ASSESSMENT OF THE RISK TO PUBLIC HEALTH RESULTING FROM EXPOSURE TO THE BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) AGENT THROUGH CONSUMPTION OF BEEF AND BEEF-PRODUCTS

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Food Standards Australia New Zealand
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1.0 EXECUTIVE SUMMARY

Variant Creutzfeldt-Jakob Disease (vCJD), a progressive and fatal neuro-degenerative disorder in humans, was first diagnosed in the United Kingdom in 1996. It is now accepted that vCJD can develop as a result of ingestion of food containing a transmissible agent that causes Bovine Spongiform Encephalopathy (BSE). BSE, commonly known as "mad cow disease", is a chronic degenerative disease affecting the central nervous system of cattle. This risk assessment analyses the available data to estimate the risk of Australians developing vCJD from exposure to food containing the BSE agent.

There have been approximately 131 cases of vCJD diagnosed worldwide to date, a very small number relative to the large number of people likely to have been exposed to the BSE agent in food. Although the number of cases is relatively small, the uncertainties about the incubation period between exposure to the BSE agent and the subsequent development of vCJD, as well as significant gaps in knowledge about the BSE agent, its characteristics and the transmission factors, dictate a conservative approach to the assessment of the potential health risk.

Transmission of the BSE agent to humans appears to be dependent on a number of factors. They include the likelihood of an animal with BSE entering the food chain, the part and amount of the animal carcase consumed and the infectivity of the tissues. The central nervous system of clinically affected cattle contains most of the infectious agent.

Experimental studies indicate that a single exposure to contaminated feed is sufficient to cause BSE in cattle; however it is not known the extent or frequency of exposure to the BSE agent in food that is required to result in vCJD in humans. Current scientific evidence indicates the BSE agent is extremely resistant to physical and chemical treatments that are normally used to inactivate proteins and micro-organisms. The thermal conditions used in processing of beef products such as canned meat and smallgoods will not inactivate the BSE agent. However, at least one manufacturing process, the sodium hydroxide treatment in gelatine production, can inactivate BSE agent infectivity.

Contamination of uninfected meat by an infected carcase is possible in abattoirs and must be considered in estimating risk. Contamination can occur even when extensive control measures have been implemented to reduce cross-contamination.

Not all beef products pose the same risk of transmitting the BSE agent to humans, because some products appear to contain more of the agent than others. Some products, such as the brain, can contain the agent in very high concentrations. Animal products that were identified in the risk assessment as containing significant amounts of the BSE agent include so-called specified risk material (SRM) such as the brain tissue and spinal cord, and mechanically recovered meat. The risk assessment also examined the potential for the BSE agent to be transmitted to consumers via edible collagen, edible tallow, gelatine, and milk and dairy products.

SRM and mechanically recovered meat may be used in the preparation of canned beef products and smallgoods in some countries and therefore are considered to pose a potential hazard to Australian consumers. If sourced from an animal infected with BSE, the potential for these products to contain the BSE agent is high, because the raw materials are often sourced from older cattle with potentially high levels of the BSE agent.

Food-grade gelatine that has been produced from porcine skins and bovine hides is considered to pose a negligible risk to consumers, because skins and hides from these animals have been shown to contain negligible levels of the BSE agent. Where gelatine is derived from bones using a process that includes a sodium hydroxide step, the risk has also been shown to be negligible. However gelatine manufactured from bones where there is not a sodium hydroxide step results in a higher level of risk.

Edible collagens are also produced from food-grade bovine hides. Collagens which have not been contaminated with SRM during slaughter, are also assessed as posing a negligible risk. The current use of edible tallows by the food industry as an ingredient in products such as cakes, biscuits and pastries is estimated to pose a negligible risk of transmission of the BSE agent.

Current evidence indicates that milk and dairy products do not contain the BSE agent.

1.1 Summary of key findings

1. Epidemiological evidence indicates that BSE and vCJD are caused by the same agent. Strain typing evidence (both biological and molecular) strongly suggests that vCJD and BSE are caused by an agent with similar biological and molecular properties.

2. The oral route is the most likely route of exposure to the BSE agent due to consumption of beef-derived products contaminated with the BSE agent. The risk of exposure to the BSE agent is dependent on the likelihood of an infected animal entering the food chain, the type of tissue used in the product and the amount of BSE infectivity present.

3. While some of the tests and bioassays may not have high enough sensitivity to detect low levels of the agent, experimental data have shown that infectivity is not found in up to 50 tissues of cattle with BSE, including most tissues that are used in the human diet, such as muscle and milk.

4. BSE exposure risk is highest from specified risk material (SRM), and in these tissues the central nervous system tissues from clinically-affected cattle would present the highest risk. Approximately 95% of the total infectivity is estimated to be in the central nervous system of clinically affected animals. The removal of SRM from a clinical BSE case will reduce the infectious load by about the same amount.

5. The amount and distribution of the BSE agent in an animal changes over time and therefore the age of animal at slaughter will influence the level of BSE infectivity entering the food chain from an infected animal. Risk of exposure to the BSE agent is low early in the incubation period and rises rapidly later in the incubation period.

6. The incubation period is such that clinical BSE is rarely detected in animals younger than 30 months.

7. Experimental studies indicate that a single dose is sufficient to cause disease in cattle but the effect of cumulative doses on humans is unclear. Repeated exposure to infection could increase the absolute numbers of individuals that might receive an infecting dose and thus the likelihood that exposure would result in infection.

8. Heating in the presence of moisture can reduce BSE infectivity but cooking cannot be relied upon to completely inactivate the BSE agent.

9. Most traditional methods for inactivating microorganisms, including ionising, ultraviolet and microwave radiation, will not inactivate the BSE agent, but sodium hydroxide and buffered sodium hypochlorite, under particular laboratory conditions, will inactivate the agent. Some of these treatments are suitable only for use in by-product industries, such as the manufacture of gelatine and soap.

Fresh meat and processed meat products

10. The slaughter practices which have a greater risk of contamination of carcases with SRM occur during the stunning procedure through dissemination of brain tissue and during carcase splitting through dispersal of spinal cord material. There is also a risk of contamination during butchering where SRM have not been removed. Other steps in the slaughtering process that may involve a risk of cross-contamination of carcases with SRM are head processing and carcase trimming.

11. In non-European countries, SRM and mechanically recovered meat may be used in the preparation of smallgoods and canned beef products. If these products are sourced from an animal incubating BSE, the risk from these products is increased as raw materials may be sourced from older animals.

12. The conditions to which meat and meat products are exposed during processing do not inactivate BSE infectivity. The thermal conditions used in processing of beef products such as canned meat and smallgoods are insufficient to completely inactivate the BSE agent. The conventional unit operations, such as filtration and washing, used in the food-processing industry, do not eliminate the BSE agent.

Milk and dairy products

13. Experimental studies to date have shown no detectable infectivity in the mammary gland of:
    - BSE-affected cows or goats;
    - Suffolk sheep with natural scrapie,
    - Colostrum of Suffolk sheep in the pre-clinical phase of natural scrapie, or
    - Milk from cows with BSE or goats with natural scrapie.

14. The main epidemiological studies have suggested some low-level maternal transmission of BSE, enhanced in the late stages of maternal incubation, although no mechanism of transmission has been identified. Epidemiological studies examining the maternal transmission of BSE in a specific cohort study have been complicated however by the contamination of cattle feed after the implementation of the feed ban.

15. A study of BSE-infected cows in beef herds showed no evidence that colostrum or milk consumption during rearing was a risk factor for occurrence of BSE in the progeny.

16. The weight of available evidence does not support a conclusion that bovine milk is a risk factor in the occurrence of vCJD cases. Milk is the lowest risk animal product that is consumed widely by the population.

Gelatine

17. The BSE infective agent has been found to occur in the nervous tissue associated with some bovine bones. It has also been found in bovine bone marrow in the clinical phase of experimental BSE. Furthermore, there is some evidence that during slaughter the infective agent can contaminate parts of the hide that are later used in gelatine manufacture.

18. There has been no work to show the efficacy of the gelatine production process in its entirety in reducing the level of the TSE agent. However, studies on the majority of unit operations of gelatine manufacture have shown that the conventional acid bone and limed bone treatments reduced infectivity by more than 99.9%. Addition of a sodium hydroxide step, which is not in current commercial use, gives an infectivity reduction of more than 99.99999%.

19. On the basis of experimental evidence, the degree of inactivation achieved by the routine manufacturing procedures is considered to be sufficient to have inactivated as much BSE infectivity as might be present in bones under worst-case scenarios (i.e. bones from one complete BSE-infected carcase). The addition of a sodium hydroxide step, if undertaken, provides a further level of assurance.

20. Cross-contamination of hides during slaughtering is not likely to carry any more infectivity than bones into the start of the gelatine manufacturing process.

Tallow

21. Bovine tallow produced for edible purposes in Europe is routinely made from discrete adipose tissue. However in other parts of the world other animal tissues are used to make edible tallow. Adipose tissue in cattle has been shown to be free of BSE infectivity.

22. Tallow produced by rendering other animal tissues is usually subject to further refining involving neutralization of free fatty acid by sodium hydroxide, filtration and deodorisation before use in edible applications. This may further reduce any BSE infectivity.

23. On the basis of the available evidence, edible tallow used as an ingredient in a processed product present a negligible risk of carrying BSE infectivity. Bulk tallow may present a higher risk of exposure to BSE infectivity because, if produced by the rendering of animal tissues, further refining may not occur and the resultant tallow may be incorporated into a range of products.

Collagen

24. The parts of the hide used for making collagen do not include those which may be contaminated with SRM during slaughter. Collagen presents a negligible risk of carrying BSE infectivity.

2.0 INTRODUCTION

The purpose of this risk assessment is to systematically examine the available scientific data and information to estimate the risks to human health from consumption of beef and beef products containing the bovine spongiform encephalopathy (BSE) agent. The Australia New Zealand Food Authority (ANZFA) conducted this risk assessment in consultation with national and international experts.

Variant Creutzfeldt-Jakob disease (vCJD), a rare and fatal human neuro-degenerative disorder, was first reported in the United Kingdom in 1996. The disease affects younger people (average age 29 years) and is characterized by neurological symptoms including psychiatric symptoms, difficulty in walking, involuntary movements and loss of speech. The emergence of vCJD has caused worldwide concern about the use of bovine materials from BSE infected animals in the production of food for human consumption. BSE, commonly known as "mad cow disease", is a chronic degenerative disease affecting the central nervous system of cattle. Information on the BSE epidemic in cattle is contained in Appendix 1.

The aim of the risk assessment is to:

(i) examine the impact of vCJD on human health;
(ii) evaluate the current scientific data which implicates the BSE agent as the causative agent for vCJD; and
(iii) estimate the risk of exposure to the BSE agent through food consumption in order to develop risk management options.

There are four components to the assessment:
hazard identification, hazard characterization, exposure assessment, and risk characterization.

Hazard identification describes the BSE agent and examines its association with developing vCJD. The hazard characterization estimates the relationship between the dose of the infectious agent and likelihood of humans developing vCJD. This estimation has been extrapolated from the available data on the infectious dose that results in the development of BSE in cattle. The physical and chemical characteristics of the BSE agent are examined. The exposure assessment estimates the likely intake of the BSE agent resulting from exposure through food and examines the effect of processing conditions on the activity of the BSE agent. The risk characterization estimates qualitatitively the overall risk of developing vCJD as a result of exposure to the BSE agent via food by integrating the above information.

This risk assessment has been based on the currently available evidence. As results of further studies become available, the assessment will be reviewed.

3.0 HAZARD IDENTIFICATION

The hazard being evaluated in this assessment is the BSE agent and this section examines the evidence indicating that the BSE agent is the causative agent for vCJD.

3.1 Variant Creutzfeldt-Jakob Disease (vCJD)

Creutzfeldt-Jakob Disease (CJD) is a generic term for a group of rare, progressive and ultimately fatal disorders of the central nervous system. It belongs to a group of disorders known collectively as transmissible spongiform encephalopathies (TSE). TSEs usually affect adults, are all fatal diseases and have long or very long incubation periods, measured in years or decades. Other than scrapie in sheep and chronic wasting disease in deer, TSEs are not contagious. TSEs are caused by unconventional agents that are very resistant to inactivation using processes that are lethal to conventional micro-organisms such as bacteria and fungi. The disease specific form of PrP is insoluble and is partially protease resistant. These properties are utilised in diagnostic tests.

All TSEs show spongiform change in the brain and the appearance in the brain of unique fibrils called scrapie-associated fibrils (SAF). TSEs are also characterised by the presence in the brain of prion protein (PrP) which is unique to these diseases and may be the infectious agent. PrP is a glycoprotein present on nerve cell membranes and contains many O- and N-linked glycosylation sites (Brown, 1990). The protein is anchored to the neuronal surface by glycosylphosphatidylinositol suggesting a role in cell signalling or adhesion (SEAC 1994). The disease specific form of PrP (PrP^{Sc}, read as 'PrP scrapie') is post-translationally derived from the normal cellular form (PrP^{C}) by an unknown process. The process involves a change in the secondary/tertiary structure in which α helix is converted into β sheet conformation (Howard, 1996; Horwich and Weissman, 1997).

Sporadic CJD, the commonest form of CJD, occurs worldwide at a constant, rare incidence of one to two cases per million per year (Holman et al, 1995). Familial CJD and Gerstmann Sträussler Scheinker syndrome are hereditary forms caused by mutations in the *PrP* gene, *PRNP* (Lugaresi et al, 1986; Prusiner, 1995). Iatrogenic CJD has occurred in a small number of human patients as a result of historical medical accidents that have transmitted CJD from one human patient to another. The vehicles have been incompletely decontaminated surgical instruments and stereotactic depth electrodes, corneal grafts (collectively fewer than 10 patients), *dura mater* grafts and human, cadaver-derived pituitary growth hormone and gonadotrophic hormones (Richard et al, 1994; Ellis et al, 1992). Contaminated *dura mater* and growth hormone have been responsible for most cases of iatrogenic CJD. Two other very rare prion diseases are fatal sporadic insomnia and fatal familial insomnia (Collins et al, 2001).

The other human TSE is kuru, now virtually eliminated from a population of Fore-speaking people inhabiting villages in the Eastern Highlands of Papua New Guinea (Alpers, 1987). The disease was transmitted between people by endocannibalism, the consumption of the whole body after death as part of the funeral rite. After cannibalism stopped in the late 1950s, the disease has virtually disappeared, with the few cases that have been recently reported having incubation periods of over 40 years.

In 1996 a new type of CJD, now commonly known as variant CJD or vCJD, was first described in the United Kingdom. It is the only human TSE that is considered to be derived from animals. The evidence from kuru supports a minimum incubation period for vCJD of 3 to 4 years and a maximum of 30 to 40 years. vCJD differs from classical CJD in several aspects (Will et al., 1996):

- the early age of onset (with a median age at death of 29 years (range 12 to 74 years) compared with 65 years for classical CJD;

- the duration of illness—it takes on average 14 months between the onset of clinical symptoms and death, compared with 4 months with classical CJD;

- the atypical electroencephalogram trace. The brains of vCJD patients show other distinctive changes in addition to the classical spongiform changes seen in the brains of classical CJD patients (Roberts and James, 1996); and

- the tissue distribution of infection and PrP. In vCJD, the lymphoreticular tissues are implicated notably the tonsils, lymph nodes and spleen.

Risk factors for vCJD

In humans there are two common forms of PrP with either methionine or valine at amino acid 129. All of the human cases of vCJD so far evaluated have been methionine homozygous at amino acid 129 of the PrP gene, although these individuals form less than 40% of the population (CJD, 2001; Ironside et al, 2000; Will et al, 2000). Methionine homozygosity for amino acid 129 in the PrP gene is known to be a risk factor for other human TSEs, such as kuru and sporadic CJD (Cervenakova et al, 1998). It is likely that methionine homozygotes are more susceptible to vCJD infection and/or have shorter incubation periods than other genotypes (Ghani et al, 2002). However, this does not mean that the other possible genotypes at this amino acid, namely valine/valine or methionine/valine are not susceptible. They may be, but with longer incubation periods (Lee et al, 2001). A study on patients with kuru revealed that homozygotes at codon 129 displayed a reduced average age of onset and duration of illness compared with methionine /valine heterozygotes (Cervenakova et al, 1998).

The methionine homozygosity, residence in the United Kingdom and a young age are considered risk factors for developing vCJD (Will 2001). Population subgroups considered to be “immunologically at risk”, such as infants, pregnant women and individuals on immunosuppressive treatments, are likely to be no more susceptible to vCJD than the rest of the population (Brewer, 2001).

Size of vCJD epidemic

As at July 2002, there were 131 vCJD cases worldwide with the majority of cases (122) occurring in the United Kingdom. There have been a number of attempts to estimate the potential size of the human epidemic of vCJD in the United Kingdom resulting in a wide range of estimates due to uncertainties surrounding the route of infection, infectious dose, incubation period and the role of genetic susceptibility. In

constructing statistical models, the epidemiology of kuru, another human TSE linked with the consumption of infected material, has been examined. As kuru has been studied for over 40 years, it is seen as a useful model to estimate epidemic development and the likely range of the incubation period.

It has been predicted the number of vCJD cases in the UK will double every three years (Will, 2001). The upward trend in vCJD continues to be statistically significant with an increase of 21% per year for disease onset and 23% for deaths and the possibility of 32 deaths occuring in the next twelve months from the disease (CJD, 2002). The most recent estimates of the size of the vCJD epidemic in the United Kingdom predict a range from a minimum of about 200 cases to a maximum of 136,000 cases by 2040 based on an age susceptibility factor (Ghani et al., 2000a). In the first five years of the epidemic, the age distribution of those affected with the disease has remained constant, thereby limiting the size of the population to be considered to be at risk of developing vCJD (Ghani et al, 2002). On this basis, the predicted total number of cases would reduce to approximately 20, 000 if the average annual incidence of vCJD during the period 2000 to 2003 is less than 15 cases. Another study has predicted 80,000 cases over a 25-year period (Alperovitch, 2001) and another study (Valleron et al., 2001) has predicted only 200 to 400 cases, suggesting that a considerably greater amount of the infectious agent is required to infect humans compared to cattle. This estimation was based on the following assumptions:

- the risk of developing vCJD decreases exponentially with age after age 15 ie. the young are more susceptible to infection;
- all infections occurred between 1980 and 1989; and the distribution of the incubation period is lognormal with a mean duration of 16.7 years.

This model predicts the peak of the epidemic would be during 2000/2001 and the annual number of cases should decrease after this time.

The studies by Ghani and Valleron assume that genetic susceptibility is a high risk factor for developing vCJD and do not take into account the possibility of a longer incubation period for individuals with other genotypes at amino acid 129.

There are still insufficient data to determine the period between infection and the onset of clinical symptoms of the disease, which makes it impossible to predict the size of the epidemic. If the incubation period is short, cases should have already developed symptoms however if the incubation period is longer than 20 years, cases will only be reported in the future. It is estimated that 54,000 infected animals entered the human food supply in the United Kingdom before the disease was recognised in 1985, and that approximately 750,000 infected animals entered the food chain between 1974 and 1995 (Anderson et al., 1996). This data suggests that there has been significant human exposure to the BSE agent.

Treatment for vCJD

There are no effective treatments or prophylaxis for vCJD or other human and animal TSEs. There are drugs that, when administered to experimental animals at or around the time of experimental challenge, prolong or shorten the incubation period of the TSE (Fraser, 2002). If it can be determined how these operate at the molecular level there may be the potential to develop more effective drugs to assist in the care of at-

risk patients or those in the very early stages of clinical disease. However, at present there is no way to arrest the progress of CJD.

3.2 The BSE disease in cattle

There have been over 180,000 confirmed cases of BSE in cattle in the UK and over 1500 cases have now been reported in continental Europe. Recent developments indicate that BSE may be present but undetected throughout Europe and it has recently been reported in Japan.

The clinical, neurological signs of BSE are insidious in onset, and progressive; they include changes in sensation and behaviour, mental status, posture, locomotion and general signs. The most important signs are apprehension, hypersensitivity to touch, sound and light, and gait ataxia especially of the hind limbs. General signs include loss of condition and milk yield (Davis et al, 1991). Characteristics of the disease are vaculolation of neurons and neuropil, neuronsal degeneration and astrocytosis, associated with the accumulation of a protease resistant form of prion protein in the brain. BSE has an estimated mean incubation period of around 60 months and most cases occur in 4 to 6 year old cattle.

Pathological examination of the brain reveals there are no gross lesions. Microscopic lesions are confined to the brain and include, loss of nerve cells, spongiform change in the grey matter, vacuolation of nerve cells and enlargement of supporting cells called astrocytes. In addition, prion protein can be detected in association with the vacuolation (Davis et al, 1991).

Clinical examination provides sufficient evidence to suspect BSE and make a clinical diagnosis. However not all clinical suspects are confirmed by laboratory testing, and in the UK all clinically identified cases are subjected to confirmatory testing. Microscopic examination of the brain is the method of choice. This can be supported by immunohistochemical examination of tissue sections of brain to confirm the presence of PrP^{Sc}. PrP is a protein of 250 amino acids which is specific for the particular animal, however the amino acid sequence for PrP and PrP^{Sc} is identical. PrP can be detected by immunoblotting and a variety of other immunological tests (such as Rapid tests). Finally, scrapie-associated fibrils (SAF) can be detected by electron microscopy.

