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SCIENTIFIC REPORT ON STUNNING METHODS AND BSE RISKS

(THE RISK OF DISSEMINATION OF BRAIN PARTICLES INTO THE BLOOD AND CARCASS WHEN APPLYING CERTAIN STUNNING METHODS.)

PREPARED BY THE TSE BSE AD HOC GROUP

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REPORT ON STUNNING METHODS AND BSE RISKS
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THE RISK OF DISSEMINATION OF BRAIN PARTICLES INTO THE BLOOD AND CARCASS WHEN APPLYING CERTAIN SLAUGHTER METHODS.

I. BACKGROUND AND MANDATE

1. In the Opinion of 9 December 1997 *Listing of Specified Risk Materials: a scheme for assessing relative risks to man*, bovine, caprine and ovine lungs from animals above 12 months are included in the suggested list of specified risk materials to be excluded from the food and feed chains except when derived from a BSE free country with a negligible risk, if there is contamination from brain via blood when animals are killed by pithing or certain stunning methods.

2. It has been shown that the use of certain types of captive bolt guns to stun cattle causes brain tissue to enter the blood stream and it could, therefore, be disseminated throughout the carcass. (Garland, Bauer and Bailey (1996); Anil *et al,* 1999, 2001; Grandin, 1997; Schmidt *et al,* 1999).

3. Following the adoption on 13-14 April 2000 of the opinion on the *Safety of ruminant blood with respect to TSE risks*, pithing was forbidden (in addition to the already forbidden pneumatic bolt stunning) as a slaughter technique.

4. In a communication of 22.01.2001, the Government of the Federal Republic of Germany raises the question whether since the adoption on 13-14 April 2000 of the opinion on the safety of ruminant blood, new data became available on the possible distribution of central nervous tissue material (e.g., lacerated fragments of brain and/or spinal cord) in blood, other tissues and organs, like the heart and lungs following stunning by a penetrative captive bolt pistol without pithing[1]. Moreover, in a Notification of 19 April 2001, the Government of the Federal republic of Germany provided documents which it interprets as suggesting a hazard of contamination of the head, the blood, the lungs and the heart resulting from [penetrative] captive bolt stunning[2].

5. There are preliminary (unpublished) data (D.Harbour, 23.02.2001, personal communication[3]) that also without pithing brain material is detached in the trajectory of a penetrating captive bolt. The estimated amounts are in the range of 10-35 grams of brain material (results obtained on a sample of 10 animals. It was not determined what the fate was of this material, but it is considered to possibly contaminate the blood and other body tissues via the blood stream, in the first place the heart and then the lungs.

   Studies performed by Horlacher et al. (2001, in press) and by Martin et al. (2001, in press), collectively could not unequivocally demonstrate dissemination of brain tissues to the hearts and/or lungs of 735 cattle cattle after captive bolt stunning without pithing. However, in the Horlacher *et al* (2001) study on cattle stunned without pithing, at least 1,2% of lungs were - albeit weakly - NSE/GFAP positive. Moreover, the total number of emboli found differed markedly between the Horlacher et al 2001 and Martin *et al* (2001) studies; it cannot be excluded that this might indicate that cattle slaughtered in different abattoirs might have variable risks of the development of emboli even when stunned in the same way. A possible

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1 Note: There exist non-penetrating captive bolt pistols. These are widely used in Australasia and clearly there is no possibility for penetration to occur.

2 . The Notification does not specify whether it concerns penetrative and/or non-penetrative?

3 At the occasion of a presentation on 23 February 2001 to staff of Commission services on contamination of beef carcasses by CNS material during splitting.

explanation is indicated in the publication of Martin *et al* (2001) showing that the number of "emboli" found is depending on the time delay between stunning and examination of the lungs.

6. The Scientific Steering Committee was therefore invited to (1) list for each of the most commonly applied slaughter methods in the European Union, the tissues and organs including the whole head that are at risk to become contaminated with CNS material, (2) rank the various slaughter methods according to the risk and possible level of contamination to (3) provide a justification of the proposed ranking on the basis of available scientific or technical evidence and (4) indicate the level of risk to consumer health associated with each method, taking the age of the animal into consideration. The SSC is also invited to carry out a reflection on alternative stunning methods, with due regard for animal welfare considerations.

II. SCOPE OF THE REPORT

Only risks applicable to ruminants are considered. This means cattle, sheep and goats. There are no specific known data from the slaughter of goats. Therefore it will be assumed that wherever sheep are mentioned it will mean sheep and goats unless otherwise specified.

TSE risks to slaughtermen, abattoir workers and the environment (especially of the abattoir) are also excluded from this discussion, as well as the possible contamination of the carcass resulting from operations subsequent to stunning (e.g. sawing**,** cleaning, cutting, etc.

The report also does not address the safety of meat from fighting bulls killed by a sword thrust between the skull and the neck vertebrae.

III. SALIENT ANATOMICAL AND PATHOLOGICAL FEATURES OF THE RUMINANT ANIMAL.

III.1. Circulatory considerations

a. Oxygenated blood from the lungs enters the left atrium, passes through the left ventricle and thence is distributed to all organs, including the heart via coronary arteries and to the lungs via the bronchial arteries. De-oxygenated blood returns from all the body organs, including the heart and lungs, to the right atrium, passes through the right ventricle to exit via the pulmonary arteries to be re-oxygenated in the lungs.

b. The consequences are that should cerebral emboli develop such as a result of pre-slaughter stunning, in theory at least, they would be most likely to enter cerebral veins and sinuses and return to the right atrium via the *vena cava cranialis*. Some emboli might get trapped in the crevices of the right atrium, around the valves or in the *chordae tendinae*. Successful traverse through the right heart is followed by exit through the pulmonary arteries where the larger emboli might be trapped, or in tributaries thereof. Smaller emboli would be trapped in the capillaries. Emboli smaller than the calibre of the capillary bed and soluble brain material could traverse the capillary bed of the lungs and thus be distributed to any part of the body including the heart and, for a second time, the lungs via the bronchial artery. The liver too would receive emboli smaller than the calibre of the lung-capillary bed and soluble material directly through the hepatic artery and also via the portal vein and, like the lungs, have the possibility to receive infectious doses via two separate blood supplies. These small emboli and dissolved material could in theory re-circulate *ad*

infinitum. This assumes that no vascular damage occurs that is sufficient to rupture vessels and allow the contents to escape to the tissue spaces. Such events do happen in healthy cattle at slaughter, for example in some animals killed for kosher meat. This is believed to be due to a rapid rise in blood pressure following cutting the throat. It is recognised by multiple small haemorrhages into the musculature. Such meat is aesthetically unpleasing and would normally be diverted to manufacturing.

c. Arterio-venous anastomoses or shunts are direct connections between arteries and veins that bypass the capillary bed supplied by the artery. They are most frequently found in the extremities such as the digits and ears. Their function is to increase the supply of warm blood to the extremities and come into operation in the cold or when the circulatory system is stressed. In these circumstances emboli in the arterial circulation could bypass the capillary bed and be re-circulated by the venous system. However, overall they would not be expected to significantly alter the distribution of brain emboli from that anticipated from the basic anatomy because they would already be in the arterial circulation. These shunts do not play a role, as they are only relevant for such extremely small emboli that did already pass the capillaries of the lung.

d. Congenital abnormalities of the heart occur in animals including cattle and have an incidence of, on average, about 0.5% (Ducatelle, 1997). Some are so severe that they are incompatible with post-natal life. During growth there may be secondary effects on the circulatory system of significance, which result in culling or death of the young animal. Other defects of the heart have minor or no detectable remote effects. Patency of the *foramen ovale* is one of the more common defects (Carlton and McGavin, 1995) that can persist into adulthood without significant secondary effects. According to the literature the *foramen ovale* is found not to be anatomically closed in 16 % of adult cattle (Ducatelle, 1997; Jubb *et al*, 1993), but it is functionally closed because left atrial pressure exceeds right atrial pressure Analysing 2043 calves, it was found that 22% had a still open *foramen ovale*, and 49 % a still open *foramen ovale* with a still open *ductus arteriosus Botalli* (Herzog, 1961). In 1039 examinations of cattle and calves a *foramen ovale persistens* was found in 0.48 % of the animals. (Schmidt and Mickwitz, 1964). True atrial septal defects lead to pulmonary hypertension and congestive heart failure, and are therefore only very rarely observed in adult cattle. Post mortems systematically performed by the University of Ghent, Belgium, on approximately 3000 adult cattle did not show a single case of true *foramen ovale persistens* defect, causing pathological lesions (Vanopdenbosch, personal communication, 2001). Patency of the *foramen ovale* and possibly other congenital abnormalities can theoretically result in an embolus, particularly if small, entering the right side of the heart and crossing to the left side and thus enter the arterial circulation without passing through the lungs. The frequency of such an occurrence is unclear but in all probability is extremely low. However, such an event could account for the occasional detection of brain emboli in the arterial supply of organs like the liver and kidney.

e. The conclusions are that in theory large emboli would be trapped in the head if arterial, and in the cavities of the right ventricle or pulmonary artery and its tributaries if venous. Emboli larger than the diameter of the capillary bed will be trapped in the capillary beds of the head if arterial and lungs if venous. If the emboli are smaller than the diameter of the capillary bed they could

circulate *ad infinitum* or be released with the blood that results from sticking (killing the animal by severance of the major vessels at their point of entry into the thoracic cavity). Once the animal is stuck any size of embolus and soluble material could exit to the blood trough.

f. In practice, there are several other issues that may influence the distribution of brain material after stunning, especially the type of stun gun used, whether pithing is practiced or not, and the efficiency of bleeding out, that is facilitated by a short interval between stunning and sticking. The effects of some of these factors have been investigated in ruminant species (the only species considered) killed for food and are reported below.

III.2. Aspects of central nervous system tissue and the TSE agent.

In the context of the effects of stunning and dissemination of TSE infected material, the following features should be taken into account.

a. TSE infectivity in central nervous tissue is highest in grey matter at least of sheep and goats (Hadlow *et al*, 1980, Hadlow Kennedy and Race, 1982) and there may be differences in the distribution of infectivity in an infected brain or spinal cord that are dependent on the stage of incubation, the agent and perhaps in sheep, the prion protein (PrP) gene genotype. Schmidt *et al* (1999) particularly noted the occurrence of histologically identified spinal cord tissue (as distinct from brain tissue)[4] in clots in the right ventricle of the heart but only in two of 150 hearts from cull dairy cows or bulls with thick, dense skulls stunned with a pneumatically operated stun gun that injected air into the cranial cavity and which was continued for an extended period. These emboli were 10 and 13 cm long. In TSE diseases it is not unusual for infectivity to be detectable in CNS first in the spinal cord, then in the brainstem and lastly in the fore-brain. Thus TSE risks could occur even if no infectivity was present in the brain but was present in the cervical spinal cord.

b. Where a TSE risk exists, all CNS material should be considered infective. This can only be confirmed by bioassay. Titrations are necessary to determine the titre of infectivity.

c. It is generally regarded that detection of the disease-associated PrP (PrP^{Sc}), in CNS of ruminant animals is indicative of infection. However, absence of detectable PrP^{Sc} is not evidence for absence of infectivity.

d. The presence or increased levels or activities of other markers such as enzymes and glial fibrillary acidic protein (GFAP, an astrocyte marker that is over-expressed in TSEs) in the blood may strongly indicate brain damage but may not necessarily indicate that infection is present in the blood, though it may be.

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[4] Schmidt looked at 15 plants and in one plant examined 150 hearts. These came from cull dairy cows and bulls which were stunned using the pneumatic Hantover stunner. The animals were centrally restrained (as distinct from using a knocking box) so the operator could inject massive amounts of air to achieve complete stunning. (This is not possible in the conventional knocking box as the live animal falls immediately to the ground out of the operators reach and the gun is immediately disengaged). Only two of the hearts showed spinal cord (confirmed histologically) and extended air injection was claimed to be the cause. They were massive pieces 13 cm long. 23% of hearts in this plant showed (blood) clots in the right ventricle that may or may not have had CNS material trapped inside. Only 1% of cattle stunned with captive bolt without pithing had clots and none was reported to have spinal cord present.

