning to atmospheric air (Raj and Gregory, 1990b). Carbon dioxide induced analgesia may last longer than the period of unconsciousness and, therefore, response to comb pinching may not be a reliable indicator of return of consciousness (Zeller *et al.*, 1988). In any case, even 26 sec of unconsciousness would not be sufficient to avoid a return of consciousness and sensibility either before neck cutting or during bleeding. There are no scientific publications regarding the use of carbon dioxide for stunning turkeys, but 2 min exposure to 49% by volume of carbon dioxide has been known to kill them (Raj and Gregory, 1994).

Exposure of chickens for 2 min to a mixture of argon (it could also be nitrogen rather than argon, or mixtures of argon and nitrogen) and 10, 20 or 30% by volume of carbon dioxide resulting in 5% by volume of residual oxygen has been reported to cause death in 76, 99 and 100% of birds respectively (Raj, Gregory and Wilkins, 1992). The exposure time required to stun rather than to kill poultry and the duration of unconsciousness induced with these gas mixtures are not known. There are no scientific publications regarding the use of this gas mixture for stunning turkeys, but 2 min exposure has been known to kill them (Raj and Gregory, 1994).

Exposure of chickens for 2 min to a mixture of 40% by volume of carbon dioxide, 30% by volume of oxygen and 30% by volume of nitrogen resulted in death in 3 out of 17 birds. Returning the survivors to atmospheric air resulted in recovery of response to comb pinching as early as 30 sec after exposure (Raj *et al.*, 1998). As mentioned earlier, a bleed out time of 25 sec would be necessary to cause brain ischaemia through blood loss to avoid a return of consciousness. Therefore, if a carbon dioxide-oxygen mixture is to be used for stunning chickens, the birds should be exposed to the gas mixture for longer than 2 min and both the common carotid arteries must be severed within 5 sec of birds exiting the gas mixture to prevent them regaining consciousness. However, this suggestion needs to be evaluated scientifically and confirmed prior to implementation because it is doubtful whether all the birds in a batch could be shackled and neck cut within 5 sec. Alternatively, new rapid bleeding techniques could be developed that do not necessitate prior shackling

There are no scientific publications regarding the use of this gas mixture for stunning turkeys.

When broilers were exposed to 45% carbon dioxide in air for 2 min and returned to atmospheric air, the majority of them died and the survivors responded to comb pinching in two different manners (Raj and Gregory, 1990b). One group responded positively, on average, at 90 sec (time ranged from 26 to 290 sec) before they opened their eyes, and, although the other group opened their eyes between 200 and 300 sec, they did not respond to comb pinching performed for up to 6 min after exposure to the gas. This suggests that the analgesic effect of carbon dioxide could be prolonged beyond the resumption of consciousness (Zeller *et al.*, 1988). Together, these results imply that the addition of oxygen to carbon dioxide reduced the time taken to resume consciousness as well as reduced the duration of the analgesic effect of carbon dioxide.

Concerning carbon dioxide / oxygen mixtures, a different opinion can be found in a paper by Hoenderken *et al.* (1994). The authors refer to AVMA (1986), van den Bogaard, Dam and Weekers (1985), van Luytelaar *et al.* (1993), and Fenwick and Blackshaw (1989), who claim that adding oxygen to carbon dioxide, in contrast to using carbon dioxide in air, reduces signs of asphyxia and excitation in rats and can therefore be beneficial for anaesthetising rats (see papers by Leach *et al*. that showed that in choice tests, rats found levels of carbon dioxide more than 20% aversive). In the other series of experiments with chickens, the conclusion drawn was that adding oxygen to carbon dioxide atmospheres and moistening the very dry technical gases are beneficial for animal welfare. Also, Coenen *et al.* (2000) recommend supplementation of oxygen (comparing 40% carbon dioxide, 30% oxygen, 30% nitrogen in relation to 40% carbon dioxide, 15% oxygen, 45% nitrogen) to minimize signs of agitation and asphyxia until birds lost consciousness.

Exposure of poultry to hypoxia (residual 2% volume of oxygen in argon, nitrogen and their mixtures) and 40% by volume of carbon dioxide in air results in wing flapping (clonic convulsions), leading to a tonic phase before the onset of respiratory arrest. However, convulsions due to hypoxia (wing flapping) occurring after a loss of consciousness can be an aesthetically unpleasant sight. Ernsting (1965) reported that, under hypoxic conditions, depression of activity in the mammalian brain extends progressively from the telencephalon to the diencephalon and then to the mesencephalon. Convulsions result from the lack of modulation of the caudal reticular formation from higher centres, particularly the cerebral cortex and rostral reticular formation (Dell *et al.*, 1961; Ernsting, 1965). The implication of this is that the onset of convulsions themselves can be used as an indicator of loss of consciousness. This interpretation is supported by the fact that effective head-only electrical stunning and captive bolt stunning, in which there is a profound brain damage, leads to severe wing flapping in poultry (Raj and O'Callaghan, 2001). Similarly, decapitation and neck dislocation also induce wing flapping.

It is important to note that hypoxia-induced convulsions occur as spinal reflexes and not as a result of epilepsy in the brain. Nevertheless, Woolley and Gentle (1988) reported that, based on the occurrence of slow waves in the EEG of chickens, the birds are unconscious during the occurrence of wing flapping when hypoxia was induced gradually. Raj, Gregory and Wotton (1991) provided further evidence on the basis of changes in the EEG and absence of response to comb pinching that convulsions occurred in unconscious poultry.

The composition of gas mixtures and precise exposure times that would be required to stun chickens and turkeys, without killing any bird, and the duration of unconsciousness induced and the blood vessels to be cut to prevent a return of consciousness during bleeding are not yet known. Therefore, it is not possible to make any firm recommendations on the use of gas mixtures for stunning poultry. Moreover, if crates and conveyors are used for gas stunning, poultry will be leaving the stunning units in large numbers and at the exit the unconscious poultry will have to be shackled before neck cutting can be performed. Under this situation, the sum of time interval between the end of exposure to a gas mixture and onset of death after neck cutting is likely to be longer than the duration of unconsciousness induced with a gas mixture. For example, when crates are used, there will be a significant delay in time between the end of gas stunning and neck cutting between the first and the last bird. When conveyors are used, it will be difficult to shackle poultry in the sequence in which they exited the unit.

There is no scientific evidence concerning the time to onset of death due to bleeding after gas stunning in poultry. However, considering the prolonged interval between gas stunning and shackling and the time it takes for bleeding to induce brain ischaemia in poultry, it is doubtful whether a return of consciousness could be avoided in gas stunned poultry.

Description of effective use

In the absence of scientific evidence concerning the depth and duration of unconsciousness induced with gas mixtures, minimum conditions could not be recommended.

Owing to this, no monitoring points, advantages and disadvantages are known.

10.6. GAS MIXTURES FOR STUN / KILLING

The only difference between gas stunning and gas stun / killing is that the birds are exposed to gas mixtures until they are dead. Therefore, any delay in neck cutting and the blood vessels cut become irrelevant.

Various bird welfare concerns associated with the stress of induction of unconsciousness with gas mixtures and the scientific details listed under gas stunning above are obviously also relevant to this gas stun/kill methods.

It is emphasised that, since one of the objectives of stun / kill with gas mixture is to alleviate the pain and suffering associated with shackling conscious poultry under water bath stunning and killing systems, this method must be limited to birds contained in crates or on conveyors only. The pain and suffering induced by shackling are also very likely to mask the signs of distress that may be caused by induction of unconsciousness with gas mixtures.

These gas mixtures are used in abattoirs and have also been extensively researched. The results of scientific studies are presented in the following paragraphs.

Gas mixtures used for stun / killing poultry:

- A minimum of 2 min exposure to argon, nitrogen or other inert gases, or any mixture of these gases, in atmospheric air with a maximum of 2% residual oxygen by volume.

- A minimum of 2 min exposure to any mixture of argon, nitrogen, or other inert gases with atmospheric air and carbon dioxide, provided that the carbon dioxide concentration does not exceed 30% by volume and the residual oxygen concentration does not exceed 2% by volume.

- A minimum of 1 min exposure to a mixture of 40% carbon dioxide by volume, 30% oxygen by volume and 30% nitrogen by volume, immediately followed by 2 min exposure to a minimum of 80% carbon dioxide by volume in air for killing chickens. However, there are no peer reviewed scientific publications available to assess the welfare aspects of the use of this gas mixture for stunning or stun / killing of turkeys.

Gas mixtures that are being evaluated to stun / kill poultry under commercial conditions:

- Minimum of 1 min exposure to a mixture of 40% carbon dioxide by volume, 30% oxygen by volume and 30% nitrogen by volume, immediately followed by 2 min exposure to a minimum of 80% carbon dioxide by volume in air for killing chickens. However, there are no peer reviewed scientific publications available to assess the welfare aspects of the use of this gas mixture for stunning or stun / killing of turkeys.

- Increasing concentrations of carbon dioxide in air at around 20% for approximately 30 sec, then 40% for 50 sec, and finally 50% for 30 sec.

- Minimum of 2 min exposure to 45 to 55% carbon dioxide in air.

- Under commercial poultry processing conditions, a mixture containing 80% nitrogen and 20% argon or carbon dioxide is currently being used in chickens and turkeys (in one member state). With both mixtures, a residual oxygen level of less than 2% by volume is maintained and birds are exposed for a minimum of 2 min. In this system, crates containing poultry are carried through a tunnel containing one of these gas mixtures. The time between the end of gas killing and neck cutting is longer than the corresponding time under electrical stunning or killing systems. However, studies involving chickens and turkeys revealed that the delay between the end of gas killing and neck cutting does not impede blood loss at slaughter, provided that neck cutting is performed within 3 and 5 min of killing chickens and turkeys, respectively (Raj and Gregory, 1991a; Raj, Gregory and Wotton, 1994 and Raj and Johnson, 1997).

A previous report considered the experimental evidence, available in 1997-98 (SCAHAW, 1998), and reported on the gas mixtures used and on those listed below:

- Method 1: 40% carbon dioxide, 30% oxygen and 30% nitrogen.
- Method 2: 30% carbon dioxide, 20% oxygen and 50% air.
- Method 3: increasing concentrations of carbon dioxide in air at around 20% for approximately 30 sec, then 40% for 50 sec, and finally 50% for 30 sec
- Method 4: Method 1 followed by exposure to 80% carbon dioxide in air.
- Method 5: 45% carbon dioxide and 55% air.

Since the publication of the previous report in 1998, a number of studies have evaluated the killing of poultry with gas mixtures listed under Methods 1 and 4, and some concluding remarks were published recently as a symposium report (Barton-Gade *et al.*, 2001). These remarks can be summarised as follows.

Lambooij (see Barton Gade *et al.*, 2001) compared the reactions occurring in chickens during exposure to 90% argon in air, 70% argon and 30% carbon dioxide, 60% carbon dioxide in air and 40% carbon dioxide and 30% oxygen in air. The results indicate that the number of birds showing gasping, head shaking and convulsions are less using 90% argon in air than the other gas mixtures.

Coenen *et al.* (2000) compared the bird welfare implications of exposing chickens for 1 min to either (a) a mixture of 30% carbon dioxide, 60% argon in air, (b) a mixture of 40% carbon dioxide, 30% oxygen and 30% nitrogen, or (c) a mixture of 40% carbon dioxide, 15% oxygen and 45% nitrogen. They concluded that convulsions occurred in conscious poultry during exposure to the carbon dioxide-argon mixture and that exposure to carbon dioxide - oxygen mixture resulted in convulsion-free induction of unconsciousness within 1 min. These observations are in contradiction with other scientific reports due to variations in the criteria to determine unconsciousness that were used. For example, Raj, Wotton and Gregory (1992) reported that exposure of chickens to a 30% carbon dioxide and 60% argon mixture in air resulted in a suppressed EEG and abolition of SEPs (an objective measure of brain dysfunction) on average at 11 and 19 sec respectively, and that convulsions started on average at 21 sec, which is after the loss of SEPs in the brain.

Exposure of broilers for 2 min to a mixture of 40% carbon dioxide, 30% oxygen and 30% nitrogen failed to abolish SEPs in all the chickens (Raj *et al.*, 1998). However, Coenen *et al.* (2000) reported that, on the basis of subjective interpretation of reduction in the amplitude of EEG signals interspersed with epileptiform spikes, broilers became unconscious in 43.5 sec during exposure to the same mixture of gases. This is not in agreement with Raj *et al.* (1998), who found that exposure of broilers to this gas mixture resulted in a suppressed EEG in only 8 out of 14 birds and that the epileptic spikes occurred only in 6 out of 14 birds. Together, these results imply that the magnitude of reduction in the EEG signal amplitude by the carbon dioxide and oxygen mixture is inadequate to abolish evoked potentials in the brain. Considering that exposure of chickens to 45% carbon dioxide in air resulted in suppression of EEG signals and abolition of SEPs on average at 11 and 26 sec, respectively (Raj, Gregory and Wotton, 1990), EEG suppression occurring during exposure to the carbon dioxide and oxygen mixture, in the presence of SEPs, may not represent unconsciousness and insensibility in poultry. Understandably, the conclusions reached in these studies differ due to the differences in criteria used to determine the state of consciousness and sensibility.

Method 2: No peer reviewed scientific publication exists.

Method 3: There are no peer reviewed scientific publications available on the multiphase systems involving various concentrations of carbon dioxide for killing chickens or turkeys. Existing knowledge indicates that exposure of poultry to 20% carbon dioxide for 30 sec would not be sufficient to induce unconsciousness and, therefore, subsequent exposure to 40% carbon dioxide may be aversive. The average time to onset of unconsciousness during exposure of chickens to 19 and 23% by volume of carbon dioxide in air is reported to be 322 and 251 sec, respectively (Bogdanov *et al.*, 1979). In spite of this concern, scientific and the technological development of multiphase carbon dioxide killing systems should consider using distinctly separated (compartmentalised) concentrations in order to separate not yet unconscious birds from higher and aversive concentrations of carbon dioxide.

Hypoxia induced with argon or nitrogen should be considered for stunning poultry before killing them with a high concentration of carbon dioxide. Poultry should be exposed to hypoxic atmosphere for 1 min before killing them with an aversive gas mixture.

Unfortunately, recommendations for multiphase systems cannot be made without knowing the time to loss of consciousness (e.g. loss of SEPs) or onset of death during exposure to these systems. Further research is needed to substantiate any claims relating to these systems.

The four methods listed below would kill chickens if exposed for a sufficient time in crates or conveyors:

- Hypoxia induced with argon or nitrogen may have no negative welfare implications because these gases are apparently not detectable by the birds and, given a free choice, chickens and turkeys spontaneously enter such hypoxic atmospheres and die.

- Mixtures of carbon dioxide with argon seem aversive as indicated by gasping and head shaking during the induction of unconsciousness.

- Gasping and head shaking occurs during exposure to 15% by volume or more of carbon dioxide in air, as a result of respiratory stimulation effect (Bogdanov *et al.*, 1979).

- On the other hand, aversiveness to carbon dioxide seems to occur probably as a result of the pungency of the gas, which increases in severity when the carbon dioxide level is 40% by volume or more, at least in humans (Gregory *et al.* 1990). Moreover, the lungs of birds have intrapulmonary chemoreceptors that are acutely sensitive to carbon dioxide and insensitive to hypoxia which may make it worse for birds than humans (Ludders, 2001). Therefore, the use of gas mixtures containing carbon dioxide for stunning or stun / killing of poultry raises welfare concerns.

Scientific evidence suggests that concentrations of more than 40% carbon dioxide is aversive and induction of unconsciousness with a high concentration of this gas is distressing to poultry. Hypoxia induced with inert gases appears to be the best option on birds' welfare grounds.

Control of temperature and humidity of any gas mixture used for stunning or stun / killing poultry could improve the welfare of birds because inhalation of warm and humidified air helps to alleviate physical discomfort and distress and this concept is widely used in artificial respiration systems.

Although poultry are killed with the gas mixtures, a residual heartbeat can persist in birds for a short period of time after exiting the gas mixtures. However, the cardiac function may not be adequate to result in resuscitation or recovery of consciousness (Raj and Gregory, 1991a). Carcasses should not show any breathing or wing flapping during bleeding.

Pending availability of further data concerning the welfare of birds during the induction of unconsciousness, the gas mixtures listed below are suggested as being effective in killing poultry. The criteria used to establish loss of consciousness in these gas mixtures were abolition of SEPs and occurrence of a profoundly suppressed EEG.

At present, dead on arrival (DOA) birds that are in rigor are removed during shackling and suspicious carcasses are removed after the feather removal (plucking) on the basis that they retained feathers and showed congested skin indicative of poor bleeding.

10.6.1. Description of effective use

Live poultry shall be conveyed into the gas mixtures either in transport crates or on conveyor belts (these also apply to gas stunning methods, if one is developed in the future).

Birds should be lowered into a chamber or tunnel containing one of the following gas mixtures:

(a) Minimum of 1 min exposure to 40% carbon dioxide, 30% oxygen and 30% nitrogen, followed by a minimum of 2 min exposure to 80% carbon dioxide in air; or

(b) Minimum of 2 min exposure to any mixture of argon, nitrogen or other inert gases with atmospheric air and carbon dioxide, provided that the carbon dioxide concentration does not exceed 30% by volume and the residual oxygen concentration does not exceed 2% by volume; or

(c) Minimum of 2 min exposure to argon, nitrogen, other inert gases or any mixture of these gases in atmospheric air with a maximum of 2% residual oxygen by volume.

Compressed gases must be vaporised prior to administration into the chamber.

Under no circumstances, should solid gases with freezing temperatures enter the chamber.

Gas mixtures should be humidified.

Appropriate gas concentrations must be monitored continuously at the bird levels inside the chamber.

The exposure time should be extended, if necessary, to kill 100% of birds.

10.6.2. Monitoring points

All the birds shall be killed with the gas mixtures and under no circumstances should they show signs of recovery of consciousness.

Adequate application of the method is indicated by:

- Completely relaxed carcass.
- No corneal or papillary reflexes.
- Cardiac fibrillation.

10.6.3. Advantages

Killing poultry in transport crates with gas mixtures eliminates the need for live bird handling at the processing plant and killing birds on conveyor belts eliminates the welfare concerns associated with live bird shackling under electrical water bath methods.

Anoxia induced with inert gas mixtures is non-aversive to poultry.

10.6.4. Disadvantages

Induction of unconsciousness with gas mixtures containing aversive concentrations of carbon dioxide is a welfare problem.

10.7. CAPTIVE BOLT

Mechanical devices have been developed specifically to kill, rather than stun, poultry. These are penetrating or non-penetrating captive bolt devices and are fired using either cartridges or compressed air (Hewitt, 2000; Raj and O'Callaghan, 2001).