Differences between the BSE agent and scrapie agents

Strains of TSE agents can be identified by biological means. Strains are identified by inoculation of a panel of mice and determining the incubation period, lesion profile (distribution of vacuolation) in the brain (mouse bioassay) and physical symptoms. In this way, some 10 or so strains of scrapie agent have been detected, and many but not all, are adapted to laboratory species such as hamsters and mice, so that they can be maintained in laboratory animals. The BSE agent from cattle in the UK, Switzerland and France appears identical and is quite different from any of the strains of scrapie that have been identified.

Molecular methods have also been developed to determine differences in fragment sizes and glycosylation ratios in PrP^{Sc} following treatment of concentrated brain

extracts with proteinase K. Different molecular strains can be identified and may explain differences in the development of the disease in different species (EC2000a). Based on lesion profiles, BSE is caused by a single strain of the agent however a number of strains are responsible for variations in the disease phenotype of scrapie and more that one scrapie strain can be isolated from a single sheep (Wells, 2001).

3.3 Evidence linking BSE and vCJD

The main evidence linking BSE and vCJD comes from epidemiological investigation and the transmission and molecular properties of the causal agent (Ironside, 1999; Dormont 2000; Zeidler and Ironside, 2000).

The epidemiological evidence

Epidemiological evidence has shown that vCJD is a new disease not observed before 1994 in any country. Furthermore, from that time, when 10 cases had been identified, and until now (July 2002), when 130 definite or probable cases have been reported, there are temporal and geographical correlations that indirectly support the view that exposure to the BSE agent is the probable cause of vCJD and that the agent is transmitted via food.

There is a strong temporal correlation between the appearance of vCJD in humans and exposure to BSE-infected meat. vCJD was first diagnosed in the United Kingdom in 1996, approximately ten years after BSE was reported in their cattle population. During the period 1994 to 1996, vCJD was firmly identified as a single clinical and pathological entity distinguishable from all other forms of CJD and prion disease in humans (Will et al., 1996). Before the specified bovine offals (SBO) ban was introduced in the UK in 1989 (Appendix 3), there was clear evidence that infected tissues could and did enter the human food chain, thus resulting in exposure of the human population to the BSE agent. However the extent of consumption of brain or spinal cord is not known nor the tissues with the potential to have highest titre and which might deliver a human oral infective dose. It is known that mechanically recovered meat (MRM) was produced from, among other tissues, bovine vertebral column. Thus, it was entirely possible that remnants of spinal cord and dorsal root ganglia found their way into MRM. MRM was used in meat products such as some burgers, pies, and sausages. All the necessary ingredients for human exposure were therefore present probably from around 1983 until 1989 when the SBO ban would have curtailed, but did not stop, human exposure. The youngest reported case of vCJD was 12 years of age and was only 3 years of age when the SBO ban was implemented (Verity et al, 2000).

The geographical evidence stems from a demonstrated association between the consumption of beef products and the occurrence of vCJD in 5 people who had lived within a five kilometre radius of each other in Leicestershire, UK. This study remains the only statistically significant cluster of cases in the UK. The key findings were:

- a geographical cluster of vCJD cases;
- the potential for BSE-affected cattle to enter the human food chain; and
- a possible explanation for the cluster through the potential for cross-contamination of muscle meat with brain tissue.

These practices for slaughtering animals and carcase trimming were typical of traditional butchering practices in the UK until the 1980s (Leicestershire Health Authority, 2001). This study also provided the first evidence that the incubation periods of vCJD are in excess of 10 years.

There is also a regional variation in the incidence of vCJD with approximately twice the number of vCJD cases reported in the north of the United Kingdom compared with the south (Will, 2001). The basis of this finding is unclear and may be related to dietary consumption of high risk products containing mechanically recovered meat, however this pattern does not reflect the geographical distribution of BSE cases in cattle (Stevenson et al, 2000).

The transmission evidence

The crucial evidence was obtained by biological strain typing the BSE agent from humans with vCJD, other cases of human TSE disease and cattle with BSE. The incubation period and lesion profile of the agent isolated from three human patients with confirmed vCJD were compared with isolates from cattle with BSE, humans with sporadic CJD before and during the BSE era, and with isolates from farmers with sporadic CJD who had BSE confirmed in their herds. These studies showed that vCJD isolates were similar to each other and to isolates from cattle with BSE (Bruce et al., 1997). They were clearly different from isolates from sporadic CJD. Bruce et al. (1994) had previously shown that isolates from cattle with BSE were different from those from sheep with scrapie but similar to those from cats with feline spongiform encephalopathy, and a greater kudu, a nyala, and sheep, goats and pigs that had been experimentally challenged with BSE and developed a spongiform encephalopathy.

The results of these studies showed conclusively that there was a close similarity in the biological strains of agent, as determined by incubation period and lesion profiles, derived from humans with vCJD and cattle with BSE. This evidence strongly suggests that both diseases are caused by the same agent.

The molecular evidence

The prion theory of infection postulates that the amino acid sequence of PrP from different species is different, and this is indeed the case in cows and man, each having a specific sequence depending upon the nucleotide sequence of DNA in the coding region of the *PrP* gene (Prusiner, 1995). Thus, the PrP derived from human patients with vCJD is not necessarily chemically identical in primary structure to PrP in cattle with BSE. However, the prion theory proposes that prions can adopt multiple conformations and different conformations of the infectious form of the protein (PrP^{Sc}) are responsible for transmitting strain-specific information.

PrP from diseased humans and animals can be distinguished by molecular analysis of the protein (Collinge et al., 1996; Hill et al., 1997). The essential process is to extract the protein from infected tissue (usually brain), treat with proteinase K and identify digested fragment using western blotting. Differences in the fragment sizes produced by the enzyme and the specific amounts of mono-, di- and non-glycosylated fragments are characteristic for the PrP derived from the brains of all patients examined who have vCJD confirmed by other methods according to standard criteria.

Patients with various other forms of CJD or prion disease have glycosylation patterns that are clearly distinguishable from those found in the brains of patients with vCJD. The PrP characteristic of vCJD has a uniform glycotype (PrP^{Sc} type 4) which is distinct from that of sporadic CJD (Ironside et al, 2000).The glycosylation patterns found in the brains of patients with vCJD are closely similar to those found in cattle with BSE. These data provide strong evidence that vCJD was induced by the BSE agent. Furthermore, experimentally induced BSE in macaques and mice, and a naturally infected domestic cat with feline spongiform encephalopathy, show indistinguishable glycoform patterns to those in vCJD (Collinge et al., 1996).

3.4 Conclusions

The available evidence from epidemiological data, strain identification and molecular characterization of the infective agent strongly suggest that vCJD is caused by the transmission of the BSE agent to humans.

Epidemiological evidence indicates that BSE and vCJD are caused by the same agent and strain typing evidence (both biological and molecular) strongly suggests that vCJD and BSE are caused by an agent with similar biological and molecular properties. There is an excellent correlation between biological and molecular typing of BSE and vCJD isolates and these have a unique molecular profile. This enables vCJD isolates to be clearly distinguished from isolates from other forms of human prion disease.

The occurrence first of BSE, then of vCJD in humans, initially in the United Kingdom, strongly suggests that vCJD has been derived from the consumption of beef and beef products derived from cattle with BSE. The most likely tissue origin for vCJD is central nervous tissue from BSE-affected cattle or from other tissues that have been significantly contaminated with such tissue.

A similar conclusion was drawn at a joint WHO/FAO/OIE (World Health Organization/Food and Agriculture Organization of the United Nations/Office International des Epizooties) Technical Consultation on BSE, which was held in Paris in 2001. The consultation confirmed that BSE is a risk to public health because the disease is transmissible to humans and scientific consensus confirms that food is the main avenue of exposure.

4.0 HAZARD CHARACTERISATION

In this section, the modes of transmission of the BSE agent, the infectious dose of the BSE agent for humans and the physical and chemical characteristics of the agent are discussed. The impact of processes used during food production on the infectivity of the BSE agent are examined in section 5 – Exposure assessment.

4.1 Transmission of the BSE agent

Dietary transmission

The main source of transmission of the BSE agent from animal to animal is considered to have occurred through contaminated feed (Wilesmith et al, 1991).

The oral route is clearly the most common and possibly the only route of effective exposure in cattle. This is supported by experimental oral challenge. The nasal, respiratory and conjunctival routes cannot be rejected with total certainty, but if they are effective they probably play a minor and probably insignificant role. Possibilities for iatrogenic spread of infection such as via contaminated medicinal products, vaccines, catgut, surgical instruments are theoretical, and there is no substantiated case for this in cattle.

A few studies have examined the dietary transmission of BSE-infected tissue using brain and cerebral spinal fluid, extraneural tissues and milk (Barlow and Middleton, 1990; Middleton and Barlow, 1993; Taylor et al*.*, 1995).

Barlow and Middleton (1990) were able to show that the BSE agent could be transmitted orally to mice using infected brain and cerebral spinal fluid. In this study, infected tissue sourced from four BSE-affected cattle was fed to mice over an 8-day period. Mice exposed to infected feed subsequently developed BSE, the first five infected cases dying 15 to 18 months after the feeding study began. This 'baseline' study established that the BSE agent could be transmitted experimentally through feeding, the most simple and natural route of transmission.

The epidemiological evidence demonstrates that the major route of transmission of the BSE agent to cattle is through the consumption of contaminated meat and bone meal. The evidence for transmission of the BSE agent to humans has been examined in section 3. As mentioned previously, experimental studies have confirmed that the agent can be transmitted to animals (including humans). Epidemiological evidence has shown a very strong association between the people who developed vCJD and the consumption of beef from butchers who used practices which resulted in cross-contamination of meat with brain.

Maternal transmission

While the incidence of BSE declined after the feeding of meat and bone meal was prohibited in the United Kingdom, new cases were also confirmed which suggested that there might have been another source of the BSE agent. A second possible route of transmission of the BSE agent is maternal transmission and several studies have investigated this possibility. Maternal transmission refers to the transmission of

infection from dam to the offspring in utero or in the immediate post-natal period. Possible mechanisms of maternal transmission include transfer of the infective agent across the placenta during gestation (direct transmission) or via milk, colostrum or reproductive tissue after birth (indirect transmission). The key experimental and epidemiological studies conducted to date which have examined maternal transmission and the infectivity of milk and/or colostrum are:

- Wilesmith et al. (1997) conducted a long-term cohort study to assess the risks of non-feed borne transmission of BSE. This study suggested milk was an unlikely source of infection if maternal transmission had occurred because of the absence of BSE cases among the offspring of BSE-affected pedigree suckler cows, a group with higher exposure to milk. The majority of the pairs in the cohort study were from dairy herds which receive only colostrum for the first 24–48 hours of life and no milk thereafter.

- Donelly et al. (1997b) undertook further statistical analysis of the data from the cohort study. This analysis showed a significantly enhanced risk of infection in exposed animals born close to the onset of clinical signs of infection in the dam (either after or less than 150 days before the onset of clinical signs). These temporal observations could not be explained by genetic susceptibility alone and are purported as evidence for low-level direct maternal transmission in the late stages of the incubation period.

- Donelly et al. (1997a) confirmed the presence of an enhanced risk of BSE in calves born to dams that had the disease or developed it soon after giving birth. The extent of the increased risk was related to the BSE incubation stage of the dam when calving.

- A further study by Donnelly (1998), using the data from the Wilesmith and Ryan (1997) pedigree suckler cow study, proposed a maternal transmission rate of 17.3% (at the 95% confidence limit) over the last 6 months of the maternal incubation period, though no possible mechanism of transmission was indicated.

- Wilesmith and Ryan (1997) conducted a further study to examine the risk of developing BSE for offspring of all BSE-affected pedigree suckler cows in the UK. A total of 132 offspring from beef suckler herds which had suckled their dams for at least one month was investigated. It was estimated that these animals, collectively, consumed 111,500 litres of milk. None of the cattle developed BSE. On this basis it was concluded that milk was highly unlikely to be a source of infection for cattle.

- A case-control study by Hoinville *et al.* (1995) further investigated the evidence for direct transmission (maternal and /or horizontal) of BSE to cattle born after the feed ban. This study found that the number of BSE cases in the offspring of cattle that were subsequently diagnosed with BSE was not statistically significant and that direct transmission could not account for the majority of BSE cases born after the feed ban. These later cases would most likely be attributed to feed contaminated with the BSE agent or indirect routes of transmission between cattle.

- Ridley and Baker (1995, 1996) propose that there is much stronger evidence for a genetic basis for susceptibility rather than maternal transmission. Ridley and Baker (1996) emphasise that the numerical data used in the cohort study cannot differentiate between genetic susceptibility and maternal transmission, nor can exposure to contaminated feed be discounted. They emphasise that there is no established mechanism by which maternal transmission would occur and that there is no evidence for maternal transmission in other spongiform encephalopathies, including scrapie.

- Braun et al. (1998) and Fatzer et al. (1998) reported on a study of the offspring of BSE-affected dams in Switzerland involving clinical and neurological examination of 182 offspring and included testing of blood, milk, urine, rumen fluid and cerebrospinal fluid. Clinical, histological and immunohistochemical examination of the animals were all negative for BSE, though other neuropathological lesions were found in seven animals. While this study did not support maternal transmission, it was limited in that only half of the animals examined had survived for sufficient time to develop signs of the disease. Additionally, most of the animals were born in the early stages of the dams' incubation of the disease, an increase in risk of developing BSE having been shown in the later stages of maternal incubation (Donelly *et al.* 1997a).

Maternal transmission of other TSEs

Scrapie

It has been widely accepted that vertical transmission, through the placenta *in utero* or by contact with the placenta after birth, is the main route of transmitting infection of natural scrapie (SEAC, 1994; EC 1999). Studies which have examined tissue infectivity from sheep and goats with natural scrapie have shown no detectable infectivity in milk and colostrum or in reproductive tissues when bioassayed in mice (EC 1999).

CJD and kuru

Kuru was found to affect more women and children than men. This was because women and children primarily consumed the brains in ritual ceremonies while the men mainly consumed muscle and other tissue (Alpers, 1987). Kuru declined after cannibalism ceased in the late 1950s, and no children born after this time to any women who subsequently developed kuru have developed the disease (Alpers, 1987). This includes children suckled by mothers with clinical kuru at the time of lactation.

There is one report in the literature of transmission of infectivity of spongiform encephalopathy through colostrum from a woman in early lactation (fourth day after delivery) who had been diagnosed with CJD during her pregnancy. (Tamai et al., 1992). The colostrum, along with other tissues, was tested for infectivity through intracerebral injection into mice which were then observed for a minimum period of 600 days. The colostrum was reported as demonstrating infectivity, both in the first and second passage of transfer, however this study has been invalidated because further examination found no sign of disease (no spongiform change or PrP^{Sc}) on the

first passage from human colostrum to mice. Transmission of disease was found only in mouse to mouse transmission in the second passage which could not be attributed to colostrum (EC 2001a).

Conclusion

vCJD and BSE are not contagious diseases and there is no direct evidence for the occurrence of horizontal transmission. Comparison of the expected number of infected calves born since 1990 and the actual number of infected calves provide some evidence for direct maternal transmission at a low level (reported at approximately 10% late in the incubation period) but no mechanism for maternal transmission of BSE has been identified (SEAC 2001). As very few cases of BSE have appeared in the United Kingdom since the MBM ban in 1996, the true incidence of maternal transmission is likely to be lower than 10% transmission (SEAC 2001). No infectivity has been detected in milk, ovary, embryos, placental cotyledon, amniotic and allantoic fluids, uterine caruncle, mammary gland or in embryos transferred from BSE-affected cows (EC, 1999).

4.2 Infectious oral dose of the BSE agent for humans[1]

A critical determinant for assessing the level of risk resulting from exposure to a particular agent is the infectious dose. However, the infectious dose of the BSE agent that induces vCJD cannot be determined from human studies. The infectious dose can be deduced from studies examining the following factors:

- the titre of infectivity in the tissue measured by the i/c route;
- the mass of infected tissue consumed at one sitting;
- the difference in efficiency between the i/c and oral routes;
- the strength of the species barrier;
- cross-contamination by tissues with a higher infectivity; and
- any concentration of infectivity that might result from processing.

The titre of infectivity would ideally be measured in the species under consideration (human) by the most efficient route (i/c) to maximise the chance of detecting low levels of infectivity and determining the level of risk across a species barrier. It is clearly impossible to conduct a bioassay in humans, so reliance must be placed upon i/c titrations in the donor species. Mice are chosen because it is relatively quicker and cheaper to assay infectivity in this species and they can be bred to be genetically uniform, but bioassays in cattle can detect lower levels of infectivity that mice cannot. Titres in infected brain from cattle can be underestimated by a factor of 500 when measured in mice rather than cattle (Wells, 2001).

The species barrier

The species barrier denotes the relative efficiency with which a disease is passed to an animal of a different species compared to an animal of the same species. A greater amount of infectious agent is required to infect an animal from a different species

[1] Further explanation of the definitions and concepts relevant to this discussion of infectious dose is in Appendix 2.

compared to the amount required to infect an animal from the same species. The species barrier is influenced by the interaction of two variables: the strain of agent and the donor species effect. In the case of BSE and vCJD the donor species effect seems to be determined, at least in part, by the sequence differences of the *PrP* gene in the donor and recipient species (SEAC, 1994). The greater homology in sequence between the PrP^{Sc} and PrP of the recipient species, the greater likelihood of the recipent species developing the disease. The bovine and human form of PrP differ by approximately 20 amino acids (EC2000a). A study inoculating normal mice and transgenic mice containing hamster genes with hamster PrP^{Sc} demonstrated that the normal mice rarely developed the disease but the transgenic mice acquired the disease in only a few months (Prusiner, 1995). In transmission to humans (and in sheep) there are naturally occurring polymorphisms in the *PrP* gene that also appear to influence the incubation period and possibly the occurrence of infection.

A range of 1 to 10,000 for the cattle to human species barrier has been indicated for use in risk assessments of human exposure to BSE-contaminated products (EC 2000a).

Data from studies on BSE infectivity titres in different mice genetic lines indicate an intra-species barrier of the order of 1000 (Collinge et al, 1995) which could be assumed to be the minimal barrier for transmission of the disease between species.

The route of exposure

The main routes of experimental challenge with TSE agents that have been used are, in order of decreasing efficiency : i/c, i/v, i/p, s/c and oral/intragastric. At least four other routes have been investigated including intraocular, conjunctival (Scott, et al., 1993), dental (Ingrosso et al., 1999). and skin scarification (Taylor *et al*., 1996c). There is no reported evidence that the respiratory system is a route of infection.

Experimental studies in rodents have demonstrated that the intracerebral (i/c) route is the most efficient means of transmitting infection and that the oral route is the least efficient. Studies using experimental scrapie in hamsters showed that oral transmission was very inefficient, requiring about 10^9 times the i/c dose to effect transmission within the same incubation period (Prusiner *et al*., 1985), as determined by incubation time rather than end point titration of BSE infectivity.

Ttransmission studies of BSE-infected cow brain into mice (i.e. across a species barrier) demonstrated that, in RIII mice, the incubation period was shorter when the route was intraperitoneal (i/p) than if the route was i/c or a combination of i/c and i/p (Fraser *et al.,* 1992; Taylor *et al.,* 1994). This was attributed to the need for the material inoculated from the foreign species by the i/c route to require peripheral processing before returning to the brain to produce disease. This may not be required for within-species transmission from host-adapted TSE agents and the significance should be considered when assessing the risks from BSE-infected cattle tissue for man.

Some studies have compared the i/c and the oral route, using the same agent and the same inoculum, allowing a direct comparison of the efficiencies of the two routes. The relative efficiencies of the different routes in mice using the 139A strain of

scrapie were: i/c = 1, intra-venous (i/v) = 9, i/p = 430, sub-cutaneous (s/c) = 24,500 and oral, i.e. intragastric = 100,000. These data indicate that infection via the oral route would require 100,000 times more material than by the i/c route. Further studies showed that, using hamsters and the 263K strain of scrapie, the comparative efficiency of the i/c to i/p route was 40,000 and suggesting that there might be species/strain effects (Kimberlin and Walker, 1988a,b, 1989). Kimberlin (1994), using the historical data on murine scrapie and new BSE data across the species barrier from cow to C57 black mice, showed that the efficiency of the i/c compared with the oral route was of the same order of magnitude (about 10^5 mouse to mouse for strain 139A scrapie and 2×10^5 cow to mouse for BSE). However, Taylor *et al.,* (2001) concluded that transmitting the BSE agent via the oral route was 700 times less efficient than the intracerebral route, significantly less than the factor of 200,000 calculated from other studies in C57 black mice by Kimberlin (1994). The reason for this difference is not understood though one possibility proposed by Taylor *et al.* (2001a) was that there are more replication sites for the agent in the intestine of RIII, compared with C57 black mice.