Correlations between the two should ideally be shown, as measurement of these indicators in general is easier, cheaper, quicker and more efficient than detecting infectivity or PrP.

e. It is not known what properties the infectious agent would have in blood. It could be associated with large tissue fragments. Alternatively percussive forces (similar to the effect of sonication) could result in disruption of agent-associated structures to near the smallest operational size, which filtration studies suggest is between 10 and 25nm. The size of the infected particles and the solubility and binding properties both of the agent *per se* and of any associated tissue may affect where the agent is eventually trapped.

f. Few data are available to determine whether CNS emboli can occur in a homogenized form or just as structured tissue fragments. In cattle and sheep, when pneumatic stunners are used that inject air into the cranial cavity, CNS tissue can be identified both macro- and microscopically (Garland, Bauer and Bailey (1996), Schmidt *et al (*1999), Anil *et al,* (1999, 2001)). Determination of soluble CNS markers (Love *et al,* 2000) such as syntaxin 1-B revealed elevated levels in jugular blood after pneumatic stunning or conventional stunning with (Anil *et al,* 1999) or, in sheep, without (Anil *et al,* 2001) pithing (Anil *et al,* 1999). It could be that homogenized CNS tissue might enter the arterial circulation, as it was shown that marker bacteria placed on the captive bolt pistol could be recovered from the spleen, and those placed on the pithing rod were found in both spleen and muscle (Mackey and Derrick, 1979). This latter finding may have also some significance in regard to the TSE risk in uninfected animals that are penetratively stunned immediately after a brain-infected animal is stunned using the same captive bolt pistol.

IV. METHODS OF STUNNING OF RUMINANT ANIMALS

Current practices and stunning methods are listed and commented on in Annex 1.

The frequency with which CNS tissue enters the bloodstream depends on the method of stunning. The frequency of occurrence of neural emboli in 15 cattle was 33% after powered air injection stunning (emboli were detected in 4 and 1 had raised syntaxin 1-B and annexin V levels in jugular blood (Anil *et al,* 1999)). The same authors report about 6% (1 of 16) of cattle after captive bolt stunning with pithing; no evidence of embolism was found in 15 cattle stunned with a non-penetrating captive bolt or in 14 cattle stunned with a penetrating captive bolt without pithing (Anil *et al,* 1999).

Schmidt *et al* (1999) described blood clots in the right ventricle in 33% of hearts after pneumatic stunning, whereas only 1% of hearts had clots after cartridge-fired stunning. However, the observation of visible clots cannot be considered as direct measure for the presence of CNS tissue. There was no difference in incidence of neural embolism in jugular vein blood (and by inference in the heart and lungs) of sheep stunned either with conventional cartridge operated stun pistols or pneumatically operated stun guns that inject air in a study conducted by Anil *et al* (2001). The frequency of neural embolism was >13% in each group (2 of 15 sheep in each case). No evidence for the occurrence of neural emboli was found in the arterial circulation but the authors recommended that further work should be done because the samples analysed were small. Regarding the question of how much brain tissue enters the blood, Anil *et al* (1999) report that the quantities of soluble proteins syntaxin 1-B and annexin V detected were in the nanogram range per ml. Microscopic brain fragments in buffy coat derived from 250 ml of blood were

visible after staining; some appear to be quite large, probably in the milligram range. The extremely large brain-emboli in the pulmonary arteries reported by Garland, Bauer and Bailey (1996) could be measured in grams (T. Garland, personal communication) but may have been restricted to a relatively small proportion of the total cases. Schmidt *et al (*1999) reported two spinal cord emboli in the right ventricle that were very large (10-13 cm long) but only after prolonged stunning with pneumatic-powered air injection stunners.

Based on the currently available data, it is very difficult at this stage to get an estimate of the total amount of CNS in blood when embolism occurs but there is clearly a range from very large (estimated >10g) down to undetectable.

Regarding the dilution of the brain tissue in the blood, the report of Anil *et al* (1999) shows clearly that contamination occurs within the first 40 seconds after stunning the animal. After that levels become insignificant. But it is considered unlikely that in the majority of cattle sticking (severing of the major vessels of the neck to kill by bleeding out) is accomplished within 20-40 seconds of stunning, and especially so if pithing is practised. According the Humane Slaughter Association (HSA), it is more likely to exceed one minute (HSA, 1995). Thus in the event that sticking is not completed within 40 seconds of stunning, the majority of cerebral emboli will already have traversed the heart or be trapped in the heart or lungs which accords with the findings in practice (Garland *et al*, 1996); Schmidt *et al*, 1999).

From an average cow up to 10 litres of blood are collected but this is collected over a few minutes so it might reasonably be expected that most emboli would be trapped elsewhere than in the blood. However, it is unwise to assume that the blood would be free of risk from emboli derived as a result of using severe stunning methods even when sticking is delayed. Depending on the size of the abattoir and the size of collecting vessels blood from up to 100-200 animals can be pooled. Blood is collected in tanks and is stirred and anticoagulants are added. Thus the pooled blood appears to be well mixed. If one animal in the pool is BSE-infected, and if brain tissue from this animal entered its bloodstream, brain contamination is further diluted by a factor of at least 100. Thus any infectious dose would be diluted, but a larger volume would become contaminated. Sheep and goat blood is less frequently collected for animal or human consumption and amounts are correspondingly smaller.

Horlacher et al. (2001, in press) examined the lungs of 323 cattle after stunning with cartridge driven captive bolt without pithing. Emboli found in the lungs (in total 358 emboli found in 194 lungs) were collected and pooled. They were examined using GFAP and NSE. From the weak positive NSE results found in 2 of the pools and 2 larger individually collected emboli the authors concluded that not more than 1,2 % of all cattle brain tissue may have been mobilised.

Martin et al. (2001, in press) examined the lungs and hearts of a total of 726 cattle stunned with cartridge driven captive bolt. 412 were stunned without pithing and 314 with pithing.

Emboli found were examined using NSE (neuronspecific enolase).

Only two emboli showing a weak NSE signal were found in the group of cattle which was stunned and where pithing was applied.

Conclusions:

Essential parameters for assessing the risk are incomplete, especially for goats. There is no obvious reason why the risk of CNS embolism in goats is likely to be different from that in sheep. If captive bolt devices are used some horned sheep and goats may have to be stunned at a different site from polled animals due to obstruction by the horns. The effect of stunning at different sites on embolism is not known. Most sheep and goats however, are stunned by electro-narcosis.

Following penetrative stunning, an accurate estimate on the total amount of CNS material in the blood circulation and in different tissues is not known. The size range of emboli is not known. The infectivity titre of brain and spinal cord tissues of animals incubating TSE is not known. Furthermore since some of the studies rely upon the detection of soluble materials there is currently no way of knowing how the results of such studies relate to TSE infectivity. On the other hand it is clearly evident that if visible CNS material is found (and it has only ever been reported in the right ventricle and pulmonary arteries of the lungs) it is clear that if this tissue was TSE-infected the organ in which it resides presents a TSE risk. Before embarking on answers to the posed questions it might be helpful to make some other positive statements.

Order of stunning and killing methods used in the EU in ruminants ranked by decreasing TSE risk because the brain is damaged with decreasing severity (assuming a killed animal although healthy was incubating a TSE):

- **Type B** pneumatic stunner
- **Type A** pneumatic stunner (For definitions: see Annex 1)
- Captive bolt stunner with pithing
- Captive bolt stunner without pithing; free bullet (limited data available for the latter – see Munro, 1997).[5]

Negligible risk (because the brain may not be significantly structurally damaged):

- Non-penetrative stunner
- Electro-narcosis.

Note: traditional Kosher and Halal killing is not preceded by stunning, no hole is thus made in the skull, concussion to the head is minimised and these methods are considered for comparative purposes to create the lowest risk (and possibly absent risk) of embolism in slaughter animals.

The level of risk will vary according to the specific equipment used and criteria to be taken into account are: depth and velocity of penetration, amount of brain material damaged and possibly displaced, the location of the stun, etc. The ranking order of tissues (in descending order risk from neural embolism) in which a risk of TSE infectivity might arise in a brain-infected animal as a result of the use of penetrating stunning methods:

[5] Based on the Harbour (2001) personal communication.

Definite risks:

- Blood collected within 40 seconds from stunning.
- Pulmonary arteries and lung
- Right atrium and ventricle of the heart (in practice it may be difficult to distinguish levels of risk in heart and lungs unless macroscopically visible tissue pieces are present)
- Blood collected after 40 seconds from stunning

Parts of the head may become contaminated during stunning and slaughter.

It is noted also that blood collected at slaughter using penetrating stunning methods may become contaminated with brain material exuding from the stun hole. This may be extremely significant if an animal has to be stunned twice and particularly if a pneumatic gun is used that injects air under pressure.

Absent, negligible or lower risks: Any other organ.

Other points and clarifications emanating from the open consultation:

(1) In the abattoir, the additional risks to inherently TSE infection-free tissues like blood, heart and lungs could occasionally emanate largely, if not entirely, from cross contamination by TSE infected brain emboli. Such contamination can only occur with the use of certain stunning methods described in this paper. No method of collecting blood can avoid this risk if a risk method of stunning is used, emboli result and the brain of the animals is infected with a TSE agent.

(2) Dilution (e.g. of blood) is not an acceptable way to protect public or animal health in the context of the risks described in this paper.

(3) Delaying the collection of blood for 40 seconds after stunning is not an acceptable way to protect public or animal health in the context of the risks described in this paper.

(4) If animals are killed or believed to have been killed by alternative methods not described in this paper but where there is the potential risk of brain or spinal cord embolism or other TSE-infected tissue resulting from traumatic damage to any part of the central nervous system, its associated ganglia, the eye or any other SRM such animals might pose a TSE risk to consumers particularly if fed infected feed.

(5) There is currently no evidence for TSE-infectivity being distributed into the systemic circulation following stunning by methods currently regarded as presenting negligible risks for venous blood, heart and lungs. However, this could be investigated within a research programme and when the results are known the opinion presented here could then be endorsed or modified.

(6) In regard to cattle the UECBV have indicated that information on the welfare aspects and cross contamination by different stunning methods and by stunning pistols under abattoir conditions in the EU is incompletely reported and could be extended with industry participation. This is welcomed. UECBV is invited to proceed / start / support confirmatory research as indicated in its contribution.

(7) In particular it is noted that the data upon which the current Opinion is based are inconsistent, sometimes meagre and lacking in clarity. Of greatest importance in cattle is to further investigate whether or not stunning by penetrative methods alone create a risk of cerebral embolism, whether this risk is dependent on the

design of the stunning pistol or the cartridge used to discharge it or on the age or type of animal presented for slaughter.

(8) It is also important to ensure that any alternative methods of stunning that do not rely upon penetration of the skull are both compatible with good animal welfare and, as anticipated, do not create an unexpected risk from cerebral embolism.

V. AGE AND RISK.

A background document and risk assessment are provided in annex 2.

V.1. GENERAL

For the determination of the age at which brain material may contain infectivity, the following elements should be taken into account:

a. The interpretation given to the stage into the incubation period at which infectivity can be detected in central nervous system tissues.

b. The numbers of animals in a defined stage of incubation and changes (increasing or decreasing) in this figure over the course of a BSE epidemic, as estimated from epidemiological studies, which will depend, amongst other factors upon the level and duration of implementation of BSE risk measures in the cattle population.