Birds should be restrained in cones, shackles, crushes or by hand (provided that operative safety is not compromised by the design of the gun) to facilitate accurate shooting. Captive bolts must be fired perpendicular (at right angles) to the frontal bone (Raj and O'Callaghan, 2001).

Killing birds with a penetrating or non-penetrating captive bolt can be performed using either blank cartridges or compressed air. Spring-loaded captive bolts have also been used to kill poultry in Germany (Schutt-Abraham, Knauer-Kraetzel and Wormuth, 1992b). However, severe wing flapping occurring due to captive bolt shooting is not conducive to operator's safety, neck-cutting or good carcass and meat quality (Lambooij *et al.*, 1999b).

In general, poultry slaughtered for human consumption are very young and their skull bones are not fully ossified. Therefore, unlike red meat species, it is unlikely that concussion of the brain can be induced in poultry by shooting with captive bolts. In addition, their skull bones are fractured during shooting with non-penetrating captive bolts, although this has no adverse welfare implications. Needless to say, both penetrating and non-penetrating bolts induce severe structural damage to the brain and immediate death, provided the bolt parameters are adequate. Therefore in principle they both destroy the brain and similar conditions apply. Research carried out in the Netherlands involving broiler chickens and a penetrating bolt with a diameter of 5 mm and a length of 25 mm (Hillebrand *et al.,* 1996), showed that captive bolt shooting can be effective in inducing unconsciousness in birds.

More recently, a pneumatically operated penetrating captive bolt was evaluated for broilers. The results indicated that the bolt must be fired perpendicular to the skull and the ideal parameters should be a minimum of 6 mm bolt diameter delivering an impact energy of not less than 21 J and a penetration depth of 10 mm. (Raj and O'Callaghan, 2001). In these broilers, loss of visual evoked potentials (VEPs) occurred immediately after shooting. Broilers shot with these parameters died immediately but showed very severe wing flapping. Furthermore, it was found that, unless the bolt was fired perpendicular (right angle) to the surface of the skull, it did not always stun or kill the birds, a disconcerting feature from a welfare point of view.

It has been reported that, based on the spontaneous behaviour, when chickens were shot using a bolt fitted with a plastic concussive head, effective killing was achieved (Hewitt, 2000). Owing to the plastic concussive head, the velocity of this bolt is high and firing on the heads of chickens and turkeys causes severe structural damage to the skull and brain. This bolt can be fired using compressed air or cartridges and, based on this and further research, an equipment manufacturer in the UK is marketing a concussive device for casualty / emergency slaughter of poultry.

Although the commercially produced captive bolt gun is used for shooting chickens and turkeys, there are no peer reviewed scientific publications available to be considered.

10.7.1. Description of effective use

Captive bolts must be fired perpendicular to the frontal bone

Bolt diameter shall be a minimum of 6 mm and deliver impact energy of a minimum of 21 J, in any cases, appropriate to the species of poultry to destroy the skull and brain.

Only mechanical devices should be used (manual blow to the head may not be consistently accurate)

Captive bolts should be maintained according to manufacturer's recommendations.

10.7.2. Monitoring points

Adequate application of the method is indicated by:

- Completely destroyed skull and brain.
- Immediate onset of apnoea.
- Dilated pupils.

- Absence of corneal reflex.
- Severe wing flapping.
- Bleeding through the wound.

10.7.3. Advantages

This method can be used for slaughter and as a backup method to kill poultry in slaughterhouses if other methods fail.

10.7.4. Disadvantages

Severe wing flapping occurs following shooting, which is not conducive to efficiently performing neck-cutting or dislocating necks.

10.8. OTHER METHODS

The use of needle bolts (injection of air into the cranium of poultry; Lambooij *et al.*, 1999b) and microwave irradiation (Zeller, 1986 and Zeller *et al*., 1989) have been tested experimentally and have disadvantages on animal welfare grounds.

11. STUNNING AND STUN / KILLING METHODS FOR HORSES

The methods used for stunning and killing of horses for slaughter are captive bolt and free bullet. Although captive bolt stunning is the most used method in European abattoirs, scientific investigations in mechanisms and effectiveness of this method in horses could not be found. Chest sticking is used in most cases.

11.1. CAPTIVE BOLT

Captive bolt shots lead to instantaneous collapse of the animals, followed by seizures (spasms and leg movements). The use of captive bolt in horses is not a stun / killing method and therefore needs to be combined with pithing or bleeding by cutting the common carotid arteries (Schatzmann, 1997).

Practical experience has shown that post-stun behaviour is similar to that shown in cattle, although horses often make a small forward movement (leap into the air) following shooting. This can be dangerous for the slaughterman if standing in front of the horse.

The ideal shooting position for horses is perpendicular to the frontal bone 2 cm above the intersection of a diagonal line from the middle of the base of the ear to the middle of the opposite eye (Figure 11-1) (TVT, 2002).

A stun-to-stick interval of 41-50 sec after the application of captive bolt stunning has been reported to be satisfactory (Meat Hygiene Service (UK), Animal Welfare Review, March 2002).

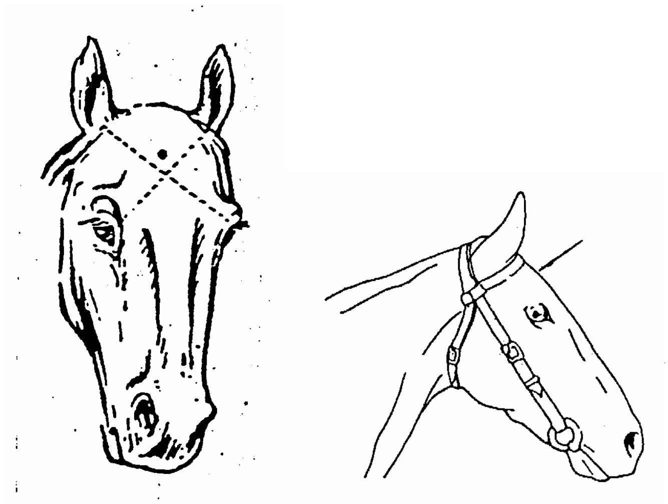


Figure 11-1. Shooting positions for horses

11.1.1. Description of effective use

Horses must be adequately restrained.

The ideal shooting position is 2 cm above the intersection of a diagonal line from the middle of the base of the ear to the middle of the opposite eye.

To avoid skidding of the bolt along the skull and facilitate proper penetration of the bolt it is recommended to shoot at right angles to the frontal bone.

The bolt should damage the brain.

Bleeding or pithing should be performed within 41 sec.

Both common carotid arteries should be severed to keep the time to onset of death as short as possible.

11.1.2. Monitoring points

The following signs indicate an effective stun:

- Horses will collapse immediately.
- Tonic – clonic seizures.
- Immediate onset of apnoea
- The position of the eyeball is fixed (*i.e.* facing straight ahead).
- Fixed eyes with dilated pupils

11.1.3. Advantages

When performed correctly, captive-bolt stunning is an effective method of stunning horses and loss of consciousness is immediate.

11.1.4. Disadvantages

In excited and uncontrollable horses, it can be impossible to use this method.

11.2. FREE BULLET

Free bullet shots lead to instantaneous collapse of the animals without any virtual movement afterwards (Oliver, 1979). The basis for this killing method will be described in more detail in chapter 13. It should not be used in confined spaces without adequate safety measures.

This method should only be attempted by individuals trained in the use of firearms (Oliver, 1979; UC Davis, 2001).

Ideal shooting position for horses is the point of intersection of a diagonal line taken from the base of each ear to the medial canthus of the eye on the opposite side (differs from captive bolt) (Oliver, 1979). The firearm should be aimed in a direction down the neck, perpendicular to the front of the skull, and held at least 5-15 cm away from the point of impact (UC Davis, 2001).

A 0.22-caliber rifle is recommended, but a 9 mm or 0.38-caliber handgun will be sufficient for most horses. The use of a hollow-point or soft nose bullets will increase brain destruction and reduce the chance of ricochet. If a shotgun is the only available firearm, the use of a rifled slug is preferred (UC Davis, 2001).

11.2.1. Description of effective use

Horses must be adequately restrained.

A 0, 22-caliber rifle or a 9 mm or 0,38-caliber handgun with hollow-point or soft nose bullets should be used.

Ideal shooting position for horses is the point of intersection of a diagonal line taken from the base of each ear to the medial canthus of the eye on the opposite side.

The firearm should be aimed directed down the neck, perpendicular to the front of the skull, and held at least 5-15 cm away from the point of impact.

11.2.2. Monitoring points

If the shot is effective horses will collapse immediately without movements afterwards. Normal rhythmic breathing will stop. The position of the eyeball is fixed (*i.e.* facing straight ahead).

11.2.3. Advantages

When performed correctly, free bullet is an effective method of stunning and killing horses and loss of consciousness is immediate.

Distance from the horse can be maintained, which can have benefits in violent or uncontrollable horses.

11.2.4. Disadvantages

It cannot be used in confined spaces without adequate safety measures.

12. STUNNING AND STUN / KILLING METHODS FOR FARMED FISH

12.1. PAIN FEAR AND SENTIENCE IN FISH

Until recently, there was a widely held belief that fish did not have the mental capacity to experience pain and were thus incapable of suffering. This belief was based on the view that fish had not evolved the salient biological characteristics that are hypothesized to permit sentience. It was proposed that because awareness of pain in humans apparently depends on functions of specific regions of the cerebral cortex, and because fish lack these brain regions, it is untenable that fish can experience fear or pain (see Rose (2002) for a recent review of these arguments).

However, these rather simplistic arguments are being increasingly challenged. Teleost fish do have marked differences in some aspects of brain structure and organisation as compared to mammals. But, they demonstrate behavioural similarities and show a level of cognitive development suggestive of sentience.

Consciousness of any animal is not yet directly accessible to scientific investigation. However, an array of indirect evidence is used to infer whether these subjective states are likely to exist in animals. Briefly, examples of the type of evidence generally accepted to indicate that fish have cognitive development suggestive of sentience are that:

- they form declarative representations of their environment (Topál and Csányi, 1999);
- they show observational learning and use information gained in this way to direct their future behaviour in flexible and adaptive ways (Oliveira *et al.*, 1998; McGregor *et al.*, 2001 and Doutrelant and McGregor, 2000); and
- they communicate with each other during social interactions (O'Connor *et al.*; 1999, Höglund *et al.*, 2000).

Most recent interpretations of behavioural data suggest that affective states of pain (see in particular Sneddon, 2003, for recent experimental evidence of pain perception per se in fish), fear and stress are likely to be experienced by fish in similar ways as in mammals.

The arguments expounded by Rose (2002) are based on neuro-anatomical comparisons and it is useful to consider how the specific brain structures and neural systems associated with emotions and motivated behaviour in mammals are represented in fish. The limbic system is accepted in mammals to have functions in emotional behaviour, memory, and learning (Kötter and Meyer, 1992; Hildebrand, 1995; Ono *et al.*, 2000). The exact neurophysiological basis of emotions is not fully understood. But, most importantly, research supports the view that emotions involve relatively "primitive" brain circuits that have been preserved through evolution (Butler and Hodos, 1996; LeDoux, 2000). Despite differences in phylogeny (Ito and Kishida, 1978; Munro and Dodd, 1983; Kálmán, 1998; Butler, 2000), there is a growing body of anatomical, behavioural, and pharmacological evidence to suggests that the brain structures and neural systems associated with motivational-affective states in mammals are present in fish. For example, behavioural effects of telencephalon ablation in fish resemble lesion experiments on the mammalian limbic system (Davis and Kassel; 1983; Portavella *et al.*, 2002). It has therefore been proposed that the fish telencephalon, along with other brain structures, is functionally homologous to limbic structures found in tetrapods (Ohnishi, 1997; Mok and Munro, 1998). Similarly, dopaminergic innervation of the fish telencephalon has been shown to mediate motivational states and behaviour in similar ways as in mammals (Lett and Grant, 1989; Mattioli *et al.*, 1995; Mattioli *et al.*, 1997). Thus, recent studies have demonstrated the existence of sensory pathways in fish which reveal that fish have the necessary functional organisation of the brain which is a prerequisite to suffering (Meyer *et al.*, 2002) (see Chandroo *et al.*, 2003, for a recent detailed exploration of sentience and suffering in fish).

In summary, most recent interpretations of the results of many studies lead to believe that fish have the structures necessary and the capacity to experience fear and pain and can thus suffer and therefore, welfare considerations for farmed fish should take these into account.

12.2. PRACTICAL WAYS TO RECOGNISE WHETHER FISH ARE CONSCIOUS, UNCONSCIOUS OR DEAD

The need for killing methods to induce immediate unconsciousness or, if unconsciousness is induced slowly, the reason why the process should be without pain or fear, is discussed in chapter 5. Therefore it is important for people involved in fish slaughtering operations to be able to recognise whether a stunning operation has rendered a fish rapidly unconscious.

Laboratory methods for measuring EEGs in fish are available that determine the onset of unconsciousness with some precision (Kestin, Wotton and Gregory, 1991; Robb *et al.*; 2000b; Van de Vis *et al.*, 2003a; Lambooij *et al.*, 2002) and the general principles outlined earlier in the document with respect to mammals and birds apply.

EEGs are relatively complex to carry out and best suited to a laboratory. More practicable methods are available for use in the field or at sea. As with mammals and birds, these are based on measurements of spontaneous behaviour, responses to stimulation and reflexes (see section 13.1 above). The relationship between some of these behavioural measurements and EEG activity has been established for a few species of commonly farmed fish (salmon trout and eels), but the behavioural measures need to be used with caution in other species

Field methods for determining the state of consciousness of fish:

If a fish reacts coherently to potentially painful or fearful events, it is able to detect and respond to external stimuli. This suggests the presence of at least some brain function, possibly sufficient brain function to suffer. Thus, if a fish is showing co-ordinated swimming responses, or co-ordinated escape behaviour, or regains equilibrium when inverted (turned on its back while in water), or reacts to painful stimulation with a needle or tail pinch, it cannot be considered to be unconscious (swimming is a sustained rhythmic motor activity, whereas escape is a 'one-shot' response that is much more rapid and forceful than swimming).

With most killing methods, fish that do not show these behaviours or responses could be considered unconscious. But caution needs to be exercised as some slaughter methods induce sedation, and possibly paralysis, but without analgesia or anaesthesia before unconsciousness. Fish killed by these methods could experience suffering but are unable to demonstrate it behaviourally. Carbon dioxide narcosis could cause such an effect (Kestin, Van de Vis and Robb. 2002).

As explained in the section on mammals and birds, reflexes mediated by the brain stem such as rhythmic breathing or corneal reflex are widely used to assess the slaughter of mammals and birds. These reflexes are the first overt signs of recovery from a stunning insult (Anil 1991) and are widely accepted as relatively robust indicators of brain function (Gregory and Wotton 1983). When they are absent, it can safely be concluded that the animal is unconscious (Anil 1991). In fish, the vestibulo-ocular reflex (VOR) (commonly called Eye roll*)* and breathing reflexes have been found to be similarly robust indicators of brain function (Kestin, Van de Vis and Robb, 2002). VER data support the proposition that these reflexes are lost only when a fish is unconscious. Thus, it can be concluded that if the Eye roll/VOR and 'Breathing' reflexes are absent, a fish is probably dead or unconscious (but note that VOR can be absent in some species when they are conscious, e.g. after live chilling, see below).

In the case of eye roll (VOR), the movement of the eye is observed when the fish is rocked from side to side. In a dead fish, the eye remains fixed in the skull. In a fish retaining some brain function, the eye rotates dorso-ventrally when the fish is rocked. Similarly, in the case of 'breathing', the operculum of the fish is observed when the fish is placed in water. In a dead fish, the operculum remains still. In a fish retaining some brain function, rhythmic movement is seen, similar to breathing in mammals and birds.

At present there are no behavioural methods for differentiating fish that are paralysed (but still conscious) from those that are unconscious. Both fish can show VOR and respiratory movements. Therefore, to avoid suffering, it is necessary to assume that a fish that shows these reflexes is conscious. The only fish that can be assumed not to be capable of suffering are fish that are dead (not showing breathing or VOR).

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Table 12-1. Method for assessing the state of consciousness of fish at slaughter (Kestin, Van de Vis and Robb, 2002)

	Self initiated behaviour		Response to Stimuli			Clinical reflexes	
Name	Swimming	Equilibrum	Handling	Pin prick	6V shock	Eye roll	opercula movement
Behaviour/ reflex	Swimming behaviour	Righting ability	Response to handling	Response to prick on lip	Response to stimulation on lip	Vestibulo-ocular reflex (VOR)	Rhythmic opercular activity
Observation place	In water	In water	In water or air	In air or water	In air	In air	In water or air
Procedure	Observe spontaneous swimming behaviour	Invert fish, observe righting response	Attempt to catch by tail and administer tail pinch, observe response	Prick lightly on lip with enough pressure to cause pricking sensation to human, observe response	Stimulate carefully on lip with 6V DC, observe response	Observe eye movement when fish is rolled from side to side through the vertical	Observe opercula for rhythmic movement (similar to breathing in mammals and birds)
Sequence of observation	1	2	3	5	6	7	4
Score 0*	No swimming	Unable to right	No response	No response	No response	Eyes fixed relative to head	No opercula movement
Score 1*	Slow or abnormal swimming e.g. upside down	Slow to right	Only slow or feeble response after tail pinch(s)	Slow and reduced response	Slow and reduced response	Partial VOR or one eye shows VOR	Slow or irregular movement
Score 2*	Normal swimming	Quickly rights	Immediate vigorous escape attempt on first touch/pinch	Head shake or escape attempt	Head shake or escape attempt	Eyes roll relative to the head whilst attempting to remain upright when fish is rolled	Regular opercula movement
				Some species show no response even when fish is fully conscious	1. Direct stimulation of muscles. 2. Some species show no response when fish is fully conscious	Needs careful observation see in some species	Needs careful observation in some species

*General comments, possible artefacts: This scoring system is too simplistic, *i.e.* all the reflexes are either present or absent. Some comments regarding the presence of combinations of reflexes and their interpretation will be helpful.

In summary:

- If care is taken that fish are neither paralysed, nor exhausted (*i.e.* if electrical stimulation or immobilisation due to live chilling or immersion in carbon dioxide enriched water have been avoided), if they do not show any muscular activity and do not show the Eye roll and 'Breathing' reflexes, then they can be considered unconscious. Where paralysis or exhaustion have occurred, this influences the responses and is discussed later

- If the fish shows Eye roll and Breathing reflexes but no co-ordinated activity or response to painful stimulation, it may be unconscious or just paralysed. It should then be given the benefit of the doubt and considered conscious.