A study conducted to investigate the possible cumulative effect of low infectivity brain material, showed that, following oral titration of infection in hamsters, the relative efficiency of the i/c route to the oral route was between 10^5 and 10^6 (Diringer *et al*., 1998). These studies confirm the oral/intragastric route is relatively inefficient compared with parenteral routes and especially the i/c route.

These studies demonstrate that transmission of the BSE agent to humans can occur orally (via food consumption), however this route is relatively inefficient in transmitting infection.

Experimental BSE oral attack rate study in cattle

Currently the infectious oral dose for humans or cattle is not known. An oral attack rate study in cattle (Dawson 1993) provides some insights. The study used untreated brain material from a pool of brains derived from cattle with clinically confirmed BSE. Groups of cattle were exposed to four different treatment regimes: 100 g of untreated brain material given orally on three occasions, or 100 g given on one occasion; 10 g given on one occasion; or 1 g given on one occasion. The infectivity titre of the brain pool used in this study was about $10^{3.5}$ mouse i/c ID_{50}/g (refer to Appendix 2 for explanation of this unit; Dawson *et al.,* 1993; Wells *et al.,* 1994, 1996, 1998, 1999). Estimates for the ID_{50} of BSE-affected cattle brain tested in mice range from 10^3 to 10^5 per gram of brain (EC2000a).

All four groups developed disease. The attack rate was not 100% in the two, low-dose groups (1 g and 10 g) and the incubation period was longer than in the high dose groups (G.A.H. Wells and S.A.C. Hawkins, personal communication). It can be concluded that 1 g of brain with an infectivity titre of $10^{3.5}$ mouse i/c ID_{50}/g is probably not a limiting oral dose for cattle. Since cattle were never exposed in the field situation to untreated brain material but rather to cooked and dried brain, in the form of meat-and-bone-meal (MBM), it might reasonably be expected that an infective oral dose of 1 g of fresh brain might now be accommodated in a mass of about 0.1 g of dried brain, assuming that the cooking process did not provide for any inactivation and there was no dilution by mixing. An infectious oral dose could be

present in a very small amount of MBM as a result of the effective titre increasing due to concentration.

A further transmission study has examined the effect of oral inoculum dose on the attack rate and incubation period of BSE in cattle. Interim results from this study suggest a bovine oral ID_{50} can be contained in 0.38 g of brain but the 95% confidence intervals are wide (Wells, 2001).

To more precisely determine the limiting infective oral dose for cattle a second study is in progress using doses of brain of 1 g, 0.1 g, 0.01 g and 0.001 g. The results of this study are not yet available.

Oral infective doses

A cautious best estimate of infectivity of bovine brain is that it contains 10 bovine oral ID_{50}/g or 1 ID_{50} would be contained in 0.1 g (e.g. DNV, 1997). If the species barrier between cattle and humans is 1 (i.e. there is no species barrier, which is most unlikely), the risk for man would be the same as the risk for a cow following consumption of 0.1 g of brain. Consumption of this amount of brain by each of a group of individuals would be expected to result in infection and the ultimate death from vCJD in 50% of them, provided they lived through the incubation period (which in humans is unknown but is expected, on average, to exceed 10 years). The risk of death from vCJD for a person consuming 0.1 g of such brain would be 50%.

If the species barrier is actually greater than 1 (more likely), the risk would be diminished accordingly. One estimate assumes 10 bovine oral ID_{50}/g and assuming a cow to human species barrier of 10, indicating that every gram of cow brain (and spinal cord) contains 1 human oral ID_{50}. In an opinion of the Scientific Steering Committee of the EC (EC, 2000 a), a whole cow with BSE would contain 8000 oral ID_{50} units. An evaluation of a worst case scenario assuming a cow to human species barrier of one concluded that there would be from 10 to 1000 human oral ID_{50}/ gram in the brainstem and for other tissues, excluding the distal ileum, at least 1000 times less infectivity.

If the cattle to human species barrier was assumed to be 10,000 then 1 kg (more than two adult cattle brains) would need to be consumed at one sitting to deliver a 50% risk of contracting vCJD. Such an event would be most unlikely to occur in practice. However, this illustration suggests a problem might still exist for any consumer eating a small proportion (say 10 g) from the two brains, assuming a species barrier difference of 10,000. If the infectivity were evenly distributed in the two brains, and if there is a threshold dose below which infection will not occur, an infective dose could not be consumed. However the BSE agent is not evenly distributed as it is sticky, insoluble and tends to aggregate.

Currently there are no data on the titre of infectivity (measured in cows by the i/c route) for brain or other infected tissues at intervals during the incubation period of BSE to determine the risk from consuming meat from healthy but incubating cows.

There is also a lack of data on the titre of infectivity in different cattle tissues at different stages of incubation, the efficiency of the oral route of exposure across the

species barrier between cattle and humans, and whether the species barrier is large, small or absent.

It is most likely that there is a species barrier between cattle and humans but an estimate of its strength cannot reliably be made.

4.3 Cumulative dose

When infectivity is administered by the oral route, a considerable part of it is believed to pass straight through the gut and to void in the faeces. A proportion of any infectivity that might be absorbed may be destroyed or sequestered and later eliminated by the action of normal cellular mechanisms. If infectivity is established in a susceptible animal, as evidenced by replication of the introduced agent, it is likely that the rate of replication will eventually exceed that of destruction, sequestration and elimination, leading finally to the onset of clinical disease. There are few data to show the efficiency of any clearance mechanism for TSEs. Hadlow et al, (1979) reported the case of a Montdale sheep affected with natural scrapie, where infectivity was detected by bioassay of brain (that had lesions of spongiform encephalopathy) but was undetectable in any other tissue. This argues for a natural mechanism for inactivating or eliminating infectivity in peripheral tissues or, alternatively, that an extra-neural phase of replication is not always necessary, which seems unlikely. Inactivation or elimination of infectivity might be a more efficient process if the infecting dose is small rather than large.

An EC expert group investigated the cumulative dose effect from literature study (EC, 2000b). They concluded there were three possibilities:

- accumulation is unnecessary and is an insignificant factor;
- at least some cases of TSE could result from one or more administrations of a dose below the ID_{50} ; or
- there is a threshold dose, and administering repeated doses below this level at short intervals could result in accumulation of infection and eventually an infectious dose could be achieved.

The expert group concluded that the risks resulting from (low) residual infectivity should be, at present and until further evidence is available, calculated as fractions of a population that would be exposed and possibly infected. In addition, repeated exposure would increase the risk in absolute numbers of cases and the likelihood that exposure would result in infection.

4.4 Inactivation of TSE agents, with special reference to the BSE agent.

TSE agents are characteristically resistant to physical and chemical inactivation procedures proven to be effective against most common conventional infectious agents, like bacteria, viruses and fungi.

Other than studies involving rendering, where brain material from naturally occurring cases of BSE or scrapie were used as spiking materials, most inactivation studies have

used mouse, hamster and guinea pig adapted strains of scrapie, CJD or BSE strain 301V. Strain 301V is the mouse-adapted BSE strain derived originally from cattle and selected in mice. It is the most thermostable strain of agent that has been isolated to date.

Different TSE agents may show different responses to inactivation procedures but, generally, they have the same basic properties that vary only in degree. The BSE agent should be regarded as at least as resistant to any of the inactivating procedures to be described, as any of the agents used in decontamination studies (Schreuder et al, 1998). Macerates should be regarded as more resistant than homogenates of tissues.

The available information on the inactivation of TSE agents has been recently reviewed (Taylor, 2000).

Physical methods for inactivation of TSE agents

Ionising, ultraviolet and microwave irradiation have little effect on TSE agents and have no practical application to their inactivation (Taylor, 2000)

Dry heat is not totally effective in inactivating TSE agents. Brown et al. (1990) demonstrated limited survival of a lyophilised homogenate of hamster scrapie brain following ashing under anoxic conditions at 360ºC for 1 hour. On the other hand, Taylor et al. (1996b), could find no detectable infectivity in macerated mouse brain infected with ME7 scrapie after dry heating to 200ºC for 1 hour, but did detect infectivity after heating at 200ºC for 20 minutes or at 160ºC for 24 hours. Hamster scrapie and 301V BSE brain material partially survived dry heating at 200ºC for 1 hour, however. These studies point to unpredictable differences of thermostability in different strains of TSE agent and the difficulty under practical conditions of determining a dry heat temperature that guarantees that no detectable infectivity is present.

Wet heat, in the form of gravity displacement autoclaving (in which air is expelled by incoming steam) at temperatures in the range 121ºC to 132ºC for periods up to 4.5 hours, enables 5 to 6 log reduction in titre of the hamster-adapted scrapie strain 263K. However, the same studies reviewed by Taylor et al. (2000) also show that significant titres, sometimes in excess of 3 logs, could survive when using some agents and time–temperature combinations.

Wet heat in the form of porous load autoclaving (in which the autoclave chamber is evacuated before filling with steam) using a single cycle of 134–138ºC for 18 minutes hold time at temperature (HTAT), or six separate cycles for 3 minutes HTAT, has been recommended for inactivating any infectivity on instruments that might be used on patients suffering with CJD (DHSS, 1984). However, more recent studies have shown that the higher temperatures in the range are less effective than the lower ones. The 301V murine BSE agent, for example, could survive 138ºC for 1 hour (Taylor, 1999a). This anomaly is attributed to the higher temperature ‘fixing’ the agent and thereby increasing its thermostability. The greater thermostability of infectivity in macerates than in homogenates can be attributed to the faster ‘fixation’ of the former smears that are firmly attached to conducting surfaces. The long known property of formalin treatment (Taylor and McConnell, 1988) to enhance TSE agent

thermostability is similarly attributed to a 'fixation' process. Following incompletely effective autoclaving, resistant sub-populations of infectious agent are produced that are incompletely inactivated by a second round of autoclaving (Taylor et al., 1998). It can thus be concluded that, while autoclaving can reduce infectivity if the right conditions are selected, it cannot be guaranteed to be 100% effective.

Chemical methods for inactivation of TSE agents

Acids (other than formic acid used to inactivate TSE in fixed tissue in pathology laboratories) have little inactivating effect. However alkalis can be very useful. Boiling macerated tissue in 1M NaOH for 1 minute (Taylor et al., 1999b) or autoclaving in 2M NaOH at 121ºC for 30 minutes (Taylor et al., 1997a) completely inactives TSE agents. This last method can be adapted and used as a treatment for non-fatty rendered material and if KOH is substituted the end product is useful as a fertiliser (Taylor, 2001). Alkylating agents including formaldehyde, organic solvents, most oxidising agents, salts and detergents (other than sodium dodecyl sulphate which has some inactivating effect) have little effect. Amongst proteolytic enzymes, pronase, proteinase K and Quiagen™ can partially inactivate TSE agents under some conditions. Of the chaotropes reviewed by Brown et al. (1986), 4M guanidine thiocyanate is relatively effective. For practical use, amongst the halogens, sodium hypochlorite providing up to 25,000 ppm of available chlorine, is highly effective. Application of sodium hypochlorite to two sources of cow brain naturally infected with BSE resulted in no detectable infectivity when the brains were bioassayed in mice after treatment (Taylor *et al.,* 1994). However, cross-species testing could theoretically result in small amounts of residual infectivity. Other chlorine-based disinfectants (such as dichlorisocyanurate) and iodine-based disinfectants are far less effective and cannot be considered reliable, probably because insufficient halogen is released (Taylor et al., 1994).

4.4 Conclusions

The available data demonstrates that the BSE agent is transmissible to humans and food is the main route of exposure to the agent.

Experimental studies indicate that a single oral dose of the BSE agent is sufficient to cause the BSE disease in cattle, however there is still uncertainty concerning the oral dose required to cause vCJD in humans. The most recent experimental data indicate an infectious dose for cattle of 0.38g of brain material containing $10^{3.5}$ mouse i/c ID_{50}/g from BSE-affected cattle. A similar amount of brain material from BSE affected cattle could contain an infectious dose for humans if there was no species barrier, however this is highly unlikely. There is currently insufficient evidence available to speculate about the possibility of a cumulative dose effect. The risk to humans would be expected to be higher if cumulative dose effect could occur.

Most traditional methods for inactivating microorganisms, including ionising, ultraviolet and microwave radiation, will not inactivate the BSE agent. Heating in the presence of moisture can reduce BSE infectivity but cannot be relied upon to inactivate the BSE agent. Sodium hydroxide and buffered sodium hypochlorite, under particular laboratory conditions, will inactivate the agent.

5.0 EXPOSURE ASSESSMENT

In this section the human exposure to the BSE agent is assessed by examining the relative infectivity of various cattle tissues, the consumption of various products that may be implicated in transmission of the BSE agent and the impact of processes used during food production on the BSE agent.

5.1 Which tissues carry the BSE agent in an infected animal?

It has not been practical to study the pathogenesis of BSE under natural conditions, because the within-herd incidence of BSE is very low (Bradley and Wilesmith, 1993), it is not possible to determine the date of exposure of any individual animal (and therefore one cannot calculate the stage of incubation) and there are no tests to detect infectivity in the living, infected animal. Although a study of the distribution of infectivity in tissues of naturally infected cattle has been made (Dawson *et al*., 1990, 1991, 1993; Bradley, 1999; MAFF, 2000), the distribution of infectivity in tissues during the incubation period of BSE in cattle has been investigated only following experimental oral challenge of cattle with 100 g of brain from confirmed clinical field cases of BSE. A distinction has therefore to be made between the findings in natural forms of the disease and in cases in which the disease has been experimentally induced.

5.1.1 *Distribution of infectivity in tissues in cattle with natural BSE*

The only cattle tissues that have shown to be infected with the BSE agent following bioassay by the intracerebral (i/c) and intraperitoneal (i/p) routes in susceptible mice have been the brain (Fraser et al., 1994) (also positive after challenge by the oral route (Middleton and Barlow, 1993), the cervical and terminal spinal cord (and by inference the whole of the spinal cord), and the retina (Bradley, 1999). Around 50 other tissues have been similarly bioassayed and no detectable infectivity has been found (Fraser and Foster, 1994; MAFF, 2000). The list contains most of the tissues important in the human diet.

Middleton and Barlow (1993) examined the transmissibility of a number of BSE-infected tissues in mice—mammary gland (plus milk), supramammary lymph nodes, spleen, carcase and mesenteric lymph nodes, and foetal membranes (cotyledons and intercotyledary trophoblast). Mixed brain, including cerebral spinal fluid, was also included. Mice were observed for a cumulative period of three and a half years, over which time none of the mice that were fed the infected extraneural tissues developed BSE. Mice that had been fed infected brain did develop spongiform encephalopathy. In this study, the extraneural tissues used failed to transmit BSE even though they were consumed in larger quantities (up to 10 times larger weights) than infected brain tissues.

Taylor et al. (1995a) studied the infectivity of milk from cattle with BSE through both feeding and injection. In this experiment, milk samples were collected from six cows showing clinical signs of BSE at varying stages of lactation—early, mid and late lactation. Each sample was then either fed to mice over a 40-day period or injected by combined intracerebral and intraperitoneal injection. The mice that drank milk

consumed, on average, 300 mL and showed no signs of BSE over a 702-day observation period. The mice which had been injected were observed for up to 653 days and also showed no signs of disease. In this study, milk failed to demonstrate any BSE infectivity.

Studies have been conducted on bovine embryos from cows with BSE (Wrathall and Brown, 1991; Wrathall 2000) and placenta by the oro-nasal route (Dawson et al., 1991) both of which have revealed no detectable infectivity.

5.1.2 *Distribution of infectivity in cows challenged orally with BSE agent*

In this study 30 calves, each 4monts-old, were challenged orally with 100 g of brain material from clinical cases of confirmed BSE. There were 10 undosed control animals. Three challenged and one control animal were killed at approximately 4 month intervals, starting 2 months after dosing and over 40 different tissues were collected for bioassay. The samples from individual tissues from the challenged animals were pooled before inoculation (Dawson et al, 1994).

The results showed that there was no infectivity detectable in any tissue from cattle at 2 months after challenge. Infectivity was detected in the distal ileum from 10 to 18 months post-challenge and again from 36 to 40 months post-challenge. The reason for the absence of detectable infectivity between 18 and 36 months in this tissue has yet to be explained. The earliest onset of clinical signs in cattle (of those surviving to that time) was 35 months. The earliest detection of infectivity in neural tissue (spinal cord, dorsal root and trigeminal ganglia) was at 32 months post-challenge. The earliest time that spongiform change and PrP^{Sc} were detected was also 32 months, though the spongiform changes were not consistently present. From 36 months onwards (up to 40 months) infectivity was found throughout the brain, spinal cord and aforementioned ganglia. At 38 months post-challenge but not at 36 or 40 months post-challenge (i.e. in the clinical phase of disease only), infectivity was detected in the sternal bone-marrow (Wells *et al.,* 1994, 1996, 1998, 1999; Wells, 2001). Further studies involving bioassay in cattle are awaited before this result can be completely interpreted.

5.1.3 *Conclusions*

In natural clinical cases of BSE, infectivity is confined to the brain, spinal cord and retina but infectivity cannot be excluded in other closely associated central ganglia. In experimental BSE cases, infectivity is found through most of the incubation period in the distal ileum (a short portion of the terminal part of the small intestine) and part of the brain, spinal cord and spinal ganglia from about 3 months before clinical onset.

While some of the tests and bioassays may not have high enough sensitivity to detect low levels of the agent, experimental data have shown that infectivity is not found in up to 50 tissues of cattle with BSE, including most tissues that are used in the human diet such as muscle and milk.

The whole central nervous system (CNS) and associated central ganglia are infected in the clinical phase of disease and approximately 95% of the total infectivity is estimated to be in the central nervous system of clinically affected animals. The conclusions drawn from available data indicate the following tissues, designated

specified risk material (SRM), would carry infectivity. For bovine animals the SRM are the skull including the brain, eyes, trigeminal ganglia, tonsils, thymus, spleen, and the spinal cord of animals over 12 months of age; the vertebral column (including dorsal root ganglia) of animals over 12 months of age, and the intestines (from duodenum to rectum) of animals of any age. The absence of an age limit for intestines resulted from experimental data demonstrating BSE infectivity in the distal ileum of animals very early after challenge with the BSE agent. The current list of SRM for the European Union is detailed in Appendix 3.

5.2 Risk of contamination of the carcase during slaughter

Abattoir practices were examined to determine whether the infective agent is spread to other tissues during slaughter and, if so, to identify the potentially critical points during animal slaughter for risk of contaminating with the BSE agent those tissues which are not infected naturally. Our approach here is to compare Australian and European slaughter practices. European slaughter practices have been examined as almost all of the cases of BSE are of European origin. The assessment of European slaughter practices has been based on present European Union (EU) legislation and has used worst case situations in which any process concerning handling of SRM is considered a potential risk of spreading SRM onto products.

5.2.1 *The Slaughter Process*

5.2.1.1 Australian slaughter processes

Several slaughtering methods are used, and the steps in the slaughtering process are summarised in Appendix 4.[2] The larger throughput export and domestic abattoirs tend to use more automated equipment than smaller establishments. Meat for domestic consumption can be produced in domestic or export abattoirs.
Most abattoirs produce carcases to meet the definition of the Australian Standard Carcase, as defined by AUS-MEAT (Appendix 5). Although not all of the domestic abattoirs are accredited by AUS-MEAT, similar standards of carcase preparation are met. Preparation of a standard carcase includes removal of the head between the skull (occipital bone) and the first cervical vertebrae but does not include removal of the spinal cord. In Australia, there is no regulatory requirement for removal of spinal cord at domestic or export abattoirs, although this would usually be done.[3]

The steps in the slaughtering process that may involve a risk of cross-contamination with SRM between carcases are stunning, carcase splitting, head processing, and carcase trimming. These steps are described below.

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[2] Minimum requirements for slaughtering practices in Australia specified in the Australian Standard for Hygienic Production of Meat for Human Consumption. Export-registered abattoirs comply with the additional requirements of the Export Meat Orders.

[3] The Australian Standard for Hygienic Production of Meat for Human Consumption also specifies removal of certain items from a carcase (mammary glands, pizzle, spermatic cords.

Stunning

Most cattle are stunned using a captive bolt which penetrates through the skull and about 70 mm into the brain. Leakage of brain tissue from the stunning would occur onto a small area of hide around the stunning wound (facial part of the hide) and this risk would be increased if a repeat stunning was necessary to kill the animal. Although the stunning process could lead to contamination of other animals with brain tissue, the risk is very small. Contamination by brain tissue at this stage is on the outside of the hide only. It is unlikely that brain tissue leaking from the stunning wound could be transferred to edible carcase tissue. Brains from animals stunned by a captive bolt cannot be used for food.

Alternative stunning methods

Halal stunning.

Animals that are slaughtered by means of Halal ritual slaughter are not stunned with a penetrating bolt. They are either electrically stunned or stunned with a percussive blow to the forehead. Percussive stunning may break the skull but leakage from the wound does not usually occur.

Pneumatic stunning.