In all naturally occurring prion diseases, PrP^{Sc} and infectivity are found in the CNS at least during the clinical phase of disease and, based mainly on experimental studies, almost certainly for at least a short period before clinical onset. Conversely, neither PrP^{Sc} nor infectivity has been reported in the CNS in susceptible animals at birth or indeed in young animals (for example sheep below one year and cattle below two years of age) with very few exceptions (for these see Dickinson *et al* 1965, Hourrigan *et al*, 1979, Renwick and Zlotnik, 1965 in sheep; Collee and Bradley, 1997 for cattle and the Opinion of the SSC on listing of SRM December 1997). Consequently, if it were possible to determine the minimum interval between exposure and the age at which infectivity in the CNS is first detectable for each ruminant species infected naturally with TSE, then penetrative stunning would not be a hazard (in respect of embolism or contamination of head meat) provided slaughter occurred before the end of this interval. If exposure occurred at birth (improbable for most exposures from feed) the interval would equate with the actual age of the animal. Between this age and the age of clinical onset there can be assumed to be an increasing risk of infectivity being present with time in exposed animals. Also the closer to clinical onset slaughter occurred, the higher the titre of infectivity in the brain would likely to be, so the risk might be proportionately greater. The difficulty is in determining the interval (or range of intervals) between exposure and occurrence of CNS infectivity in natural disease and the earliest age at which infectivity in the CNS could thereby be present. There is also a difficulty in determining the proportion of exposed animals that would have infectivity in the CNS at the shortest interval and how rapidly this proportion increases as the interval between exposure and infection of the CNS, increases. In other words, in practice there is likely to be a range of intervals between exposure and detectable infectivity in the CNS and a range of ages at which this occurs. Factors that could influence the interval and age include the species exposed, the age at exposure, the strain of agent, the dose, the route and, in sheep and goats, the *PrP* genotype. The main issues will now be discussed in regard to cattle and sheep.

V.2. CATTLE

a. In naturally occurring BSE in cattle, the age at which brain material may contain infectivity is unknown and it is not possible to predict with certainty when a natural case of BSE will show infectivity in the brain. The limited available data from experimental oral infection of cattle suggest that that infectivity would become detectable in the central nervous system only at a late stage of the incubation time, some months before clinical onset. In an experimental pathogenesis study of BSE in cattle after oral exposure, in which the lower limit of the incubation period range was 35 months, evidence of infectivity in the CNS was detected at 32 months, but not at 26 months after dosing (Wells *et al* 1998), but it is uncertain whether these data can be applied to natural disease cases where variation in dose results in a wide range of incubation periods (mean 60 months, and cases may occur in animals aged 20 months to lifetime). The date of first detection of infectivity in the CNS in these experiments [by conventional mouse bioassay] (32 months after exposure) relates to a minimum incubation period of 35 months and a probable mean incubation of some 43 months (preliminary estimate from dose response data of cattle infected orally with BSE - G. A. H. Wells, unpublished data). However, based on the overall knowledge gained from natural incidents of TSEs in animals it seems not unreasonable to accept that infectivity may be first *detectable* in the CNS in natural BSE in advance of clinical onset, and this might be as little as 3 months before clinical signs. BSE infectivity has been assayed in mice and cattle, providing evidence for a cattle-to-mouse species barrier of about 500 fold ($10^{2.7}$) (G. A. H. Wells, unpublished data) As the cattle-to-human species barrier is yet unknown (E.C., 1999), no assessment of infectivity risk for man from an estimated onset of detectable infectivity in cattle brain (whether 3 months, or earlier or later) can be made. However, on the basis of available data one could propose, as a "worst case scenario", that the BSE agent is present in the CNS in cattle during the last third of the incubation period.

   The age of the youngest naturally occurring case of BSE is 20 months. Since in the experimental pathogenesis study the earliest age of clinical onset was 35 months and infectivity was found in the CNS three months earlier than this, an estimate of when the brain of the 20 months old natural case became infected is required. This could be at 17 months of age (3 months earlier) or, taking a worst scenario view, when 2/3 of the incubation period was complete, namely about 13 months of age in this solitary case. This would mean that it would be most unlikely that the brain would be infected in cattle under 12 months old, even in those with a low age of occurrence such as 20 months (which is exceedingly rare).

b. The proportion of BSE cases in UK cattle[6] aged 24 months or less at onset is less than 0.006% (or 10 animals out of approx. 177.500 cases). (0.05% or 81 cases for animals of or under 30 months of age; 0.17% or 307 cases for animals of or under 35 months of age). BSE is thus at a very low incidence in cattle under 30 months of age.

[6] Estimates provided in the SSC opinion of 12 January 2001 on the questions submitted by EC services following a request of 4 December 2000 by the EU Council of Agricultural Ministers regarding the safety with regard to BSE of certain bovine tissues and certain animal-derived products

Donnelly (2000) analysed the likely size of the French BSE epidemic and compared the number of cattle less than 30 months of age entering the feed chain in France and in the UK in the year 2000. The number of cattle in a late stage of incubation in France was estimated to be two. In the UK the number of cattle below 30 months within one year of the onset of clinical signs was estimated to be 1.2 (range 0 – 4) in 2000 and was expected to further decrease in 2001 (Donnelly *et al,* 2000). It is noted, however, that these estimates concern 2 countries with different surveillance system and assumed risk management that has been gradually improving over the years.)

Note:

The current rapid post mortem tests detect PrP^{Sc}, not infectivity. Detection of PrP^{Sc} by any method is considered as evidence for the presence of infectivity. But absence of detectable PrP^{Sc} is not necessarily providing evidence of absence of infectivity. However, correlations have been made between PrP^{Sc} detection (though not always by the rapid tests), spongiform encephalopathy and infectivity when clinical disease is evident in natural and experimental BSE and sometimes at least in other circumstances. A negative PrP^{Sc} test result, including that from a rapid test, does not mean that the animal, or even the brain, is devoid of infectivity, whether currently detectable or not.

Because of this low incidence of clinical cases of BSE in cattle under 30 months of age and the sensitivity of current *post mortem* testing methods the effect of testing this population[7] on the TSE exposure risk from CNS emboli is likely to be minimal, both in terms of identifying affected animals for elimination and in terms of reducing the number of cases that might be identified by testing of the over 30 months old cattle population. Notwithstanding the lack of reported experimental data on the effectiveness of the rapid tests detecting BSE in clinically normal incubating animals, a positive result would eliminate the animal from the food and feed chains. However, if the result of the rapid test was negative it cannot be concluded that the animal (including the brain) is devoid of detectable infectivity. Thus it is not inconceivable that a animal with a negative rapid test result could, if stunned by a method that produced emboli, still have TSE-infected emboli dispersed through the venous blood stream, the lungs and the heart as described in the report. There is no current way of estimating the magnitude of this risk. However, in its contribution to the public consultation of the preliminary opinion EAPA supplied the SSC with a quantitative risk assessment (EAPA, 2001). The SSC has not verified this assessment. (Secretariat) It would certainly be lower when methods other than penetrating methods and pithing are used.

V.3. SHEEP

The use of rapid tests evaluated in sheep, even when available, would not be the most effective way of reducing the risk from potentially infectious emboli induced by stunning. This is because whilst some data are available for the pathogenesis of natural scrapie, they are not yet available for the pathogenesis (and timing of CNS infection) of experimental BSE in sheep and variation in the factors which determine pathogenesis of TSE's in sheep are more variable than is the case with BSE in cattle. The way forward would be to eliminate penetrative stunning

[7] 30 months and under, fit for slaughter for human consumption (excluding casualty slaughter animals.)

methods in small ruminants[8]. Furthermore the wide peripheral distribution of infectivity in sheep of certain genotypes means that a different approach might be required to fully protect public health especially if BSE were to be found in sheep. In this situation penetrative stunning should not be considered a feasible method of stunning small ruminant species.

The issue of BSE in sheep should it be found under domestic conditions, can partly be addressed on the basis of scrapie data (Hadlow, Kennedy and Race, 1982) and partly on data now coming forward from experimental BSE in sheep (see Annex 2 for details). The mean age of occurrence of scrapie in sheep is younger and the mean period of incubation period of natural scrapie and experimental BSE is shorter in sheep than for cattle with BSE. In sheep affected by experimental BSE after oral exposure, PrP^{Sc} can be detected from 16 months and the first clinical signs appear between 21 and 26 months: in this case, infectivity is first detected 62%-76% of the incubation period and the phase of infection of the central nervous system represents less then half of the remaining incubation period. Cases of verified clinical scrapie seems to be reported in a lamb as young as 7 months of age (Sigurdarson 1991), but the animal was probably not genotyped. Joubert (1972) described scrapie in ten months old french lambs. Out of the 10 cases, four had a paralytic form without pruritus and without tremor, while six had pruritus and tremor. Neither in Norway nor in the USA, no verified "natural" scrapie cases in lambs below 12 months of age have been found. The youngest actual clinical case in the USA was 17 months of age but infective tissues have been found in animals below 12 months. (L Detwiler, personal communication, 27.07.2001; M.Ulvund, personal communication, 9.08.2001). Results from the French epidemiological scrapie surveillance network shows that some sheep develop scrapie infection in the brain before they are one year old (minimum age: 8 months) (AFSSA, 2001).
In natural scrapie flocks, the earliest traces of PrP^{Sc} in medulla oblongata (vagal nucleus) was reported to be around 9 months of age, with few morphological changes (Andreoletti et al 2000), and also in spinal cord thoracic segments T8-T10 in naturally infected lambs at the age of 10 months (van Keulen et al 2000). Jeffrey et al (2001) also detected PrP^{Sc} in the tonsils at 8 months of age in a natural scrapie flock. Preliminary results of Ersdal et al. (2001) (M.Ulvund, personal communication, 9.08.2001) indicate the detection of PrP^{Sc} in three 12 months old lambs, one had PrP^{Sc} deposits in lnn retroph. only, the other two in medulla oblongata as well as lnn retroph.
The situation is more complex for sheep than for cattle and there are more uncertainties because key data do not exist. For example, although the *PrP* genotype and route of exposure affect the length of the incubation period, the dose and resultant attack rate from BSE under natural conditions are not known because naturally occurring BSE has not been reported in sheep or goats. Importantly also there could be a difference in the incubation period on a second passage of the bovine agent within sheep if maternal transmission occurred following a natural and initial introduction of the disease from a cattle-adapted agent *via* feed.

Furthermore iatrogenic scrapie has occurred in sheep (but not in cattle) following parenteral inoculation of locally prepared, non-commercial vaccines and this also could affect the incubation period especially if the origin of the infection was already host-adapted.

[8] See the SSC opinion of 8-9 February 2001: "Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions."

Currently, any risks to consumers from penetratively-stunned, small ruminants is negligible since, even if scrapie infections existed in the CNS, this is not regarded as a human pathogen. This view may have to be re-appraised following the identification of a French sheep scrapie isolate with similar biological properties to a single case of sporadic and a single case of iatrogenic CJD (Lazmézas *et al*, 2001). Contrariwise, if BSE is found in sheep the situation would change dramatically because BSE is regarded as a human pathogen. However, other factors including the probable wide distribution of BSE infectivity in different parts of the carcass (lymph nodes for example) would lead immediately to the necessity to protect public health from all possible sources of infection in small ruminants including that from penetrative stunning. For a detailed discussion of the issues see Annex 2.

VI. ALTERNATIVE METHODS TO PENETRATIVE STUNNING

Electrical stunning (electro-narcosis) – applicable to sheep and widely used. It is also in use in cattle in the UK (Wotton *et al*, 2000). It is also acceptable as a prelude to religious slaughter (see below).

Carbon dioxide is [currently] not applicable to ruminant species.

Non-penetrating stun guns are possibly applicable to all ruminant species but especially cattle. This method is widely used in some other countries.

Free bullet is not applicable for normal slaughter but might be necessary under some circumstances such as with 'wild' fractious animals.

Traditional Kosher and Halal religious slaughter methods alone do not employ stunning and are [currently] not applicable to any species for welfare reasons.

VII. ANSWERS TO THE QUESTIONS

The following draft answers to the posed questions may be proposed taking into account all the knowledge in papers consulted.

VII.1. QUESTION 1: LISTING FOR EACH OF THE MOST COMMONLY APPLIED STUNNING METHODS IN THE EUROPEAN UNION, THE TISSUES AND ORGANS THAT ARE AT RISK TO BECOME CONTAMINATED WITH CNS MATERIAL.