- If a fish shows any co-ordinated activity or responds to painful stimulation, it is conscious.

12.3. ASSESSING THAT THE KILLING PROCESS IS NOT AVERSIVE.

One of the fundamentals of humane slaughter is that an animal should die without pain or fear. Stunning methods that induce immediate unconsciousness have the capacity to be humane, provided that no fish is allowed to recover after stunning. However, some methods do not induce unconsciousness immediately. It is therefore important to determine that in this case, the animal does not experience pain or fear whilst unconsciousness is being induced.

In practice, the behavioural responses of the animal, supported by some biochemical measurements, are usually used to identify that the animal is not experiencing pain or suffering during the killing process. If, for example, fish show increased activity after the start of the procedure - in particular agitated swimming or escape activity -, as salmon do during the early stages of commercial carbon dioxide narcosis (Robb *et al.* 2000b, Roth *et al.*, 2002), it is assumed that the process is aversive to the fish. However, absence of such activity does not necessarily mean absence of aversion. Some methods could induce immobilisation or exhaustion before unconsciousness, as ice slurry killing of sea bream and application of insufficient current during electrical stunning (Van de Vis *et al* 2003a) or rapid live chilling of Atlantic salmon. In this case, it may be necessary to examine the effect of the process on changes in stress hormone secretion or heart function. For example, cooling fish rapidly (as occurs in 'live chilling') leads to elevated plasma cortisol levels (Donaldson, 1981; Skjervold *et al.*, 2001), indicating that it may be stressful, and over time to disturbance of plasma osmolarity (Rorvik *et al.*, 2001). Such rapid live chilling also results in a marked decrease in the muscle pH, indicating increased muscle activity, which could be a sign of aversive activity (Skjervold *et a.,l* 2001). It has also been observed that cardiac rhythm in eels subjected to live chilling is irregular whilst the fish is apparently immobilised by the cold which indicates that the process may be stressful (Van de Vis *et al.*, 2003a).

Commercial practices:

Fish are often treated as one species when it comes to regulations and legislation governing welfare during farming or at slaughter. But, it is important to realise that a very wide number of species of fish are farmed, with an equally wide variety of ecological adaptations and evolutionary developments. These differences mean that different species of fish react differently to similar situations. For example, at a given environmental temperature, some species like trout die relatively quickly when removed form water into air, whilst others like eels or marine flatfish can take several hours. Similarly, in electrical stunning situations, eels require a much larger amount of stunning current than trout or salmon to render them unconscious. Species differences need to be taken into account when adopting particular procedures. Processes must be developed and optimised with respect to welfare specifically for each species. For example, it would be as unreasonable to assume that a process developed for killing trout in freshwater would be suitable for killing tuna in the sea as it would be to assume that a system developed for quail would be effective on ostriches.

General principles:

One of the differences in the environmental conditions for aquatic and terrestrial animals is that aquatic animals have a limited supply of oxygen. Depending on the habitat, fish have adapted to tolerate to various degrees hypoxic and hypercapnic environments. In general, freshwater species have a much higher tolerance to hypoxia and hypercapnia due to more variation in their environment. The main mechanisms for surviving in such environments is that besides being ectothermic, and thus with a reduced oxygen demand relative to endotherms, fish can maintain metabolic and neural activity by anaerobic glycolysis and use of aminoacids. Hypercapnia causes cerebral vasodilatation and increased cerebral blood flow in mammals to sustain neuronal activity and these effects are mediated via nitric oxide. By contrast, hypercapnia (75 mm Hg) induces these effects in rainbow trout (*Oncorhynchus mykiss*) independent of nitric oxide and has no effect on these variables in crucian carp (*Carassius carassius*) (Soderstrom and Nilsson, 2000).

Fish have also developed mechanisms to prevent metabolic acidosis induced depolarisation of neurons, by using the buffering capacity of elevated GABA in their brain. The degree of tolerance differs between species. Some fish species like eel, tilapia, cyprinids and goldfish are, compared to other fish, extremely tolerant and can survive at least 1 hour in hypoxic or hypercapnic water, while rainbow trout only survive 10 min (Kestin, Wotton and Gregory, 1991). Eel, turbot and African catfish can survive in air (out of water) for several days.

Whilst terrestrial animals like mammals and poultry are individually restrained before stunning, fish are often treated in large batches through transport, hauling / pumping, chilling, stunning and killing. With smaller species, as many as 3000 individuals can be stunned and killed as one batch (Wall, 2002). Individual handling or restraining fish prior to stunning to enhance treatment is rather difficult, but not impossible, depending on the size of the fish. However, it usually means that the fish has to be brought out of the water, which is known to release primary and secondary stress responses (Mitton and McDonald, 1994; Robb and Kestin, 2002).

In comparison to the slaughter of mammals and birds, very little research work has addressed the welfare issues of stunning and killing of fish. Even for widely farmed species like trout and salmon, there are many gaps in our knowledge relating to humane killing that need to be addressed before processes can be prescribed. For many farmed species, there has been no investigation into killing methods, humane or otherwise, on which to base sound judgement.

12.4. CLASSIFICATION OF THE METHODS

In this section, methods used to kill fish have been classified into 2 groups:

- stun/killing methods,
- killing without stunning.

Stunning methods that rely on exsanguination to effect the death of the fish are almost unused in fish because they are relatively slow to lose brain function after exsanguination (Robb *et al* 2000b) and few stunning methods induce a sufficient period of insensibility. It is important to note that some methods, such as percussive stunning and electrical stunning, can be primary stunning methods or stun / killing method depending on the parameters applied but; in practical application, they are almost always a stun / killing methods.

12.4.1. Stun / killing methods

12.4.1.1. Percussive killing

Percussive stunning is commonly used in the salmon industry by halibut fish farmers and by anglers to kill fish. The fish are removed from water, restrained, and a blow or repeated blows are delivered to the top of the head above the brain by a club or hammer until the fish is presumed dead. Typically, fish are out of water for 5 to 10 sec while they are restrained and positioned before the blow is administered.

The blow can be applied manually with a plastic club or 'priest', though semiautomatic percussive stunning devices (for example the MT4 or Si5) are becoming widespread in the salmon industry. In the MT4 apparatus, a pneumatic hammer with a flat head approximately 20 mm in diameter is housed within a support. The fish are manually pushed head first into a guide and when the snout of the fish touches a trigger, the hammer delivers a hard percussive blow to the head of the fish. The fish is immediately rendered unconscious, providing the blow is of sufficient force (Kestin and Robb, pers. comm.). Percussive stunning using these devices is reported to be irrecoverable in more than 99% of cases providing it is applied correctly. The carcass is slid out of the apparatus and exsanguinated. This is performed for flesh quality reasons, though it may also help to prevent recovery in inadequately stunned fish. When correctly set up, semi-automated percussive killing is highly effective at reliably rendering fish unconscious rapidly and without undue preslaughter stress. Current developments with semi automatic percussive killing include methods for percussively killing fish in water and encouraging fish to swim into the apparatus voluntarily and without need for an operator.

The impact energy required to stun or kill is dependent on the shape of hammer, where a flat hammer is more efficient than a round or cone head hammer (Roth, pers. comm.).

When correctly carried out, percussive killing is an efficient and humane way to slaughter fish. In addition, there are consistent reports that fish killed by percussive blows show reduced physical activity at slaughter, slower post mortem muscle acidification and slower onset of rigor mortis, compared to other commercial methods of killing farmed fish (Azam, Mackie and Smith, 1989; Marx *et al.*, 1997; Robb, 1998; Morzel *et al.*, 2002). These are all features associated with relatively little muscular activity ante-mortem indicating exposure to relatively little stress before and during the killing process

Currently percussive killing requires individual handling of fish. This requires more labour than batch killing methods and so may not be economically viable for some low value species of fish. However, research is progressing into automated systems of percussive killing, which may reduce labour requirements.

When the blow is correctly applied and is of adequate force, loss of movement and VERs can be immediate and permanent in salmon and trout (Kestin, Wotton and Adams, 1995; Marx *et al.*, 1997; Robb *et al.*, 2000a). When applied incorrectly or with insufficient force, unconsciousness is not immediate or consciousness is recovered after a short period of unconsciousness (Kestin, Wotton and Adams, 1995; Robb *et al.*, 2000b) and injuries to the fish can result, which will seriously compromise welfare. Maturing salmon (especially grilse, fish that mature after only 1 sea winter) require much more force to kill than immature salmon, due to changes in skull morphology during maturation, and therefore require a much greater impact energy. Due to variations in anatomy, not all fish species are suited for percussive killing. In some species of fish such as sea bream, African catfish or eels, skull morphology appears to prevent sufficient energy reaching the brain to render the animal unconscious (Van de Vis *et al.,* 2003a). In general, salmonids and flatfish can be successfully and humanely killed by percussion.

Description of effective use:

Operatives should be trained, examined and certified in the humane application of this technique.

The blow should be above or immediately adjacent to the brain.

When killing novel species, it is important that the location of the brain is determined by careful dissection and related to external anatomical markers so that the blow can be correctly targeted.

The blow should be of sufficient force to immediately render the fish unconscious. The adequacy of the blow may be determined subjectively by initially testing the device on a small number of fish.

The head of the fish should be free to move slightly in the direction of the blow in order to achieve the stun (percussive stunning works by accelerating the brain relative to the skull, not by crushing the brain).

After stunning, fish should be observed for several min (e.g. 10 min) for signs of recovery of reflexes (VOR and breathing) or motor function. If activity is observed, the fish should be re-stunned and the force of blows used to stun subsequent fish increased.

Monitoring points:

Adequate application of the method is indicated by:

- Immediate loss of VOR.
- Immediate cessation of respiratory movements (opercular activity).

- Clonic seizures, if any, is limited to one or two flaps of tail and minor muscular tremors.

- No reaction during bleeding.

Advantages:

Immediate onset of death can be achieved if adequately used.

Disadvantages:

Mis-hits occurs and conduce to poor welfare of the fish.

Defined criteria for effective use are currently limited to a few species.

The method is not adequate for large numbers of fish, unless it is mechanised.

12.4.1.2. Mechanically applied spiking, coring or iki jime

This method is similar to captive bolt stunning of mammals.

The fish are lifted from the water and a spike is driven into the brain through the top of the head using a pneumatically operated pistol.

Spiking is a killing method that needs good restraint of the fish prior to spiking and accuracy of application. It is not as easy to perform as percussive killing and is therefore only useful in species such as tuna, where percussive killing devices have not yet been developed. As with fish killed by percussive killing, fish killed by spiking show reduced physical activity at slaughter and consequently have slower post mortem muscle acidification and slower onset of rigor (Lowe *et al.*, 1993; Mochizuki and Sato 1994; Ottera *et al.*, 2002; Van de Vis *et al.*, 2002).

When spiking is correctly and very accurately applied, salmon or eels lose movement and consciousness immediately (Robb *et al.*, 2000a; Van de Vis *et al.*, 2002). As accuracy is very important, use of anatomical markers that allow the brain to be targeted accurately, such as the pineal window in tuna, are important. However, with smaller fish, such as salmon, the brain is harder to target and, as the fish make vigorous attempts to escape during spiking, the system can be prone to misapplication, with the spike being driven into the fish but not fully disabling the brain. In this case, depending on the amount of damage done to the brain, fish may be merely injured and disabled but not rendered unconscious (Robb *et al.*, 2000a).

Spiking without the aid of a mechanically operated device has been manually applied to tuna for many years. The fish are lifted from the water and a spike driven into the brain through the top of the head. In some cases, the fish are subsequently pithed with a rod or wire to destroy the upper part of the spinal cord and reduce carcass convolutions (Robb and Kestin, 2002). The period between capture and removal from water and spiking can vary from about 10 sec to up to a min. Death, as a result of manual spiking, is slow to achieve and the technique should not be used.

Modifications to spiking include so called captive needle stun / killing systems. These involve pneumatically firing a captive needle into the brain and injection of compressed air. It can cause immediate loss of SERs in eel (Van de Vis *et al.*, 2003a) and African catfish (Van de Vis, pers. comm.). Problems associated with normal spiking, such as the high precision of application required, apparently do not arise when stunning catfish by the captive needle method (Van de Vis *et al.*, 2003b). Unconsciousness and death is induced immediately (Van de Vis *et al.*, 2003b). Currently there are no commercial applications for this method

Description of effective use:

Only mechanical devices specifically designed for stun / kill of fish should be used.

High precision is required in its application. Such precision cannot be achieved in small fish, therefore this method should only be applied on larger fish (> 10 kg) which are individually restrained.

Operatives should be trained in the humane application of this technique.

The spike should be inserted deep into the brain to destroy the brain completely.

After stunning, fish should be carefully observed for recovery of reflexes or motor function for several min (e.g. 20 min) after stunning. If activity is observed, the fish brain should be macerated and the technique improved by greater focus on anatomical accuracy of stun and on training of operatives. Even so, some species will remain unsuitable for the use of the technique for anatomical reasons.

If recovery from spiking is observed, consideration should be given to pithing the fish after spiking.

For large fish weighing more than 10kg, such as tuna, pithing should be undertaken as a matter of course immediately after spiking to prevent recovery.

Fish may be bled after spiking but bleeding is undertaken for meat quality reasons, not to hasten death

Monitoring points

Adequate application of the method is indicated by the following signs:

- Immediate loss of VOR (check both eyes and restun if necessary).
- Immediate loss of respiratory movements.
- Clonic seizures, if any, is limited to one or two flaps of tail and minor muscle tremors.
- No reaction during bleeding.

Advantages

Immediate onset of death can be achieved if adequately used.

Disadvantages

Fish need to be taken out of water.

The methods requires considerable skill. It is very difficult to achieve 100% accuracy in many species of fish which conduce to poor welfare.

Misapplication leads to poor welfare of the fish

Investigation of the effectiveness of the method is lacking for many species.

12.4.1.3. Electrical stunning or stun / killing systems

In this section, electrical processes that deliver sufficient current to induce immediate unconsciousness, *i.e.* a stunning or stun / killing situation, are discussed. In practice, this process can be a stunning or a stun / killing method, depending on the parameters used and the species of fish. Both stunning and stun / killing processes are described together in this section.

Electrical processes that do not deliver sufficient energy to induce unconsciousness immediately, *i.e.* a sub-stun situation, are discussed in the section on 'electro immobilisation' below.

Electrical stunning systems are not at present widely used to slaughter farmed fish commercially, though small scale apparatus is available in some countries for farm gate sales. Recently, as a result of research, apparatus has become available for killing some species like eel, and considerable research is being undertaken to develop commercial systems for salmon and trout.

In most electrical stunning conditions, fish are stunned whilst in water, though semi dry systems (drained) have been used. Typically, a tank with electrodes attached to opposite sides is filled with water and fish. A current is passed between the electrodes, using the water and the fish within as a conductor. Provided certain parameters are met, the fish are immediately stunned. After a fixed period of electrical stunning, the current is turned off and the fish removed from water. In some systems (mainly those used for salmon), the fish are subsequently exsanguinated to kill them. In other systems (mainly those used for trout and eels), the fish are killed by the current and do not require exsanguination. The mechanism of death as a result of electrical application is not known. It does not appear to be related to fibrillation of the heart (Kestin and Lines, pers. comm.) as normal cardiac rhythm could be recorded post stun for a prolonged period. Death in these fish could be due to respiratory arrest or complete and irreversible depolarisation of the nervous system.

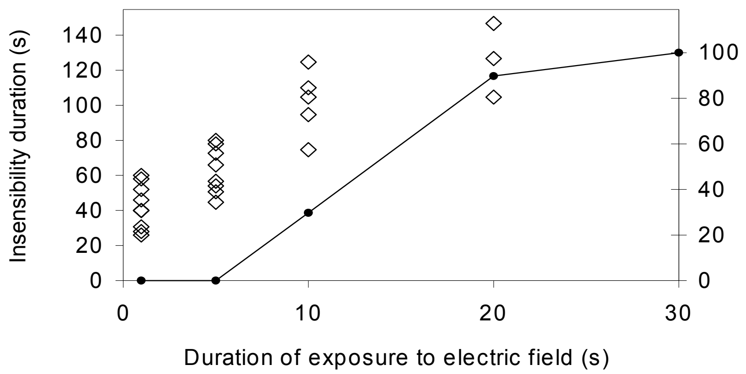
Semi automatic continuous throughput electrical stunning devises are being developed. There are numerous system designs and permutations of stunning duration, voltage and frequency that have been investigated or are currently in use for electrical stunning of fish. Some of the more consistent findings with these systems are reviewed below.

In most systems, 50 Hz AC is passed through a waterbath in which the fish are contained. Provided the electrical field is uniform and high enough to stun the fish (see below), loss of movement (Marx *et al.*, 1997; Robb *et al.*, 2002) and VERs or SERs is immediate (Kestin, Wotton and Adams, 1995; Van de Vis *et al.*, 2003a). If the current passed through each animal is not sufficiently large to stun the fish, they are immobilised during current flow and, after the current is switched off, strong aversive reactions are seen (Kestin, Wotton and Adams, 1995; see section on electro-immobilisation below).

If the fish are stunned and evoked responses lost, the fish enters a stage of mild tonic and clonic spasms that last approximately 20 – 50 sec in trout and salmon (Kestin, Wotton and Adams, 1995; Robb and Roth, 2003) and eel (Lambooij *et al.*, 2002) and African catfish (van de Vis unpublished results). A proportion of fish may be killed by this method. If they are not killed by the process (see below), recovery of consciousness is gradual, with most trout being fully recovered after 3 min (Robb *et al.*, 2002). In trout and eel stunned in fresh water (500 µs/cm), the effect of stunning parameters (electric field, frequency, duration etc) on induction and maintenance of unconsciousness, and mortality, has been investigated in some detail (Lines *et al.,* 2000; Robb *et al.*, 2002; Lambooij *et al*, 2002; Roth, 2003).

Recent experimental evidence indicates that it is important in most species that the fish are stun / killed by the set up. This is because studies show that it takes approximately 4.5 min for salmon to loose brain function as a result of exsanguination after gill cutting (Robb *et al.*, 2000a). Morzel *et al.* (2002) reported that turbot took more than 15 min before behavioural responses to stimulation were lost after exsanguination, whilst the period of insensibility induced by electrical stunning can be much shorter.

Table 12-2. The effect of duration of exposure to electric field on time to recovery of rhythmic gill motion (◊) and on mortality (-). Electric field 230 V/m RMS, 50 Hz AC 500 µs conductivity. 10 fish per exposure duration. From Lines *et a.,l* 2003



In general depending upon the species of fish:

- Higher stunning currents and longer electrical application times are associated with longer periods of unconsciousness and a larger proportion of fish killed by the process.