Pneumatic stunning involves injecting air into the wound made by the captive bolt to immobilise animals after stunning. It has been reported that high pressure (over 100 psi) pneumatic stunning can cause brain tissue to be distributed to other parts of the body, particularly to the heart and lungs. A low air pressure (about 30 psi) pneumatic stunning system has been tested in Australia and no evidence of brain tissue was found in other parts of the stunned animals.

The practice of pithing, ie.'the laceration after stunning of central nervous tissue by means of an elongated rod-shaped instrument introduced into the cranial cavity' is not banned in Australia but is generally not used. There is a risk with this technique that the lacerated brain tissue could be distributed to other parts of the body.

Carcase Splitting

In most abattoirs a band saw is used to divide carcases into sides. Band saws have a fine blade and teeth and produce very little sawdust. The saws are generally fitted with water sprays that wash dust down the carcase. AQIS Notice Meat 96/06 sets out requirements to minimise the chance of cross-contamination between carcases caused by the use of water sprays.

In general the spinal cord is left intact in one side of the carcase after splitting with a band saw, as the saw blade tends to travel down one side of the spinal cord. The alternative to the band saw is the reciprocating saw, used in smaller abattoirs. This has a wider blade and larger teeth than the band saw. It cuts a wider path through the backbone and is more likely to damage the spinal cord because of the wider cut.

Carcase trimming

Spinal cords are generally removed from carcases by hand on the slaughter floor as part of the final carcase trim between final inspection and weighing. There is no regulatory requirement in Australia to remove spinal cord from carcase sides, but the spinal cord is usually removed to improve carcase appearance. Quality assurance procedures at export establishments usually include the removal of spinal cords from carcases if the carcase is split, to reduce possibility of spoilage caused by remaining bone particles in the spinal canal. Some spinal cord may be retained if the backbone is not cut near the centre line and the cord is not exposed.

Some abattoirs collect spinal cord for food processing but it is more common for spinal cord to be added to the other material that is trimmed from carcases and rendered.

Head processing

The skinned heads are usually removed from the body after hide removal. The heads remain correlated with the carcases while they are washed, trimmed and inspected. It is very uncommon in Australian abattoirs for brains to be collected for food. If brains are collected for food, they are recovered by splitting the skull in half and extracting the brain by hand. During head processing, brain tissue is not exposed apart from at the cut made between the skull and the first neck vertebra and there is little risk of contamination of carcases or offal with brain tissue.

After removal of the tongue, cheeks and jaw, the whole head including brain and eyes is consigned to rendering. Brains cannot be collected from animals that have been stunned with a penetrating bolt to the forehead. The Export Meat Orders and the Australian Standard for Hygienic Production of Meat for Human Consumption require that tonsillar tissue be removed from edible product. Tonsils are removed from the tongue before the tongue is washed and are discarded to inedible rendering.

Further processing of carcases

After chilling, carcases may be boned on site or transported off site to butchers shops or other boning rooms. Since the spinal cord is mostly removed on the slaughter floor, there is generally no SRM associated with chilled carcases. Product for domestic use is boned into a variety of boneless and bone-in cuts. If carcases are fully boned into boneless cuts, all bones are collected for rendering. If there is any residual spinal cord in the vertebrae it will be rendered. There are some bone-in cuts that could include residues of spinal cord. These cuts are bone-in shortloin from which T-bone steaks are derived and bone-in rib. It is possible that pieces of spinal cord could be left in these cuts. It is unlikely that spinal cord or any other SRM could be associated with other boneless or bone-in meat apart from carcase sides or quarters.

In summary, pneumatic stunning, and presumably pithing, can cause fragments of brain to enter the circulatory system and be pumped through the heart into the lungs Garland et al, 1996). There is a question of whether captive bolt stunning as used in Australia can have the same effect. If fragments of brain dislodged by the penetrating bolt entered blood in

the brain sinuses they could be drawn back to the heart and lungs. It is likely that the fragments would be trapped in the lungs and would not get into muscle tissue.

5.2.1.2 European slaughter processes

Slaughter practice at larger European abattoirs that present a high risk of cross-contamination of carcases with SRM are summarised below. Additional information on stages in the slaughter process that present a much lower risk of carcase contamination with SRM and the legislative requirements introduced into the EU to minimise this risk of cross-contamination has been supplied in Appendix 6.

Stunning

Captive bolt stunning used to stun cattle prior to slaughter results in haematogenous dissemination of CNS tissue (Fisher et al, 2001). Cerebral tissue may be spread in the air either as an aerosol or as particles from the shot hole caused by the pressure during the shooting using a bolt pistol and contamination of the frontal lobe from leakage of cerebral tissue is a risk. There is no available data to indicate that the use of a bolt pistol for stunning involves a risk of spreading cerebral tissue to the muscles (as is seen when a pithing rod is used) but it cannot be totally excluded.

CNS tissue has been detected in the jugular venous blood in one of a group of sixteen cattle stunned using a captive bolt gun followed by pithing. These studies used immunocytochemistry and ELISA to discriminate non-CNS tissues and CNS tissue (Love et al., 2000). At some slaughterhouses the frontal lobe is cut off and disposed off, but this cannot be considered to be a sufficient measure against contamination. If the shot hole is plugged at the receiving table, cerebral tissue will not ooze onto the surroundings. Application of electrical stunning will entirely eliminate the above risks of contamination from cerebral tissue.

The prohibition of pithing to immobilise carcases has at some abattoirs been replaced by electrical immobilising. During the stimulation, the muscles of the carcase contract and the blood pressure rises. The rise of blood pressure will increase the risk of leakage of cerebral tissue from the shot hole in the skull.

Carcase splitting

The final mechanical process in dressing cattle carcases is splitting down the median plane to form two carcase sides (Fisher et al, 2001). During this process the blade of the splitting saw cuts through the spinal cord, with the risk of contamination of the vertebral column with SRM. The blade is sprayed with water which will run down the carcase into the abdominal and thoracic cavity, and onto the neck and floor, with the risk of spreading SRM (sawdust from the vertebral column and spinal cord). There is also a risk of the spinal cord remaining in on half of the carcase due to uneven splitting of the vertebral column.

The present rinsing/sterilisation of the saw blade is not sufficient to inactivate the BSE agent, if present . The wash is nevertheless very important since it minimises the presence of potential SRM however, accumulation of material cannot be entirely avoided. Sawdust from carcase splitting contains risk material (spinal cord). Avoiding

carcase splitting by sawing through the spinal canal/spinal cord will eliminate some of the potential risks of contamination on the slaughter line.

Head processing

As the brain and medulla oblongata contain the highest concentrations of the BSE agent in positive animals, the process of dehorning should be considered as high risk Laying bare the membrane of the brain involves no risk of spreading of SRM, but exposure of the brain itself involves a high risk of spreading highly infected cerebral tissue onto other parts of the head (jaw meat). When the head is washed with water, the SRM particles present will be spread all over the head. Wash water will flow over the tonsils and the possibly exposed brain, with the risk of spreading SRM to the jaw meat.

The trimming of jaw meat will involve a high risk of exposure to the BSE agent, if present, in the animal. The thymus is removed manually with a knife, either in connection with cutting free the trachea or simultaneously with the pluck removal. When loosening the thymus, there is a risk of incision with the knife which may cause the spread of risk material onto the neck meat and into the surroundings.

When removing the tonsils from the hyoid bone before cutting off the tongue, there is a risk of incision into the tonsils with subsequent spreading of the BSE agent.

Further processing of carcases

Removal of the spinal cord is performed manually with a knife, by vacuum suction or with a rotating cutter. When the spinal cord is removed, there is a risk that the remains of the cord may be left on the vertebral column and the muscle of the back.
All material from the spinal canal must be removed completely and collected in a separate container. Although the BSE agent is not destroyed during rinsing/sterilisation of the equipment used, the washing is crucial to minimise the presence of accumulated potential risk material.

As mentioned previously, uneven splitting of the vertebral column will increase the risk of inadequate removal of SRM (spinal cord). The subsequent control procedure must ensure that unevenly split carcases are retained while the back is split and spinal cord removed, otherwise there is a risk of spinal cord remaining in products.

Effective removal of the spinal cord and sawdust from the vertebral column at the slaughter line will greatly reduce the risk of spreading SRM between products. The vertebral column is removed as part of the general deboning process. The need to remove the vertebral column is a result of the risk of the BSE agent being present in the dorsal root ganglia. Nevertheless, there will be no cross-contamination by the BSE agent in the dorsal root ganglia unless the vertebral column is split and the dorsal root ganglia exposed.

At some abattoirs, the vertebral column is removed and cut through each joint into individual vertebrae with the aim of improving the yield. This procedure creates a greater risk of contamination of SRM from the exposed dorsal root ganglia.

5.2.2 Conclusions

The available information indicates there is still a likelihood of a risk of contamination of carcase meat with BSE infectious tissue as a result of the slaughtering process, even with the implementation of extensive control measures. The major routes for potential contamination of carcases with CNS are:

- dissemination of brain tissue during the stunning procedure using captive bolt guns or pneumatic stunning using air injection;
- laceration after stunning of central nervous tissue by introduction of a rod into the cranial cavity (pithing), and
- dispersal of spinal cord material during splitting of carcases. In the majority of abattoirs, carcases are split using a band saw, resulting in significant contamination with spinal cord in the area adjacent to the spinal canal (Fisher et al., 2001).

Recent developments in technology aimed at reducing the amount of SRM contamination during the slaughter process are detailed in Appendix 7.

5.3 Risk of contamination of meat products

The amount and distribution of the BSE agent in an animal changes over time and therefore the age of the animal at slaughter will influence the level of BSE infectivity entering the food chain from an infected animal. Risk of exposure to the BSE agent is low early in the incubation period and rises rapidly later in the incubation period. The incubation period is such that clinical BSE is rarely detected in animals younger than 30 months. Meat cannot be exposed to conditions known to inactivate the BSE agent.

5.3.1 *Risk of contamination of fresh meat*

SRM account for 99.4% of the cumulative load of BSE infectivity. The removal of SRM from an animal with clinical BSE case will reduce the infectious load by more than 95% and subsequently the exposure to the BSE agent will be reduced. BSE infectivity has not been found in the blood of BSE-infected cattle (Wells et al, 1998; Bradley, 1999).

5.3.2 *Risk of contamination of mechanically recovered meat*

Mechanically recovered meat (MRM), also known as mechanically deboned meat, mechanically separated meat and finely textured beef, is obtained by recovering the residual raw meat adhering to bones under high pressure after other boning processes have been completed. MRM has been identified as a high risk tissue for BSE infectivity due to the likelihood of contamination with spinal cord tissue. The various types of mechanical deboning machines all apply pressure to squeeze bone against a screen of fine holes. The soft tissues forced through the screen are the MRM. The harder tissues are ejected from the equipment and are usually rendered.

Only selected bones are used to make mechanically recovered meat. They are generally forequarter bones including vertebrae and rib bones. The large marrow bones of the legs are not usually used. Since the spinal cord is usually removed from

carcases there should be little spinal cord associated with these. However, there could be residual pieces of spinal cord in vertebrae used to make MRM.

If spinal cord is in bones used to make MRM, the spinal cord could be included in the soft tissue fraction from the deboning machine. The machines can be adjusted to give different yields and fat content. If a machine is set for high yield, it is more likely that spinal cord could be included in the MRM. However, high yield settings are not necessarily used because they result in a high fat content. At lower yield settings it is less likely that spinal cord would be in the soft tissue fraction.

5.3.3 Conclusions

The risk of exposure to the BSE agent from fresh meat that does not contain SRM is relatively low. Products containing MRM present a high risk of exposure to the BSE agent if spinal cord is in the bones used to make the MRM. The level of contamination in the product will depend on the type of deboning machine used and how the machine is adjusted. The amount of infectivity that is present in the product wil be dependent on the number of infected animals used in the production run to extract MRM. If sourced from an animal incubating BSE, the risk from these products is increased as raw materials may be sourced from older animals containing high levels of BSE infectivity. MRM is considered as SRM because of these two routes of exposure to the BSE agent.

5.4 Impact of processes used in preparing beef-based food products on the inactivation of the BSE agent

In order to understand the processing conditions for the range of beef products under investigation, the products were grouped into the following categories based on common processing conditions:

- canned beef products
- smallgoods
- beef extract
- dry beef products including soups and flavours
- cooked, chilled and frozen beef products.

Although the processing procedures described are routinely used, it is acknowledged that some manufacturers may use markedly different methods. The European Community prohibits the use of MRM from cattle, sheep and goat bones for incorporation into meat products for human consumption, however this is not the case throughout the world. The processes described relate to Australian production but would be applicable to overseas production.

5.4.1 *The canning process*

Raw materials

a) Beef flesh, perhaps beef offal and MRM, usually from older cattle. The meat is usually supplied frozen and boneless in cartons but sometimes comes directly from boning operations.

b) Minor ingredients include thickening and binding agents such as starches, vegetable proteins and gums, and salt, spices and flavouring agents.

c) Cured products may use nitrite, polyphosphates, erythorbates and ascorbates.

Preparation
Frozen meat is thawed or partly thawed (tempered) and cut to the required size. Uncured products are prepared by mixing the cut meat with thickening and binding agents which form a gravy when the product is heated to about 100°C in stirred tanks before filling into the cans, or heated in the closed cans during the heat sterilisation. Cured loaf products are prepared by mixing the finely chopped meat with thickening and binding agents and curing salts, and filling into the cans. Water which separates from the meat during heat sterilisation of the closed cans is absorbed by the thickening and binding agents to give a loaf product.

Canned corned beef in Australia is made by scalding the chopped beef in water at about 100°C. Scalding releases water from the meat protein before can-filling, therefore preventing it from separating during heat sterilisation. Curing salts are then mixed with the scalded meat which is then sealed into the cans; the pink, cured colour develops as the cans are heat sterilised. Some corned beef is produced overseas without scalding the raw meat; the water that would have been lost during scalding forms a brine in the can surrounding the beef during heat sterilisation.

The watery soup which separates from beef during scalding is the raw material for the manufacture of beef extract, discussed below (section 5.4.4).

5.4.2 *Heat processing*

The heat sterilisation processes applied to the closed cans are designed to make the product commercially sterile, i.e. to inactivate possible contaminating mesophilic organisms which may otherwise grow and spoil the finished product. Heat processes are usually designed to inactivate spores of *Clostridium botulinum*, a moderately heat-resistant bacterium of major public health significance. These processes have a large safety margin and, over several decades, have been proven to give safe products.

The heat processes usually involve heating the cans in steam in retorts (autoclaves) at temperatures of about 120°C. The time of heating depends on the size and shape of the cans, the thermal properties of the product and often on how the cans are cooled at the end of heating. Typical retorting conditions for a 450 g can of stewed steak are 70 minutes at 120°C, or 240 minutes at 120°C for a 2.7 kg can. The sterilising power of canning processes is measured in F_o units, which are defined as the number of minutes at a reference temperature of 121.1°C that would give the same amount of inactivation as the heat processes applied to the cans. The two processes specified have F_o values of about 4 minutes, which means that the heat treatments applied give the same amount of sterilisation as if the centres of the cans were heated instantly to 121.1°C, held at that temperature for 4 minutes and then instantly cooled to sub-lethal temperatures. The F_o system is based on a heat-resistance curve for the target bacterial spores which has a slope (z) of 10 Celsius degrees, i.e. thermal death time decreases by a factor of 10 for each 10°C increase in temperature from 90°C to about 140°C.

Traditionally, the minimum treatment for low-acid canned foods has an F_o value of 2.5 minutes, which corresponds to the probability of a spore surviving to 1 in 10^{12}. Uncured beef products are usually processed to F_o values of 4 minutes or more, as some are cured products. Other cured beef products may apparently receive processes giving F_o values as low as about 1 minute. Ultra-high-temperature processed milk, another low-acid canned food of bovine origin, is usually processed at about 135°C for about 10 seconds by direct steam injection; this process gives an F_o value of about 4 minutes.

These basic principles of heat sterilising low-acid canned foods apply regardless of whether the product is packed in metal cans, glass jars, plastic pouches or aluminium trays.

It is essential to know the kinetics of the inactivation process to assess the effect of heat processes in inactivating prions. Studies on the heat inactivation of a scrapie agent has been shown to proceed in an exponential fashion, i.e. were consistent with first-order kinetics (Taylor, 1999b; Kimberlin et al., 1983). This log-linear relationship applies in general to the heat inactivation of other microorganisms, including bacterial spores and allows the heat resistance of the organisms and prions to be compared in terms of their 'D' or decimal reduction times, i.e. the time at a constant lethal temperature required to reduce the population by a factor of 10. Although the literature in this area is sparse, D values for prions were estimated for comparison with the usual D value used in calculating heat-sterilising processes for inactivating sporing bacteria in food-processing operations; the results of these calculations are in Table 3. It should be noted that log-linear inactivation curves may not be obtained in some circumstances, for example, the heat inactivation of the 263K scrapie strain was biphasic and indicated the agent was not inactivated after many hours at 134°C (a D_{134} value of many hours) (Taylor et al., 1998).

The D values in Table 3, despite the uncertainties associated with their estimation, indicate that prions vary in their apparent heat resistance with type and other factors. In relation to heat processes used in food processing, prions have D values that are probably at least an order of magnitude larger than the D values for mesophilic bacterial spores such as those of *C. botulinum*, at temperatures tradionally used to treat low-acid canned foods.

The minimum heat sterilisation process for low-acid (pH > 4.6) canned foods is traditionally accepted as giving 12 decimal (12D) reductions in the target population of bacterial spores, however this treatment would be insufficient to reduce the probability of a prion surviving to 1 in 10 on the basis of the data in Table 3. The usual heat processes applied to low-acid canned foods, which include many canned meats, would not inactivate the BSE agent.

Canning processes incorporate a large safety margin to protect consumers against botulism; the expected level of contamination by botulinus spores is probably fewer than 10 per can, so a 12D process would give a probability of survival of less than 1 in 10^{11}. As vCJD results in neural degeneration and eventual death, the heat processes to inactivate the BSE agent in foods should also have a large safety margin. If processes reducing the probability of the BSE agent surviving to 1 in 10^6 (6D) were

adopted as the standard for inactivating the BSE agent, the equivalent canning processes would need to have F_o values of 105–340 minutes on the basis of the D values for CJD in the third line of Table 3.

The major sources of uncertainty in assessing the effectiveness of heat sterilisation processes are the number and heat resistance of individual target microorganisms or prions initially present in the product. The probability of having individual target organisms of unusually high heat resistance increases as the population increases. There is clear evidence that the heat resistance of prions may be markedly altered by exposure to a sub-lethal heat treatment, or the physical form of the sample being heated or by changes in the chemical environment (Taylor et al. 1998). The heat resistance may increase or decrease but often the causes of these changes are unknown.

Table 3. D values for TSE agents and mesophilic spores

Temperature (°C)	Estimated D value (min)	Agent	Reference and nature of substrate
121	0.21	Mesophilic spores	Traditional value for foods with pH > 4.6
121	11–16	Scrapie 263K (hamster	J. Virological Methods, 1993, 41, 193–202. Brain homogenate
121	3.7–12	CJD (guinea pig)	Journal of Infectious Diseases. 1986, 153, 1145–48. Brain homogenate
126	30–60	Scrapie 139A (mouse)	Journal of Neurological Sciences, 1983, 59, 355–69. Brain macerate
126	27	Scrapie 22A (mouse)	(As above)
132	9–12	Scrapie 263K (hamster)	Journal of Virological Methods, 1993, 41, 193–202. Brain homogenate
132	3.7–12	CJD (guinea pig)	Journal of Infectious Diseases, 1986. 153, 1145–48. Brain homogenate
132	8.8	Scrapie 263K (hamster)	(As above)
134	5.7(a) 9.4(b)	Scrapie 263K	Journal of Infectious Diseases, 1990, 161, 467–472. (a – intact brain) (b – extracted fibril)
134	5.5(a) 10.6(b)	Scrapie 263K (hamster)	Veterinary Microbiology, 1998, 64, 33–38. Brain macerate (a) initial 18 min at 134^{C} (b) repeated 18 min at 134^{C}
134	2.5–4.1	Scrapie 263K (hamster)	Archives of Virology, 1994, 139, 313–326. Brain macerates
136	3–4	Scrapie 139A (mouse)	Journal of Neurological Sciences, 1983, 59, 355–69. Brain macerate
136	1	Scrapie 22A (mouse)	Journal of Neurological Sciences, 1983, 59, 355–69. Brain macerate
138–140	1 second	Scrapie 263K (hamster)	Rohwer et al. (2001) Intermediate data…gelatine manufacturing process. Full details needed

5.4.3 *Meat Preservation*

Raw materials

The raw materials of smallgoods are beef flesh, offal and perhaps MRM.:.

a) Some meat may be from older cattle; it is usually supplied as frozen selected boneless cuts in cartons but could be supplied directly from boning operations.
b) Minor ingredients include binding agents, salt, nitrite and nitrate, erythorbates and ascorbates, polyphosphates, spices and flavouring agents and acidifying agents.