Irrespective of the type of penetrative stunning, the tissues and organs likely to be contaminated, in decreasing order of risk, are considered to be:

Definite risks:

- The external surface of the head but not necessarily the tongue, particularly following contact with heads from other cattle whilst in transit to a cutting/head-deboning plant (Note: the head has the potential for becoming contaminated even if Kosher/Halal methods are used but the risk is increased if penetrative stunning is used because the number of exits from the brain is doubled.)
- Blood collected within 40 seconds from stunning.
- Pulmonary arteries and lung
- Right atrium and ventricle of the heart (in practice it may be difficult to distinguish levels of risk in heart and lungs unless macroscopically visible tissue pieces are present)
- Blood collected after 40 seconds from stunning.

Parts of the head may become contaminated during stunning and slaughter. Following a request from Commission Services, the SSC will address this as part of an overall opinion on the safety of the head including skeletal muscle, tongue, and associated innervation should be considered as specified risk material.
Absent, negligible or lower risks:

- Any other organ.

It is noted also that blood collected at slaughter using penetrating stunning methods may become contaminated with brain material exuding from the stun hole. This may be extremely significant if an animal has to be stunned twice and particularly if a pneumatic gun is used that injects air under pressure (Grandin, 1997).

VII.2. QUESTION 2: RANKING OF THE VARIOUS STUNNING METHODS ACCORDING TO THE RISK FOR AND POSSIBLE LEVEL OF CONTAMINATION.

The following ranking is proposed of stunning and killing methods used in the EU in ruminants in decreasing order of TSE risk because the brain is damaged with increasing severity (assuming a killed animal although healthy was incubating a TSE and infectivity is present in the brain):

- Type B pneumatic stunner (that injects air)
- Type A pneumatic stunner (that does not inject air)
- Captive bolt stunner with pithing
- Captive bolt stunner without pithing; free bullet (no data available for the latter)

Minimal or absent risk (because the brain may not be significantly structurally damaged but there is a lack of data on the remote effects of these methods):

- Non-penetrative stunner
- Electro-narcosis

The level of risk possibly associated with penetrative methods will vary according to the specific equipment used and criteria to be taken into account are: depth and velocity of penetration, amount of brain material damaged and possibly displaced, the location of the stun, etc.

VII.3. QUESTION 3: JUSTIFICATION OF THE PROPOSED RANKING ON THE BASIS OF AVAILABLE SCIENTIFIC OR TECHNICAL EVIDENCE.

The justification for the proposed ranking is based partly upon the scientific publications of Garland, Bauer and Bailey (1996), Munro (1997) and Schmidt *et al* (1999) using data from slaughterhouse material and the experimental studies of Anil *et al* (1999, 2001) and unpublished data from Harbour (D.A.Harbour, personal communication, 2001 - See section I: Background and mandate) and partly on an analysis of the blood circulation in ruminants. Collectively, the authors of the studies on stunning have shown in cattle a relatively high risk resulting from stunning with a pneumatic stun gun that injects air under pressure particularly if air is injected over an extended period or any stunning method accompanied by pithing (at a lower but still significant level), and no evidence of risk from the other methods (though it cannot be completely excluded particularly from any form of penetrative stunning). In sheep (and by inference goats) stunning with a cartridge activated captive bolt or by pneumatic stunning that injects air shows relatively high incidences of CNS embolism (>13%) using either method but no evidence of embolism following electro-narcosis. It follows that if pithing is used following conventional captive bolt stunning in sheep or goats (see the Figure in the Annex),

the embolic effect would be unlikely to be less than for penetrating captive bolt stunning without pithing, and is likely to be greater.

Based on unpublished and in-print work (Anil *et al*, 2001; D.A.Harbour, personal communication, 2001), the pneumatic stun gun that injects air produces less than half the incidence of emboli in sheep (c.13%) than is produced in cattle (33%) by the same method. Furthermore, in sheep stunned with a conventional cartridge operated penetrating captive bolt the incidence of cerebral embolism is no different (c.13%) from the incidence following pneumatic stunning that injects air. This contrasts with the situation in cattle where formal proof of embolism following penetrative stunning without pithing is absent and the studies on 199 cattle reported by Munro (1997) based on macro- and microscopic examination of lungs support the view that if it occurs at all, it is a rare event. Also Horlacher et al. (2001, in press) and Martin et al. (2001, in press) could not unequivocally prove embolism of CNS tissue in cattle using captive bolt stunning without pithing in 323 and 412 cattle respectively under slaughterhouse conditions.

In practice however, there will be lower societal risks from cerebral embolism from sheep and goats because the majority are not stunned by penetrating methods, but rather by electro-narcosis.

VII.4. QUESTION 4: INDICATION OF THE LEVEL OF RISK TO CONSUMER HEALTH ASSOCIATED WITH EACH METHOD, TAKING THE AGE OF THE ANIMAL INTO CONSIDERATION.

There is a minimum interval ('The Period') between exposure and when detectable infectivity is found in the CNS. If exposure is at birth, the age at which detectable infectivity reaches the CNS will be the same as the time that the CNS becomes detectably infected. However, for most animals exposed by the oral route from feed, the exposure will be at varying intervals after birth and may exceed one year, so the age at which the CNS becomes detectably infected will be increased. Several factors will influence the age at which the CNS becomes detectably infected including, the species exposed, the age at exposure, the strain of agent, the dose, the route and in sheep and goats the *PrP* genotype.

In practice, as for each species there is a range of ages at which the CNS becomes detectably infected and the risk increases with age (*i.e.* there is a continuum from the end of 'The Period', with younger ages on average having a lower risk than older ages) it is only possible to provide a judgement, based on epidemiological and experimental evidence detailed in Annex 2, of the level of risk for three different age classes: from birth up to 12 months (see paragraph V.1.a), from one year to 30 months and over 30 months. The following, conclusions can be drawn:

1. Non-penetrative stunning methods in any ruminant species of any age are unlikely to increase the risk of cross contamination and risk for the consumer from that occurring following traditional Kosher/Halal slaughter which, because stunning is not employed at all, is assumed to present the lowest possible risk in practice and the base for comparison.

2. Penetrative stunning methods increase the risk of cross contamination and risk for the consumer (unless additional measures are taken) as follows:

- **Cattle under one year old and cattle from GBR I countries and cattle between 1 year and 30 months old in GBR II countries:** negligible risk.
- **Cattle above 30 months old in GBR II countries and cattle younger than 30 months old in GBR III countries:** very low risk.
- **Cattle above 1 year in GBR IV and cattle over 30 months of age in GBR III:** the risk is higher than indicated above and, on average, will rise with increasing age of the animals stunned. However, if evidence exists that a given sub-population is highly unlikely or unlikely, but not excluded, to be infected with BSE, the risk is negligible or very low as indicated for GBR levels II and I.
- Also **for sheep and goats**, should BSE be present under domestic conditions in these animals, the risk, on average, will rise with increasing age. For animals less than 1 year old the risk is estimated to be very low or low but not zero because occasionally scrapie has been observed in young animals below 12 months.
- **In regard to risks from parenteral exposure,** as distinct from oral exposure upon which this risk analysis is based, these might be higher in sheep than in cattle. This is because at least two iatrogenic outbreaks of scrapie have occurred in sheep as a result of the use of a scrapie-contaminated non-commercial vaccine administered by a parenteral route, one in the BSE era. Otherwise the risks would be similar to those described for cattle.

Note:

It is noted that under abattoir conditions it is not practical to decontaminate captive bolts guns between successive animals. Furthermore in cattle BSE risks in the current European situation are likely in general to be higher in cattle >30 months old than in cattle <30 months old. When a healthy animal that nevertheless has infectivity in the brain is stunned using a penetrative method there is the possibility that any infectivity that contaminates the bolt of the gun could introduce that infectivity into one or more sequentially stunned animals, stunned with the same gun, as has been described for bacteria by Mackey and Derrick, (1979). Where mixed ages of cattle from the above two groups are sent for slaughter for human consumption and are stunned by brain-penetrative methods, the risk of exposure for animals <30 months old could be greater than otherwise would be the case (Royal Society, 2001). Post mortem rapid testing would eliminate the whole carcass from the food chain if the test is positive but not the potentially contaminated animals. It is further noted that the delay between stunning and the test result being available is at least several hours and that if any action was required on contiguous carcasses the order in which animals were stunned and the gun that was used would have to be recorded. This theoretical risk is noted but not further commented upon. The use of non-penetrative stunning methods largely eliminates the problem of cross-contamination of other cattle from the stunning gun.

VII.5 ON ALTERNATIVE METHODS TO PENETRATIVE STUNNING

The report lists and briefly comments on a number of non-penetrative stunning methods. The TSE/BSE *ad hoc* Group considers that further research on stunning methods and their effects in regard to embolism, especially in cattle should be undertaken.

Experts in the field of animal slaughter procedures and welfare of animals should be consulted to determine the effects of implementation of any advice emanating from this report. They may need also to amplify the section dealing with alternative stunning methods.

VII.6. FINAL COMMENT AND RECOMMENDATION

On the basis of the limited observations available, lungs, heart and blood can pose a TSE risk if penetrative stunning[9] is used on ruminant animals (cattle, sheep and goats) from the age at which infectivity can first be detected in the central nervous system, because production of emboli composed of CNS tissue at slaughter cannot be completely excluded with penetrative stunning.

Should these observations be confirmed, then the possible risk resulting from penetrative stunning of cattle and small ruminants [particularly if evidence of BSE were found in small ruminants[10]] below a given age (see section VII.4 above), may be considered to be very low. For cattle and small ruminants above this age, the risk is not negligible, even if the outcome of a rapid post mortem BSE test is negative. Therefore heart, lungs and blood could become risk tissues if penetrative stunning or mixed stunning methods (penetrative and non-penetrative) are used. For cattle above that given age, the banning of either penetrative stunning of animals or excluding blood, heart and lungs could be considered. For small ruminants, the wide peripheral distribution of infectivity in sheep of certain genotypes means that a different approach might be required to fully protect public health especially if BSE were to be found in sheep. In fact, because of this wide distribution of infectivity it is not excluded that almost the whole animal may have to be considered as a SRM and in this situation the risk reduction by banning penetrative stunning would not result in additional safety.

Because the recent studies have involved few observations, the SSC recommends that these be expanded as quickly as possible to improve the confidence in current results. It is noted that additional studies funded by the EC and UK authorities are, however, in hand though the detail is not known. It is also noted that a verification of these recent studies could be rapidly carried out at little cost by existing laboratories specialised in veterinary pathology.

The TSE/BSE *ad hoc* Group further recommends:

- Because very few reports have been found on the possible occurrence of neural embolism following stunning by electro-narcosis, the use of non-penetrating captive bolts or conventional Halal or Kosher slaughter in cattle, studies should be done in order to verify the expectation of an absent incidence of embolism when these methods are used. This would permit possible alternative stunning methods with lower TSE risks to be recommended or developed for use in

[9] For pneumatic stunning or stunning with pithing: see the SSC opinion of 13-14 April 2000 on the The Safety of ruminant blood with respect to TSE risks

[10] See the SSC opinion of 8-9 February 2001: "Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions."

ruminants. It is noted that electro-narcosis does not appear to produce cerebral embolism in sheep (Anil *et al* 2001) and that non-penetrative stunning of cattle does not produce detectable embolism (Anil *et al* 1999).

- Because little work has been done to verify that, in practice, the arterial circulation and organs other than the jugular venous blood, heart and lungs are devoid of risk, studies on this aspect should be extended.
- The possible implications and drawbacks in terms of safety, meat hygiene, religious and animal welfare of abandoning penetrative stunning in favour of other methods (e.g., mushroom head bolt stunning) be checked with the appropriate experts.
- In the event that rapid tests are applied a positive test in any case will result in condemnation of the carcasses, all organs and tissues including blood. This should include also any animal, organ or tissue that could be cross contaminated such as pooled blood from several animals.
- Consideration should be given to the possible risks that might emanate from using a potentially brain-contaminated penetrative stun gun on sequentially stunned animals.
- As the risk is age-dependent, the introduction of separate slaughter lines for younger and older animals should be considered where appropriate.