- Higher frequencies (up to 2000 Hz) are associated with shorter periods of unconsciousness and lower mortality (Roth, 2003).

Eels have been shown to be particularly resistant to electrical stunning and require high currents for at least 5 min to achieve reasonable periods of unconsciousness. If the water is simultaneously flushed with nitrogen, eels die without recovering consciousness (Lambooij *et al.*, 2002).

Summarising, in terms of welfare and commercial processes, electrical stunning has some potential advantages compared to some the other stunning methods:

- Large batches of fish can be stun / killed with a minimum of handling and restraint.

- Fish can be stun / killed in water, so the stressful event of removal from water is avoided.

However there are problems associated with electrical stun / killing systems:

- Carcass damage can be induced by electrical stunning and this is commercially problematic.

- Care must be taken to ensure that the fish are really stunned and not just electro-immobilised.

Requirements for effective use:

It is not really possible to fully define the correct parameters for effective use because adequate scientific information is lacking for many species of fish, however:

- Operators should be trained in the humane application of this technique.

- Equipment and procedures used should be in good working order to ensure efficiency.

- Equipment and procedures have to be properly and critically evaluated to ensure that the process is effective in humanely stunning or stun / killing the fish.

- The ratio between water and fish must be adequate for fish not to be deprived of oxygen during the period between loading and stunning. As a guide, no fish should be seen to be gasping or in respiratory distress in the period up to the application of stunning current. The duration between loading and stunning must be kept to the minimum.

- The electrodes must extend to the full length of the bath to ensure a uniform current distribution.

- The current applied must be adequate to immediately (< 1 sec) stun or stun / kill fish. This should be evaluated by measurement of EEG or signs of breathing and eye role or swimming behaviour from the end of application of electricity.

- Owing to the lack of published scientific information, it is not possible to describe the exact amount of current required to stun / kill each species of fish.

- The duration of the stun must be sufficient to ensure a distinct and sufficient period of unconsciousness. If fish are to be exsanguinated to kill them, then unconsciousness should last until death from blood loss. In practice, it is unlikely that this can be achieved. If fish are not exsanguinated, then the electrical parameters should stun and kill all the fish.

- Under stun / killing systems, no fish should survive the treatment.

- After stunning (and exsanguination if performed), a sample of fish should be carefully observed for recovery of EEG or reflexes or motor function for several min in fresh water. If any activity is observed, fish should be stunned immediately using a backup method such as percussion and the stun parameters should be adjusted so that no recovery is observed in subsequent batches.

Monitoring points:

Adequate application of the method is indicated by:

- Immediate loss of VOR.

- Immediate loss of respiratory movements.

- No recovery of VOR or respiratory movements.

- Tonic and clonic seizures present for a period after a short electrical stun period when current is turned off.

- No signs of recovery during handling or bleeding.

Advantages

- If properly performed, this can be an effective method of stun / killing.

- Immediate loss of consciousness.

- In a well designed system, preslaughter handling and restraint can be minimised or eliminated with consequent welfare improvement.

- High rates of stun / kill are possible.

Disadvantages:

- Very limited information is available for most species regarding the electrical parameters required to ensure systems are humane. Even for trout, salmon and eels, where there is the greatest experience some critical data is lacking.

- There are wide differences in stun / kill parameters for different species

12.4.1.4. Specialised stun / killing methods for large tuna

a) Shooting:

In Australia and Spain, some large tuna are killed by shooting (Villarejo *et al.*, 2002). The fish are crowded in the cage net and then, either shot in the head whilst free swimming or caught with a gaff (a long pole with a sharp hook on the end), pulled to the surface and then shot in the head with a 12 bore shotgun or 0.357 Magnum. The period between gaffing and shooting is not known but is likely to be about 30 sec. Shooting should result in immediate death if the shot is accurate. The system was developed to kill high value fish quickly to prevent damage and stress during escape attempts. There are, however, reports that the noise of the gun detonation causes vigorous escape attempts in the other fish in the nets (Robb and Kestin, 2002).

Description of effective use:

- Gaffing should be avoided for welfare reasons.
- Shot should be carefully aimed at brain.
- Second shot may be required if any signs of inaccuracy.

Monitoring points:

- Immediate loss of VOR.
- Immediate loss of respiratory movements.
- No recovery of VOR or breathing.
- No reaction during bleeding.

Advantages: not known.

Disadvantages:

- Gaffing causes pain.
- Fear caused by noise of the shot is stressful.

b) Electric Harpoon:

This method is being developed for large tuna. Two experienced divers enter the cage or pen the fish are contained in, one of them carrying the harpoon and the other an underwater switch. The harpoon head is connected to a power supply on board a ship through an insulated wire. A metal plate placed close to the ship acts as the earth electrode. The diver carrying the harpoon selects a tuna to be slaughtered and fires the harpoon into the tuna. If the operation is successful, the other diver switches on the power. The equipment delivers around 150 V with a current of between 1 and 7 A. The frequency and waveform that apparently achieve unconsciousness without causing unacceptable carcass damage are said to be 'a Shape Controlled Low Frequency

Complex' (Villarejo *et al.*, 2002). When the fish is stunned or dead, the diver releases the switch.

No information has been published relating to the welfare consequences of this killing method, but to ensure adequate current flows through the brain to achieve unconsciousness, it would be important for the harpoon to hit the fish near the head.

Description of effective use:

- Harpoon should be carefully aimed at the head.
- Second harpoon may be required if any signs of inaccuracy.

Monitoring points:

- Immediate loss of VOR.
- Immediate loss of respiratory movements.
- No recovery of VOR or breathing.
- No reaction during bleeding.

Advantages and disadvantages: not known

12.4.1.5. Potential stun / killing methods

a) Hydraulic shock

There are numerous accounts of explosive devices being used to kill fish and the welfare and quality implications of this killing method have been investigated (Robb and Kestin 2002). In these trials, the shock wave resulted in a stun provided fish were sufficiently close to the detonation (within the stunning range). Serious carcass damage in the form of haemorrhages within the flesh was however induced in areas adjacent to hollow gas filled organs (e.g. the swim bladder and gut). It was reported that the carcass damage incurred by the fish was of such a magnitude as to adversely affect the overall quality of the fish. This was mainly due to haemorrhages within the flesh. Fish exposed to the shock wave beyond the stunning range were disabled, suffered internal damage which would probably have been fatal, but were not rendered immediately unconscious.

Description of effective use: not known.

Monitoring points: not known.

Advantages and Disadvantages: not known.

b) Hypoxic water baths

Rainbow trout show signs of becoming unconscious after a few min (4 to 6 min) of exposure to hypoxia (Hylland *et al.* 1995). Experimental attempts have been made to kill trout or other freshwater fish, in water from which all the oxygen has been removed, either by degassing the water or by displacing the oxygen with an inert gas such as nitrogen or argon. These studies have shown that it is difficult to remove sufficient oxygen from the water to induce unconsciousness quickly (Kestin, Wotton and Adams, 1995). Maintenance of the hypoxic water is also difficult, because fish activity and the process of adding the fish enable atmospheric air to become dissolved in the water. In these studies, the fish showed aversive reactions during induction of unconsciousness albeit less than were induced in fish killed by carbon dioxide narcosis.

Description of effective use: not known.

Monitoring points: not known.

Advantages: not known.

12.4.2. Killing without stunning

12.4.2.1. Carbon Dioxide narcosis

Carbon dioxide is highly soluble in water and has a narcotic effect on fish placed in water saturated with the gas. Under commercial slaughter conditions of salmon and trout, carbon dioxide is bubbled continuously into a tub, tank or bath of water (sea water if appropriate). The pH of the water falls as it becomes saturated with carbon dioxide, and when it stabilises at about pH 4.5, the water is judged to be approaching saturation with the gas (Anon, 1995). Fish are then netted or pumped into the water and are left in the bath until movement stops. They are then removed and exsanguinated.

Modifications to the process outlined above include cooling the carbon dioxide saturated water to about 1°C, by the addition of ice. This has been found to result in a faster loss of physical activity in Atlantic salmon, but activity still continued for about 1 min (Robb, pers. comm.).

Carbon dioxide narcosis is an easy method to mechanise and requires little labour to manage. For this reason, it is popular in some countries where labour is expensive. Loss of consciousness in salmon stunned in carbon dioxide (judged by loss of VERs) takes approximately 6 min to induce (Robb *et al.*, 2000a) but fish species that are more resistant to hypercapnia can survive for much longer, eels and sturgeon were reported to show escape behaviour for more than an hour (Marx *et al.*, 1997; Robb, pers. comm.).

There is a substantial body of evidence to indicate that fish find immersion in a hypercapnic environment aversive. On immersion in the carbon dioxide saturated water, trout (Kestin, Wotton and Adams, 1995), salmon (Wall, 2002; Robb *et al.*, 2000a; Roth *et al.*, 2002), carp and eels (Marx *et al.*, 1997), show vigorous aversive reactions, swimming very rapidly and making escape attempts. This behaviour can last for about 3 min in salmon and trout (Robb *et al.*, 2000a; Kestin, Wotton and Adams, 1995 respectively) but up to 1.8 hours in eels (Marx *et al.*, 1997). Carp, trout and eels are all reported to show signs of increased mucus production during carbon dioxide narcosis (Marx *et al.*, 1997) which could be further indications that the process is irritating. The aversive reactions to carbon dioxide stunning have been reported to cause injury and scale loss. (Robb *et al.*, 2002; Akse and Midling, 1999; Roth *et al.*, 2002). There is no evidence to show that carbon dioxide has any analgesic or anaesthetic effects, just narcosis which does not imply any reduction in pain or fear.

Since killing facilities do not usually exchange the water during the killing process, it is likely that the fish are also exposed to hypoxia and this has been proposed to be the main aversive effect. However, similar behavioural reactions have been reported in fish exposed to high levels of carbon dioxide in a hyperoxic environment (Bernier and Randall, 1998). Based on this, it is suggested that fish find immersion in a bath of water saturated with carbon dioxide per se very aversive. Other parameters in the water are also likely to be aversive to the fish. These include the presence of highly active fish, elevated ammonia levels caused by the high metabolic activity. The high activity in the carbon dioxide stunning bath routinely results in gill haemorrhage, (Robb and Kestin, pers. comm.)

Because fish become immobile before loss of consciousness (Robb *et al.*, 2000a), there is a risk that fish could be exsanguinated or gutted whilst still conscious. Industry codes recommend that the fish should be left in the water for at least 4.5 min before exsanguination (Anon, 1995), but observations indicate that fish are often removed when all carcass movements stop after 2 to 3 min (Robb, pers. comm.). Under most practical applications of carbon dioxide narcosis, the fish are not rendered unconscious by the process and are killed by subsequent exsanguination, (Robb, pers. comm.). Failure to exsanguinate the fish effectively (which also routinely occurs) results in fish with some level of consciousness passing to the next stage of the operation and being eviscerated (Robb, pers. comm.).

If fish are removed from the carbon dioxide bath before all respiratory movements have been lost (usually before the fish has lost brain responsiveness), the fish can recover if placed in well oxygenated water. However if fish are not placed in clean water, or are left in the carbon dioxide solution for a prolonged period, the process leads to death. But as mentioned above, in commercial practice, fish are rarely left in the carbon dioxide bath for long enough to die (Robb, pers. comm.). Carbon dioxide narcosis is potentially a killing method but in commercial practice it is usually only a sedation method.

Description of effective use:

This method does not allow good welfare during killing and it is therefore difficult to prescribe conditions that would reduce suffering.

It should not be used on fish resistant to hypercapnia like eels, carp and marine flat fish.

Operators should be trained in its use and equipment setup to achieve the best possible results with this method.

Water should be fully saturated with carbon dioxide before fish are introduced and maintained saturated by further addition of carbon dioxide.

pH should be monitored at all times to ensure water remains saturated.

Fish should be left in the carbon dioxide bath for sufficient time to be killed (at least 6 min in the case of salmon).

Fish should be exsanguinated immediately on removal from the bath.

If fish are returned to water to bleed out, this water should also be saturated with carbon dioxide.

Monitoring points:

When carbon dioxide narcosis is used, the water should be kept clean and free from blood, if necessary by frequent replacement of water (to avoid fish experiencing this aversive situation before loss of consciousness).

Carbon dioxide levels should be monitored and maintained at or near saturation (to ensure induction of unconsciousness is as rapidly as possible).

Fish should be left untouched in the carbon dioxide bath for a period after all loss of carcass movement to avoid processing them whilst still aware.

In the case of salmon, the exposure period should be at least 6 min before the fish are exsanguinated.

If the system is taking longer than 2 min to stop all activity, killing must be stopped and the system parameters checked.

Advantages:

There are no welfare advantages.

Disadvantages:

There is a delayed loss of consciousness.

The method exposes fish to an aversive environment before loss of consciousness.

The method causes immobilisation, potentially allowing fish to be processed before loss of consciousness.

12.4.2.2. Asphyxiation

Fish are killed by this method simply by removing them from water and leaving them to die in air. This is a killing method and not a stunning method. Asphyxia is usually achieved by netting the fish from the water or pumping fish through a 'de-waterer' and placing them in free draining bins or boxes. No special equipment is required. Fish are left to die and when movement has ceased, they are processed. Most species of fish will eventually die when held in air, because their capacity for gaseous exchange is compromised when the gill lamellae collapse. Asphyxia by removing the fish from water is probably the most common method used for killing fish around the world (Robb and Kestin, 2002). Smaller farmed fish with low individual economic values like trout (*Oncorhynchus* and *Salmo spp.*) or tilapia (*Oreochromis spp.*) tend to be killed by this method. This method is commonly used when fish are brought on board fishing vessels.

Within the fish farming industry, death by asphyxia is a common method used for emergency killing. (Roth, pers. comm.).

The time required for the fish to die (based on abolition of VERs) is dependent on both species and temperature.

Table 12-3. The effect of killing by asphyxiation in air on time to loss of brain function and carcass movement

Species	Temp ˚C	Time to loss of brain function (min)	Time to loss of carcass movement (min)
Rainbow trout	14	3	28.6
Rainbow trout	20	2.6	11.1
Gilthead sea bream	22	5.5	4

From Kestin, Wotton and Gregory, 1991; Robb and Kestin, 2002; Van de Vis *et al.*, 2003a.

Because carcass movement ceases after consciousness is lost, this criterion cannot be used as an indicator of death. Removing fish from water is highly aversive to fish. In most cases, violent attempts to escape are made and a maximal stress response is initiated (Robb and Kestin, 2002). The procedure is widely used as an experimental stressor in studies of the stress response of fish (see for example Donaldson, 1981).

Description of effective use:

Because unconsciousness is not induced immediately, and the fish find the method aversive, this method cannot be considered humane whatever the circumstances.

Monitoring points:

Complete cessation of rhythmic respiratory movements and heartbeat. No VOR.

Advantages:

There are no welfare advantages.

Disadvantages:

The method causes a maximal stress response, maximal aversive reactions and physical activity.

There is a delayed loss of consciousness.

Handling and aversive reactions can cause injury to fish.

Loss of movement may occur well before loss of consciousness so unless care is taken, fish may processed while still sensible, substantially affecting their welfare.

12.4.2.3. Asphyxia in Ice / Thermal shock

In this section, asphyxia in ice/thermal shock or live chilling as a killing method is discussed. Asphyxia in ice means transfer from water at ambient temperature into different water or slush ice at a significantly lower temperature (temperature differential usually at least 10˚C), often followed by a draining of the water. The aim is to simultaneously chill, sedate and kill the fish by asphyxia.

Chilling of fish prior to killing by another method like exsanguination or carbon dioxide narcosis followed by exsanguination is also practised as a preslaughter handling step to sedate or condition fish and is discussed below in the section 'Preslaughter sedation'. By slow chilling is meant the gradual lowering of the temperature of the water the fish are contained in by refrigeration (at the rate of approximately 1.5˚C per hour), whilst the fish are supplied with sufficient oxygen to maintain consciousness. The aim in this application is to chill and sedate the fish whilst maintaining it conscious and alive.

Fish are killed by rapid chilling by first cooling them rapidly and then depriving them of oxygen. Fish are netted or pumped through a de-waterer and added to a relatively small tank or bin of chilled brine or ice/water slurry. If added to an ice / water slurry, the water is sometimes drained off after a period, leaving the fish surrounded by ice. The aim is that by depriving the fish of oxygen, either by draining the water or because the quality of the melting ice / water is sufficiently low, the fish will succumb to hypoxia.

This killing method is commonly used for farmed species such as rainbow trout (*Oncorhynchus mykiss*) (Kestin, Wotton and Gregory, 1991), gilthead sea bream (*Sparus auratus*), sea bass (*Dicentrarchus labrax*) (Smart, 2002), barramundi (*Lates calcarifer*) (Frost, Poole and Grauf, 1999), turbot (*Ctalurus punctatus*) (Robb, unpublished observation, Boggess *et al* 1973), African cat fish (Robb and Kestin, 2002) and eel at fish processors (Van de Vis *et al.*, 2003a). Some fish, such as turbot and gilthead are harvested direct from the cage / tank and packed live in polystyrene boxes and covered with ice (Morzel *et al.*, 2002).

Temperate species of fish take longer to lose brain function when killed in ice than air Compare Table 12-3 above with Table 12-4 below. In situations where the ambient temperature is low and the fish are already cold adapted (as happens in winter for rainbow trout for example), the fish will suffer no effect of the ice slurry and will die by anoxia in the water.

Table 12-4. The effect of killing by asphyxiation in ice on time to loss of brain function and carcass movement.

Species	Temp ˚C	Time to loss of brain function (min)	Time to loss of carcass movement
Rainbow trout	2	9.6	198
Eels	1	>12	>1
Gilthead sea bream	0.1	5.0	
African catfish	0	12	> 1 min

From Robb and Kestin, 2002; Lambooij *et al.*, 2002; Van de Vis, unpublished results

Table 12-4 indicates that asphyxiation in ice does not result in immediate unconsciousness. It has been proposed that when the differential between the ambient temperature of the fish and the ice slurry is relatively great, thermal shock may shorten time to loss of brain function. There is limited evidence to support this from the above tables. Sea bream at an ambient temperature of 22˚C were killed in air or ice slurry and both groups lost brain function at approximately the same time. If loss of consciousness had followed the same pattern as trout, the group killed in ice slurry would have been expected to retain brain function for much longer than fish killed in air. Thus thermal shock may have played a role in shortening the time to loss of brain function in this case. More work is required in this area to confirm these effects.