Preparation

Smallgoods are generally made by chopping the meat ingredients using high speed blades to give an emulsion. This is done at temperatures of about 0–10°C. Non-meat ingredients may be added at some stage during chopping or at a later stage. The products are then filled into natural or synthetic casings. Fermented sausages are stored under controlled conditions of temperature and humidity to allow the product to dry and reach an acidity of about 1% lactic acid (pH about 5) to ensure shelf life. Smoking at temperatures of about 45°C is also used.

Other smallgoods are heat processed to produce the desired quality characteristics and improve the shelf life. The heat processes may be applied during smoking or by immersion in sprays or baths of water at about 80°C to give internal product temperatures of about 70°C. These treatments are intended to coagulate the meat proteins without breaking the emulsion, to set the colour in cured sausages and to inactivate many vegetative microorganisms. A third group of smallgoods includes fresh products such as breakfast sausages which are simply raw emulsions packed into casings; these products have a short shelf life even in cool storage and are intended for cooking by the purchaser.

5.4.4 *Other Processes*

Beef extract is made from the watery soup released from beef when, for instance, it is scalded in the preparation of canned corned beef. Similar watery extracts are obtained from crushed bones and liver to product bone and liver extracts, respectively. The extraction conditions usually involve up to 30 minutes at neat boiling temperatures, but sometimes pressurised kettles are used at about 115°C. The watery extract is usually centrifuged to remove fat, and filtered to remove solid materials. It is then partly concentrated under vacuum and reduced to a moisture content of about 20% in shallow open pans at temperatures below 100°C. Sometimes the clarified watery extract is heated to about 130°C to inactivate thermophilic bacteria before it is concentrated.

Dry beef products like jerky are usually made by low-temperature drying of strips of beef that have been salted or marinated. Dried meat powder may be an ingredient in dry soups and similar products; it is likely to be made by processes that involve temperatures of less than 100°C such as by spray or freeze-drying. Beef flavours are usually based on beef extract described above or made from non-meat components such as amino acids and sugars. Canned soups are prepared in essentially the same way as the canned beef products described above.

Cooked chilled and frozen beef products are prepared in industrial kitchens using conventional cooking procedures such as roasting, frying or boiling. Although the surface temperature of the meat may exceed 100°C towards the end of roasting or frying, when free water has evaporated the internal temperatures may be as low as 70°C, or even lower if the meat is still rare.

5.4.5 Conclusions

Shelf-stable canned beef products and beef extracts receive heat treatments which are often described as severe in that they inactivate bacterial spores. These processes are equivalent in sterilizing effect to holding all parts of the product at 121.1°C for at least a few minutes, however these conditions will not inactivate BSE infectivity. Most other beef products receive no heat treatment or heat treatments of low severity; in most cases some or all of the product is heated to less than 100°C. The conventional unit operations, such as filtration and washing, used in the food-processing industry, would not eliminate the BSE agent. Processes use in meat preservation would not inactivate the BSE agent, however the use of nitrate may have some impact on the BSE agent as nitrate is known to form a spore inhibiting material (P. Board, pers. comm.). These products, such as smallgoods, would present a high potential risk of exposure to the BSE agent through the inclusion of brain and MRM in the raw materials

5.5 Estimated consumption of fresh meat and processed meat products in Australia

The consumption of fresh meat and processed meat products for different age groups in Australia was calculated using ANZFA's dietary modeling computer program, DIAMOND, that accesses food consumption data from the 1995 Australian National Nutrition Survey (NNS). The NNS uses a 24-hour recall dietary survey of people aged 2 years and over. The mean consumption of fresh meat was 137 g/day and 376 g/day for high consumers and varied from 60g/day (2-4 years) to 161g/day (20-29 years).

In the case of processed meat products, the amount consumed was lower and the consumption pattern among the population was different to fresh meat. The mean consumption was 93 g/day and 287 g/day for high consumers and varied from 63 g/day (2-4 years) to 100 g/day (over 40 years). These figures includes foods such as corned beef, beef sausages, frankfurts, salami and luncheon meats consumed on their own and incorporated into foods such as sandwiches and pizzas.

Consumption of fresh meat and processed meat products occurs in all age groups, although at lower rates of consumption in the 2-4 year age group.

5.6 Milk processing

Milk and milk products are generally processed using one or more of the following treatments:

- pasteurisation
- ultra-heat treatment

- drying
- concentration
- fermentation

Apart from fermentation, these are largely thermal processes used to control bacterial populations and the physical characteristics of the product. The temperatures typically used in these treatments are summarised in Table 4.

The BSE agent is very resistant to treatments that have conventionally been used to inactivate biological agents such as bacteria and viruses. While some loss of infectivity of the BSE agent occurs at temperatures above 100ºC, even autoclaving may not be sufficient for complete inactivation, particularly if a high level of infectivity is present. It is unlikely, therefore, that the processing treatments generally used in the production of milk and milk products would inactivate, if present, the BSE agent to any significant extent.

Table 4. Thermal treatments used in the processing of milk and milk products

Processing treatment	Thermal parameters typically used*
Pasteurisation	72ºC for 15 seconds (or other combination of time/temperature to achieve an equivalent level of bacterial reduction)
Ultra heat treatment	132ºC for 1 second (minimum) for milk Higher temperatures (133–140ºC) for cream
Drying	The heat treatments used in the spray-drying of milk powders vary depending on the type of powder. Preliminary heat treatment of the liquid milk using temperatures of 85-95ºC for several minutes or 125ºC for 20 seconds are widely used for the production of whole-milk powders. The milk is then concentrated and spray-dried, the temperature of the dry particles never exceeding that of the cooled outlet air (70–95ºC).
Concentration	Concentrated or evaporated milk products commonly use pre-heating temperatures in the range 65.5–77°C and evaporate under vacuum. The milks may be then pasteurised before packaging (79.4ºC for 25 seconds) or canned.
Retorting	The retort temperatures used for canned evaporated milk are typically 136ºC for 30 seconds to 150ºC for <1 second.

* Varnam and Sutherland (1996)

5.7 Preparation of gelatine

5.7.1 *What is gelatine?*

Gelatine is defined in the *Australia New Zealand Food Standards Code* (ANZFA, 2000) as a protein product prepared from animal skin, bone or collagenous material, or any combination thereof. The protein product is a mixture of polypeptides obtained by partial hydrolysis of the collagen contained in bones and hides, mainly from bovines and/or skins from pigs after successive treatments: degreasing, acid treatment and/or alkaline treatment (liming), washing, filtration, ion exchange and sterilisation (EC, 2000d).

5.7.2 *How is gelatine used?*

The worldwide production of gelatine is 220,000 tonnes, of which 44% is produced in Europe[4]. In Europe, the total amount of raw material transformed annually into gelatine is estimated to be about 500,000 tonnes with 100,000 tonnes of gelatine produced (52% from porcine skins, 21% from bovine bones and 27% from bovine hides) (EC, 2000c). In western Europe, about 35,000 tonnes of gelatine are used in edible and pharmaceutical applications. In Australia, New Zealand and South Africa, gelatine is also sourced from sheep skins (Schrieber and Seybold, 1993).

Most gelatine for use in food is sourced from porcine skins and bovine hides. On a global basis, very little bone gelatine is used in food because the physical and chemical properties that make it useful in the pharmaceutical industry offer no advantage over cattle hide gelatine in food applications. In addition, bone gelatine is more expensive to produce than hide gelatine (T.Akred, personal communication). A.H. Grobben (personal communication) suggested that the approximate breakdown of edible gelatine by source material is: 70% porcine skins, 20% bovine hide, and 10% bovine bone. Gelatine manufactured from bones is predominantly used by the pharmaceutical industry, along with porcine hide gelatine and to a lesser extent, bovine hide gelatine (T. Akred, personal communication). A.H. Grobben (personal communication) reported the approximate breakdown of source materials for pharmaceutical gelatine to be: 50% bovine bone, 40% porcine skins and 10% bovine hide.

Gelatine is one of the most versatile food substances and is the only natural protein capable of producing clear, thermo-reversible gels in water in the body temperature range. As such, it is used in foods such as confectionery, jellies and ice cream, where it performs a number of different functions. In confectionery, for example, it acts as a gelling agent (fruit jellies, wine gums), foaming agent (marshmallows), emulsifier (fruit chews), adhesive (liquorice all sorts) or controller of sucrose crystallisation (fruit chews) (Anonymous, 2000).

Gelatine is produced and distributed similarly in different countries, including Australia (T. Akred, personal communication). Usually, gelatine from different source materials is produced in separate plants. Gelatine as a food ingredient is almost

[4] Information from the Gelatine Manufacturers of Europe (GME), which represents most of the gelatine producers in the EU.

always sold individually by source material (e.*g.* bovine bone or bovine hide gelatine) and it is extremely rare for a manufacturer to sell a blend. Blends are usually used only by the pharmaceutical industry.

5.7.3 *The gelatine process*

In Australia, there is a need to assess the safety, with respect to the BSE agent, of gelatine both in the form of raw gelatine and as a food ingredient.

The production of gelatine varies according to the type of source material. The GME reported that production involves three main processes and three types of raw material: an acid process for bovine bones, hides and porcine skins; an alkaline process for bovine bones and hides; and a heat/pressure process for bones (EC, 2000d). Raw material from one production plant may originate from several sources and may be a mixture of materials from different slaughterhouses and suppliers. In addition, various parts of the production process may be spread over several locations. There are many critical control points (CCPs) in the production chain from source material to final product, and the GME states that monitoring of these CCPs is not always easy or evident (EC, 2000d).

The first step in all gelatine manufacturing processes involving bone is degreasing of the bones. The bones are finely crushed and the bulk of soft tissue and fat is removed by cooking in hot water (80 to 85ºC). The bone chips are dried by hot air and the finer material is removed by sieving. The calcium phosphate in the bone is then extracted by treating with dilute hydrochloric acid. The acid is applied over 4–5 days in a sequence of increasing concentrations, the highest concentration being 4%, giving a product called ossein (Taylor et al., 2001b).

The initial steps in the production of hide and skin gelatine involve treatment of the source material with lime (calcium hydroxide) or sodium hydroxide for 4–10 days. Treatment with sodium hydroxide requires the lower time period in this range, while lime treatment takes up to 10 days. From this stage on, processing is the same regardless of the source material (T. Akred, personal communication; Taylor et al., 2001b).

Acid process

The ossein or treated hide is soaked overnight at below pH 4 and the gelatine is then extracted with warm water.

Alkaline/liming process

The ossein or treated hide is treated with a supersaturated solution of lime (pH 12.5) for 45 days. The limed ossein or hide is treated in an extraction step with sulfuric acid until the pH remains below 6, and then water-washed and extracted several times with warm water (>50ºC) until the gelatine concentration is between 3 and 8%.

Heat/pressure process

The bones or treated hides are autoclaved for approximately 20 minutes with saturated steam at a temperature of more than 133°C and a pressure of more than 3 bar, followed by extraction with hot water. The cycle of autoclaving and extraction is repeated eight times, after which no more gelatine is extracted.

Finishing unit steps

A crude gelatine extract obtained from each of these processes contains 2–8% gelatine. These solutions are filtered through cellulose cake or diatomaceous earth, undergo a salt removal step using ion-exchange chromatography, are concentrated in a vacuum evaporator, and finally ultra-high temperature (UHT) sterilised for at least 4 seconds at 138 to 140°C. When sterilised, the gelatine solution is cooled to form a gel, which is dried by warm air.

5.7.4 *Improved processing methods*

Various studies have been conducted to assess the efficacy of the gelatine production process and other treatment processes in reducing the BSE infective agent. These studies have either observed the effect of processing steps on the BSE agent, PrP^{Sc}, or on other TSE agents. Crucial information relating to reduction studies is the likely initial infectivity of the source materials used in commercially produced gelatine. There are few data in the literature relating to initial infectivity, but Taylor et al. (2001b) noted that typical starting titres in inactivation studies of about 6 $\log_{10}ID_{50}$ are about 100,000 times higher than would be expected for raw materials used in gelatine production. Based on this estimate, the starting titre in raw materials would be 10 ID_{50}, which is in agreement with the starting titres used for brain and skull in the EC's draft quantitative risk assessment on the use of vertebral column for gelatine production (EC, 2000c). The estimate of Taylor et al. (2001b) is a conservative one which assumes contamination of source materials with infected dorsal root ganglia only (A.H. Grobben, personal communication). Possible contamination with bone marrow was not considered.

Manzke et al. (1996) showed that, during the degreasing step, 98–99% of proteins of nervous origin were removed. It was also shown that total proteins were reduced by 82% after degreasing. After the subsequent acid step (4% hydrochloric acid for 4–5 days), specific nerve proteins could not be detected.

Inveresk Research International (1998b) reported on the effects of two processing steps—an acid treatment and a liming treatment—for the reduction of ME7 scrapie agent. Scrapie-infected mouse brain was inoculated intracerebrally into susceptible mice after initial concentrations of infectious agent underwent each treatment. The starting titre for the acid step was 7.44 $\log_{10}ID_{50}$ and for the alkaline step was 7.90 $\log_{10}ID_{50}$. After 18 months inoculation, the acid treatment was shown to reduce infectivity by 1.17 $\log_{10}ID_{50}$. The alkaline treatment was performed for 20 days, 45 days and 60 days, and gave reductions of 2.33 $\log_{10}ID_{50}$, 2.23 $\log_{10}ID_{50}$ and 2.10 $\log_{10}ID_{50}$, respectively. Clearly, the efficacy of the alkaline treatment did not appear to increase as treatment time increased.

Inveresk Research International (1998c), using the same infectious agent as in the previous study, used a combined acid and lime treatment to study reduction of

infectivity. The initial titre, 7.90 $\log_{10}ID_{50}$, was reduced in the combined treatment by 2.84 $\log_{10}ID_{50}$, measured by mouse bioassay over an 18-month period. This reduction was less than that predicted by the previous study if the effects of acid and lime processes are assumed to be additive.

In order to study the effects of sodium hydroxide rather than lime in the alkaline step of gelatine production, Inveresk Research International (1999) again observed the effects of treatment on the infectivity of ME7 scrapie agent. Two treatments, 0.25M sodium hydroxide for 5 days at 15ºC and 0.30M sodium hydroxide for 7 days at 15ºC, reduced infectivity from a starting titre of 7.80 $\log_{10}ID_{50}$ by 4.82 $\log_{10}ID_{50}$ and 5.25 $\log_{10}ID_{50}$, respectively. Sodium hydroxide appeared to reduce infectivity to a greater extent than did lime in previous studies.

The EC (2000d) commented that the studies conducted by Inveresk Research International used scrapie-infected brain material rather than inoculated bones, the latter being a better representative of the starting material in gelatine production. However, there are methodology problems which must be overcome in order to inoculate mice with processed bone material (Inveresk Research International, 1999). More importantly, the EC also suggested that future studies investigate the efficacy of the entire gelatine production process and not just isolated processing steps.

Taylor et al. (2001b) described interim results on studies on the potential of the initial steps of gelatine production potential to reduce infectivity of a BSE agent. The results were based on observations at about 390 days post-inoculation on acid and alkaline manufacturing processes, which were scaled down for validation to occur under laboratory conditions. Fresh, crushed bone was spiked with mouse brain infected with the 310V strain of mouse-passaged BSE agent. Spiking was achieved by injection of infected mouse brain into bovine spinal cord or by smearing of infected material onto the surfaces of crushed bones (Table 5).

Gelatine was then extracted using the following steps:

- degreasing (immersion in degreasing solution for 20 minutes at 77– 85°C and hot air drying at 69–83°C); and
- demineralisation (several days in 36% HCl).

The resulting ossein then underwent liming (48 days in supersaturated lime followed by nine hours in 2M sulphuric acid), extraction (performed at 60, 70 and then 80°C in the alkaline process and at 60 and 70°C in the acid process) or a short treatment with sodium hydroxide (two hours in 0.3 M sodium hydroxide and pH 13 followed by addition of HCl to give pH 2).

Table 5. Interim results on the inactivation of the BSE agent by gelatine processing steps (Taylor et al., 2001b).

Processing step	**Initial infectivity** ($\log_{10}ID_{50}$/mL)	**Final infectivity** ($\log_{10}ID_{50}$/mL)	**Reduction of infectivity** ($\log_{10}ID_{50}$/mL)
Limed process	8.1*	4.9	3.2
Standard acid process	9.8*	6.0	3.8
Acid + NaOH step	8.1	0	≥7.6

* The initial infectivity in each case was derived from different brain pools, making comparison difficult if there is any brain pool effect.

There was an apparent contradiction in the results found for the reduction of the acid process (3.8 $\log_{10}ID_{50}$) and the limed bone process (3.2 $\log_{10}ID_{50}$) in that previous studies have showed the alkali process to be more effective that the acid process. However, at the time of publishing the interim results, the starting titre if the brain pools for each assay was not known. The limed bone process could still give rise to the greater reduction depending on the outcome of assays on the infectivity titre of the starting material.

By subtraction, demineralisation (91.5 – 103 hours in 36%HCl, following degreasing) reduced infectivity by 1.2 $\log_{10}ID_{50}$, and the combination of demineralisation and liming reduced infectivity by 2.8 $\log_{10}ID_{50}$. Reduction by degreasing was therefore between 0.4 and 2.6 $\log_{10}ID_{50}$.

The authors concluded that:

- including a short sodium hydroxide step after demineralisation resulted in no detectable BSE agent;
- the acid bone manufacturing process reduced infectivity by about 3.8 $\log_{10}ID_{50}$;
- the limed bone process reduced infectivity by at least 3.2 $\log_{10}ID_{50}$ (and a higher reduction is expected as the titration is not yet complete); and
- reductions reported are before the gelatine undergoes filtration, ion-exchange and sterilisation.

Rohwer et al. (2001) presented interim data on the removal and inactivation of hamster-adapted 263K scrapie in artificially spiked bovine gelatine solutions (Table 6). Infectivity was measured by bioassay in hamsters and the data were based on clinical observations of the titration of animals after at least 400 days. The animals were to be held for another 150 days before the titrations were terminated. Different industrial-scale manufacturing processes were scaled down in the laboratory so as to maintain essential process conditions. Three individual operations at the end of the process, the 'finishing unit steps', were tested singly or in tandem for their ability to remove or inactivate the scrapie agent. The authors noted that results applied to gelatine manufactured from hides as well as bones.

The following 'finishing unit steps' were investigated for efficacy in reducing scrapie infectivity:

- filtration (cellulose filter cake);
- ion-exchange of the filtrate;
- ion-exchange of re-spiked filtrate (infected hamster brain added to filtrate before ion-exchange); and
- UHT sterilisation (maximum temperature of 140°C and above 133°C for 5.3 seconds).

Table 6. Interim results on the inactivation of 263K scrapie by gelatine processing operations (Rohwer et al., 2001).

Finishing unit step	Initial infectivity ($log_{10}ID_{50}$/mL)	Final infectivity ($log_{10}ID_{50}$/mL)	Reduction of infectivity ($log_{10}ID_{50}$/mL)
Filtration	6.72	5.13	1.6
Ion-exchange of filtrate	5.13	4.93	0.2
Filtration + ion-exchange	6.72	4.93	1.79
Ion-exchange, re-spiked	6.70	5.96	0.74
UHT sterilisation	7.47	3.27	4.2

The authors demonstrated significant removal of infectivity by filtration and UHT steps, but not by ion-exchange chromatography. The workers commented that removal by ion-exchange was probably mechanical rather than chemical.

UHT sterilisation was very effective, and was thought to act as an inactivation step rather than a removal step. The equivalent of 138 to 140°C reduced infectivity by 4.2 $log_{10}ID_{50}$.

Presuming that UHT sterilisation occurred independently of the filtration and ion-exchange removals, all steps combined would give a cumulative removal of 6.0 $log_{10}ID_{50}$.

Rohwer, R.G. (2001), reported on the continued observations of the work initiated in Rohwer et al. (2001), after a period of 412 to 445 days post-inoculation. As predicted, there was no change in infection status of any animal since the previous report in October 2000. At the conclusion of his study, Rohwer concluded that the purification steps gave an overall reduction of infectivity of 10^4 to 10^6-fold. It was also concluded that filtration and ion-exchange alone may not remove infectivity left from earlier treatments, and that UHT-sterilisation provides a potentially secure inactivation step (Vermeulen, pers. comm.).

5.7.5 Estimated consumption of gelatine in Australia

Exposure to gelatine can come from a number and variety of sources. Consumption of gelatine was estimated for the whole population, as well as for a number of age groups; 2-12, 13-18, 19-64 and 65+ years and include uses of gelatine as an ingredient in foods such as dairy desserts, jelly and mousse (Table 7). These foods are assumed to present a similar level of risk of exposure to the BSE agent, as the processes used in their preparation would not reduce BSE infectivity. There were only two respondents (out of 13 858) to the NNS who reported consuming gelatine as gelatine, rather than as an ingredient in mixed foods.