The Working Group further recommends that the most recent scientific data, including on the pathogenesis of TSEs in ruminants, be analysed as soon as they become available in order to further refine current knowledge on the age at which different TSE agents can be expected to be present in CNS tissues of each ruminant species used for food.

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ANNEX 1: METHODS OF STUNNING OF RUMINANT ANIMALS

1 CURRENT PRACTICES

Stunning of animals is applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover until killed by bleeding out. A second reason for stunning is to produce sufficient immobility to facilitate the initiation of exsanguinations, (Blackmore & Delany, 1988). Bleeding out must be accomplished after stunning without delay.

According to current legislation, ruminant animals must be stunned before slaughter. Animals must be restrained in an appropriate manner, as to spare them any avoidable pain, suffering, agitation, injury or contusions. Animals must not be suspended before stunning or killing.

Currently permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis (Wooton *et al*, 2000) and 4) exposure to carbon dioxide. However, in practice exposure to carbon dioxide is inappropriate and not used to stun ruminant species.

The Hantover type of pneumatic stunner (referred to below as Type B) is not used in the UK (Taylor, 1996; MLC, 1996) and on the basis of a small survey (Prof Piva, personal communication) probably also not in Italy. Since 1993 (93/119/EEC) it is prohibited for use in ruminant animals in the EU. Pithing is also prohibited in the EU since 2000.

2. STUNNING METHODOLOGY

Captive bolt stunning is widely used for all ruminant animals. Explosive cartridges, compressed air and springs under tension have been used to drive bolts through the skull of animals. The ideal shooting position is frontally on the head. The site for puncture is at the intersection of a line joining the medial canthus of the eye to the horn bud of the opposite side. In naturally polled cattle the position of the absent horns should be estimated as if they were present. Their position is just medial to the ears when viewed from the front.

Where slaughter lines move fast, animals are killed at a fast rate and cartridge driven pistols become very hot and need time to cool down. Furthermore, recharging with new cartridges causes unacceptable delay. To overcome these difficulties captive bolt pistols have been devised that are re-cocked by air pressure (Type A pneumatic stunner). Others like the Hantover (Type B pneumatic stunner) not only used air pressure to re-cock the pistol but also inject air under pressure into the cranial cavity and scramble the brain and sometimes the spinal cord. Scrambling the brain in this way increases the safety for the slaughterman as he shackles the hind limbs for hoisting and subsequent carcass dressing procedures. The same can be achieved following conventional captive bolt stunning by using a pithing rod that is thrust up and down into the stun hole to causes severe damage to the brain structure. This process is called pithing. Pithing is not recommended for hygienic reasons independent of TSE risks (Mackey and Derrick, 1979).

3. EFFECTS OF PENETRATIVE STUNNING ON BRAIN TISSUE

In humans it is well recognised that severe penetrating injuries to the brain and head trauma from any cause (even during parturition, (Ogilvy *et al* 1988)) can cause such severe brain damage that cerebral emboli are produced which most frequently are found in the lung at autopsy. For example cerebral tissue emboli have

been found in the lungs following gun shot wound to the head (Hatfield and Challa, 1980, Ogilvy *et al* 1988) and that lung transplantation can cause acute graft dysfunction due to cerebral embolism derived from the donor whose death was due to cerebral trauma (Rosendale *et al,* 1992).

Penetration of a missile into the brain causes injury in the following three ways, depending on its velocity and shape by:

- laceration and crushing at a low velocity (< 100 m/s)
- shock waves at a high velocity (about 100 to 300 m/s)
- temporary cavitation at a very high velocity (> 300 m/s) (Hopkinson & Marshall, 1967).

The velocity of a bolt of a captive bolt pistol is about 100 m/s in the air. This low velocity and shape of the bolt should crush the brain cortex and deeper parts of the brain either by the bolt itself or by forward shock waves (Lambooij, 1982).

When the bolt penetrates the cavity of the skull, the capacity of which cannot be increased due to the inflexible bony skull, some brain tissue has to leave the cavity via the hole produced by the bolt. Some of this may be maintained within the air-filled sinuses of the frontal bones but some may escape to the exterior, including the trough where blood is collected. If a second shot is required to effectively stun an animal (a not uncommon event according to Grandin, (1997)), the force of penetration through a second hole may cause a significant release of brain material through the first hole. This will be potentially very large if a pneumatic stun gun Type B is used (EC, 1998).

4. EFFECTS OF PENETRATIVE STUNNING IN SITES ANATOMICALLY REMOTE FROM THE BRAIN

When stun gun Type B is used, emboli consisting of macroscopically visible brain tissue has been observed in the pulmonary arteries of 2.5% – 5.0% of cattle slaughtered in the US (Garland, Bauer and Bailey, 1996). The validity and relevance of this report in regard to cattle killed for human consumption in the UK where the pneumatic stun gun that injects air into the cranial cavity was not and is not used (MLC, 1996) was questioned by Taylor (1996).

In another study to determine the extent of dissemination of CNS tissue in the heart approximately 2,000 carcasses in commercial beef slaughterhouses in the US were screened (Schmidt *et al,* 1999). These authors reported also finding spinal cord tissue but only in the right ventricle of the heart from two animals in a slaughterhouse specialising in the slaughter of cull dairy cows and bulls where a Type B stunner was used with a prolonged holding to the head.

Multiple fragments of brain tissue were detected in the jugular vein blood in 4 out of 15 (and one other had evidence of embolism by detection of syntaxin 1-B and annexin V by assays of jugular blood), 1 out of 16 and 0 out of 15 cattle slaughtered after the use of powered air injection stunners, cartridge penetrating stunner with subsequent pithing and captive bolt stunner, respectively. Brain tissue was not observed in the jugular vein blood after concussion stunning of cattle (Anil et al, 1999).

Munro, (1997) reported no macroscopically or microscopically detectable emboli in the lungs of ten healthy cattle stunned with captive bolt pistol. In a subsequent study involving 1 bull stunned with a free bullet, 199 cattle stunned with captive

bolt pistols [of which 140 (70%) were pithed], none showed any evidence of pulmonary embolism with brain tissue.

Dr Alan Harbour and colleagues in the UK, Ireland and France are currently conducting research, funded under the EC FAIR Programme, into 'Measures to reduce contamination of meat and environment with CNS tissue during slaughter and processing of cattle and sheep' (FAIR CT97-3301) and 'Contamination of meat and exposure of abattoir workers by CNS material during standard butchering processes prevalent in the Members States of the EU' (FAIR-PL98-7004). Some of this research is relevant to the current item under discussion. Additional studies funded by the UK Food Standards Agency are also in hand.

The first project has already developed innovative techniques to assess directly or indirectly, the level of cross contamination with CNS material. Sensitive assays for the detection of syntaxin 1-B, glial fibrillary acidic protein (GFAP) and PrP have been developed and used to measure CNS contamination of carcasses (Anil *et al*, 1999 and Love *et al*, 2000). Spiking the captive bolt gun or inoculation of the brain through the stun hole, immediately after stunning with marker organisms showed no significant difference in distribution through the arterial circulation. These methods have shown that marker organisms can be recovered from the spinal cord, kidney, liver, lung and M. *trapezius cervicis* and from the exsanguinated blood. Spiking the captive bolt gun or pithing rod with marker bacteria has shown that these organisms can be recovered from the spleen and muscle (Mackey & Derrick (1979))].

The second project has confirmed that neural emboli are found in sheep and cattle (using syntaxin 1 B as a marker) following stunning with a pneumatically operated stun gun, and with a conventional captive bolt gun followed by pithing. In cattle neural embolism was not found when a conventional captive bolt alone was used, nor with a non-penetrating captive bolt [pistol].

In sheep, Anil *et al.* (2001) found no difference in the incidence of neural embolism in the jugular vein blood following stunning with a pneumatic gun that injected air or with a conventional cartridge operated captive bolt pistol. The incidence of embolism in sheep (c. 13%) was less than half that found in cattle (33%) stunned with a pneumatic pistol. By contrast no emboli were reported following electro-narcosis of sheep which is the common method of stunning used in this species.

In these studies emboli were only sought in the jugular blood, not in the heart or lung. They were detected by histology supplemented by immunohistochemistry for S100 protein in buffy coat samples or by ELISA for syntaxin 1-B and annexin V in jugular blood. By inference, if emboli were present in jugular blood, the blood itself would contain emboli and unless released from the body by bleeding out, would enter and probably traverse the heart and be trapped mainly in the lung (Anil *et al*, 2001).

5. STUNNING METHODS IN PRACTICE IN THE EU

A study in the EU-Member states in the 1970s showed that the captive bolt was used for more than 90% for cattle with a few slaughterhouses using concussion stunning. Electrical stunning was mainly used for sheep and in a few cases for bobby calves. Pneumatic stunning was not used for cattle and sheep (Mickwitz & Leach, 1977). It can be expected that these figures have not changed dramatically in the last 20 years. Religious **Kosher and Halal** slaughtering (bleeding without

stunning) is limited in the Member states and operates under the responsibility of the official veterinary surgeon (OVS).

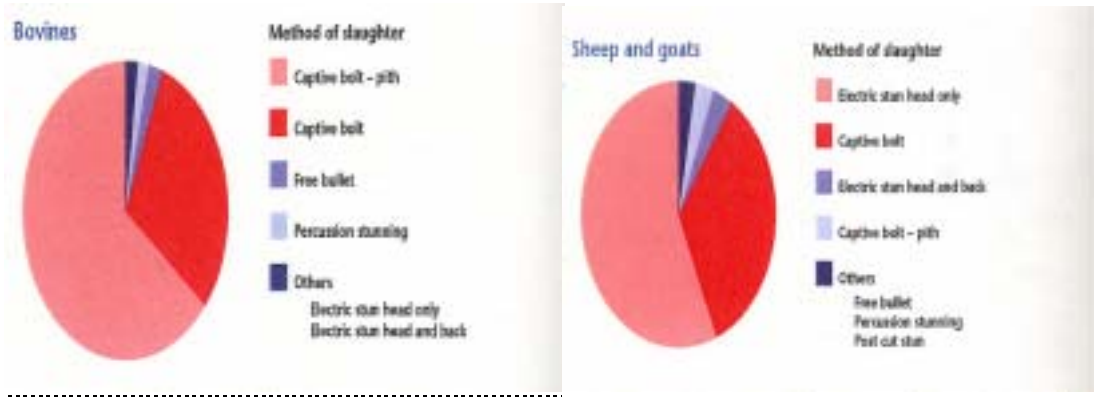

Figure: In the UK, the Meat Hygiene Service reported the distribution of stunning methods in the period 1997/1998 during an animal welfare survey. (Figure: Crown Copyright , MHS 1998).

6. THE SLAUGHTERING OF CATTLE

Stunning

a. Captive bolt pistols

- Instruments must be positioned so as to ensure that the projectile enters the cerebral cortex. In particular, it is prohibited to shoot cattle in the poll position. When using a captive bolt instrument, the operator must check to ensure that the bolt retracts to its full extent after each shot. If it does not so retract, the instrument must not be used again until it has been repaired. Animals must not be placed in stunning pens unless the operator who is to stun them is ready to do so as soon as the animal is placed in the pen. Animals must not be placed in head restraint until the slaughterman is ready to stun them.

  This method is only permitted using a mechanically-operated instrument which administers a blow to the skull. The operator must ensure that the instrument is applied in the proper position and that the correct strength of cartridge is used to produce an effective stun without fracture of the skull.

  Animals which are stunned or killed by mechanical or electrical means applied to the head must be presented in such a position that the equipment can be applied and operated easily, accurately and for the appropriate time. The competent authority may, however, in the case of cattle, authorize the use of appropriate means to restrain head movements.

  - There is a wide variety of captive bolt stunners available. Non-penetrative stunners have a "mushroom-headed" bolt which impacts with the skull but does not enter the skull or brain. It causes stunning by concussive forces alone.
  - Penetrative stunners cause insensibility due to the concussive blow to the skull, and the physical damage resulting from the entry of the bolt into the brain.
  - There is in addition a particular model of penetrative stunner which provides a blast of air through the centre of the bolt following entry into

the brain which is intended to also "pith" the animal immediately after the stun (Type B pneumatic stunner) – see above.

b. Electro-narcosis

Electrodes must be so placed that they span the brain, enabling the current to pass through it. Appropriate measures must also be taken to ensure that there is good electrical contact, in particular by removing excess wool or wetting skin.