In general, many species of fish are adapted to survive in cold waters for many days by controlling their metabolism, as happens in nature during winter months. When fish are introduced to water at ambient temperature, they continue to swim actively. But, when introduced to an ice slurry, responses can be variable. Some species move around before slowing and becoming immobilised as their muscles cool. Other species like eel, gilt-head seabream, and African catfish, show vigorous attempts to escape on introduction to the ice slurry (Van de Vis, pers. comm.). Some species of fish like trout can acclimatise to water at near freezing but this process requires gradual cooling and takes several days.

There is a growing body of evidence that fish find introduction to iced water stressful. Elevated plasma cortisol levels have been reported (Donaldson, 1981; Skjervold *et al.* 2001), and over time plasma osmolarity is disturbed (Rorvik *et al.,* 2001). Also, the muscle pH drops markedly with introduction of salmon to cold water (Skjervold *et al.,* 2001), indicating increased activity during induction, probably related to aversive reactions. Some species of fish show escape behaviour on introduction to ice slurry (e.g. eel, African catfish, and gilthead sea bream). Other indices of stress like an increase in heart rate have also been observed (Lambooij *et al.*, 2002; Van de Vis *et al.*, 2003b). However, because of the progressive muscle paralysis induced by cooling, it is difficult to use behavioural indices to determine whether fish find rapid cooling aversive at later stages of the procedure.

Loss of brain function due to cooling can be reversed if the fish are removed from the cold water too soon. Fish transferred from iced water immediately after loss of VERs or SERs to water at normal temperatures recovered brain function and subsequently muscular movement quickly (Robb and Kestin, 2002).

Description of effective use:

Because unconsciousness is not induced immediately and the fish appear to find the method aversive, it should not be used.

Monitoring points:

Complete cessation of rhythmic respiratory movements and heartbeat. No VOR.

Advantages:

There are no welfare advantages.

Disadvantages:

The method causes a stress response, aversive reactions and physical activity.

The method can cause immobilisation allowing fish to be processed before loss of consciousness.

There is a delayed loss of consciousness.

Handling and aversive reactions can cause injury to fish.

12.4.2.4. Dry salt or ammonia bath

Eels are difficult to kill, and it has been commercial practice to kill them by placing them in a bath or tub and pouring dry salt (sodium chloride) or a 1% ammonia solution over them. The main aim of this process is to cause a desliming of the fish, as the slime interferes with later processing. They react very vigorously to the introduction of the chemical and writhe around in the tub, slowly losing motor function over a period of tens of min. Copious quantities of slime are produced. When the fish are limp and still, they are removed and processed, usually after approximately 15 min. The main purpose of the chemicals is to aid removal of the slime from the fish, but they also render the fish immobile and suitable for processing (Van de Vis *et al.* 2002) but if left long enough the fish are rendered unconscious and dead. However, in commercial practice, it is the processing (evisceration and filleting) which actually kills the fish by exsanguination. These are cheap, easy and relatively labour free ways to render eels suitable for processing and to simultaneously remove the slime.

Eels make extremely vigorous attempts to escape from a salt (Van de Vis *et al.*, 2003a) or ammonia bath (Kuhlmann and Munkner, 1996). Eels killed in salt take a long time to lose consciousness (based on VER data, more than 10 min (Van de Vis *et al.*, 2003a) based on behavioural data and reaction to stimulation greater than 25 min (Van de Vis, pers. comm.). Eels killed in ammonia solution apparently take 15 min to be killed, based on behavioural observations (Kuhlmann and Munkner, 1996). In both cases, it is probable that body movements stop due to muscular exhaustion. If the animals ultimately die as a result of the process, it is probably osmotic shock that kills them, but it is likely that most eels treated with salt are processed before they are dead. Killing eels in salt or ammonia is now considered inhumane in Germany and the Netherlands and since April 1999 has been prohibited in Germany (TierSchiIV, 1997 and 1999) and will be prohibited from 2006 in the Netherlands (Van de Vis, pers. comm.).

Description of effective use:

Because unconsciousness is not induced immediately, and the fish appear to find the method very aversive, it should not be used.

Monitoring points:

Complete cessation of rhythmic respiratory movements and heartbeat. No VOR.

Advantages:

There are no welfare advantages

Disadvantages:

The method causes a maximal stress response, maximal aversive reactions and physical activity.

The application of salt or ammonia appears to be painful.

There is a prolonged period of suffering.

The method is extremely distressing

The method can cause immobilisation, allowing fish to be processed before loss of consciousness.

There is a delayed loss of consciousness

Handling and aversive reactions can cause injury to fish.

12.4.2.5. Bleeding out / Exsanguination

Many large fish such as Atlantic salmon (*Salmo salar*) and tuna (*Thunnus spp.*) are commonly exsanguinated after stunning or killing to improve carcass quality, but exsanguination without stunning is also routinely used in some regions to kill fish e.g. salmon (Robb *et al.*, 2000a), large rainbow trout, cod, turbot (Robb, pers. comm.), and channel catfish (Boggess *et al* 1973). To achieve exsanguination, the gills are cut or manually pulled out, or the main blood vessels in the tail cut in the case of turbot, and the fish returned to water to bleed for a period of 10 to 15 min (Wardle, 1997). In some cases the isthmus is cut or the heart pierced with a knife. Flat fish are often bled by cutting the main blood vessels in the tail.

In the case of large salmonids, in commercial practice, exsanguination is the main cause of ultimate death in some of the procedures outlined in this document, e.g. carbon dioxide narcosis and rapid live chilling.

Robb and Roth (2003) both indicate that a functioning heart is not necessary for an efficient bleed-out and that provided major vessels like the gill arches, isthmus or heart are cut, there is little difference in the efficiency of exsanguination. Exsanguination of fish after stunning would appear to improve welfare without compromising quality.

Exsanguination without stunning is a relatively slow method for killing fish, Atlantic salmon killed by exsanguination took 4.5 min to lose VERs after gill cutting without prior stunning (Robb *et al.*, 2000a). The fish were reported to show clear signs of aversive behaviour for the first 30 sec whilst bleeding. Similarly, Morzel *et al.* (2002) reported that turbot took more than 15 min before behavioural responses to stimulation were lost after exsanguination, and Ruff, FitzGerald and Cross (2002b) reported aversive reactions in turbot after exsanguination and that it was 1-1.5 hours before the fish were dead. After gill cutting, brain function in African catfish lasted more than 10 min (Van de Vis, unpublished results).

The time for the fish to die by exsanguination appears to be temperature related, with salmon at lower temperatures taking longer to die (Robb *et al.*, 2000a).

Description of effective use:

Exsanguination without stunning is not humane and should not be used. When exsanguination is performed after effective stunning, major vessels must be cut to ensure rapid bleed out. Severance of all gill arches on both sides of the fish, or the isthmus, or piercing the heart directly, would appear to be the best method (Robb *et al.*, 2002). It is also essential that a sharp knife is used to cut the vessels. Pulling the blood vessels manually or cutting with a blunt knife could result in partial occlusion of the vessels and a slower subsequent bleed out.

Monitoring points:

Complete cessation of rhythmic respiratory movements and heartbeat. No VOR.

Advantages:

There are no welfare advantages to exsanguination before the onset of unconsciousness.

After a non-lethal stun, exsanguination can be used to ensure no recovery from the stun.

Disadvantages:

The method causes a maximal stress response, aversive reactions and increased physical activity.

The method can cause immobilisation allowing fish to be processed before loss of consciousness.

There is a delayed loss of consciousness.

Handling and aversive reactions can cause injury to fish.

12.4.2.6. Electro-immobilisation / Electrostimulation / Physical exhaustion using electrical shocks

In this section, electrical processes that do not rendered fish immediately unconscious are discussed. Electrical processes that deliver sufficient energy to induce immediate unconsciousness, *i.e.* a stunning situation, are discussed in the section Electrical stunning / Electrical stun / killing systems.

Some electrical systems used in Europe to kill trout and eels passes low voltage AC waveforms (< 1 V/cm) through a solid mass of live fish for several (> 5) min (Kestin, pers. comm.). The fish are not rendered unconscious but are electro-immobilised due to the electro-stimulation of the muscles. The muscles of the fish become completely exhausted and the fish are immobile when they are processed 10 min later. If fish are removed from the mass during the early stages of electrical stimulation (in trout up to 3 min, in eels up to 0.5 min) they are seen to have respiratory movements and, if returned to fresh water, they swim away immediately (Lambooij *et al.*, 2002; Van de Vis *et al.*, 2003a; Kestin, pers. comm.).

The method prescribed in the legislation of one Member State for electrical stunning of eels appears to be, in essence, an electro-immobilisation system (Lambooij *et al.*, 2002). Based on behavioural observations made on trout in 500 µs water, voltages less than 2 V/cm are likely to electro-stimulate trout rather than stun them.

This killing method apparently does not cause carcass downgrading problems sometimes associated with electrical stunning. However, energy reserve depletion caused by the electrical stimulation leads to conditions of low pH immediately post mortem and rapid rigor onset (Azam, Mackie and Smith, 1989), similar to fish which have undergone vigorous exercise immediately premortem (Robb *et al.*, 2000b).

Description of effective use:

This method cannot be considered humane.

Monitoring points:

Complete cessation of rhythmic breathing movements and heartbeat. No VOR.

Advantages:

There are no welfare advantages.

Disadvantages:

The method exposes fish to painful electric shocks.

Fish are paralysed during the application of the current, masking any signs of aversive reactions and physical activity.

The method can cause immobilisation, allowing fish to be processed before loss of consciousness.

There is a delayed loss of consciousness.

12.4.2.7. Decapitation

Decapitation is currently used as a means of killing eels on a small scale at fish mongers. Eels are held on a board and the head is completely severed. The heads are discarded and the carcasses processed after movement stops. Decapitation is unsuitable as a killing method for many species of fish as their body shape makes adequate manual restraint difficult and prevents its effective application.

Verheijen and Flight (1997) report loss of reactions in severed eel heads 30 min after decapitation and Van de Vis *et al.* (2003a) reported that it took 13 min before brain function, determined using EEGs, was lost after decapitation. Thus, decapitation would appear to expose eels to considerable periods of suffering.

Van de Vis (2003a) established that no recovery in brain function occurred when African catfish were decapitated after a 5 sec electrical stun, thus decapitation after stunning would appear to improve welfare.

Description of effective use:

Decapitation without prior stunning cannot be considered humane and there are no criteria for effective use.

Monitoring points:

Complete cessation of rhythmic breathing movements and heartbeat. No VOR.

Advantages:

There are no welfare advantages.

Disadvantages:

Restraint induced stress.

Pain is due to neck cutting in conscious animals.

There is a delayed loss of consciousness.

12.5. PRESLAUGHTER IMMOBILISATION METHODS USED TO REDUCE MOVEMENT TO FACILITATE KILLING OR PROCESSING

Some commercial killing operations incorporate a preslaughter handling step immediately prior to the application of a killing method which aims to reduce the activity of the fish. There are two main reasons for this:

- Firstly, reducing the activity of fish during the application of a killing method can make the operation easier and thus, the accuracy of the process can be improved (it is much easier to percussively stun, spike or exsanguinate a still fish than one who is fighting to escape).

- Secondly, reducing fish activity at slaughter leads to improved carcass and meat quality.

This pre-slaughter handling step does not induce unconsciousness and is therefore not a stunning method. It must be followed by a humane stunning or killing method.

12.5.1. Preslaughter sedation with anaesthetics

No use of pre-slaughter anaesthetics for fish is permitted for fish produced or imported into the EU from countries where the practice is currently allowed (Council Directive 2001/82/EC; Council Regulation, EEC/2377/ 90.)

Fish anaesthetics or sedatives based on eugenols have recently been developed and marketed outside of the EU for use as an aid to killing fish. One particular combination is marketed under the trade name AQUI-S™ (AQUI-S New Zealand). When introduced into the water at an approximate concentration of 17ppm, salmon lose motor function and responsiveness to stimulation after about 30 min (Robb *et al.*, 2000b). The fish are then netted and killed by percussion or spiking and show no physical activity or aversive reactions to handling (Robb, 1998; Goodrick *et al.*, 1998). AQUI-S™ is used commercially in Australia, Chile and New Zealand as a preslaughter sedative during salmon killing. Fish like eels, which are relatively resistant to anaesthetics, require higher concentrations (Van de Vis *et al.*, 2002).

Isoeugenol (the anaesthetic compound in AQUI-S) has true anaesthetic properties (Robb, pers. comm.) and fish sedated before slaughter appear to suffer far less distress than normal fish when removed from water for stunning. Unlike induction of anaesthesia with some anaesthetics (e.g. MS222) induction of sedation with AQUI-S does not appear to be stressful, based on observation of behaviour (Kestin, Robb and Van de Vis, pers. comm.) though at high concentrations eels showed attempts to escape from the tank, which is indicative for aversion (Van de Vis, pers. comm.)

There are several reports that aspects of flesh quality in salmon and rainbow trout killed after sedation with AQUI-S™ are improved. (Goodrick *et al.*, 1998; Robb *et al.*, 2000b; Jerret, Stevens and Holland, 1996; Van de Vis *et al.*, 2002).

Note that according to the EU regulation 2377/80, the use of Clove oil and thus Aqui - S is not permitted for use on food grade fish.

12.5.1.1. Description of effective use

This method is not a stunning or killing method. It should only be considered as a preslaughter sedation step.

Operators must be trained in the effective use of the method in order to achieve best practice.

Fish should be exposed to an adequate concentration of Isoeugenol for sufficient time to induce full sedation. Several batches of fish at a time can be exposed to the anaesthetic, as salmon have been shown to survive for over 3 hours under full anaesthesia and recover with no signs of distress.

Water quality, especially oxygen, must be monitored throughout and supplementary oxygen must be available if required.

Typically, fish should show no response to handling and will tend to show complete loss of equilibrium.

Once adequately sedated, fish should be stunned by an accepted humane method.

12.5.1.2. Monitoring points

Depth of sedation should be monitored by examining responses to stimulation. Fish are adequately sedated when all responses to stimulation are lost.

12.5.1.3. Advantages

Reduced stress associated with handling before slaughter.

The method can render fish unconscious before major handling steps.

12.5.1.4. Disadvantages

These substances are not avalaible for use on fish produced or imported in the EU because there is no evaluation on food safety aspects.

12.5.2. Preslaughter sedation by slow live chilling

In this section, live chilling of fish prior to killing as a preslaughter handling step to sedate or condition fish is discussed. Live chilling means the lowering of the temperature of the water the fish are contained whilst the fish are supplied with sufficient oxygen to maintain consciousness. The aim with this process is to chill and sedate the fish whilst maintaining it conscious and alive.

Live chilling followed by asphyxia in ice as a killing method is discussed above. The aim with this process is to simultaneously chill and sedate and kill the fish by asphyxia.

Fish are ectotherms and as such can adapt to natural fluctuations in ambient water temperature. But, this process can take several days; they cannot adapt to more rapid changes. Therefore, if fish are chilled relatively rapidly from an ambient temperature of over 10°C to one close to 0°C, they become cold-paralysed.

Chilling as a preslaughter conditioning / sedation step is achieved in two ways.

Salmon are sometimes conditioned and sedated prior to killing during transfer from production cages to a slaughter station in well-boats.

Chilling is achieved by gradual reduction of the temperature of the water the fish are contained in by refrigeration. The rate of temperature reduction is approximately 1.5˚C per hour (Michie, pers. comm.). In some cases, oxygen or air is supplied to the fish during the journey to prevent hypoxia. In other cases, carbon dioxide levels from respiration are allowed to rise apparently to further sedate the fish.

As a result of cooling, the fish are said to become sluggish without signs of aversion and do not subsequently respond vigorously to handling. The fish lose motor function but are not rendered unconscious (Roth, 2003).

More rapid live chilling is achieved by pumping fish from a cage, either at a farm or more commonly at a holding facility (lairage), into water between 1 and 5°C, in a killing and processing station. In this case, oxygen and carbon dioxide may or may not be supplied and after a period of chilling, the fish are transferred to a bath for carbon dioxide narcosis or are exsanguinated by cutting the gills (Robb, pers. comm.).

In this case, if the ambient temperature of the water from which the fish are pumped is high (over 10°C), the large, rapid drop in temperature results in the fish being stimulated into vigorous activity on entering the chilled water. The fish then become exhausted over a period of about 15 to 20 min, though they may retain some activity for up to 30 min. If the drop in temperature is low (as may happen in the winter with salmonids and other temperate species of farmed fish) the fish do not react, but nor do they become affected by the cold in terms of slowing muscle activity down. This means that at the next stage of the process (normally exsanguination), they are fully conscious and active and respond with great vigour to the cutting of their gills (Robb, pers. comm.).

A low dose of carbon dioxide and oxygen is sometimes added to the water, the carbon dioxide to provide a narcosis, while the oxygen to attempt to keep the fish quiet during the chilling process. There is no convincing evidence that either of these methods significantly reduce fish activity in response to the aversion (see above for effects of addition of gas to the water).

Rapid live chilling leads to release of primary stress responses, such as increased plasma cortisol levels (Skjervold *et al.,* 2001), and over time also disturbance of plasma osmolarity (Rorvik *et al.* 2001). It also causes a large drop in muscle pH indicating a large amount of muscle activity (Skjervold *et al.,* 2001). Nor is there any evidence to show that it stuns salmonids, and many fish are exsanguinated or processed fully conscious (Robb, pers. comm.). Roth (2003) showed that when salmon were exposed to 2°C in carbon dioxide saturated seawater, aversive behaviour and flight reactions were present. Erikson (2002) reports the use of chilled seawater at 1°C for up to 4 hours to subdue Atlantic salmon prior to carbon dioxide narcosis. In this case, the fish were torpid on removal from the chilled water and induction of carbon dioxide narcosis. Live chilling thus raises many questions with regard to welfare.

Note that after live chilling the VOR reflex may be reduced or absent and respiratory movements are very slow, but depending on the species, the fish may still retain some consciousness (assessed by EEGs, Van de Vis, unpublished results). Therefore, caution needs to be exercised when assessing fish that may have been paralysed when monitoring reflexes.

12.5.2.1. Description of effective use

Because unconsciousness is not induced, and the fish appear to find the method aversive, it should not be used.

12.5.2.2. Monitoring points

None.

12.5.2.3. Advantages

There are no welfare advantages.

12.5.2.4. Disadvantages.

The method causes a stress response, aversive reactions and physical activity.

The method can cause immobilisation allowing fish to be processed before loss of consciousness.

There is no of consciousness.

Handling and aversive reactions can cause injury to fish.