Table 7. Estimated consumption of gelatine by Australians (1995 NNS)

Age	Number of consumers	Consumers as % respondents	Consumption (g/day) Respondent mean*	Consumer mean**	95th %ile
2-12	118	5.7	0.15	2.7	5.6
13-18	21	2.3	0.08	3.7	11.2
19-64	129	1.5	0.05	3.5	2.8
65+	46	2.3	0.07	2.8	5.6
All	314	2.3	0.07	3.1	8.4

* This is the mean consumption of all respondents in each category regardless of whether they consumed gelatine.
** This is the mean consumption of only those respondents who reported consuming gelatine and gelatine-containing foods.

The percentage of individuals in the 2 to 12 year olds consuming gelatine (5.7%) was more than double that of the other age groups. The 13-18 year old age group were the highest consumers of gelatine on a grams per day basis, with a mean consumption of 3.7 g/day for those respondents who did consume gelatine. The mean consumption of gelatine for all consumers of gelatine was 3.1 g/day, 8.4 g/day for high consumers (95^{th} percentile).

5.7.6 *Conclusions*

The BSE infective agent has been found to occur in the nervous tissue associated with some bovine bones and in the clinical phase of experimental BSE in bovine bone marrow. There is some evidence to suggest that some parts of the hide that are later used in gelatine manufacture could be contaminated during slaughter. However if this did occur, the amount of BSE infectivity would be considerably less than the level of infectivity present if sourced from bovine bones of an infected animal.

There has been no work to show the efficacy of the gelatine production process in its entirety in reducing the level of the TSE agent. However, studies on the majority of unit operations of gelatine manufacture have shown that the conventional acid bone and limed bone treatments reduced infectivity by 3.8 $\log_{10}ID_{50}$ and 3.2 $\log_{10}ID_{50}$, respectively (more than a 99.9% reduction). Addition of a sodium hydroxide step, although not in current commercial use, gave an infectivity reduction of 4.4 $\log_{10}ID_{50}$, higher than that for the limed bone treatment (total reduction of more than 99.99999%). An additional 2 – 4 $\log_{10}ID_{50}$ reduction in infectivity could be achieved through the finishing steps of filtration and UHT sterilisation.

Cross-contamination of hides during slaughtering is not likely to carry any more infectivity than bones into the start of the process.

5.8 Preparation of tallow

5.8.1 What is tallow?

Tallow is a term used to describe a range of fats produced by many different techniques dependent on the tissues used as raw material and the product end use. In Australia dripping or edible tallow is the product prepared by rendering the fatty tissues, attendant muscles and bones of bovine animals or sheep or bovine animals

and sheep, fit for human consumption. The product may be refined (ANZFA, 2000). Any rendered animal fat that is solid at room temperature is likely to be called tallow. Australian tallow is predominantly rendered bovine fat. Depending on the producer, tallow could be 100% beef, 100% ovine, a mixture of bovine and ovine, or a mixture of bovine, ovine and porcine fat. There may be other tallows that include a proportion of fat or oil from other species such as poultry, horse and fish.

In Europe, tallow is defined as fats obtained by extraction and separating by melting or by processing such as rendering and extracted by pressing, drawing off or centrifugation. The source material can be certain ruminant discrete adipose tissue masses (reserves of fat which can be removed readily during slaughter in the abattoir or at meat-cutting plants, e.g. fat trimmings but not lipid extracted from mechanically recovered meat), trimmings, bones, and certain slaughter offals. It can be further purified by centrifigation, filtration or treatment with phosphoric acid, or by thermal refining (EC 2001b). The composition of raw material rendered in the UK is estimated to be as follows: -

pigs	–	18%
cattle	–	54%
sheep	–	28%

The difference between tallow and other fats and oils is the species of origin, which determines the fatty acid composition of the triglycerides that characterise the fat. The practical distinction between tallow and other fats is the melting point of the tallow. Tallow is one of the hardest fats and has a melting point of about 42°C, (tallows and other fats and oils do not melt at a specific temperature, they melt over a temperature range). Mutton tallow has a higher melting point than beef tallow. Rendered pork fat may be referred to as tallow and is solid at room temperature. Edible pork fat, when not mixed with fat from other animal species, is called lard. Other rendered animal fats (poultry, horse and fish) are liquid at room temperature and are usually referred to as oils.

5.8.2 *How is tallow used*

The quality of tallow is determined mainly by the type (i.e. freshness and cleanliness) of raw material processed. There are a range of commercial terms used to describe tallow (eg. Prime tallow, No. 1 tallow etc) however the American Fats and Oils Association has a set of specifications that are used in international tallow trading. Some of these specifications are shown in Table 8. Australian tallows do not necessarily fit in with the specifications shown in Table 8. The main criteria of Australian tallow is free fatty acid which is normally less than 2% and maximum insoluble impurities of 0.05%. Insoluble impurities includes any remaining protein component from the raw materials and this would be a potential route for exposure to the BSE agent.

The total production of tallow in Australia is approximately 450,000 tonnes per year and about 70,000 tonnes of tallow is used in edible applications per year.[5] About

[5] Report for the Meat Research Corporation in 1996.

25,000 tonnes of edible tallow products are exported and Australia has no need to import the raw commodity.

The main use of tallow in the food industry is as frying fat, though it may also be used as a shortening agent in products such as biscuits, cakes and pastries. Tallow as produced at a rendering plant is almost always further refined as described above before it is used in edible applications. Edible tallow can be used either for edible or inedible applications but inedible tallow cannot be used for edible purposes.

Table 8. Examples of AFOA specifications for tallow

Grade	Titre (minimum °C)	Free fatty acid maximum	FAC maximum	R & B maximum	MIU
Edible tallow	41	0.75	3	–	*
Top white tallow	41	2	5	–	1
Bleachable fancy tallow	40.5	4	–	1.5	1
Prime tallow	40.5	6	13–11B	–	1
No. 2 tallow	40	35	–	–	2

Notes: Titre is the melting point of fatty acids in the tallow. FAC is colour scale usually used to measure the colour of unbleached tallow. R&B is a colour measurement applied after the tallow has been bleached. MIU is the combined measurement of moisture, insoluble impurities and unsaponifiable material.
*There is no MIU specification for edible tallow, but the maximum moisture is 0.2% and the maximum insoluble impurities is 0.05%.

5.8.3 *The tallow process*

There are a number of systems use for the production of tallow:

a) Melting of fatty tissues—the raw materials are fresh slaughter-fats (discrete adipose tissues) fit for human consumption and dedicated processing is widely used. In Europe the highest quality grade of tallow, Premier Jus, is produced with the fat melting system for food applications (such as soups, sauces, margarines, frying oils; Table 9). Although the legal value for insoluble impurities is 0.15%, the European Fat Processors and Renderers Association (EFPRA) purifies the tallow for edible fat for food to below 0.02% insoluble impurities (EFPRA, 2001a,b,c).

Discrete adipose tissue, directly associated with the digestive tract of cattle, sheep and goats, could contain intestine-associated tissue. However, if slaughter practices ensure the removal of this mesenteric tissue, the rest of the adipose tissue in this area should not be considered an SRM. BSE infectivity has not been found in the distal ileum in natural BSE cases and has only been under experimental conditions 10 months post challenge (see Section 5.1.2). The tissues that theoretically could carry BSE infectivity associated with discrete adipose tissues are the mesenteric nerves and nodes. The mesenteric lymph nodes are embedded in fat and located at the ventral part of the mesentery and close to the intestine. These tissues have been taken from clinical BSE cases and bio-assayed in mice and cattle. The results demonstrated

absence of BSE infectivity. Similar assays using mesenteric lymph nodes from experimentally infected BSE cattle showed no detectable infectivity at any stage of incubation of the disease. These results indicate that the midrum fat is a low risk tissue for BSE infectivity.

b) Rendering—a mixture of tissues such as bones, trimmings, meat rests and slaughter offals are rendered to release tallow at temperatures that could be below 100^0C followed by a purification step. This is the typical process for tallow production in Australia and is described in further detail below. A sterilisation process may or may not be incorporated. Tallow produced by rendering is used for feed, pet food and technical applications and purified to below 0.15% insoluble impurities. The raw materials can either be animal by-products fit but not destined for human consumption or high risk materials. A sterilisation step is used to produce meat and bone meal.

c) Fats pressed after rendering at 133°C for 20 minutes under 3 bar pressure - a mixture of bones, trimmings, meat rests and slaughter offals.

There are two major types of rendering systems used in Australia: dry and wet. These systems either render edible animal fatty tisues into edible fats for human use or render inedible animal byproducts into fats and protein for animal feed and non-edible applications. Inedible tallow is produced in grades appropriate to the category of use. Dry rendering systems are by far the most prominent style of rendering. The distinction between dry and wet rendering systems relates to the condition of the rendered material when the tallow is separated from the solids. In dry rendering, raw materials are boiled in their own juices until most of the water in the raw material has been evaporated. Heating continues but the solids are fried in tallow and more moisture is evaporated from the mixture. The end of the process occurs when the solids are about 10% moisture. At this stage the tallow and dried solids are separated. Dry rendering can be a batch or continuous process.

In wet rendering, raw materials are heated in their own juices, with or without steam injection. The temperature could be 60 to 100°C but in Australian conditions the temperature is usually 95 to 100°C. Water is not evaporated from the materials in the wet rendering process. After the heating stage, liquid including tallow and free water is separated from the wet solids by centrifugation or pressing. The wet solids are dried separately from the tallow. The term wet rendering is used because tallow and solids are separated while the total material is still wet. Wet rendering processes used in Australia are continuous processes.

In Australia, tallow produced at rendering plants is further refined to make edible products. The specifications for tallow used for further processing to make edible product are usually less than 1% free fatty acid, colour 11A or less, and moisture and impurities less than 1%. According to the *Food Standards Code*, the raw materials used to make tallow for edible use should be fit for human consumption and edible tallow must have an acid value of less than 4 (free fatty acid less than 2%).

Tallow is refined for edible use by treating with caustic soda to neutralise free fatty acids. The tallow is water washed to remove soap and acid oil, vacuum dried, heated to 95°C and mixed with activated bleaching clay. The tallow is filtered through a bed of diatomaceous earth and bleaching clay and then deodorised by heating to at least

230°C and holding for 15 minutes. The total deodorising time is about 100 minutes and involves moving the tallow through stages from 60 to 230°C and back to 60°C. The deodorised product is passed through a 5 micron filter which would theoretically reduce BSE infectivity by a factor of 100. The level of insoluble impurities resulting from these steps would be much less than 0.15% (B. Spooncer, personal communication). The level of insoluble impurities in unrefined 1% free fatty acid tallows is generally less than 0.07%.

Taylor has examined tallow production processes using abattoir waste spiked with 10% BSE-infected bovine brain and shown that the most effective process for inactivating the BSE agent was autoclaving the raw material at 145°C for 18 minutes (Tayloret al, 1995; Taylor et al , 1997b). This produced MBM with no detectable infectivity, and mice injected with filtered and unfiltered tallow did not develop any signs of neurological disease. A separate experiment using a worst-case scenario resulted in the MBM containing levels of infectivity similar to those of the untreated raw material but the tallow fractions injected into mice yielded no signs of neurological disease. EFPRA (2001a) estimates that the infectivity in tallow should be 10^7 less likely than infectivity in MBM, based on the result of Taylor's studies. This was based on the assumption that the level of infectivity in the tallow was calculated to be below $10^{-2.5}$ cattle oral ID_{50}/mL and the corresponding level of infectivity in the MBM was $10^{5.0}$ cattle oral ID_{50}/g. This result must be considered as an estimate only, given the limitations of available data and inherent problems of extrapolating results from laboratory-scale experiments. Ultrafiltration to reduce insoluble impurities to 0.02% would reduce BSE infectivity by a factor of 100.

A recent study has indicated that autoclaving is less effective with increasing amounts of lipid present, resulting in survival of the TSE agent. The authors suggested that BSE infectivity would have a tendency to associate with tallow, rather than MBM, during the rendering process because of the hydrophobicity of PrP^{Sc} (Appel et al., 2001). However the study was not performed using the normal conditions used in rendering and demonstrated only the effect on inactivation and not on infectivity through the use of biochemical methods. Appel's experiments added prions directly to animal fat, whereas rendering entails separation and purification steps during the process.

Tallow can contain up to 0.5% of insoluble solids that have been estimated to contain up to 85% proteinaceous material. (EFPRA has quoted 5–16% protein levels in insoluble impurities. A level of 0.15% insoluble impurities corresponds to 0.127% of proteinaceous impurities in final product (based on 85% crude content assumption).

Recently the SSC has evaluated the effect of the fat extraction and sterilisation processes on the safety of tallow. Fat extraction procedures exclude most proteinaceous material, and therefore most infectivity, unless there is selective partitioning of infectivity into the fat phase. There is little evidence for or against this possibility. Filtration steps in the process may trap some infectivity, if infectivity follows the bulk of proteinaceous material. However, it is probable that significant reduction of infectivity occurs during processing by reducing the non-fat matter during filtration and purification stages.

The efficiency of reducing BSE infectivity by subjecting tallow to a sterilisation step has not been clearly demonstrated as some TSE strains are not completely inactivated at 133°C for 20 minutes even when fully hydrated. In general, TSEs are more readily inactivated by wet than dry heat. The hydration state of protein, or specifically TSE infectivity, after the fat extraction procedure is not known; neither is it known how it might change when exposed to wet heat sterilisation at 133°C. If the extraction procedure dehydrates the infectivity then it is more likely to survive.

Ruminant-derived tallow that has been heat treated at 133°C for at least 20 minutes at 3 bar would give an F_o value of about 300 minutes if it is assumed the target agents are in a wet environment (EC, 1998). Studies have shown scrapie 263K survived 1 hour at 360°C in a dry substrate (Brown *et al*., 1990). Although prions are proteinaceous and therefore likely to separate from the lipid phase of melted tallow, it may be possible for them to be dispersed in lipids even if the tallow is wet. Prions may behave similarly to fat-encased microorganisms, by displaying unusually high heat resistances. The data in Table 3 indicate that the process of heating at 133°C for 20 minutes would reduce the probability of survival of prions in wet conditions by a factor of 100 – 1000.This range is consistent with the SSC estimate of a 1000-fold reduction in infectivity titre of tallow compared to the titre of the MBM fraction.

In Australia there are small amounts of tallow that are used as frying fat without any further processing. These tallows are made only from fresh soft fats such as boning room fats, kidney fats and caul fats. Use of other material is constrained because, without further processing, the odour of tallow made from other tissues would be unacceptable for use as an edible frying fat.

Table 9. Specifications for ruminant tallow production in the European Union

Type of tallow produced	**Edible tallow, Premier jus, FFA max 0.50% (legally max 0.75%) Edible tallow, others, FFA max 1,25% Tallow for refining, FFA max 3.0%**	**Ruminant tallow, FFA max 1% to max 15%**
Type of industry	Fat processors	Renderers
Legislation	92/5/EEG (amended and updated 77/99/EEG)	90/667/EEG
Intended use (tallow)	Human or animal use	Animal or industrial use
Animal class	Approved fit for human consumption	Approved fit for human consumption (cat.3: low risk material)
Animal by-product class	Fit for human consumption	Fit for animal consumption : low risk material (dead animals banned from feed as from 1 March 2001)
Type animal by-products	Fresh slaughterfats from bovines: fatty tissues from the kidney area, << mesogastrium >> mesentery and cutting fats (minimal) readily removed during slaughter in the slaughterhouse or cutting plants (fat content fatty tissue from kidney area : 80% fat)	Animal by-products from bovine, ovine and caprine. All other ruminant tissues (SRM excluded) : cutting fats, bones, etc.
Bones as raw material	Bones are not used as raw material	Bones are used as raw material. In specific cases, bones are processed separately to produce bonemeal (40% protein) and bonefat (e.g. bone processors)
Species dedicated{?}	Always applied	Is occurring in specific cases
Other products produced	Wet greaves, proteinwater, greavesmeal (80% protein)	Meat meal, meat and bone meal, bonemeal (protein 40–60%), greavesmeal (80% protein)
Tallow production process	1. Wet melting process (premier jus Mincing, direct steam injection (95°C), purification by decantation, centrifugation and filtration (bag filters, aid free) 2. Dry melting process Mincing, indirect heating (e.g. 135°C; disc dryer), purification by decantation, pressing, centrifugation and filtration	1. Dry rendering process : the tallow is separated from the proteins after indirect drying; mincing, indirect heating (e.g. 135°°C disc dryer), purification by decantation, pressing centrifugation and filtration. Pre- or post-sterilisation can be applied. 2. Wet rendering process : the tallow is separated from the proteins before removal of the water; mincing, heating, purification by decantation, centrifugation and filtration. Pre- or post-sterilisation can be applied.
Sterilisation 133°C/20 minutes/3 bars	Applied only on animal protein destined for feed (temporary	For both methods pre-sterilisation on raw material or

Type of tallow produced	**Edible tallow, Premier jus, FFA max 0.50% (legally max 0.75%) Edible tallow, others, FFA max 1,25% Tallow for refining, FFA max 3.0%**	**Ruminant tallow, FFA max 1% to max 15%**
	banned)	post sterilisation on purified tallow and/or meal is used. The sterilisation of animal proteins destined for petfood and tallow from LRM destined for feed/petfood is not required (derogations on sterilisation 1999/534/EC are applied in practice).
Residual insoluble impurities	Max 0.02%	Max 0.15%
Applications tallow	Food : soups, sauces, margarine, frying medium Feed : mainly calf milk replacers Petfood : petfood ingredient	Feed : feed ingredient Petfood : petfood ingredient Industrial : oleochemistry, cosmetics, soaps, detergents, fuel
Remarks	For commercial reasons tallow is refined or deodorised (e.g. removal of FFA, odour, colour and impurities) Refined tallow FFA max. 0.30%	

5.8.4 *Estimated consumption of tallow in Australia*

Information from the food industry on the use of oils by major fast food chains in Australia indicate that in many cases foods, such as potato chips and hamburgers, are fried in vegetable oil or, where animal fats are used, the tallow is sourced in Australia. Tallow is not used by major producers of biscuits, cakes and pastries as current practice in Australia and New Zealand is to use vegetable shortening in all these food categories. Where these major companies have manufacturing plants overseas, the same recipes are used as in Australia or New Zealand.

The amount of imported biscuits (savoury and sweet, including snacks) reported in Australian Quarantine Inspection Service (AQIS) records is 47 047 751 kg per year over a time period from January 1999-June 2001 (average of 18 819 100 kg per year). The 2001 Australian Grocery Marketing Guide indicates that the volume of total biscuit sales (savoury and sweet, including snacks) for 2000 was 112 056 200 kg. Based on these figures, imported biscuits, cakes and pastries represents ~17% total biscuit market, with the single largest imported category being shortbreads and other 'premium' biscuits. The average biscuit consumption per person per year based on grocery sales and an assumed Australian population of 19 million people is 16.1 g/person/day (2.7 g/day imported biscuits). This figure accords well with reported total biscuit consumption for the whole population from the 1995 National Nutrition Survey (NNS) given below (12.8 g/person/day). It is not possible to derive similar figures for the percentage of imports of the cakes or pastry categories because a much higher proportion of these are made on site to be sold fresh at bakeries or other retailers and as such are not reported in the Retail World publication. However,

the percentage of imports is expected to be lower than that for biscuits because these products tend have a short shelf life and are less likely to be imported.

Estimates of actual biscuit, cake and pastry consumption (Table 10) were derived from ANZFA's dietary modelling computer program, DIAMOND.

Table 10. Estimated consumption of biscuits, cakes and pastries and the intake of fat from biscuits, cakes and pastries in Australia (1995 NNS)

Food	Food consumption (g/day)		% of total fat contribution
	Respondent mean*	Consumer mean**	
Biscuits	12.8	34.4	3.3
Cakes	12.6	86.0	3.4
Pastries	30.3	171.9	6.0
All foods	-	-	100% = 75.5g/day

* This is the mean consumption from respondents on day of survey regardless of whether they consumed biscuits, cakes or pastries

** This is the mean consumption of only those respondents on day of survey who reported consuming biscuits, cakes or pastries.

Total dietary fat intake and fat intake from biscuits, cakes and pastries was also estimated for the Australian population. The estimated mean total dietary intake of fat for all respondents was 75.5 g/day. Of this total amount, biscuits, cakes and pastries were estimated to contribute <10 g fat/day. If it is assumed that the maximum percentage of imported goods is 17% for these categories, then 1.6g fat per day may come from imported foods in these categories.

5.8.5 Conclusions

When evaluating tallow, the risk of exposure to the BSE agent stems from the potential for insoluble impurities to be present in the tallow. Tallow used for edible purposes can be made by melting adipose tissues, which has been shown to be free of BSE infectivity, or rendering other animal tissues. The reduction of BSE infectivity by the various processes has not been fully validated except in the case of the process of 133°C/3 bar/20 minutes, which reduces TSE infectivity by at least a factor of 1000. Edible tallow involves further processing of the raw tallow before it is made into a margarine or used in a meat pie. Subsequent refining involves neutralisation of free fatty acid with sodium hydroxide, bleaching and filtering and deodorisation by exposure to temperatures greater than 230°C for more than fifteen minutes. However these processes would not guarantee elimination of the BSE agent, if resent due to contamination with protein impurities. In addition, it is possible that not all tallows are subject to some or all of the refining steps before they are used in edible application such as frying fat. Further refining involving neutralisation with sodium hydroxide reduces BSE infectivity and the filtration steps theoretically reduce BSE infectivity by a factor of 100 and further reductions in infectivity can be achieved by undergoing a process of 133°C/3 bar/20 minutes. This latter treatment is not usually used for the production of edible tallow.