The following specific methods are subject to authorisation by the competent authority, which must ensure in particular that they are used by duly qualified staff and respecting the animal.

c. Free bullet pistol or rifle are occasionally used particularly for bulls and fractious animals.

Killing with stunning

Animals are killed by bleeding out.

For animals which have been stunned, bleeding must be started as soon as possible after 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 the animal regains consciousness. A target interval between stunning and sticking (severing the major blood vessels at the lower end of the neck) is about 20 seconds. However, in practice a minute or more may elapse especially if pithing is done but it must be as short as is practically possible. The UK Humane Slaughter Association (HAS, 1995) gives a mean time of 73.6 seconds.

All animals, which have been stunned must be bled by incising at least one of the carotid arteries or the vessels from which they arise.

The person that is responsible for the stunning, shackling, hoisting and bleeding of animals must carry out those operations consecutively on one animal before carrying them out on another animal**.**

Animal welfare legislation requires that the animal be rendered unconscious until death. Death will occur by loss of brain function as a result of blood loss caused by cutting both carotid arteries. The heart will continue to beat until the animal dies and there will be circulation (of sorts) until then. ('Of sorts' because with both carotids (and jugulars) severed there will be an effect on venous return to the heart and on blood pressure that likely would drop considerably. Also, the animal is usually suspended from a hind limb at this stage. The most risky period from the perspective of the current opinion would probably be the interval between stunning the animal and cutting the arteries as the blood circulation is fully intact then. The "animal welfare directive" (93/119/EEC) mentions that animals should be stunned before slaughter or killed *instantaneously*. Bleeding of the animals, must be started *as soon as possible*. For sheep and goats the directive mentions a time limit of 15 seconds between bleeding and shooting. For cattle no mention of exact values is given but it is estimated that it would take at least 30 seconds[11] (the animal falls out

[11] Intervals between stunning and sticking of about 1 minute in total have been observed under field conditions (R.Bradley, pers.comm, 2001). In a number of EU countries, including Germany, 60 seconds is in the duration indicated in the legislation.

of the stunning box, a hoist is attached to its leg and it is raised in the air - then the arteries are severed)

Usually, blood destined to human use is collected with a hollow knife directly from the animal. This collecting procedure is more hygienic than collecting from the blood trough, the source of pooled blood from many animals that may be used for animal feed and other purposes.

Post-mortem inspection of carcasses is carried out downstream of the blood collection point. Therefore, if the carcass is not declared fit for human consumption, blood of the same carcass is no longer separable from blood of the other animals.

Killing without stunning

This method is used by Jewish (Kosher slaughter) and Moslem (Halal slaughter).

Note: These are not methods of stunning but rather of killing. They are included here for reasons of completeness but are otherwise irrelevant to this report.

All the precautions able to avoid excitement, pain or suffering of the animals must be adopted. Slaughtering without stunning is usually only allowed under the law for religious reasons. It must be carried out by trained and qualified personnel. In some countries it has been agreed locally between the religious leaders and those responsible for legislation in abattoirs that certain types of stunning are acceptable even for religious slaughter. This usually means the use of a non-penetrating stun gun prior to killing by bleeding out. Such agreements are desirable to be included in legislation in order to improve animal welfare at slaughter.

Under the *Jewish ritual*, the rabbi, using a razor-sharp knife (about 46 cm long by 3.5 cm wide), makes a swift cut from side to side to sever both jugular veins and the two carotid arteries in a single stroke without burrowing, tearing or ripping the animal. The head is then raised further as the blood spurts forth. For beef, the animal is to be cut horizontally across the throat, severing the trachea and the oesophagus.

The *Muslim ritual consists of* cutting simultaneously, with a sharp knife, the throat, windpipe and the blood vessels in the neck causing death, but without cutting the spinal cord. The blood has to be drained before the head is removed.

ANNEX 2: THE IMPACT OF AGE ON THE TSE RISK ARISING FROM STUNNING BY METHODS THAT RESULT IN THE DISSEMINATION OF CENTRAL NERVOUS TISSUE INTO THE BLOOD AND OTHER ORGANS AND TISSUES.

I. BACKGROUND

The basic issues - BSE and vCJD

Cattle are naturally susceptible to bovine spongiform encephalopathy caused by a single biological strain of TSE agent called the BSE agent. No other strains of TSE agent have been isolated from cattle with the natural disease. The same biological strain of agent has been isolated from human patients with variant CJD (vCJD) and therefore BSE is regarded as a zoonosis. Although there is no direct proof, it is generally believed that consumption of specified risk materials (mainly central nervous system (CNS) tissue) from infected cattle, or other products contaminated by such tissues, are the vehicles of transmission of vCJD to humans. Strict measures are now in force throughout the EU to protect the public (and animals) from exposure to the BSE agent. Although BSE has been experimentally transmitted to sheep and goats by the oral route using untreated brain material, no natural cases of BSE have been confirmed in small ruminants. However there are certain risk-reduction measures in place in the EU to minimise any risks there may be. Thus 'BSE in sheep' is currently a hypothetical disease.

Additional risks from CNS emboli

There is an additional risk that CNS tissues in the form of emboli may be distributed to the blood stream, lungs and heart (and possibly into other tissues) as a result of the use of particular penetrative stunning methods. If healthy cattle that were incubating BSE were stunned with these methods, and if the infective agent were detectable in the CNS, there would be a potential risk that humans consuming tissue containing CNS emboli might receive an infectious dose of the BSE agent. The critical factors that determine the TSE risk from this source will be the type of stunner used, the amount of CNS damage caused, size and frequency of the emboli produced, the interval between stunning and sticking (releasing the blood from the main blood vessels of the head and neck), the infectivity titre in the CNS material and the stage of incubation of the source animal. However, most of these data will not be known for any individual animal. Therefore in countries at risk from BSE, to protect public health from risk, other steps need to be taken. One such measure is to ban the use of pneumatic stun guns that inject air into the cranial cavity and pithing following conventional cartridge-operated, penetrative captive bolt stunning.

Other factors

Polymorphisms in the bovine *PrP* gene do occur but are not associated with disease occurrence. All cattle are therefore assumed to be susceptible to the disease. The mean incubation period of the natural BSE is 60 months. Oral experimental challenge of cattle with a single dose consisting of 100g of brain material from natural confirmed cases of BSE results in clinical onset of disease at about 35 months after dosing. Three months prior to the onset of clinical signs of disease (32 months after dosing), infectivity is detected in the spinal cord and some parts of the brain. As part of the public health protection measures, first in the UK and subsequently in some other countries, cattle over 30 months old are not permitted into any food or feed chain. With the occurrence of BSE in native-born cattle in

Denmark, Germany, Italy and Spain for the first recorded time during 2000 and the early part of 2001, additional measures were applied throughout the EU.

Rapid testing for BSE by the use of tests that detect PrP^{Sc}

Sensitive and specific rapid tests for BSE have now been developed and evaluated in confirmed cases of BSE and cattle free of the disease (Moynagh and Schimmel, 1999). These approved tests that must be done in approved laboratories, seek to identify the presence or absence in CNS tissue of the disease specific form of the prion protein (PrP^{Sc}). Commission Decision 2001/8/EC determines that from 1 January 2001 no part of any cattle over 30 months of age intended for human consumption and including emergency slaughter animals over 30 months of age shall be permitted for any use unless the that animal passes an approved rapid test. All parts of any cattle that fail the rapid test must be destroyed.

Age criteria for cattle for slaughter for human consumption

Thus at the present time (May 2001), throughout the EU, cattle intended for human consumption and approved for export must, apart from satisfying the historical rules for slaughter and meat hygiene, either be under 30 months old, or if over 30 months old, have passed one of the approved rapid tests for BSE. There are some exceptions to this rule within the EU for beef consumed within the country of origin in countries categorised as GBR risk 1 or 2. Currently this means Austria, Finland and Sweden only.

BSE incubation period, the 30-month rule and stunning

Taking account of the estimated incubation period range of natural cases of BSE (mean 60 months; range from 20 months to perhaps lifetime) and the time at which the CNS becomes detectably infected in experimental BSE (at 32 months after infection) the Commission asks what impact the testing programme would have on the risk of becoming exposed to TSE-infection if cattle were penetratively stunned by a method that is known to produce cerebral emboli. In other words, would the 30 months rule and negative rapid testing provide adequate protection for public health? This will be discussed in the next section.

II. ANALYSIS OF THE PROBLEM

Since there appear to be no genetic resistance factors operating in cattle, where there is a risk of BSE, all cattle that might have been exposed to infection should be considered to be potentially infected unless this can be excluded by certain certification schemes (*e.g.* Date Based Export Scheme, Certified Herd Scheme, *etc*). In the course of a BSE epidemic within a particular country the effectiveness of a feed ban may not be determined with certainty until the epidemic is close to elimination. This is because the incubation period of BSE is so long that it takes a minimum of five years from the date of a ban to determine that it is indeed effective by determining the actual number of cases occurring after the date and seeking reasons for them. At the present time the BSE epidemic in the UK (that is still the largest in Europe) is clearly declining and the date of the effective enforcement of the feed ban is considered to be 1 August 1996. This date has been accepted by the EC and by Member States, as providing a sufficiently robust protection to permit the export of deboned beef from cattle satisfying the agreed conditions. In countries with smaller epidemics that are rising, or not clearly falling, it is more difficult to determine a secure date. Therefore, in countries where there remains a BSE risk for cattle used for human consumption it is even more

necessary to ensure that no additional risk for humans or animals might arise as a result of neural embolism induced by particular stunning methods.

One method is to prohibit the use of stunning methods that are known to produce CNS emboli. This has been done (Commission Decision 2000/418/EC) since 1 Jan 2001. A residual problem is the uncertainty that penetrative stunning methods (such as cartridge operated captive bolt without pithing) not banned in this Decision might nevertheless sometimes produce emboli, although no firm data are available. It is noted that the majority of cattle in the EU are believed to be stunned with this method. Since the 30-month rule (see above) came into force, older cattle than 30 months have been excluded from food and feed chains unless they have passed an approved rapid test. Because BSE typically occurs in animals much older than 30 months, there is clearly a reduced risk if older animals are excluded from food and feed chains. The factors that govern the level of the reduced risk (as compared with the risk if no testing was done) are:

1. The incidence of BSE-infected animals under 30 months old being presented for slaughter.
2. The precision with which the age of each individual animal is known.
3. The precision with which experimental evidence of the earliest detectable infectivity in the CNS can be related to incubation period (in the pathogenesis experiment, with dosing at 4 months, clinical signs were first observed 35 months after infection (Wells *et al* 1998), but the range of incubation period relative to exposure and different doses cannot be determined from this study) in experimental BSE, can be applied to natural disease with a range of incubation periods (mean 60 months; range from 20 months to perhaps lifetime).
4. The validity of the approved rapid tests for detecting clinically normal infected animals and at what interval before clinical onset of disease.
5. The validity of negative rapid tests being equated with absence of detectable infectivity in the CNS; the incidence of occurrence of rapid test positive cattle over 30 months old; and the incidence of resultant confirmation of BSE by other approved test methods in the same CNS material.
6. The likelihood of currently (May 2001) approved stunning methods producing neural emboli, and if they do, the frequency with which this is likely to occur.

The relative risks to consumers from different stunning methods by age of animal stunned are stated below.

(i) Non-penetrative methods

- Electro-narcosis: there is no experimental or other evidence to indicate that this increases the risk over that for Kosher/Halal slaughter, which methods traditionally exclude stunning prior to killing by bleeding out and are assumed to present the lowest risk achievable.
- Non-penetrative stunning: there is no experimental or other evidence to indicate that this increases the risk over that for Kosher/Halal slaughter.