Table 12-5. Killing, stunning and stun/killing methods used for fish in Europe

	Asphyxia	Ice slurry	Salt bath	Aqueous NH3 solution	Exsanguination	carbon dioxide	Electric	Percussion
Mode of action	***Kill***	***Kill***	***Kill***	***Kill***	***Kill***	***Kill***	***Stun or Stun/kill***	***Stun/kill***
Northen Europe								
Atlantic Salmon	NK	Yes	No	No	Yes	Yes	Stun	Yes
Rainbow trout	Yes	Yes	No	No	Yes	Yes	Stun/kill	Yes
Halibut	NK	Yes	No	No	NK	NK	No	Yes
Cod	No	NK	No	No	Yes	NK	No	Yes
Eel	No	No	Yes	Yes	No	No	Kill	No
African catfish	NK	Yes	No	No	NK	No	Stun	Yes
Southern Europe								
Gilthead Seabream	NK	Yes	No	No	No	No	No	No
Seabass	NK	Yes	No	No	No	No	No	No
Turbot	No	Yes	No	No	Yes	No	No	No

NK not known

13. APPLICATION OF ON-FARM KILLING METHODS FOR DISEASE CONTROL PURPOSES

13.1. INTRODUCTION

Killing of animals for disease control purpose is used to prevent the spreading of contagious diseases whether the outbreak has occurred on the farm itself or in contiguous areas (EU Scientific Veterinary Committee, 1997). This includes the complete depopulation on a herd / flock or area basis, usually in relation to OIE List A diseases.

For killing animals for disease control purposes, some important points should be considered.

To minimize the risk of disseminating disease agents and according to different Council Directives covering the control of disease, the animals affected by the disease shall be isolated and killed on-site, without delay. Killing on-site reduces the risk of the possible spread of the agent into the environment and leads to a focussing of e.g. cleaning, disinfection measures in one place.

Biosecurity is an important consideration and, for this reason, non-invasive killing methods might be preferred.

Handling and restraint requirements are different from those in slaughter facilities. Availability of restraint may make certain killing methods more practical than others.

During the application of the disease control measure, animal welfare requirements must always be safeguarded even in an emergency.

On-farm killing of animals shall not only ensure that the killing is done in a humane way, but also ensure the biosecurity and safety of personnel.

As on-farm killing of animal for disease control is a sporadic procedure, to guarantee the effectiveness, the personnel involved should be trained and undergo periodical training to maintain their skills. The killing of animals for disease control purposes should take place under the direct supervision of a veterinarian, who controls the effectiveness of procedures.

In addition to the practical problems, killing of pregnant animals on the farm or in a slaughterhouse imposes an aesthetic problem. However, recent advances in science and neurophysiological understanding of the onset of 'awareness' in fetuses tend to provide some answers to this dilemma. When pregnant animals are killed, their fetuses die in utero from hypoxia and hypercapnia. Scientific data regarding whether the fetus is able to feel pain before they die, are lacking. Close *et al.* (1996) concluded that cortical processes may become possible when the neural tube develops into a functional brain, and therefore the time at which euthanasia of the fetus should be considered is from the second third of gestation in large animal, and from the second half of incubation period in poultry. However, it has not been demonstrated that the physical response to potentially painful stimuli observed during late gestation is linked to perception of pain (Mellor and Gregory, 2003). Mellor and Gregory (2003) suggested the onset of breathing and the associated increase in blood oxygen to levels above those seen in the fetus are essential to induce arousal and awareness. Therefore, the authors concluded that immature fetuses that cannot inflate their lungs with air, or mature fetuses that are prevented from doing so, would not attain conscious state. In addition, brain electrical activity of fetuses in utero decreases or becomes flat within 1-2 min of occlusion of umbilical cord, and hence deprivation of oxygen to the brain. Therefore, the authors suggested that it is inconceivable that fetuses that are already be unaware could be aroused or could suffer due to slaughter of pregnant animals. Furthermore, within seconds of neck cutting the pregnant animals, the blood supply to the brain and all other maternal tissue, including the uterus, of the dam drops rapidly. This would induce changes in the fetuses that are similar to those occurring after the occlusion of umbilical cord. Under both scenarios, the fetuses would die without suffering. On the other hand, if mature fetuses are removed from the uterus after slaughter of the dam and allowed to breathe air and elevate their blood oxygen levels that are compatible with awareness before the complete loss of spontaneous electrical activity in their brain, then they would respond to noxious stimuli (become conscious). Whether or not the fetuses are kept in the uterus after slaughter of the dam, gasping and movement by fetuses that cannot breath air or are prevented from breathing air does not indicate suffering.

Based on these, the NAWAC (National Animal Welfare Advisory Committee, New Zealand, 2001) has provided some guidelines that are summarised below:

1) Pregnant animals must be stunned effectively prior to slaughter and they should remain so until death occurs through bleeding.

2) Shortage of oxygen in the foetal brain prevents foetal suffering and therefore, where practical, leaves the fetus in the uterus until it is dead.

3) The earliest removal time is 5 min after the maternal neck cut or chest stick.

4) Lung inflation must be prevented if a fetus is exposed to air, either by keeping its head inside the amniotic sac, by clamping its widpipe (the trachea), or placing a plastic bag full of water over its head.

5) Fetuses exposed to air may be killed by neck cut, decapitation or destruction of its brain with a captive bolt (Mellor, 2003).

13.2. MECHANICAL METHODS

13.2.1. Free bullet

Three types of firearms are commonly used: handguns, rifles, and shotguns. Handguns are ideal for shooting at close range (less than 10 cm), shotguns at a distance between 5 and 25 cm, and rifles for long distance (few meters) shooting (Longair *et al.*, 1991). Telescopic devices fitted to rifles increase the accuracy of shooting over longer distances. All three are loaded with a cartridge that contains explosive primer, gunpowder and the bullet. The impact of the firing pin on the cartridge ignites the primer and causes the powder to burn rapidly, thus discharging the bullet down the barrel. The seal created between the bullet and the surface of the barrel keeps the gas produced by the burning gunpowder trapped behind the bullet once it leaves the cartridge case. As a result, the bullet continues to accelerate until it exits the firearm at the muzzle. There are three basic determinants of the muzzle velocity: the amount of gunpowder in the cartridge, the bullet's mass, and the length of the barrel (Fargo and Miclau, 1997). The amount of gunpowder that could be used is limited by the strength of the barrel and the amount of recoil produced. The greater is the bullet's mass, the harder is to propel. The longer the barrel, the more time it takes for the gas pressure to propel the bullet, and higher is the muzzle velocity. Once the bullet exits the barrel, the gas pressure dissipates. Rifles, with longer barrel, are able to attain significantly higher velocities than handguns, and could be used for long range shooting.

Table 13-1. Type of bullet and strength of calibre used for shooting animals.

	Animal group	Bullet	Calibre (mm)
Handguns	Bulls Cattle Calves Sheep and goats Pigs < 100 kg Pigs < 100 kg Horses	Round nose Soft/hollow Soft/hollow Soft/hollow Round nose Round nose Soft/hollow	9 9 5.5 5.5 5.5 9 9
Rifle	Bulls Cattle Calves Sheep and goats Pigs < 100kg Pigs > 100kg Horses	Round nose Soft Soft Soft Round nose Round nose Soft	9 9 5.5 5.5 5.5 9 9

Rifles and handguns are classified by the calibre, which indicates the diameter of the bullet, in mm or thousandths of an inch, depending upon the country. No scientific data are available regarding the appropriate calibre to cause the immediate death according to the specie and age. Based on the recommendation of the European Scientific Committee (1997) and the Humane Slaughter Association (1999), available information is presented in Table 13-1. Generally, a 5.5 mm (0.22 inch) calibre bullet fired from either a handgun or rifle is recommended for humane killing of calves, sheep, and pigs weighing less than 100 kg. Killing of bulls, cows, horses and pigs weighing more than 100 kg requires larger calibres such as 9 mm (0.38 inch) because of thickness of the skull (Longair *et al*, 1991; Baird, 2000).

The shotgun is a weapon designed to fire through a smooth bored barrel multiple pellets, or shot, that spread in a diverging pattern after they leave the muzzle. The shotgun shell consists of a cylinder with primer and gunpowder at its base. The projectile portion of the shell, the pellets, is separated from the gunpowder by plastic or cardboard wadding, the pellets spread in flight and after hitting the target (head), they produce massive internal damage of the brain tissue.

Shotguns are classified by the diameter of the bore in mm, and by the “gauge” which indicates the weight of the pellets fired by the weapon in fraction of a pound. For killing animals, a 0.410 gauge and 12 bore shotgun should be used. (EU Scientific Veterinary Committee, 1997).

Muzzle velocity of bullets from a 5.5 mm calibre rifle and 12 bore shotgun, is 360 to 400 m/sec, which is significantly higher than the velocity of bolts fired from a penetrating or non-penetrating captive bolt (Finnie, 1997).

The outcome depends upon the degree of brain damage inflicted by the bullet, which is largely dependent on the characteristics of the firearm, the nature of the bullet and the accuracy of the shot. When the bullet penetrates the brain, it produces a permanent haemorrhagic wound cavity in the brain as the bullet passes through due to laceration and crushing. In addition, the release of energy into the structures adjacent to the path of the bullet causes intracranial overpressure and transitory cavity, enhancing the cerebral damage (Finnie, 1997). This damage will render the animal instantaneously insensible and the destruction of the brainstem (which controls breathing and the cardiovascular system) will prevent any possibility of recovery, thus killing the animal outright.

Bullets are made from a mixture of lead and tin. The appropriate bullet regarding the species is shown in Table 13-1.

Hollow point and soft nose bullets are design to expand to double the original bullet diameter on impact, greatly enhancing the mushrooming effect or expansion on impact, imparting more energy to the neural tissues and increasing tissue destruction (Fargo and Miclau, 1997). Shotguns produce multiple projectiles consisting of several lead pellets and a lead disc in a polyethylene sleeve. This bullet, once it penetrates the tissue, produces massive damage (Blackmore, 1985). Hollow point, soft point or multiple projectiles normally cause penetrating wounds (Finnie, 1997). That means that the bullet enters the cranial cavity but does not pass through it, while it is retained within the cranium. Retention of the bullet within the head sometimes causes internal ricochet producing multiple haemorrhagic tracks through the brain. These types of bullets may not be able to penetrate the skull of mature bulls and pigs with a heavy bone structure (Blackmore, 1985).

Round nosed bullets are encased within a metal jacket that avoids the deformation or fragmentation of the bullet in the tissue and increases its kinetic energy (Fargo and Miclau, 1997). This type of bullet generally produces a perforating wound. That means that the bullet traverses the cranial cavity and leaves through an exit wound (Finnie, 1997). With perforating wounds the brain damage is often focal and not necessarily associated with loss of consciousness unless some vital structure is damaged (Finnie, 1997). These bullets are recommended to kill animals with heavy bone structure.

Head injuries caused by 12-bore shotgun pellets are generally more severe than those induced by a 5.5 mm calibre firearm due to multiple wound tracks produced by individual pellets.

13.2.1.1. Species

In cattle, the ideal point of shooting is in the middle of the forehead, at the crossing point of two imaginary lines drawn between the middle of each eye and the centre of the base of the opposite horn, or to a point slightly above the opposite ear in hornless animals. In some circumstances (e.g. where close range shooting is not possible), rifles may be used to shoot animals in the temporal region but the impact of the bullet must produce death.

For polled sheep, the aim of the firearm is the highest point of the head (front or crown position) in the mid-line, pointing straight down to the throat. The ideal shooting position for horned sheep is the position just behind the middle of the ridge that runs behind the horns (poll position described under captive bolt stunning of sheep). Then the barrel should be aimed towards the throat. In some sheep, the mass of the horn on the forehead can have little or no target area. In that case, shooting through the temporal bone should be used, as in other horned species (reindeer). A shot between the eyes is too low and should not be used under any circumstances (HSA, 1999).

For pigs, there are two options: a frontal and a temporal site (Blackmore, 1985). Recommended placement of the bullet while using the frontal site is in the centre of the forehead slightly above a line drawn between the eyes. Proper placement or aim of the muzzle is particularly important since the brain is relatively small and well protected by sinuses. An alternative site is the temporal region. The pig is shot from the side of the head so that the bullet enters the skull at a point midway between the eye and the base of the ear on the same side of the head. The bullet should be directed horizontally into the skull. This method is preferred for adult pigs due to the heavier bone structure of the front of the skull.

Ideal shooting position for horses is the point of intersection of a diagonal line taken from the base of each ear to the medial canthus of the eye on the opposite side (Oliver, 1979). The firearm should be aimed towards the neck to avoid free bullets exiting. The projected trajectory of the bullet appears to be also determinant of the efficiency of killing. Precautions should be taken to ensure that the bullet does not deviate laterally from a midline path (Millar and Mills, 2000), by keeping in line with the neck. Proper placement of the bullet is essential and best achieved by holding the firearm within a close range of the intended target. The animal should be treated with a calm and reassuring manner to reduce any anxiety that they may have. It may be advisable to sedate fractious / agitated animals before shooting them. Food may be placed in front of the animal to facilitate taking aim and shooting (Longair *et al*, 1991).

Persons involved in shooting should be properly trained, competent, and licensed. The firearm and the ammunition must be inspected and maintained according to the manufacturers specifications.

For human safety reasons, free bullet firearms should not be used indoors or in confined areas with concrete flooring, but instead used outdoors on soft ground or appropriate backdrop (manure heaps, hay or straw stacks, etc) where there is no risk of ricocheting bullets. It is also necessary to make sure that no person or animal may move between the target and backdrop area.

13.2.1.2. Description of effective use of firearms

The animal's head must be suitably presented to allow the operator to kill the animals by a single shot at the indicated position.

There must be an appropriate selection of cartridge, calibre and type of bullet.

Storage and maintenance of the gun must be appropriate according to the manufacturer guidelines.

Sufficient energy is necessary to penetrate the skull and damage the brain.

Highly skilled and licensed personnel (e.g. hunters, army, police …) are required.

Death must be confirmed in each animal.

Free bullets must be use outdoors and on soft ground to avoid the risk of ricocheting bullets and safeguarding human safety.

13.2.1.3. Monitoring points

Effective killing with a free bullet produces the following outwardly signs (HSA, 1999):

- Animal collapses immediately after the shot and stops breathing.
- Carcass can be 'tonic' or relaxed.
- Eyes have a fixed and glazed expression.
- No corneal reflex.
- Convulsions may occur after a lapse of up to 1 min.
- Pigs go very fast (<5 sec) into severe clonic convulsions with uncoordinated kicking and paddling movements of the legs.
- Death is confirmed by the absence of breathing, pupillary and corneal reflexes.

13.2.1.4. Advantages

When properly carried out, the bullet causes massive brain destruction, immediate unconsciousness and consequent death.

Free bullets may require less restraint of the animal, minimising stress induced by handling and human contact. The method is suited for killing of animals that are difficult to restrain for other killing methods due to their resistance.

Under practical on-farm conditions, free-bullet weapons may be one of the few effective killing methods available (AVMA, 2000).

13.2.1.5. Disadvantages

The risk of misfired bullets increases with longer distances from the animal. Bullets that miss the vital target organ (brain, heart) may cause injury, pain and distress and may result in a flight reaction.

Availability of specialised expertise may be limited.

Killing of animals with free bullets completely destroys the brain and subsequent examination of the brain for diseases may be more difficult.

13.2.2. Penetrating Captive bolt

Penetrating captive bolt and its application to the different species and post-stun reflexes are described in earlier chapters.

Penetrating captive bolt induces immediate insensibility, and sometimes death (e.g. in poultry), by physical destruction of the cerebral hemispheres and the brainstem depending upon the species of animal and the properties of the gun (bolt diameter, penetration depth and velocity) and power of cartridge or compressed air. However, scientific data about its efficacy in inducing death in large animals are limited. Therefore, pithing should be performed immediately after the shot in order to ensure the death of the animal (Finnie, 1993), although this may be difficult to perform in young lambs and piglets. Practical experience shows that it is likely that young animals may be killed instantaneously by the use of a captive bolt, although scientific data are lacking.

As the captive bolt should be held in contact or slightly away from the head, depending upon the make and model (see stunning methods in earlier chapters), adequate restraint of the animal is necessary.

Regular cleaning and routine maintenance of the equipment, even if they have not been used, is essential to ensure their continued effectiveness and durability. During killing with captive bolt for the purpose of disease control, the time interval between successive shots could be significantly shorter than that normally occur in a commercial abattoir, because there is no shackling, hoisting or bleeding involved. Consequently the equipment may overheat and fail to function properly.

A pneumatically operated captive bolt gun (made in the USA) that also injects compressed air into the cranium is used for killing large farm animals, without having to bleed or pith. The diameter of the bolt is 15.9 mm (0.625 inch) and the manufacturer's recommendation is to use an operating air line pressure of 170 psi or 11.7 bar. However, there is no published information available regarding its application on farm animals under disease control situation. This system is less useful if many animals need to be killed, because of its reduced mobility

Captive bolt guns (e.g. humane poultry killer) driven by cartridges or compressed air are available to kill poultry species (see stun / killing methods for poultry for details).

13.2.2.1. Description of effective use

The animal should be restrained.

Pithing should be performed immediately after shooting to ensure death (except where a pneumatic gun that injects air into the brain is used).

Death must be confirmed in each animal (see brain death under Introduction).

More than 1 gun may be needed to avoid over-heating due to repeated use at short intervals. A back-up killing method is also required, in case of failure.

13.2.2.2. Monitoring points

Those are described in details in earlier chapters:

- Animal collapses immediately with its body and muscles rigid.
- No attempt to stand up.
- Normal rhythmic breathing stops.
- The position of the eyeball will be fixed with the eyes open, the eyeballs having a glassy appearance and an absence of any reaction on touching the cornea.

13.2.2.3. Advantages

When performed effectively, captive bolt causes immediate unconsciousness, followed by death.

13.2.2.4. Disadvantages

Restraint facilities on farm may not be available. As restraint may be difficult, the method may be difficult to apply in excited animals.

The hole made in the skull leads to some leaking of body fluids, brain tissue and possibly associated pathogens.

Killing of animals with captive bolts and pithing destroys the brain and subsequent examination of the brain for diseases may be more difficult.

In large animals, it is not always effective as a killing method.

13.2.3. Percussive blow to the head:

This method is not applicable to large animals. However, a blow to the top of head has been reported as a humane method of euthanasia for neonatal piglets (up to 3 weeks of age) and lambs (up to 5 kg body weight) with a thin cranium, and also for poultry (AVMA, 2000).