Imported biscuits, cakes and pastries from European countries may be a potential source of tallow as an ingredient, though this is considered very unlikely given the current world-wide trend to use vegetable shortening agents in these foods.

Historically, tallow was used as a shortening agent in Australia, though not necessarily in other countries, for example, vegetable oils were traditionally used in America and fish oils in some European countries. The Australian population may consume 1.6 g of fat per day from imported biscuits, cakes and pastries. However, as stated previously, it is extremely unlikely that this fat would be tallow, but more likely to be vegetable shortening or, in the case of shortbread biscuits, butter.

5.9 Preparation of collagen

5.9.1 *What is collagen?*

Collagen is a polypeptide with an average molecular weight of 130,000 daltons. It comprises approximately one third of the total protein in mammalian organisms, and is the highest component in mammalian proteins. Collagen is the main constituent of skin, connective tissue, and the organic substance of bones and teeth (Merck Index, 1989). Collagen formation in the mammalian body is preceded by the formation of a much larger molecule—procollagen. There are different types of collagens. However, they are all composed of molecules containing three polypeptide chains, the α-chains. The α-chains are arranged in a triple helical conformation. The amino acid sequence of the α-chain is mostly a repeating structure with glycine in every third position and proline or 4-hydroxyproline frequently preceding the glycine residues. Slight differences in the primary structure establish the differences between types of collagens. Collagen is differentiated from the accompanying fibrous proteins (elastin and reticulin) by (1) its contents of proline, hydroxyproline and hydroxylysine, and (2) the absence of tryptophan and its low tyrosine and sulfur content, but particularly by (3) its high content of polar groups originating from the difunctional amino acids. The polar groups are responsible for the swelling properties leading eventually to dispersion of collagen in diluted acid. Denaturation of collagen is the conversion of the rigidly coiled structure to random coiled gelatine. Collagen can be dissolved and isolated from its natural sources by mild extraction with dilute acid, dilute alkali, and neutral salt solution.

5.9.2 *How is Collagen used*

The film-forming ability of collagen has traditionally been utilised in the meat industry for production of edible sausage casings (Ginnadios *et al*., 1994). Various product lines are classified unofficially under the name of sausage casings including:

fresh sausage collagen

smoked sausage collagen

coloured snack sausage collagen

hand link sausage collagen

butchers sausage collagen

collagen ring casings.

The information available indicates that there is only one manufacturer of edible collagen in Australia. The company sources its bovine hides exclusively from Australia and strict measures are applied to their quality control.

It is estimated that Devro-Teepak Pty Limited produces approximately 600 tonnes of dry collagen per year in Australia. Market information from the edible collagen industry indicates that close to 170 tonnes per year of edible collagen are imported into Australia (J.Glen, Devro-Teepak Pty Limited, personal communication). Origins and amounts of imports are given in Table 11.

Commercial uses of collagen depend primarily, but not exclusively, on the physical structure and chemical reactivity of the native or intact collagen molecule. Extruded edible sausage casings, extruded sutures, and natural sutures are examples of useful intact or native collagen products.

Table 11. Current Australia importation of edible collagens

Country of origin	Amount (tonnes)
Germany	68.5
UK	47.6
United States of America	45.6
Japan	7.2
Poland	0.6
Spain	0.2

5.9.3 *The collagen process*

Ruminant hides which are the raw material for collagen production, are not considered to present a risk with respect to BSE (EC 2000e). Studies have confirmed that BSE infectivity has not been found in skin or hair. The risk of contamination of the hide during slaughter is negligible since contamination with brain tissue resulting from stunning would occur over a small area of the facial part of the hide. The underlying layer of the hide after removal of the internal fatty layer is used for making collagen and the outside layer, which may have been contaminated, is removed and used to make leather.

Two general methods have been developed for the industrial-scale production of edible collagen casings: the 'dry process' was developed in Germany; the 'wet process' was developed in North America (Ginnadios et al., 1994).
Steps in the 'wet process' are as follows.

a) Acid- or alkaline-unhairing of the hides
b) Decalcification of hide corium and grinding into small pieces
c) Mixing of ground collagenous material with acid to produce a swollen slurry (4–5% solids)
d) Slurry homogenisation
e) Extrusion into tubular casings (8–10% solids)
f) Washing casings free of salts
g) Treatment with plasticising and cross-linking agents

h) Drying.

The ‘dry process’ involves the following steps:

a) Alkaline treatment of hide corium and acidification to pH 3
b) Shredding of acid-swollen corium to preserve maximum fibre structure
c) Mixing of acid-swollen fibres to produce dough with high solid content (i.e. >12%)
d) Addition of plasticising and cross-linking agents,
e) High-pressure pumping and extrusion of dough to form tubular casings
f) Drying, conditioning, neutralising, and/or providing additional cross-linking.

The following describes the general procedure of edible collagen casing production by Devro-Teepak Pty Limited (Pers. Comm. Devro-Teepak, 2001) the largest edible collagen manufacturer in the world. In many cases, the Devro-Teepak procedure is similar to the generic ‘wet process’ described earlier.

a) Separation of corium layer – Disease free bovine hides are unhaired and limed before the separation of the collagen-rich corium from the outer grain layer.
b) Grinding – The corium collagen is ground in a series of stages through progressively smaller gaps until a fibrous pulp is obtained.
c) Acidification and fortification – The pulp is acidified and cellulose powder is added to a specific composition dependent on casing type being produced. The process is aided by applying shearing forces and homogenisation to the structure as it swells to enable the formation of a desired gel structure.
d) Extrusion and coagulation – The gel is then passed through an annular extruder to form a thin-walled tube, which is coagulated by adjusting the pH upwards to neutralise the acidic dough.
e) Remove salt and plasticising – The neutralised casing is washed thoroughly to remove the salt formed in the neutralisation step and then plasticised by the addition of glycerol, which confers flexibility on the casing after drying.
f) Drying – Drying is effected by passing the inflated tube through a hot air dryer to reduce the moisture content to approximately 15–20%. The dried casing is cut to convenient lengths and shirred before packaging in hermetically sealed containers. The casing is ready to be used in sausage manufacture, or other food packing. It has a water activity of approximately 0.8.

The process of edible collagen manufacturing involves pH adjustment, including the early stage of lime treatment to raise the pH (up to a maximum of pH 13 for 12–48 hours), and collagen extraction through acidification (down to a minimum of pH 0.8 for 6–72 hours. The production process also involves vacuum pressurisation, up to 30 MPa, and temperature variation where the maximum is at the casing drying stage. Currently there is not comprehensive experimental data on the residual infectivity levels of the BSE agent during the collagen manufacturing process, however the risk of exposure to the BSE agent is negligible since the raw material for collagen is the corium layer of the hide which is a negligible risk tissue.

5.9.4 *Estimated collagen consumption in Australia*

Consumption of collagen was estimated for the Australian population assuming that consumption would be in the form of edible collagen casings used in sausages and frankfurts. Based on industry use data, the average collagen content in casings is approximately 55%. This translates into a maximum collagen content in sausages and frankfurts of 0.55% or 5.5 mg/kg, based on the percentage of casing in a sausage. This level was used in the dietary modelling.

Estimated intakes of collagen from the consumption of sausages and frankfurts are shown in Table11. The consumption figures in Table 12 were derived from ANZFA's dietary modelling computer program, DIAMOND. Overall intakes were low, with the 95th percentile of estimated collagen consumption being less than 1 g/day for children aged 2 to 12 years, and 1.3 g/day for the whole population. Sausage consumption was the major contributor to collagen intake for both children (2-12 years) and for the whole population (Table 13).

Table 12. Estimated intake of collagen from the consumption of sausages and frankfurts for the Australian population (1995 NNS)

Age	Number of consumers	Consumers as % respondents	Consumption (g/day)		
			Respondent mean*	Consumer mean**	95th %ile
2-12	412	19.8	0.08	0.4	0.9
All	1916	13.8	0.07	0.5	1.3

* This is the mean consumption from all respondents on day of survey regardless of whether they consumed sausages or frankfurts.
** This is the mean consumption of only those respondents on day of survey who reported consuming sausages and/or frankfurts.

Table 13. Contribution of sausage and frankfurt consumption to estimated intake of collagen in Australia

Population	Food	% of total contribution
2-12 years	Sausages	86.6
	Frankfurts	13.4
All	Sausages	86.4
	Frankfurts	13.6

5.9.5 *Conclusions*

Collagen for use in food applications is manufactured from the corium layer of food-grade bovine hides. The process of edible collagen manufacturing involves pH adjustment, including the early stage of lime treatment to raise the pH (up to a maximum of pH 13 for 12–48 hours), and collagen extraction through acidification (down to a minimum of pH 0.8 for 6–72 hours). Although these conditions of collagen production would not inactivate the BSE agent, bovine hides which are the raw material for collagen are not considered to present a risk with respect to BSE infectivity. Studies have confirmed that the BSE agent has not been found in skin or hair.

6.0 RISK CHARACTERISATION

This section brings together the information on the characterisation of the BSE infective agent and the assessment of exposure via food to characterise the risk of acquiring vCJD by consuming beef and beef products contaminated with the BSE agent. vCJD is a progressive and fatal human neuro-degenerative disorder resulting from exposure to the BSE agent through consumption of beef-derived products contaminated with the BSE agent.

6.1 Fresh meat and processed meat products

Not all animal products will pose the same risk of transmitting the BSE agent to humans, because some products appear to contain more of the agent than others. Animal products that were identified in the risk assessment as containing significant amounts of the BSE agent include so-called specified risk material (SRM) and mechanically recovered meat. For bovine animals the SRM are the skull including the brain, eyes, trigeminal ganglia, tonsils, thymus, spleen and the spinal cord of animals over 12 months of age; the vertebral column (including dorsal root ganglia) of animals over 12 months of age, and the intestines (from duodenum to rectum) of animals of any age. There is a high risk of exposure to the BSE agent through consumption of specified risk material, and in these tissues the CNS tissues from clinically affected cattle present the highest risk of transmitting the BSE agent to humans. About 95% of the total infectivity is estimated to be in the CNS of clinically BSE-affected animals and for possibly 3 months prior to clinical signs of the disease.

Risk materials may be used in the preparation of certain products such as smallgoods and canned beef products. If prepared from tissues of BSE-affected cattle, these tissues present a potential pathway of human exposure to the BSE agent. The level of risk with these products is increased as often the raw materials are sourced from older animals. Tissues other than the CNS are likely to be 1000 times less infective and would require significantly higher consumption to present a similar level of risk. The age of animal at slaughter will influence the level of BSE infectivity entering the food chain as the level of BSE infectivity in the CNS is increased if the animal is slaughtered at 30 months or older, depending on the age of the animal when initially infected.

Cross-contamination of uninfected tissues with infected tissues is potentially possible in abattoirs and cutting plants and must be considered in any risk analysis. The steps in the slaughtering process that may involve a risk of cross-contamination with SRM between carcases are stunning, carcase splitting, head processing and carcase trimming. The practices with greater risk of potential contamination of beef with CNS through dissemination of brain tissue are the stunning procedure and the dispersal of spinal cord material during the splitting of carcases.

Although heating, especially in the presence of steam, can reduce BSE infectivity by a factor of 1000, there is not a heat process that guarantees complete inactivation of the BSE agent. Shelf-stable canned beef products and probably beef extract receive heat processes which are often described as severe in that they inactivate bacterial spores. These processes are equivalent in sterilising effect to holding all parts of the product at 121.1°C for at least a few minutes. Most other beef products receive either no heat

treatment or heat treatments of low severity; in most instances some or all of the product is heated to less than 100°C. Several minor ingredients are used in some beef products to improve consumer acceptability, to reduce oxidative changes, to bind water freed from heat denatured protein and other purposes. These processes will not inactivate the BSE agent.

Consumption of fresh meat and processed meat products occurs in all age groups and constitutes a significant part of the Australian diet with an average daily consumption of 230 grams of fresh meat and processed meat products. Therefore if the BSE agent was present in these products, through usage of BSE-affected materials, the Australian population would have a high likelihood of exposure to the agent due to the significant and sustained consumption of these foods. This level of exposure would be similar for processed meat products compared to fresh meat as cooking does not affect the infectivity of the agent.

6.2 Milk and dairy products

The status of milk as a possible source of BSE infectivity has been evaluated experimentally using mouse bioassay and through epidemiological studies.
The experimental and epidemiological evidence to date does not indicate that milk from BSE-affected cattle, or indeed milk from any other species affected by transmissible spongiform encephalopathies, contains any PrP^{Sc} infectivity. This risk assessment has concluded that the key studies to date have shown no infectivity in milk and no evidence for transmission from dam to calf via suckling.

The exclusion of milk and milk products from the provision in the *Food Standards Code* that requires bovine meat and food ingredients derived from bovines to be derived from animals free of bovine spongiform encephalopathy is supported by current evidence.

Further studies investigating the infectivity of milk from BSE affected cattle will be important in conclusively demonstrating that milk does not transmit infection. The UK Food Standards Agency has commissioned a study, expected to give preliminary results in early 2003, to further investigate the infectivity of milk from BSE-affected cattle.

The weight of available evidence does not support a conclusion that bovine milk is a risk factor in the occurrence of vCJD cases. Milk is the lowest risk animal product consumed widely in the population.

6.3 Gelatine

Gelatine can be manufactured from three major sources: bovine bones, bovine hides or porcine skins. An evaluation of the current scientific reveals that bovine bones and hides are potential sources of BSE contamination although the manufacture of gelatine from these sources results in significant destruction of infectivity. The contamination of bones and hides occurs through two different mechanisms. Some cattle bones are thought to be infected by association with infected nervous tissue or bone marrow. Bovine hides could become contaminated by contact with risk material

such as brain during the slaughtering process. However, the risk of the BSE agent being present is considerably higher for bones as compared to hides.

In studies to date of TSE reduction in gelatine manufacture, there has been no work to show the efficacy of the gelatine production process in its entirety. However, studies have considered most of the commercial production process, and show significant reductions of infectivity for both acid and alkali treatments (more than 99.9% reduction of infectivity). The degree of inactivation achieved by the process up to the end of the acid/alkali step is sufficient to have inactivated as much BSE infectivity as might be present in bones under worst case circumstances (David Taylor, pers. comm.). Addition of a sodium hydroxide step can add another level of safety (more than 99.99999% reduction of infectivity for sodium hydroxide and acid/lime treatments), with total inactivation of infectivity in an experiment using bones spiked with the BSE infective agent.

Advice on the safety of gelatine should apply to whole gelatine and to foods containing gelatine as an ingredient as this would contribute to the bulk of daily gelatine consumption, which has been estimated to be approximately 3 g per day. Gelatine can be present in a variety of foods resulting in an increased likelihood of exposure to the BSE agent, if it is present in the finished product. Although there exists an increased likelihood of exposure to the BSE agent, the level of infectivity would present a negligible risk due to significant reductions in infectivity resulting from the manufacturing process. Most gelatine for food use is sourced from porcine skins and bovine hides and approximately only 10 % of gelatine is produced from bovine bones which would present a higher risk than gelatine derived from hides.

6.4 Tallow

Tallow covers a broad range of products, from human edible products derived from materials fit for human consumption, to industrial products derived from rendering any part of the animal. Tallow and its derivatives have been considered to be relatively safe products with regard to BSE-risk, but this has been the subject of continuous review.

Tallow can be made from a variety of tissues from pigs, cattle and sheep, however the predominant source is from cattle. Tallow, which is produced for edible purposes in countries which have had indigenous cases of BSE, is usually sourced from discrete adipose tissue. This tissue has been shown to be free of BSE infectivity. The ultrafiltration step theoretically reduces BSE infectivity by a factor of 100 and further reductions in infectivity can be achieved by undergoing a process of 133°C/3 bar/20 minutes. This latter treatment is not usually used for the production of edible tallow.

Results from two studies have shown that inedible tallow produced under normal rendering conditions does not contain detectable infectivity, but the MBM can contain similar levels of infectivity as the untreated raw materials. Available scientific evidence indicates that BSE infectivity does not preferentially migrate into the tallow fraction during rendering but remains in the MBM, therefore the possible BSE risks would result from protein impurities that may be present in the end product. The separation of fat from proteins can be achieved through centrifugation, filtration or treatment with phosphoric acid. The quality of filtration depends on the initial tallow

quality and the type of production process. In the production of edible tallow, additional steps of filtration and deodorising are incorporated to meet the quality criteria for food applications. This should result in low levels of insoluble impurities (less than 0.02%).

The possibility of some infectivity remaining in tallow cannot be excluded. Although experimental evidence has shown that BSE infectivity remained in the MBM fraction and did not pass into the tallow, there are varying degrees of protein-containing insoluble solid contamination in tallow.

Australia imports very little bulk tallow, however imported biscuits, cakes and pastries may be a potential source of tallow as an ingredient. This useage would be extremely small and would contribute less than 2 gram of fat per day in the average Australian diet. Based on the available evidence, edible tallows used as an ingredient in a processed product would present a negligible risk of exposure to BSE infectivity. However bulk tallow would present a higher risk of exposure to BSE infectivity because, if produced by rendering animal tissues, further refining may not occur to minimise the amount of insoluble impurities (which would contain the BSE agent, if present). Since tallow can be incorporated into a variety of products as an ingredient (fats or margarine), the Australian population could be exposed to the agent from a number of sources.

6.5 *Collagen*

While edible collagens are produced from food-grade bovine hides, for a range of other uses including pharmaceutical, medical and cosmetic materials, collagen can be produced from a range of different animal tissues, including bovine tendons, calves skins, sheepskins, pigskins, sheep gut and bovine bones. On the basis of current knowledge, the parts of bovine hides used for the production of collagen do not present a risk with regard to the BSE agent, provided that contamination with potentially infected materials is avoided. The risk of contamination of the skin with the BSE agent through spillage of blood and/or central nervous system tissues during the slaughtering and skinning process is extremely small and the risk of exposure to the BSE agent in the corium layer used for collagen production is negligible.

The mean consumption of sausage casings, which would be the major way collagen is consumed, is approximately 1.3g per day for the Australian population. Collagen presents a negligible of transmitting BSE infectivity.

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DISCLAIMER - The conclusions contained in this Risk Assessment are those of the Australia New Zealand Food Authority and do not necessarily represent, in whole or part, the position of members of the 'BSE Expert Group' or any contractor who has provided services to the Authority in relation to this risk assessment.

8.0 ABBREVIATIONS AND GLOSSARY

Alleles	Any of the alternative forms of a gene that may occur at a given gene locus.
Ante- and post-mortem inspection	Inspection by a veterinarian authorised by the 'competent national government authority' before and after slaughter at an abattoir.
Beef and beef products	Products which contain bovine tissue (including cattle, buffalo and bison). This includes meat, bone and offal but excludes milk, dairy products, gelatine, rendered fats, tallow and collagen.
BSE	Bovine spongiform encephalopathy, a slowly progressive and ultimately fatal neurological disorder of cattle.
CJD	Creutzfeldt-Jakob disease.
CNS	Central nervous system, pertaining to the brain, cranial nerves and spinal cord.
CWD	Chronic wasting disease.
D value	The decimal reduction time or the time required to destroy 90% of the organisms. This value is numerically equal to the number of minutes required for the survivor curve to traverse one log cycle. It reflects the resistance of an organism to exposure to a specific temperature.
Discrete adipose tissue	Internal and external body fat removed during the slaughter and cutting process, in particular fresh fat from the heart, caul, kidneys and mesentery of bovine animals, and fat from cutting rooms. Discrete adipose tissue is not bone fat, nor is it rendered fat from multiple source tissues from multiple species whether derived from animals fit for human consumption or from other sources.
Endemic	An endemic disease is one present in an animal population at all times.
Epidemiology	The science dealing with incidence, distribution and control of a disease in a population.
EU	The European Union.

F_0	Measurement of sterilising power of canning processes. Defined as the number of minutes at a reference temperature of 121°C that would give the same amount of inactivation as the heat processes applied to the cans.
Fallen stock	Stock which die on farm or are killed as unfit for human consumption.
Familial	Tending to occur in more members of a family than expected by chance alone.
Genome	A set of chromosomes corresponding to the haploid set of a species
Genotype	The genetic makeup of an individual organism.
Heterozygous	In genetics, having two different alleles of the same gene.
Histology	The study of cells and tissues at the microscopic level.
Homozygous	In genetics, having two identical alleles of the same gene.
i/c	Intracerebral – route of inoculation directly into the brain.
Ileum	The last portion of the small intestine.
Incidence	A measure of the frequency of a disease.
Incubation	The period between infection and clinical signs of disease.
Infectious	Capable of being transmitted.
i/p	Intraperitoneal—route of inoculation into the abdominal cavity.
i/v	Intravenous
Kudu	A species of large, grey-brown African antelope (a ruminant).
Lesion profile	The pattern of brain tissue damage used as a distinguishing feature for different TSEs.