(ii) Penetrative methods

- ***Cattle***

The risks from all penetrative methods and related to the age of the animal being stunned is determined by the time at which the CNS becomes infected and the frequency with which any particular stunning method produces its remote effect in other organs and tissues from embolism.

In regard to direct contamination of the external surfaces of the head this risk will be increased over that existing in Kosher/Halal slaughter when any penetrative stunning method is used, as there is an additional source of infection namely the stun hole. If there is no CNS infection, there is no hazard and therefore no risk.

The interpreted risk is determined by the sensitivity of the tests used to assay the presence of infectivity. Bioassay in the homologous species using susceptible genotypes, or using validated transgenic rodent animals are regarded as the most sensitive methods. However, detection of PrP^{Sc} is regarded as a proxy for infectivity but with less sensitivity (currently) than bioassay. The sensitivity of PrP assays is improving all the time (Madec *et al* 1998), is already equitable with bioassays in conventional mice, (Deslys et al 2001) and is likely to improve further in the coming months and years.

In cattle with natural BSE there are no data to inform on the timing of initiation of BSE infectivity in the brain but it is certainly present at the onset of the clinical phase, and also presumably before the onset of clinical signs. How long before is not known but guidance is given from experimental studies.

In experimental, orally-induced BSE where the first onset of clinical signs was 35 months post challenge there was detectable infectivity (determined by bioassay in mice) in advance of this by some three months *i.e.* at 32 months post challenge but not at 26 months post-challenge. However, in natural BSE one confirmed case has been detected at 20 months of age and several have been detected below 30 months of age though they are far less frequent than in cattle over this age. The mean incubation period in natural cases in the UK is 60 months and the majority of cases occur in cattle aged between 4 and 6 years in all countries with BSE.

From this it can be concluded that there is a declining risk of BSE infectivity being present in the brain/CNS as the age at slaughter is reduced. Furthermore the risk of CNS infection in cattle under 30 months old is very small but not absent. If present, the risk for a consumer is unlikely to be any different in an animal under thirty months, as over 30 months, at a comparable time in the incubation period. The current BSE risk in the EU is likely to be getting smaller in under 30 months old cattle over time, as each country with BSE, applies and enforces the measures defined by European and national legislation.

Contrariwise, when a new epidemic commences, it is the oldest animals in the population (assuming a similar age structure as in the UK) that will have the lowest risk i.e. animals born before the date of first

exposure. In practice it is unlikely that this will ever be established with sufficient certainty for practical use.

In the UK for example, the mean number of cattle incubating BSE under 30 months old and slaughtered in 2001 is estimated to be <1. No matter what stunning methods are used, SRM, as defined by current legislation, are removed from all animals so any risk would apply only to the additional tissues identified as at risk in this report. However, it is noted that currently the skull rather than the head is SRM in all countries of the EU except the UK and Portugal where the head is defined. The rationale for this is not clear. As stated above there is a risk of contaminating the external surface of the head whether penetrative stunning methods are used or not.

The incidence of BSE cases in other countries is smaller than in the UK and the number of PrP positive animals detected as a result of application of rapid tests in fallen stock and in slaughter cattle over 30 months old indicates that there is no hidden large epidemic of BSE in the EU (> 2.6 million tested, 571 positive and 513 pending, all the rest negative, Commission data 25 June 2001) or Switzerland. This is notwithstanding that evidence enabling the tests to be validated for use in incubating healthy animals has not been published.

All this assumes that the exposure is resultant upon either feed exposure (infected MBM) or more rarely maternal transmission. Other potential routes of exposure such as parenteral routes, although not reported in cattle, might result in different lengths of incubation and create risks in younger animals. At the current stage of the European BSE epidemic it is unlikely that any such man-made risk (such as via commercially produced medicinal products or vaccines) would occur though it is noted that iatrogenic scrapie has occurred as a result of the use of non-commercially produced vaccines historically, and recently within the BSE era (Caramelli *et al*, 2001).

- ***Sheep***

Although some data are available for the pathogenesis of natural scrapie they are limited for the pathogenesis (and timing of CNS infection) in experimental BSE in sheep. However, it is known that in sheep affected by experimental BSE after oral exposure, PrP^{Sc} can be detected from 16 months and the first clinical signs appear between 21 and 26 months: in this case, PrP^{Sc} and assumed infectivity is first detected after 62%-76% of the incubation period had passed and the phase of detectable infection of the central nervous system represents in this instance less then half of the incubation period.

The factors that determine the pathogenesis of TSE are more variable in sheep than in cattle. Furthermore the wide peripheral distribution of infectivity in sheep of certain *PrP* genotypes means that a different approach might be required to fully protect public health from risks of TSE in sheep, especially if BSE was found in the future. Using scrapie data in Suffolk sheep (Hadlow Kennedy and Race, 1982), infectivity in the CNS was detected at 25 months of age. Most cases of scrapie occur at older ages than this but there are reports of some rare cases in the first year of life. This would mean that in those cases CNS infectivity

would be present at younger ages. As in cattle with BSE, in sheep, even using scrapie data, it is not possible to declare an age in an individual exposed animal at which CNS infectivity is absent, as this will depend upon the age at exposure, the dose, the route and the length of the period between exposure and detectable infectivity being found in the brain ('The Period') in that animal. However, it will be an age after birth and in most instances is likely to exceed one year, particularly for exposures from feed. The situation is also more complex in sheep than in cattle because of the vulnerability of sheep to maternal and horizontal transmission, at least of scrapie. What can be said is that the younger the animal, the less likely it is that there is a risk of TSE infectivity being present in the brain/CNS and, on average, the age at which the CNS is detectably infected is likely to be substantially over 12 months in most instances (see Hadlow, Kennedy and Race, 1982).

By contrast with scrapie infectivity, that might more likely to be picked up shortly after birth from the dam or a contaminated environment, BSE infectivity in sheep at primary passage from infected feed is more likely to have been established from consumption of infected feed at some significant time interval after birth, probably in many instances after weaning and may be as young adults (see SSC Opinion on infection of sheep and goats with BSE agent, Sep 1998). In these situations the risk in lambs at slaughter would be low. It is not possible to state with certainty that sheep of any age might have infectivity in the brain though it is much less likely in lambs under a year old than in older sheep. Specifically in regard to natural BSE infectivity in sheep there are no data on which to make a judgement though it is likely that a similar situation may pertain. It is important to note that secondary passage of BSE in sheep (*e.g.* by maternal transmission, is more likely to result in infection at an early age including possibly *in utero*. In this situation, were it to occur naturally, the incubation period could be shorter because the infectivity would be host adapted, the titre of infectivity could be higher, the incubation period might be shorter and the age at which the CNS was detectably infected might be younger than at primary passage (cow to sheep).

(iii) Summary and Conclusions

- ***Cattle***

  For cattle thirty months old and older there is an increasing risk with age that the brain/CNS is infected where that population of cattle has been exposed to BSE infection. Assuming that any BSE exposure has been by the oral route, current risks of CNS infectivity in cattle under 30 months of age are likely to be very small in countries that have introduced the appropriate measures and they have been effectively enforced for at least 30 months. However, if a rare risk might occur it would be no different from the risk in an older animal that had infection in the CNS.

  It is simply not possible to guarantee on scientific grounds that any individual animal is devoid of risk. The lower the age selected for cut off, the lower the risk is likely to be. However on a population basis it is possible to say that the number of cattle under 30 months of age that is likely to be at risk, is very small. The TSE/BSE ad hoc Group refers

in this context to the risk assessments made in the various opinions related to the UK Date Based Export Scheme. This implies that the risk may vary between different countries whilst they are at different stages of eliminating BSE because the numbers of animals exposed and the evolution of the incidence may be different.

Risks resulting from exposure by parenteral routes might be different because the incubation periods might be shorter. Therefore if an exposure did result, the age for cut off might be lower than 30 months if young animals had been inoculated. However, it is most unlikely [there is no evidence] that commercial medicinal products and vaccines produced or legally imported into the EU would create a risk in practice at this time.

- ***Sheep***

  It is simply not possible to guarantee on scientific grounds that any individual animal is devoid of risk. The lower the age selected for cut off, the lower the risk is likely to be. However on a population basis it is possible to say that the number of sheep under one year of age that is likely to be at risk, is probably very small.

  In regard to risks from parenteral exposure these might be higher in sheep than in cattle if only for the fact that at least two iatrogenic outbreaks of scrapie have occurred in sheep as a result of the use of a scrapie-contaminated non-commercial vaccine, one in the BSE era. Otherwise the risks would be similar to those described for cattle.

It could be concluded that:

For cattle under 30 months old, assuming that any BSE exposure has been by the oral route, current risks of CNS infectivity are likely to be very small in countries that have introduced the appropriate measures and they have been effectively enforced for at least 30 months. However, if a rare risk might occur it would be no different from the risk in an older animal that had infection in the CNS and that was at the same relative stage of incubation.

For cattle thirty months old and older there is an increasing risk with age that the brain/CNS is infected where that population of cattle has been exposed to BSE infection. Use of rapid tests for PrP^{Sc} could assist in eliminating from the food chain clinically healthy cattle with PrP^{Sc} in the CNS.

For sheep under twelve months of age (on a population basis), the number of sheep that is likely to be at risk of having TSE infectivity in the CNS is probably very small. However, it is simply not possible to guarantee on scientific grounds that any individual animal is devoid of risk as key data are missing especially the role, if any, of sheep to sheep transmission of BSE if BSE has been naturally introduced to the species. The lower the age selected for cut off, the lower the risk is likely to be.

III. RESPONSES TO THESE ISSUES

At this time it is not possible to give complete and clear definitive responses since much of the necessary information is not known, particularly for some countries. The following comments can however, be made.

1. The incidence of BSE-infected animals under 30 months old being presented for slaughter. This is likely to vary between countries that have different levels of BSE risk and are in different stages of BSE elimination. Donnelly (2000) analysed the likely size of the French BSE epidemic and compared the number of cattle less than 30 months of age entering the feed chain in France and in the UK in the year 2000. The number of late stage cattle in France was estimated to be two. In the UK the number of cattle below 30 months within one year of the onset of clinical signs was estimated to be 1.2 (range 0 – 4) and is expected to further decrease in 2001. Thus the residual risks are similar in the two countries. It is noted that a number of assumptions have been made in arriving at these figures and if these were inaccurately interpreted, then different (larger or smaller) figures could result. The only other countries in the EU with epidemics of a similar order of magnitude at present are Portugal (where the incidence fell in 2000 compared with 1999) and the Republic of Ireland. All in all it would not be unreasonable to assume that, at present, the risks from clinically healthy under 30 months old cattle in each Member State are of a similar magnitude.

   An additional factor of the emergency slaughter animals has to be considered. These would be more likely to be infected with the BSE agent than healthy animals judged on previous analyses initially using a rapid test made in such animals in Switzerland (Doherr *et al*, 1999, Schaller *et al*, 1999, Perler *et al*, 2000) as part of an active surveillance programme. It is likely that data from other countries is also coming forward now and these should be examined to see that the actual incidence of infection is not unreasonably high in cattle less than 30 months of age.

2. The precision with which the age of each individual animal is known. As a result of Commission Regulations 820/97/EEC and 2629/97/EC it is necessary for cattle born after 1 Jan 1998 to have a unique identification and two ear tags clearly displaying it. In the UK all cattle born after 1 July 1996 must have a passport, without which they cannot be slaughtered for human consumption. It is currently uncertain if all other Member States comply with these criteria with the same rigour as in the UK. It is quite possible they do and if so, this issue is not a critical one in determining risk. If not however, there will be a significant increase in risk as a result of some cattle over 30 months old being classified as being under that age.