In these animals, the blow to the head produces acceleration and deceleration effects, responsible of the immediate depression of the central nervous system (described in previous chapters), and diffuse brain damage (Finnie, 1997). The most important types of brain damage are diffuse axonal injury and subdural haematoma. These two lesions are the commonest cause of irreversible brain damage and death in humans (Graham *et al.*, 1995).

A poultry killer based on captive bolt has been developed ("Cash Poultry Killer"). This type delivers a percussive blow to the head, causing immediate unconsciousness and death. However, it causes very severe wing flapping, compromising operator safety (Raj and Tserveni-Gousi, 2000). This device is only suitable for broiler chickens, hens and turkeys and has not been evaluated for other species. Personnel performing euthanasia by use of a blow to the head must be properly trained and monitored for proficiency with this method of euthanasia (AVMA, 2000).

13.2.3.1. Description of effective use

This method is only to be used for young animals and poultry species and is only suitable to kill small batches of animals.

To be effective, the blow should be administered swiftly, firmly and accurately.

Death must be confirmed in each animal.

Proper training of personnel is required.

13.2.3.2. Monitoring points

Percussive blow to the head produces the same outwardly signs as non penetrating captive bolt (see earlier chapters).

Death is confirmed by the absence of breathing, pupillary and corneal reflexes.

13.2.3.3. Advantage

When properly performed, it is an efficient killing method.

13.2.3.4. Disadvantage

The method is not suitable for large animals.

Percussive blow to the head may not always result in death in small piglets and lambs.

Restraint of the animal is necessary and may be stressful.

Operator fatigue may lead to inefficient application and result in poor welfare to the animal. The method is physically exhausting for personnel.

13.2.4. Cervical dislocation

Poultry species are frequently killed on the farm by either manual neck dislocation by stretching, or mechanical neck crushing.

By manual stretching, the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull (AVMA, 2000). It severs the spinal cord and brain stem, and greatly reduces the diameter of the common carotid arteries, causing death from cerebral ischemia (Gregory and Wotton, 1990c). When carried out with force, it (inadvertently) leads to decapitation, which is also discussed below.

Mechanical neck crushing at the first cervical vertebra with a pair of pliers such as Semark pliers or the Burdizzo has been used as cervical dislocation. Neck crushing does not sever the common carotid arteries and does not reduce its diameter Therefore, it does not cause cerebral ischemia and hence loss of consciousness. If the spinal cord is severed without stopping blood supply to the brain, it results in death from asphyxia. (Gregory and Wotton, 1990c).

Gregory and Wotton (1990c) showed that, only 3 of 8 birds when the necks were dislocated by stretching, and 1 of 16 birds when the necks were dislocated by crushing, showed signs of concussion, suggesting that both methods of cervical dislocation may not induce immediate loss of consciousness. Therefore, cervical dislocation should be ideally performed in unconscious poultry.

The time to onset of brain death is longer with the crushing method (192 ± 19) than with the stretching method (105 ± 17). The stump of the neck can rupture the skin causing bleeding, which can be sprayed widely by the wing flapping.

It is not easy, or even feasible, to perform this task in heavy poultry (broiler breeders and turkeys) without causing severe pain and suffering. Therefore, the UK Animals (Scientific Procedures) Act (1986) (EEC, 1986) limits the use of this procedure to birds weighing less than 3.0 kg.

13.2.4.1. Description of effective use

Owing to the animal welfare concerns, the procedure should not be used. But if it is decided to use it for practical reasons, it should be limited to small batches of poultry weighing less than 3.0 kg.

It should be performed in one stretch.

Care must be taken to ensure complete dislocation of the neck. The vertebral column must be severed from the cranium.

Death must be confirmed in each animal.

The personnel should be properly trained.

13.2.4.2. Monitoring points

Cervical dislocation must lead to discontinuity between the brain and spinal cord and sever the trachea and blood vessels supplying the brain.

13.2.4.3. Advantages

There are no welfare advantages.

13.2.4.4. Disadvantages

The welfare concern associate with this procedure is that loss of consciousness may not be instantaneous and is difficult to apply in large turkeys.

Restraint of the animal is needed and can be stressful.

Fatigue in operatives would lead to severe compromises in bird welfare.

13.2.5. Decapitation

Decapitation is only applied to poultry and involves severing the neck, close to the head, by using a sharp instrument (Close *et al*, 1996). Research has shown that there may be visual evoked responses for up to 30 sec after decapitation (Gregory and Wotton, 1986). However, abolition of VEPs indicates brain death rather than loss of consciousness. It is worth mentioning again that neck dislocation inadvertently leads to decapitation. A major concern is the biosecurity on the farm that can be compromised by the spillage of blood.

13.2.5.1. Description of effective use

Decapitation should be used to kill small numbers of birds.

It must be performed in one cut.

13.2.5.2. Monitoring points

Separation of head from the body.

13.2.5.3. Advantages

There are no welfare advantages.

13.2.5.4. Disadvantages

Loss of consciousness may not be immediate.

Spillage of blood is worsened by severe wing flapping.

13.2.6. Maceration

Mechanical maceration (instantaneous fragmentation) in a high-speed grinder results in rapid death (less than 1 sec), and is considered as a humane method for the destruction of chicks up to 72 hours old and embryonated eggs (Bandow, 1987). For this purpose, only macerators specifically designed for disposal of poultry, which have rotating blades that turn at 6000 or more revolutions per min, should be used. The equipment should be properly maintained and must not be overloaded, since birds may be incompletely macerated under these circumstances. However, it is difficult to establish the appropriate capacity of how many birds may be handled without physical overloading of the equipment and reduced efficacy.

There are at least two types of devices used for macerating unwanted chicks in hatcheries. A crushing type has either one roller that rotates against a solid projection, or two contra-rotating rollers. The chicks are crushed and killed in a narrow, restricted gap between the rollers or projections. The ‘knife-type’ design has rapidly rotating blades, which effectively macerate the chicks. The HSA (2002) Codes of Practice for the disposal of chicks in hatcheries recommends that, in crushing type, the gap between the rollers should be less than 10 mm and the rollers must not be forced apart by the chicks. However, there is no peer reviewed scientific publication to support this claim.

13.2.6.1. Description of effective use

Apparatus must have rotating blades or knives.

Instantaneous maceration, death and pulverisation are required.

Birds must be introduced in small batches.

Equipment must not be overloaded.

Poultry other than newly hatched chicks must be rendered unconscious prior to maceration.

13.2.6.2. Monitoring points

There must be instantaneous fragmentation of the birds and eggs.

13.2.6.3. Advantages

The method ensure immediate death of the birds.

13.2.6.4. Disadvantages

On-farm systems are not currently available.

Disposal of waste can be problem.

13.3. ELECTRICAL STUN / KILLING METHODS

13.3.1. Large animals

On farm killing of cattle, pigs and sheep have been carried out by electrocution (Von Mickwitz *et al.*, 1989). The current is applied across the head to stun and simultaneously (one cycle method), or immediately after (two cycle method), across the chest to produce cardiac ventricular fibrillation (see previous chapters). Head-only stunning can be performed with high frequency but the cardiac fibrillation cycle must be applied using a 50 Hz sine wave AC.

For the species mentioned above, studies have been carried out using experimental portable equipment (Von Mickwitz *et al.,* 1989):

- A constant voltage stunner which delivers about 240, 250 or 350 V, 50 Hz AC sinus wave. The current flow depends therefore on the impedance. The electrodes become quite hot and have to be cooled frequently.

- Another device delivers constant current stunners with up to 400 V, 50 Hz sine wave AC.

The required minimum currents and voltage depends on the species and age as outlined in previous chapters. However, based on practical experience when killing on site, higher currents than those recommended for humane stunning or stun / killing are advisable to ensure death in all animals.

Based on practical experience for piglets (up to 6 weeks old), kids, and lambs, the voltage has to be reduced to a maximum of 150 V (Von Wenzlawowicz, pers. comm.). Otherwise excessive skin burning and carbonisation of the electrodes will happen. In such cases, a prolonged head-only application is first performed, followed by a second head-body application.

Table 13-2. Minimum voltages and currents (minimum application times described in previous chapters)

Animal	Minimum voltage (V) RMS	Minimum current (A) RMS
Cattle	220	1.5
Sheep	220	1,0
Lamb / Kid	220	1.0
Pigs > 6 weeks	220	1.3
Pigs <6 weeks	125	0.5

The time of current flow across the heart is a critical factor to ensure death. The current should be applied during at least 10 sec on the head and 45 sec on the heart in order to ensure death in all animals and result in relaxed carcasses.

Pointed electrodes to give good grip and penetration of the skin are recommended in order to get a good electrical contact. In sheep, pointed electrodes that can penetrate the wool and make contact with the skin are to be preferred as opposed to flat electrodes.

In sheep application of saline solution to each side of the head and to the thorax reduces contact resistance on the head and across the chest (Velarde *et al.*, 2002), and this may also apply to other species.

Fast movements of the head can lead to wrong placement of the electrodes which can cause severe pain in the animal. To ensure correct positioning of the electrodes and to maintain contact, animals must be suitably restrained so that the tongs can be accurately applied. Restraint facilities on farm may not be available.

In small piglets and lambs (< 5 kg), the resistance around the skin can be less than that across the body. Therefore, the current may not flow through the body, instead on the skin surface. In such cases, ventricular fibrillation and circulatory collapse do not persist after cessation of the current flow, and electrocution may not result in death (AVMA, 2000).

The animals subjected to electrical stun / kill must be monitored to ensure that they do not recover and to ensure that death has occurred (TVT, 2001). If an animal shows signs of recovery, another back-up killing procedure should be applied.

Electrodes must be kept clean to reduce resistance to the flow of current. The equipment must be inspected at regular intervals in order to ensure that it is operating correctly to the specification and that it is in a good state of repair.

13.3.1.1. Description of effective use

Species-specific requirements are considered in earlier chapters.

Animals must be suitably restrained so that the tongs can be accurately applied, especially in the case of one-cycle methods.

Care must be taken to ensure that the animal does not receive an electrical shock before the electrodes are correctly applied.

The current should be applied during at least 10 sec on the head and 45 sec on the heart, in order to ensure killing of all animals and result in relaxed carcasses.

The electrocuted animals must be monitored during the following 10 min to ensure that death occurred.

The method is not recommended in small piglets and lambs < 5kg

13.3.1.2. Monitoring points

Species-specific monitoring are considered in earlier chapters.

Death must be ensured by the absence of breathing, pupillary and corneal reflexes.

13.3.1.3. Advantage

When performed correctly, it induces an immediate stun / kill.

13.3.1.4. Disadvantage

Restraint of the animal is necessary and may be stressful. In small piglets and lambs (< 5 kg), electrocution may not result in death.

13.3.2. Waterbath electrical stun / killing method for poultry

Various mobile systems are available that have been used on farms (Raj, 1997).

Under these systems, the current travels through the brain and through the heart, inducing immediate unconsciousness and cardiac arrest. This can be achieved by supplying a 50 Hz sine wave AC to the waterbath. The required minimum RMS current to induce cardiac arrest is 250 mA per turkey and 150 mA per chicken, applied for 3 sec (see Chapter 10). In order to ensure death in all birds, the Scientific Veterinary Committee (1997) recommended a minimum current application time of 10 sec.

The welfare concerns associated with this system is described under Chapter 10.3.

13.3.2.1. Description of effective use and Monitoring points

As described in Chapter 10.3.

13.3.2.2. Advantages

If properly applied, it is an efficient killing method.

13.3.2.3. Disadvantages

Shackling and hanging are distressing and painful to poultry.

13.4. GAS MIXTURES TO STUN / KILLING PIGS AND POULTRY

Principle of gas stunning is detailed in Chapter 6.4 and its application to the different species is described in earlier chapters.

Gas killing methods may be used to kill on site poultry, and piglets. The gaseous euthanasing method should guarantee that the animal is dead at the end of the exposure.

Portable gas stun / killing devices for poultry are being developed in one member state. One system involves crates, and the other conveyor, to carry birds through a tunnel containing gas mixtures.

For on site gas killing, the animals may be placed in the chamber containing gas mixture either manually or be transported in crates, cages, or cradles, and removed after death. In the latter situation, the handling of the animal is minimal compared with other killing methods.

Argon and carbon dioxide are heavier than air, and may be easier to use in situ (in houses) on the farm. Carbon dioxide appears to be the preferred gas for killing animals under disease control situations, because it is readily available, it can be very effective even at less than the recommended concentration, and it is the densest gas available for this purpose. Ideally, these gas mixtures should be used to kill pigs and poultry in their houses so that the carcasses can be removed later. This will eliminate the need to manually remove live animals that can cause distress.

For this method, the heavier than air gas mixtures should be administered slowly, so that they gradually fill the houses from the floor to a level well above the heads of the animals, e.g. in a 'monolayer' housing system (e.g. pig sheds and poultry on deep litter). In houses where animals are kept in several layers, the gas should reach the ceiling. It is worth noting that heavier than air gas mixtures, when left undisturbed, are likely to settle down (stratify) and produce a gradient of gas concentrations inside the house, *i.e.* leaving the maximum concentration at the floor level and extremely low concentration or none at the top. This is particularly important in housing systems, such as battery cages or percheries used for hens, where the birds at the top tier may not always be exposed to a concentration required for killing. Under these circumstances, the concentration of gas or gases above the head level of birds at the top row of cages or perches should be monitored. A lethal concentration of gas mixtures must be present at the highest point continuously for at least 10 min to ensure that these birds are also effectively killed.

If birds are not contained in crates at the time of exposure to gas mixtures (e.g. manually carried birds are put into a chamber containing gas mixtures), care is needed to ensure that there is sufficient time allowed for each batch of birds to die before subsequent ones are introduced into gas mixtures. Otherwise, conscious birds may pileup inside the chamber and die of suffocation, rather than being killed with the gas. The animal welfare concerns associated with the use of gas mixtures, appropriate concentrations of various gas mixtures and durations of exposure necessary to kill animals are described in previous Chapters.

13.4.1. Description of effective use

The chamber in which animals are exposed to the gas and the equipment used shall be designed, constructed, and maintained in such a way as to avoid injury to the animals and allow them to be supervised, and have devices whereby the gas concentration can be easily and accurately measured.

Animals must be introduced into the chamber only after it has been filled with the required gas (mixture) concentration.

Gas concentration must be continuously monitored at an animal's head level and maintained at precise levels such that animals continuously inhale the recommended gas or gas mixture from the time of induction until death occurs.

Chambers should not be overcrowded.

Measures are needed to avoid animals climbing on top of each other while entering the chamber.

Animals should be immersed in the required gas concentration as fast as possible and remain in this atmosphere until death is confirmed

All animals must be dead before any additional animals are added

To ensure working safety, good ventilation in the building is necessary or the procedure should be carried out outside.

Additional conditions described under gas mixtures for stun / killing pigs and poultry, also apply (Chapters 9.4 and 10.5).

13.4.2. Monitoring points

There is a complete cessation of pupillary and corneal reflexes, and breathing.

Cessation of heart beat in pigs.

13.4.3. Advantages

Killing in houses and small groups contained in crates or cages has welfare advantages. Handling and restraint of the animal is minimal compared with other methods.

13.4.4. Disadvantages

Welfare problems related to the aversiveness of gas have been described in earlier chapters for pigs and poultry.

It may be difficult to verify death while the animals are in the chamber.

13.4.5. Carbon dioxide, argon, nitrogen and their mixtures

Scientific details of gas killing with carbon dioxide, nitrogen, argon and their mixtures have already been described and the welfare concern associated with the stress of induction of unconsciousness in pigs and poultry are detailed in earlier chapters (Chapters 9.4 and 10.5).

Prolonged exposure to carbon dioxide causes hypercapnic ventricular fibrillation and death (Raj, 1999). Holst (1999) reported that the inhalation of 90% carbon dioxide for 5.5 min induces death in pigs. Raj (1999) reported that all pigs exposed to a gas mixture of 30% carbon dioxide and 60% argon for 7 min died as indicated by the absence of gasping and eyelid reflexes at the exit. However, the exposure of 90% argon for 7 min was not effective in killing all pigs.

In chickens and turkeys, 2 min exposure to argon, nitrogen (Raj and Gregory, 1990b), 70% carbon dioxide, and any mixture of theses gases in atmospheric air with a maximum of 2% oxygen by volume has been reported to be effective for killing. However, chickens up to 72 hours old are more tolerant to inhalation of carbon dioxide (Close *et al.*, 1997) and may be not killed with this carbon dioxide concentration and exposure times. Therefore, birds up to 72 hours must be exposed to:

A minimum of 90% carbon dioxide by volume in air and an exposure time of 2 and 5 min for chicks and turkey poults, respectively (HSA, 2001).

A maximum of 2% by volume of oxygen in argon, nitrogen, other inert gases or mixtures of these with an exposure time of 2 and 5 min for chicks and turkey poults, respectively (HSA, 2001).

A maximum of 2% by volume of oxygen and 30% by volume of carbon dioxide in argon, nitrogen, other inert gases or mixtures of these with an exposure time of 2 and 5 min for chicks and poults, respectively (HSA, 2001).

13.4.5.1. Description of effective use

Animals should be immersed in a minimum concentration of 90% by volume of carbon dioxide. The concentration of carbon dioxide should be continuously monitored and displayed.

Other gas mixtures listed under previous chapters may be used at the appropriate levels necessary to effectively kill animals.

If animals are killed with anoxic gases or their mixtures, the concentration of residual oxygen in the chamber should remain less than 2% by volume.

The concentration of oxygen should be continuously monitored and displayed.

Animals must remain in this atmosphere until they are dead.

13.4.5.2. Monitoring points

There is a complete cessation of corneal reflex and breathing movements.

13.4.5.3. Advantages

In contrast with carbon dioxide, argon and nitrogen are odourless and tasteless and they do not induce sense of breathlessness.

13.4.5.4. Disadvantages

Inhalation of high concentration of carbon dioxide on its own or with argon or nitrogen may be distressing to animals.

13.4.6. Carbon monoxide

Carbon monoxide is a highly toxic gas and, being colourless and odourless, it is difficult to detect. The environment both inside and outside of the chamber will have to be monitored.

Carbon monoxide combines with haemoglobin to form Carboxyhaemoglobin, which prevents the carriage of oxygen by the red blood cells and the animal dies from the effects of hypoxia (Chalifoux and Dallaire, 1983). The affinity of haemoglobin for carbon monoxide is about 300 times greater than for oxygen (Blackmore, 1993).

As carbon monoxide is odourless (without taste or smell), it may not cause distress to an animal during inhalation (Blackmore, 1993). Research has shown that it is suitable for killing piglets (8 to 25 kg live weight). It has not been assessed in other species. When carbon monoxide is administered at a fast flow rate (> 90 l/hour), piglets may show severe convulsions, sometimes before they are unconscious. With a low flow rate (5,5 l/hour), the convulsions are less evident and only occurred after unconsciousness (Lambooij and Spanjaard, 1980). The same authors reported that in all animals, brain death occurred before the atmospheric concentration of carbon monoxide reached 4.5%. Blackmore (1993) reported concentrations of 6% in air to be optimal for euthanasia. Most animals exposed to these concentrations collapse and become totally immobile in less than 1 min, and their heart stops beating within 5-6 min. Exposure to 0.32% and 0.45% for 1 hour will induce unconsciousness and death, respectively (Blackmore, 1993).

Carbon monoxide may be produced by three methods: chemical interaction of sodium formate and sulphuric acid, commercially compressed carbon monoxide gas and from internal combustion engines. As carbon monoxide from petrol engine exhaust is highly irritant to respiratory tissues, it must be cooled and filtered before it can be administered for killing. There is no evidence that any species finds carbon monoxide aversive, although being placed into high concentrations of the gas can cause convulsions and exposure to low concentrations over a long period causes death due to an irreversible binding to haemoglobin.

13.4.6.1. Description of effective use

Only commercially available pure compressed carbon monoxide should be used. Animals must be exposed between 4% and 6% by volume of carbon monoxide, supplied from a source of 100% carbon monoxide (Close *et al.*, 1996).

Animals must remain in this atmosphere until they are dead.

Carbon monoxide should be monitored continuously and maintained in the chamber.

13.4.6.2. Monitoring points

There is acomplete cessation of corneal reflex and breathing.

13.4.6.3. Advantages

Loss of consciousness occur without pain and minimal discomfort and death is rapid.

13.4.6.4. Disadvantages

If used in confined space, the method is hazardous for operators.

13.4.7. Hydrogen Cyanide

Hydrogen cyanide has been used for the killing of poultry. It acts by blocking oxygen uptake by a slow uptake and slow binding to haemoglobin, causing respiratory difficulties and violent convulsions before the onset of unconsciousness and death, resulting in very poor welfare (Hatch, 1982).

It is not acceptable for euthanasia of any animal.

13.5. LETHAL INJECTION

Lethal injections are normally anaesthetic agents administrated as an overdose for euthanasia. Others may have neuromuscular blocking agents incorporated (Close *et al.*, 1996). In that case, the animal should become fully anaesthetized before the neuromuscular blocking agent takes effect, in order to prevent distress to the animal. Only those anaesthetic doses and routes of administration that cause rapid loss of consciousness followed by death should be used. In practice, barbituric acid derivates are considered as the first choice of drugs to be used for lethal injection to kill cattle, sheep, pigs, horses and poultry, while the drug T-61 is also sometimes used. The route of administration is also an important consideration (intravenous, intraperitoneal, or intracardiac). As rapid high blood concentration of the euthanasia agent is required to induce rapid loss of consciousness and death, the preferred route is intravenous. Intracardiac administration also causes high blood concentrations. However, this route of administration in conscious animals can be extremely painful if penetration of the heart is not successful on the first attempt. Therefore, intracardiac administration should only be used in unconscious animals. Intraperitoneal administration, which is easy to perform in animals in which the veins are small and difficult to penetrate, can take a very long time to induce unconsciousness and death. Intraperitoneal administration of some drugs (e.g. high concentrations of sodium pentabarbitone, > 200 mg/ml) may cause irritation of the peritoneum and hence pain and distress, which can be avoided by diluting the drug appropriately (Close *et al.*, 1996) (60 mg/ml; Morton, pers. comm.).

Lethal injections require the restraining of animals that may pose an undue risk to the operator. Fractious animals may require prior sedation.

<u>Description of effective use:</u>

Animals should be suitably restrained prior to euthanasia. The dose and route of administration should be appropriate to the drug and animal. The gauge and length of needle should be appropriate to each circumstance.

The intravenous route is preferred in conscious animals and intracardiac route may be used in unconscious animals. Intraperitoneal route is not normamally a preferred route.

Drugs must be administered by trained, competent and certified personnel only.

Animals must be kept in a comfortable environment until they are dead.

Monitoring points:

There is a complete cessation of corneal and pupillary reflexes and breathing.

Advantage:

The method usually result in a calm animal being euthanized quietly and easily.

Disadvantages:

Lethal injection necessitates careful restraint.

The method is hazardous for operator.

Scheduled drugs require licence to transport and handle, especially in large volumes.

13.5.1. Barbituric acid derivatives (barbiturates)

Barbiturates are normally used as anaesthetics, but are also effective in producing euthanasia when given as an overdose. In general, three times the anaesthetic dose causes death quickly (AVMA, 2000). It is generally used at a dosage of 200 mg/kg body weight. The action of the barbiturates is to depress the central nervous system, causing anaesthesia. When administered as an overdose, the anaesthesia is followed by depression of the respiratory centre, apnoea, cardiac arrest and death. Sodium pentobarbital is the most suitable barbiturate for euthanasia of animals, including birds.

Intravenous administration is preferred because the effect is the most rapid and reliable. Intraperitoneal administration may cause irritation of the peritoneum, pain and distress. 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 fully unconscious animals. Intramuscularly and subcutaneous routes take too long to act.

In pregnant animals, barbiturates cross the placental barrier, thus killing the fetus. This method is recommended to kill this type of animal.

13.5.1.1. Description of effective use

In cattle, sheep, pigs and horses the administration route should be intravenously using dosages based on the manufacturer's recommendations.

After the injection, the animals should be left without any disturbance until death supervenes.

Handling and use of such drugs must be restricted to trained, competent and authorised personnel.

13.5.1.2. Monitoring points

There is a cessation of respiration, heartbeat and loss of reflexes.

13.5.1.3. Advantages

Killing with barbiturate overdose usually result in a calm animal being euthanized quietly and easily and kills the unborn fetus.

13.5.1.4. Disadvantages

Each animal must be restrained.

13.5.2. T-61

T-61 is a mixture of 3 drugs:

- Embutramide (200 mg/ml): a hypnotic agent that depresses the central nervous system (CNS) and respiratory centre causing unconsciousness and hypoxia.
- Mebezonium iodide (50 mg/ml): a curariform drug that has a paralytic effect on the respiratory centre and block the neuromuscular junction of skeletal muscles, e.g. intercostals.
- Tetracaine hydrochloride (5 mg/ml): a local anaesthetic that reduces the pain related to the injection.

T-61 is administered intravenously in cattle, sheep, pigs and horses and the recommended dose is 4-6 ml/50 kg live weight. When administered by other routes, different absorption and onset of action of the active ingredients may happen (AVMA, 2000). Doses larger than recommended may cause pulmonary oedema and other tissue lesion that, are without importance if the animal is dead. It is described for dogs, that too fast or peri-venous injections of T-61 in 30% of the cases could lead to fear, pain and cramping before the onset of unconsciousness (Eikmeier, 1961).

There is concern that the curariform drug may cause cessation of respiratory activity due to paralysis of muscles associated with respiration before the onset of unconsciousness, causing distress to the animal. In a survey in veterinarians who applied T-61 in different species it was reported that, although applied according to the prescription, in 35% of the cases pain reactions or vocalisation appeared (Barocio 1983). However, another study with simultaneous recordings of the behaviour, EMG, EEG and end-tidal carbon dioxide in healthy dogs and rabbits shows that the induction of muscle paralysis and unconsciousness occur simultaneously (Hellebrekers, *et al.* 1990). These authors concluded that the muscular activity and vocal response seen in some dogs was not a conscious response.

It should be noted that in some ill or moribund animals, blood flow may be compromised and effectiveness of this killing method reduced. In addition, T-61 is formulated as an oily suspension and does not cross the placental barrier.

13.5.2.1. Description of effective use

It is the first choice of drug.

The administration route should be intravenously, in the recommended dosage and at proper injection rate.

Handling and use of such drugs is restricted to trained and authorised personnel.

13.5.2.2. Monitoring points

There is a cessation of respiration, heartbeat and loss of reflexes.

No pain or distress reaction must be observed.

It is important to monitor the rate of injection because, as it is described upper for dogs, too fast injections of T-61 could lead to fear, pain or cramping before onset on consciousness (Eikmeier, 1961).

13.5.2.3. Advantages

T-61 May be a suitable substitute when barbiturates cannot be used.

13.5.2.4. Disadvantages

Each animal must be restrained.

Para venous injection or too fast intravenous injection causes pain.

T-61 does not cross the placental barrier.

13.5.3. Other chemicals

13.5.3.1. Chloral hydrate

Chloral hydrate is a poor anaesthetic. It may be used intravenously in large animals and poultry. Death is caused by hypoxemia resulting from progressive depression of the respiratory centre. However, it is not recommended for the euthanasia of animals since it is a weak analgesic and is very slow to take effect (Close *et al.*, 1996). Death may be preceded by gasping, muscle spasms and vocalization. Administration by the intraperitoneal route results in poor welfare because large volumes are required and it causes irritation of the peritoneum (Hatch, 1982).

Description of effective use:

Chloral hydrate is not recommended in conscious animals.

If it has to be used, the administration route should be intravenously.

Monitoring points:

There is a cessation of respiration, heartbeat and loss of reflexes.

Advantages:

Not known.

Disadvantages:

There is a lack of analgesic effect and the method is very slow to take effect.

13.5.3.2. Ketamine

Ketamine is a dissociative anaesthetic. It is not considered practicable as a sole agent for euthanasia as large volumes would be necessary although there are no negative welfare consequences (Close *et al.*, 1996). The use in conjunction with xylazine permits a reduction of the ketamine dose. However, it may be used as a sedative prior to administration of other lethal injection.

Description of effective use:

Ketamine is used in conjunction with Xylazine or as a sedative prior to lethal injection

Monitoring points:

There is a cessation of respiration, heartbeat and loss of reflexes.

Advantages: not known.

Disadvantages:

For euthanasia, large volumes are necessary.

13.5.3.3. Magnesium sulphate

Magnesium sulphate is a neuromuscular, including myocardial, blocking agent and has no depressant effect on the central nervous system (Heuner and Dejohng, 1973). The animal remains conscious until the brain succumbs to the effects of inhibition of normal cardiac function. Administration of magnesium sulphate should only be used as a killing agent intravenously in an unconscious animal. The intraperitoneal route is not acceptable, because of the irritant nature of a saturated solution and its lack of effectiveness via this route.

Description of effective use:

Magnesium sulphate can only be administrated intravenously and in unconscious animals.

Monitoring points:

There is a cessation of respiration, heartbeat and loss of reflexes.

Advantages:

There are no animal welfare advantages.

Disadvantages:

For euthanasia, large volumes are necessary.

There is a lack of analgesic or anaesthetic effect.

13.5.3.4. Potassium chloride

Potassium chloride is a cardiotoxic agent that produces cardiac arrest and death by concentrations of 1 to 2 mmol/kg (AVMA, 2000). However, it is unacceptable when used in conscious animals because of the lack of analgesic or anaesthetic effect. Gasping, vocalization, muscle spasm and convulsive seizures may occur on or shortly after injection (Close *et al.*, 1996). Potassium chloride may be used intravenously or intracardiacly, in unconscious animals.

Description of effective use:

Potassium chloride must be administrated intravenously or intracardiacly in unconscious animals or in conjunction with barbituric acid derivates.

Monitoring points:

There is a cardiac arrest.

Advantages:

There are no animal welfare advantages.

Disadvantages:

There is a lack of analgesic or anaesthetic effect.

13.6. Application of On-farm Killing methods for fish disease control purposes

Emergency killing of fish is necessary in several sets of circumstances. Deformed, moribund and surplus fish can require destruction in fish hatcheries. Moribund and diseased growing fish can require killing on production farms. Fish can require culling on farms for disease control purposes and emergency killing of illegal imports may be required.

The method employed depends on the species to be killed, the number of fish to be killed, the size / stage of development of the species to be killed, and the facilities available.

Percussive stunning can be the method of choice for killing small numbers of fish. Where it is not, because the fish are too small, the fish are too large or too many fish need to be killed, other methods need to be considered.

In most cases, the preferred method in these situations is to kill the fish by immersion in an overdose of an appropriate fish anaesthetic.

Where this is not possible, electrical stunning could be considered. In this case 50 Hz sinusoidal AC is likely to be the most effective frequency to use for killing the fish. A sufficiently high voltage must be used to immediately stun and kill all fish in the batch, and the duration should be long enough to ensure that there can be no recovery.

When none of the above methods are available, other methods like carbon dioxide narcosis applied for enough time to ensure death could be used.

13.6.1. Percussive stunning

This is appropriate for killing occasional cull fish on farms when they weigh more than about 200 g and less than about 5 kg. In addition to the points outlined in the section on percussive stunning (Chapter 12.4.1.1), the following should be borne in mind when emergency killing fish.

Fish should be removed from water, often with a dip net, restrained and concussed by a sharp physical blow to the top of the head as quickly as possible to prevent distress (Anon. 1995). It is often quickest to restrain the fish in the dip net and strike it through the net. The blow can be applied manually with a plastic club or 'priest', and these should be kept close to the production facility so that they are available for immediate use, or a semi automatic percussive stunning machine such as the 'MT4' can be used.

See Chapter 12.4.1.1 for description of effective use, monitoring, advantages and disadvantages of the method.

13.6.2. Overdose of anaesthetic

Fish can be immersed in a solution of anaesthetic to kill them. Since slaughter is not considered a medicinal function, any suitable anaesthetic compatible with welfare and environmental considerations may be used, but under no circumstances may any fish killed in this way enter the food chain (Council Directive 2001/82/EC).

A solution is made up in a suitable bath or tank, using water the fish are swimming in, *i.e.* seawater for sea fish or freshwater for freshwater fish. Several factors affect the potency of anaesthetics including water temperature, water hardness and pH, size / state of maturity of fish and particularly species of fish. The appropriate concentration is dependent on the chemical used, but typically will be 2 to 4 times the dose normally used for surgical anaesthesia. The information in Table 13-3 (adapted from Ross and Ross, 2001) is a guide only and the most effective concentration needs to be evaluated for each situation.

Fish are put in the solution and become anaesthetised and subsequently die under anaesthesia. Typically, if the concentration of anaesthetic agent is correct, surgical levels of anaesthesia are achieved in 1 to 2 min and the fish are dead in 5 to 10 min (Kestin, pers. comm.). The time to induce anaesthesia should be monitored and the dose adjusted appropriately. When all signs of life are extinguished, the fish can be netted out and a fresh batch added. Fish removed form the bath should be monitored for signs of rhythmic respiratory movements, VOR, and heart beat, and if not dead, they should be returned to the anaesthetic bath for a further period.

MS222, 2-phenoxyethanol and metomidate are readily soluble in water and the appropriate quantity can be added to the bath of water and stirred vigorously. Benzocaine and quinaldine have limited solubility and must be dissolved in a small quantity of acetone or ethanol before addition to the anaesthetic bath.

If an anaesthetic bath is to be used to kill multiple batches of fish, the addition of oxygen or air will prevent fish dying of hypoxia before the induction of anaesthesia. Water quality should be monitored and water changed when contaminated with mucus or blood.

Table 13-3. Summary of dose rates (mg/l) reported to achieve anaesthesia. For euthanasia, required dose level should be at least doubled

	MS222	Benzocaine	Quinaldine	2-phenoxyethanol	Metomidate
Salmonids	50	50	60	400	6
Cyprinids	100		40	600	
Tilapia	100	100			
Eels	200				
Cod	75	40			5
Sea bass / bream	75				
Marine flat fish	250				5

13.6.2.1. Description of effective use

In general, anaesthetic concentration should be 2 - 4 times the concentration reported to sedate or anaesthetise fish.

Fish should be introduced to the euthanasia bath in batches of sufficient number not to overload the bath and so that they are not distressed by excessive stocking density before loss of consciousness.

If necessary, oxygen or air should be administered to the water to prevent fish dying from hypoxia before the anaesthetic has taken effect.

Fish should lose motor function and consciousness in 2 to 4 min.

Death should be confirmed in all fish (no VOR or *breathing*) before any fish are removed for disposal.

Water quality should be monitored and fresh anaesthetic solution used when the water becomes excessively contaminated with mucus or the period of induction becomes excessively long. Supplementary oxygen should be available to ensure that no hypoxic effects occur.

Appropriate protective clothing should be worn and operators trained in handling procedures.

Carcasses and anaesthetic solution should be disposed of carefully according to regulations.

13.6.2.2. Monitoring points

Complete cessation of rhythmic breathing movements and heartbeat. No VOR.

13.6.2.3. Advantages

When carefully applied, fish die with the minimum of stress.

Killing in small or large groups can be performed.

13.6.2.4. Disadvantages

Killing with anaesthetics may take longer than killing by other methods.

It may be difficult to verify clinical death while the animals are in the solution.

Such fish, even if undiseased and only being culled for disease control purposes, can never be allowed to enter the food chain.

13.6.3. Carbon Dioxide narcosis

Carbon dioxide can be used for emergency killing of species like salmonids that are not resistant to hypercapnia. But as the welfare of animals killed by this method is not good, this method should only be considered when other methods are not appropriate. In most cases where carbon dioxide stunning could be used, fish could also be killed by an overdose of anaesthetic, which is the preferred method.

In addition to the points outlined in the section or carbon dioxide narcosis, the following points developed in sections 13.6.3.1 to 13.6.3.4 should be borne in mind when emergency killing fish.

13.6.3.1. Description of effective use

Carbon dioxide should be bubbled into the killing tank before introducing fish to be killed for enough time to allow the water to become saturated with carbon dioxide. The rate of change of pH of the water should be monitored. When the pH is no longer reducing, the water is approaching saturation. pH should be monitored continuously to ensure carbon dioxide concentrations are maintained

Fish should be introduced to the carbon dioxide bath in batches of sufficient number not to overload the bath and so that they are not distressed by excessive stocking density before loss of consciousness.

Salmonids should lose motor function and sensibility in 4 to 6 min. Other species may take longer. Fish should be monitored for signs of death.

Death should be confirmed in all fish (no VOR or *breathing*) before any fish are removed for disposal.

Water quality should be monitored and a fresh carbon dioxide solution used when the water becomes excessively contaminated with mucus or blood.

13.6.3.2. Monitoring points

There is a complete cessation of rhythmic breathing movements and heartbeat. No VOR

13.6.3.3. Advantages

There are no welfare advantages.

13.6.3.4. Disadvantages

There is a delayed loss of consciousness. The method exposes fish to an aversive environment before loss of consciousness.

Killing may take longer than killing by other methods.

It may be difficult to verify clinical death while the animals are in the solution.

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15. ACKNOWLEDGEMENTS

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