3. The precision with which experimental evidence of the earliest detectable infectivity in the CNS can be related to incubation period and, more specifically, the prediction of the time of CNS involvement over the range of incubation periods of natural cases of BSE. In the experimental study of the pathogenesis of BSE in cattle the earliest evidence of infectivity in the CNS, as detected by mouse bioassay (32 months after exposure), precedes the earliest onset of clinical signs (35 months after exposure) by only 3 months. However, the design of this study does not allow these two observations to be correlated. A study to examine the dose-incubation period response of cattle to a range of oral exposures to the BSE agent, using a similar dose to that in the pathogenesis study, provides a mean incubation of 43 months, and a range of 33-61 months (G. A. H. Wells, unpublished data). Thus, although the majority of the animals remaining in the sequential kill schedule of the pathogenesis study (32-40 months after exposure) developed probable, if not definite, clinical signs (Wells et al., 1998) the relationship between the earliest detection

of infectivity in CNS and incubation period has a potential range of 1-29 months. It is therefore not possible to predict with certainty the time of first detectable infectivity in the CNS in a natural case of BSE with an incubation of say 60 months or another with an incubation period of 20 months. However, based on the overall knowledge gained from natural incidents of TSE in animals (especially scrapie in Suffolk sheep – Hadlow, Kennedy and Race, (1982) it seems not unreasonable to accept that infectivity may be first detectable in the CNS in natural BSE in advance of clinical onset and this period might be as short as 3 months. This means that if an animal had clinical disease at 20 months of age it might have detectable infectivity in the brain at 17 months of age. It is noted that most un-bred cattle for slaughter (other than veal calves) are actually killed when they are in excess of two years old, so this hypothetical circumstance would be very rare, *i.e.* most such hypothetical cases would still be on farm and would not reach the criteria of weight and condition for general slaughter. Alternatively if they were nevertheless submitted for emergency slaughter they would be subject to a rapid test. To overcome the difficulty of still some such animals slipping through the net it could be made a condition that any healthy animal, not complying with normal slaughter criteria (*e.g.* weight, size or condition) might be compulsorily subject to rapid testing.

4. The validity of the approved rapid tests for detecting clinically normal infected animals and at what interval before clinical onset of disease. The rapid tests have so far been shown to be effective in detecting, with 100% sensitivity and sensitivity, confirmed cases of BSE (Moynagh and Schimmel, 1999). The results of rapid tests on cattle during the incubation period have yet to be published but the Swiss experience suggests that they are effective (Doherr *et al,* 1999, Schaller *et al,* 1999, Perler *et al,* 2000). As a result of testing at risk cattle and healthy cattle sent for slaughter in some other countries (in the EU) and having any positive rapid tests confirmed by microscopic examination of the brain or other methods, there are likely to be data available that could confirm this supposition. However, it is noted that in the UK a comparison of histopathology, immunohistochemistry (IHC), Western blotting and SAF methods on the brains of clinically BSE-suspect animals that were histologically negative, 5% of 100 were declared positive by IHC (MAFF, 2000).

5. The validity of negative rapid tests being equated with absence of detectable infectivity in the CNS, the incidence of occurrence of rapid test positive cattle over 30 months old and the incidence of resultant confirmation of BSE by other approved test methods in the same CNS material. Detection of PrP-Sc by any method is not absolute evidence for the presence of infectivity nor is absence of detectable PrP evidence of absence of infectivity. However, correlations have been made between PrP-Sc detection (though not always by the rapid tests), spongiform encephalopathy and infectivity when clinical disease is evident in natural and experimental BSE and sometimes at least in other circumstances. What is also absolutely clear is that the current methods available for the detection of PrP-Sc are far less sensitive than bioassay within species. It is noted that one of the three approved rapid tests have now been developed to the stage where the sensitivity is equivalent to bioassay by the i/c route in mice (Deslys *et al,* 2001). A negative PrP^{Sc} test, including a rapid test does not mean the animal, or even the brain, is devoid of detectable infectivity. Also, there is no proven evidence available as to false positive results from

rapid tests for the detection of PrP^{Sc}. It has been claimed they do, with evidence presented from Belgium, Germany and Austria (Mackenzie, 2001), but the reasons for this are not given and the case has not been proved. The consequence is that, if true a proportion of uninfected cattle might be removed unnecessarily from the food chain. If the proportion is small this may be insignificant and of course presents no risk to public health, as the whole carcass would be removed from the food chain. However, the converse is more serious. A false negative rapid test in cattle over 30 months old could allow infected animals into the food chain and it is not inconceivable that some at least might have infectivity detectable in the brain. If methods of stunning that produce CNS emboli are produced there could be a risk to consumers from their dissemination (see separate report).

6. The likelihood of currently (May 2001) approved stunning methods producing emboli comprised of brain tissue, and if they do, the frequency with which this is likely to occur. There is no positive evidence in the literature that non-penetrating methods of stunning, no stunning at all (Kosher and Halal slaughter) or stunning by conventional, cartridge operated, penetrating captive bolts without pithing, cause a high incidence or indeed any incidence of emboli in cattle (Anil *et al*, 1999) but they do in sheep (Anil *et al*, 2001). The numbers of animals examined to derive these conclusions is small but the results from 199 studies of lung tissue from slaughter animals in the UK stunned with a penetrating captive bolt, including some with pithing, revealed no evidence of neural emboli in the lung (the only tissue examined). However, there is evidence from human medicine that head injuries can result in cerebral embolism in the lung. It would seem unwise to suggest that embolism can never arise from conventional, captive-bolt, penetrative stunning without pithing. On the other hand its frequency seems likely to be low. It is noted that there are deficits in current data in regard the effects of various stunning methods and the production of emboli, especially in cattle. To reduce any risk there may be, non-penetrative stunning offers an alternative method that is less likely on basic scientific grounds to produce emboli since no penetration of the brain occurs. However, since few studies have been done even these methods cannot be guaranteed to be devoid of risk of producing emboli.

IV. RISKS IN SHEEP AND GOATS

No data in regard to stunning or rapid tests exist for goats at all but is assumed they might have similar risks from penetrative stunning and embolism, as do sheep. There are clear risks of neural embolism resulting from the stunning of sheep by conventional, cartridge-operated captive bolt without pithing (Anil *et al,* 2001). In natural Suffolk sheep scrapie, infectivity in the brain is detected from 25 months of age (Hadlow, Kennedy and Race, 1982). Additional recent data from scrapie (van Keulen *et al*, 2000) and from in progress experimental studies indicate that in some circumstances infectivity in the brain may be detectable much earlier than this. Thus any age rule for rapid testing applied to sheep would have to take this into account assuming that natural BSE in sheep, should it occur, would follow a similar pathogenesis. It is also noted that very young (7-12 months of age) cases of sheep with natural scrapie have been reported in the literature (Detwiler, 1992) but the extent of occurrence of such young cases is difficult to judge. Hourrigan *et al*, (1979) reported one clinically normal lamb 4 months old and one goat kid 11 months of age from which scrapie agent was isolated by i/c inoculation of mice.

These rare incidents could be relatively infrequent outliers like the solitary case of BSE in a 20-month-old cow.

PrP^{Sc} testing in sheep with suspected or possible scrapie has been applied to peripheral tissues, *e.g.* tonsils and lymph nodes (Schreuder *et al*, 1998), third eyelids (O'Rourke *et al*, 2000) and blood (Schmerr *et al*, 1999) with positive results even in young sheep under one year of age. However none of these tissues have been reported to have been tested by the approved rapid tests and none of the afore-mentioned tests have been evaluated for use on an international basis. In theory the use of an approved rapid test in sheep, applied to brain tissue could identify animal carcasses at risk from emboli. However, if BSE (if it were to occur in sheep or goats) followed the same pathogenesis as scrapie and if blood infectivity in sheep is confirmed in the natural disease, emboli induced from stunning would merely add to an already existent peripheral infection. How much infectivity would be added is hard to estimate but it might be expected that emboli would mostly be in blood, lungs or heart as in cattle.

An important distinction between cattle and sheep is that the *PrP* gene of sheep strongly influences the length of the incubation period and thus so-called 'resistant' and 'susceptible' genotypes can exist and be identified by molecular genetic testing. So far it appears that resistant sheep do not show PrP-Sc and thus presumably no peripheral or central nervous system infection at all. Susceptible sheep can show evidence of peripheral infection as determined by the presence of PrP^{Sc} from an early age. Partially resistant sheep may show evidence of delayed onset of peripheral infection. This simplified overview has little practical value in an abattoir situation until some means of rapidly and cheaply identifying the *PrP* genotype of individual sheep can be found. Goats appear to behave more like cattle in respect of the influence of genotype but may mimic sheep and goats with scrapie in regard to the peripheral distribution of infectivity during incubation.

An important difference between sheep and cattle slaughtered for human consumption is the age at which sheep are slaughtered. Most sheep are slaughtered as lambs from 3 months to just over one year old. In broad terms the majority of lambs in this group is unlikely to have detectable infectivity in the brain no matter what is the genotype. However, it is clear from the literature that there are likely to be some outliers that would present a risk of being close to clinical onset despite their youth. Thus embolic TSE risks from the majority of lambs (< 1 year old) will equate more with the TSE risks in veal calves because in neither case is it very likely that at the time of slaughter there will be any detectable infectivity in the CNS.

A further important difference is that in the methods of stunning used for cattle and small ruminants. The majority of the latter are stunned by electro-narcosis, which on the basis of current evidence creates no detectable risk of neural embolism.

V. SUMMARY

The risks for the consumer from other methods of stunning no matter what the species or age are as follows:

- Electro-narcosis – there is no experimental or other evidence to indicate that this increases the risk over that for Kosher/Halal slaughter.
- Non-penetrative stunning - there is no experimental or other evidence to indicate that this increases the risk over that for Kosher/Halal slaughter.

The current risks for the consumer from penetratively stunning cattle under 30 months old and small ruminants under one year old are likely to be extremely small and so small as to be negligible. If there was nevertheless any rare risk in an individual animal, then the severity of risk would likely increase by an unknown amount in the order determined by the risk assessment for different penetrative stunning methods as described in Section IV above.

The current risks for the consumer from penetratively stunning cattle over 30 months old, increase with increasing age until at least the length of the mean incubation period for BSE and in the order determined by the risk assessment for different penetrative stunning methods as described in Section IV above.

For sheep 12 months of age and older in an exposed population, there is an increasing risk of TSE infectivity in the CNS. The risk is likely to increase with increasing age both qualitatively (more sheep with detectable infectivity) and quantitatively (increased titre of infectivity in individual sheep). However in regard to small ruminants, so long as the CNS is not infected with the BSE agent (or other zoonotic TSE agent) the risks are likely to be negligible.

VI. CONCLUSIONS

a) Non-penetrative stunning methods in any ruminant species of any age are unlikely to increase the risk of cross contamination and risk for the consumer from that occurring following Kosher/Halal slaughter that is assumed as the base for comparison.

b) Penetrative stunning methods increase the risk of cross contamination and risk for the consumer (unless additional measures are taken) as follows:

- Non-penetrative stunning methods in any ruminant species of any age are unlikely to increase the risk of cross contamination and risk for the consumer from that occurring following traditional Kosher/Halal slaughter (that does not involve stunning) is assumed as the base for comparison.
- Penetrative stunning methods increase the risk of cross contamination and risk for the consumer (unless additional measures are taken) as follows:
  - Cattle under one year old and cattle from GBR I countries and cattle between 1 year and 30 months old in GBR II countries: negligible risk.
  - Cattle above 30 months old in GBR II countries and cattle younger than 30 months old in GBR III countries: very low risk.
  - Cattle above 1 year in GBR IV and cattle over 30 months of age in GBR III: the risk is higher than indicated above and, on average, will rise with increasing age of the animals stunned. However, if evidence exists that a given sub-population is highly unlikely or unlikely but not excluded to be infected with BSE, the risk is negligible or very low as indicated for GBR levels II and I.
  - Also for sheep and goats, should BSE be present under domestic conditions in these animals, the risk, on average, will rise with increasing age. For animals less than 1 year old the risk is estimated to be very low or low but not zero because occasionally scrapie has been observed in young animals below 12 months.
  - **In regard to risks from parenteral exposure,** as distinct from oral exposure upon which this risk analysis is based, these might be higher in

sheep than in cattle. This is because at least two iatrogenic outbreaks of scrapie have occurred in sheep as a result of the use of a scrapie-contaminated non-commercial vaccine administered by a parenteral route, one in the BSE era. Otherwise the risks would be similar to those described for cattle.