Lymphoid tissue	Tissue involved in the production of lymph, lymphocytes and antibodies. Consists of lymph nodes, thymus, tonsils and spleen.
MBM	Meat and bone meal. Animal-derived protein produced by rendering, and used as a protein source in animal feed.
Mesentery	A fold of peritoneum which connects the greater part of the small intestine to the parietal peritoneum at the junction with the dorsal wall of the abdomen.
MRM	Mechanically recovered meat. MRM is obtained by recovering the residual raw meat adhering to bones under high pressure after other boning processes have been completed.
NaOH	Sodium hydroxide
Offal	Internal organs and entrails of animals.
OTMS	Over Thirty Month Rule. This rule keeps meat from cattle over 30 months at slaughter out of the food chain.
Pathogenesis	The manner of development of a disease.
Peripheral nervous system	By any route other than by the central nervous system.
Peyer's patches	An aggregation of lymph nodules on the small intestine.
Phenotype	The observable properties of an organism: produced by the interaction of the genotype and the environment.
Prion	Agent causing transmissible spongiform encephalopathies.
PrP	Prion protein—protease-resistant membrane protein. A normal, host-coded protein that becomes protease resistant in infected tissue and accumulates around CNS lesions in TSEs. There is evidence that PrP in uninfected animals has the property of mopping up harmful 'oxygen free radicals', or that it carries out some signalling function between cells.

PrP^{c}	The normal cellular isoform of PrP.
PrP gene	Gene found in mammals which encodes the amino acid sequence of PrP^{c} .
PrP^{Sc}	$PrP^{scrapie}$ is the abnormal disease-specific isoform of PrP derived post-translationally from PrP^{c}.
Risk	A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard in a food.
SAF	Scrapie-associated fibrils.
SBO	Selected bovine offals.
S/c	Sub-cutaneous. Injection under the skin.
Sporadic disease	Occurring in single cases, here and there.
SRM	Specified risk material. SRM are those animal tissues that have been shown through experimental conditions and field cases to carry BSE infectivity.
SSC	Scientific Steering Committee of the European Commission.
Strain-typing	The identification of different TSE strains by determining the length of the incubation period and the pattern of brain damage in experimentally infected animals.
Thermostable	Retaining its character or active quality at moderately high temperatures.
TME	Transmissible mink encephalopathy.
TSE	Transmissible spongiform encephalopathy. Disease of the neurological system with spongy degeneration of the brain and progressive dementia.
Weasand	Oesophagus
z value	Slope of heat resistance curve. Allows for the calculation of equivalent thermal processes at different temperatures.

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APPENDIX 1. THE BSE EPIDEMIC

Bovine spongiform encephalopathy (BSE) belongs to the same group of diseases as scrapie in sheep, goats and moufflon, transmissible mink encephalopathy of farmed mink (TME) and chronic wasting disease (CWD) of some deer species and Rocky Mountain elk. The first recorded clinical case of BSE, confirmed by neuropathological examination of the brain, was in 1986 in southern England. The first clinical case (unconfirmed pathologically) was in April 1985 also in England.

Scrapie has been known in Europe since at least the early part of the 18th century. TME and CWD have been known in the USA since the 1960s. TME has also been reported in farmed mink in Finland, Russia and Germany. CWD has not been reported outside North America.

BSE is considered to occur in 16 species: cattle, domestic cats, nyala and greater kudu (types of antelopes), gemsbok, eland, Arabian oryx, scimitar-horned oryx, ankole, bison, cheetah, lion, tiger, puma, ocelot and humans (brains from Variant Creutzfeldt-Jakob Disease (vCJD) patients). Other than feline spongiform encephalopathy (FSE) in domestic cats, all these are rare diseases in captive wild animals in the UK. Cases of FSE have also been found in cheetah in Ireland, France and Australia, but only in captive animals exported from the UK. There have not been any reports of naturally occurring cases of TSE in pigs or poultry.

The spread of BSE is associated with concentrate feeding and the vehicle of infectivity was meat and bonemeal (MBM). There is continuing controversy on the origin of BSE. There were changes in rendering practices and evidence suggest this may have changed the evolution of the epidemic.

Historically, the purpose of rendering was to produce tallow, a sought-after fatty product derived from animals and especially animal fat. Tallow was required for soap and candle manufacture and was in great demand in the 19th century. Later, as refrigeration methods and transportation improved, so the beef industry developed, resulting in a wider distribution of edible parts of the carcase. The carcase industry grew and with it the problem of disposal of around 40% of the live animal weight that was not meat. The problem became larger with time, especially as, for odoriferous reasons, paraffin wax began to replace tallow used in candles. The rendering industry responded to the problem by developing a business that efficiently collected the unusable waste and cooked it, thus reducing the water content. The fatty portion was then separated by centrifugation or pressing, to leave the solid, protein-rich material called greaves. Greaves was then ground to produce MBM. MBM had uses in animal feed and as a fertiliser. Even in Australia in the 1930s, MBM was used in dairy cattle diets and as a result increased milk yield and quality. In the 1970s, as in the UK, MBM was introduced into calf starter rations in Australia but a major difference is that scrapie does not exist in Australia (or New Zealand) and neither does BSE.

Thus, the rendering industry performs useful functions to process generally unpleasant and otherwise unwanted animal by-products, and generates valuable materials for which there was a ready market. Problems arose with the occurrence of BSE, because BSE-infected materials formed part of the raw material and the infectivity was not completely destroyed. Changes in the methodology of rendering,

prompted by improved safety requirements and increasing costs of fuel for heating, compounded the problem although the influence of changes in rendering practices remains incompletely resolved. In any case, once BSE infection became established in cattle, this led to recycling of the BSE agent through the MBM that was included in cattle rations. MBM was included in the diets of dairy calves from the 1970s in the UK and this contributed to the growth in the number of cases.

The countries with BSE in native-born cattle as at July 2002 are: Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Greece, the Irish Republic, Israel, Italy, Japan, Liechtenstein, Luxembourg, the Netherlands, Portugal, the Slovak Republic, Slovenia, Spain, Switzerland and the UK (Table 1).

A BSE risk is not restricted to western Europe, since cattle and cattle products, including MBM, have been widely exported from countries that now have reported BSE. This conclusion was clearly stated at the joint WHO/FAO/OIE technical consultation on BSE in France during 2001.

The most likely method for BSE to have arisen in Europe is from feeding infected MBM and/or from importation of live cattle from the UK, brains of which subsequently entered the rendering system. It is clear that both mechanisms of spread operated between the UK and Europe in the 1980s. From 1988 it was well known that a ban on this type of feeding had been introduced in the UK, but it was legal to feed MBM to pigs and poultry until 1996 in the UK and until 2001 in the rest of the EU. Once BSE had occurred, by whatever means, recycling of infected material via the rendering systems in the affected countries could have caused further cases, just as it did in the UK. While some countries adopted a ruminant or cattle feed ban, others did not until it became EU law in 1994. It is noteworthy that Switzerland identified BSE by 1990 but this could not be attributed to importation of either cattle or MBM from the UK, or initially from MBM prepared within its own territory, but rather to imports of MBM from other European countries.

Accidental cross-contamination of ruminant feeds with meat and bone-meal (MBM), or feeds containing MBM intended for non-ruminant species, has resulted in BSE cases occurring in various countries after the feed ban was implemented. The cross-contamination can occur from very small amounts of dried material (less than 1 g), which is far too small an amount to eliminate successfully by routine cleansing from machinery and transport used during the manufacture and delivery of compounded feeds intended for different species. This was why the mammalian MBM was compulsorily withdrawn from feed for all food animal species.

The precise origin of BSE is not known. There are many hypotheses but the main three are the prion, virino and unconventional virus hypotheses. The prion hypothesis in its purest form claims the agent is an infectious protein which can be replicated in the brain of infected species without it containing a genome (Prusiner, 1994; 1998). The virino hypothesis accepts that PrP and infectivity are closely associated but argues that the agent has a genome associated with the protein, perhaps an unidentified small nucleic acid which is protected by the host PrP (Dickinson et al, 1988). No such material has yet been found in TSE agents, and the agents survive treatments that would be expected to entirely destroy nucleic acids, and remain infectious.The unconventional virus hypothesis has only limited support but again a

nucleic acid is an essential component of viruses and none has been found. The prion hypothesis is by far the most widely accepted of these hypotheses.

In considering the prion protein as the causative agent for BSE, the following hypotheses have been proposed as to the origin:

a) BSE originated from a scrapie-like agent from sheep, but not from a single mutant strain. The scrapie strain adapted to cattle and became endemic in the cattle population with the level of exposure influenced by changes in rendering practices. It has not, however, been possible to identify from which scrapie strain it originated, if it did.
b) The alternative hypothesis that BSE originated from a cattle-adapted, scrapie-like agent in cattle is also plausible. However, there are significant scientific difficulties with this hypothesis.
c) BSE is a natural infection of a wildlife species, possibly an exotic ungulate, and the BSE epidemic was initiated by the brain of an infected animal of this species getting into the British rendering system in the 1970s, in Southern England.

Several other origins of the disease have been proposed. Possible origins from organophosphorous compounds or autoimmune processes are not plausible and are inconsistent with the epidemiology of BSE (Purdey, 1996; Ebringer et al 1997). Neither are they consistent with the response of the epidemic to the measures applied in the UK, which involved controls on feed and SRM. A review of the scientific and epidemiological evidence on the origin of BSE and recently concluded that (Horn, 2001):

- there is strong evidence that spread of the BSE epidemic arose through the use of MBM in animal feed; and
- it is no longer possible to exclude an unmodified scrapie agent as the agent responsible for BSE in light of recent estimates of UK scrapie incidence and scrapie properties.

The origin of the agent remains unresolved, and a matter of controversy.

No system of rendering used during the BSE epidemic has been completely effective in inactivating the BSE agent. Some are better than others and the most secure (but still not totally effective) is pressure-cooking (3 bar) at 133ºC for 20 minutes, the current EU standard (or other methods that give at least equivalent clearance factors). However, in recent years, a new system for treating raw material employing NaOH or KOH and heat seems extremely effective and no detectable infectivity has been found when tested with high titre, laboratory mouse-adapted BSE strains.

Most exposures to BSE which produce clinical disease appear to occur in calves. These are predominately dairy calves, removed from the dam shortly after birth and are fed on artificial milk supplemented by high protein calf nuts (meal) which often contained MBM. Calves from beef cattle, on the other hand, are reared on the dam, i.e. are suckled, often for many months and they therefore rarely receive concentrate rations that might contain MBM. BSE occurs at much lower incidence in beef than dairy cattle, and cases in adult beef cattle are believed to be due usually to feeding of MBM after calfhood.

There is no difference in the incidence of BSE in different dairy breeds in the UK. The number of cases by breed is proportional to the numerical size of the herd. Thus, whilst the highest number of BSE cases occurs in the Holstein Friesian breed, this is due solely to the size of the herd.

The major measures for controlling BSE and protect public health in high risk BSE countries are:

- the compulsory slaughter and complete destruction of clinically suspect or confirmed cases of BSE;
- a ban on feeding mammalian MBM, and latterly mammalian protein, to ruminant animals, and most recently to all food animal species;
- a ban on 'specified bovine offals' and later on 'specified risk materials' (SRM);
- a ban on use of mechanically recovered meat prepared from the vertebral column or head; and
- rendering standards for mammalian waste of pressure- cooking (3 bar) at 133ºC for 20 minutes,
- culling of herds or cohorts when a BSE case is diagnosed
- controls on the use of fallen and emergency slaughter stock

TABLE 1. NUMBER OF REPORTED CASES OF BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) IN CATTLE WORLDWIDE (EXCLUDING THE UNITED KINGDOM).

Updated: 26.07.2002 (ch)

	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Austria	0	0	0	0	0	0	0	0	0	0	0	0	1*(c)*	...
Belgium	0	0	0	0	0	0	0	0	1	6	3	9	46	18*(c)*
Czech Republic	0	0	0	0	0	0	0	0	0	0	0	0	2	0*(c)*
Denmark	0	0	0	1*(b)*	0	0	0	0	0	0	0	1	6	1*(c)*
Finland	0	0	0	0	0	0	0	0	0	0	0	0	1*(c)*	...
France	0	0	5	0	1	4	3	12	6	18	31*(a)*	161*(d)*	274*(e)*	91*(c)*
Germany	0	0	0	1*(b)*	0	3*(b)*	0	0	2*(b)*	0	0	7	125	54*(c)*
Greece	0	0	0	0	0	0	0	0	0	0	0	0	1*(c)*	...
Ireland	15*(a)*	14*(a)*	17*(a)*	18*(a)*	16	19*(a)*	16*(a)*	73	80	83	91	149*(d)*	246*(e)*	165*(c)*
Israel	0	0	0	0	0	0	0	0	0	0	0	0	0	1*(c)*
Italy	0	0	0	0	0	2*(b)*	0	0	0	0	0	0	48	4*(c)*
Japan	0	0	0	0	0	0	0	0	0	0	0	0	3*(e)*	1*(c)*
Liechtenstein	0	0	0	0	0	0	0	0	0	2*(c)*	...	...	...	...
Luxembourg	0	0	0	0	0	0	0	0	1	0	0	0	0	0*(c)*
Netherlands	0	0	0	0	0	0	0	0	2	2	2	2	20	10*(c)*
Poland	0	0	0	0	0	0	0	0	0	0	0	0	0	1*(c)*
Portugal	0	1*(b)*	1*(b)*	1*(b)*	3*(b)*	12	15	31	30	127	159	149	110	18*(c)*
Slovakia	0	0	0	0	0	0	0	0	0	0	0	0	5	...
Slovenia	0	0	0	0	0	0	0	0	0	0	0	0	1*(c)*	...
Spain	0	0	0	0	0	0	0	0	0	0	0	2	82	70*(c)*
Switzerland	0	2	8	15	29	64	68	45	38	14	50	33*(d)*	42	8*(c)*
United Kingdom	see particular table													

***** Cases are shown by year of confirmation.
... Not available

(a) ***France****: includes 1 imported case (confirmed on 13 August 1999).*
Ireland*: includes imported cases: 5 in 1989, 1 in 1990, 2 in 1991 and 1992, 1 in 1994 and 1995.*
Portugal: *includes 1 imported case (confirmed on 22 November 2000).*

(b) *Imported case(s).*

(c) ***Austria*** *- date of confirmation of the case: 13 December 2001;*
Belgium *- data as of 10 June 2002;*
Czech Republic *- data as of 30 June 2002;*
Denmark*- data as of 30 June 2002;*
Finland *- date of confirmation of the case: 7 December 2001;*
France *- data as of 30 April 2002 - Clinical cases = 17; cases detected at rendering (bovines at risk) = 47; cas detected at slaughterhouse = 27;*
Germany *- data as of 30 June 2002;*
Greece *- date of confirmation of the case: 29 June 2001;*
Ireland *- data as of 31 May 2002 - Clinical cases = 50; cases detected by the*

active surveillance programme = 115;
***Israel** - date of confirmation of the case: 4 June 2002;*
***Italy** - data as of 30 January 2002;*
***Japan** - data as of 13 May 2002;*
***Liechtenstein** - date of the last confirmation of a case: 30 September 1998;*
***Luxembourg** - data as of 30 June 2002;*
***Netherlands** - data as of 7 June 2002;*
***Poland** - data as of 6 May 2002;*
***Portugal** - data as of 31 March 2002*
***Slovenia** - date of confirmation of the case: 20 November 2001;*
***Spain** - data as of 25 July 2002;*
***Switzerland** - data as of 10 May 2002 - Clinical cases = 5; cases detected within the framework of the investigation programme = 3.*

(d) ***France year 2000** - clinical cases = 101; cases detected within the framework of the research programme launched on 8 June 2000 = 60*
***Ireland year 2000** - clinical cases = 138; cases identified by active surveillance of at risk cattle populations = 7; cases identified by examination of depopulated BSE positive herds, birth cohorts and progeny animals = 4.*
***Switzerland year 2000** - clinical cases = 17; cases detected within the framework of the investigation programme = 16*

(e) ***France year 2001** - clinical cases = 91; cases detected at rendering (bovines at risk) = 100 (out of 139,500 bovines tested); cases detected as result of systematic screening at the abattoir = 83 (out of 2,373,000 bovines tested).*
***Ireland year 2001** - clinical cases = 123; cases identified by systematic active surveillance of all adult bovines = 119; cases identified by examination of depopulated BSE positive herds, birth cohorts and progeny animals = 4.*
***Japan year 2001** - clinical cases = 1; cases detected as result of screening at the abattoir = 2.*

Source: www.oie.int/eng/info/en_esb.htm

APPENDIX 2. DEFINITIONS AND CONCEPTS USED IN THE HAZARD CHARACTERISATION.

In the investigation on *cumulative dose* only an oral cumulative dose effect will be discussed and for only one strain of agent, namely the BSE agent. In other words what is disregarded is the theoretical additive effect from consumption of two or more strains of agent such as the scrapie and BSE agents or, on the other hand, a single strain of agent gaining entry *via* more than one route. Risks from the latter situation could apply, for example, to meat-eating abattoir workers in countries with TSE in food animal species. In such a situation, infection could occur through cuts and abrasions, and possibly *via* the conjunctiva, as well as by the oral route. There has been no evidence in man suggestive of occupational exposure, or of exposure by any route other than oral.

It might be useful in this context to be aware of the *replication site hypothesis* (Dickinson and Outram, 1979). This hypothesis assumes that there is a finite number of replication sites for scrapie-like agents. When occupied with one strain of scrapie, subsequent exposures to other strains are ineffective (blocked) so that the disease produced is characteristic of the first strain rather than the second. The blocking phenomenon does not occur with the transmissible mink encephalopathy (TME) agent which does not transmit to mice. Also, low doses of infectivity are not effective in blocking subsequent high-dose exposures with a second agent. However, recent studies (Baron and Biacabe, 2001) using co-infection with mouse adapted scrapie and mouse adapted BSE strains, showed that the molecular analysis of the abnormal prion protein following co-infection was like that from mice inoculated with scrapie only. Overall, it does seem that there is competition when a susceptible individual is exposed to two different strains of agent at the same time, or consecutively.

There are insufficient data to even remotely relate that to human exposures to TSE agents. The definition of *dose* in the context of this assessment means the product of the amount of infected tissue consumed and the titre of agent per unit mass of the tissue. Thus, the cumulative dose is not the sum of the individual *masses* of tissue consumed, but rather the sum of the *whole infection* that is consumed, i.e. the product of mass times titre/unit mass of infected tissue consumed.

An infectious dose is the dose able to establish infection. Infection in this context means replication (multiplication) of the agent and can be indirectly determined by clinical, pathological, biochemical and animal transmission. If infection is established, it is probable that disease will eventually result if the exposed individual lives long enough. However, the possibility of a sub-clinical carrier state occurring cannot be excluded. The infectious dose of a TSE agent for humans cannot be calculated directly but, in practice, is related to titres determined in animal models. It is also important to note that, like viruses, TSE agents cannot replicate except in living cells. Tissue culture systems do exist but they are inefficient at replicating the agent. Thus, additional dangers from replication outside the body do not exist, though concentration of infectivity could theoretically occur as a result of processing, such as drying.

Titres are usually measured by inoculating a series of batches of susceptible, genetically uniform mice intracerebrally (i/c) with increasing dilutions of the material

under investigation (e.g. 10% homogenate of 1 g of brain) such as 1/100, 1/1,000, 1/10,000 and so on. An end point is reached at the level of dilution that kills 50% of the mice inoculated and this is the Infectious Dose 50%, written $ID_{50.}$ If it were found that 5 of 10 mice were killed by a dilution of 1 in 10,000 the titre would be 10^4 mouse i/c ID_{50}/g.

A practical example might be 'the infectivity titre in the brain of a cow was 10^5 mouse i/c ID_{50}/g' as measured in *Sinc* S_7S_7 RIII mice. Sometimes it might need to be more specific still, by specifying the part of the brain that was assayed and whether it was mainly grey or white matter, because the titres might vary in different parts of the brain. In the example given, if we diluted the brain homogenate 100,000 times there would be sufficient infectivity in each 1/100,000 g or mL of the diluted brain to infect 50% of the animals challenged by the i/c route i.e. this amount of inoculum would contain sufficient infectivity to kill 50% of the inoculated mice. If exactly the same protocol and mice for bioassay are used for brain material from other cows, or tissues other than brain from the same cow, a direct comparison of the titre can be made in each instance. The titre in the brain cannot be determined except by bioassay, neither can it be judged from the stage of clinical disease or severity of the clinical signs. In practice, there may be up to a difference in titre of 10^3 mouse i/c ID_{50} between different brains harvested in the clinical phase of disease (Hadlow *et al.,* 1979, 1982). Incubation period assays have been used effectively to determine titre in certain experimental situations but are not regarded as reliable as end-point titrations as described above. Incubation period assays involve establishing a dose-response curve for the agent under investigation (say hamster scrapie strain 263 K) in in-bred Syrian hamsters. When this is done, one can read off the titre from the dose response curve from knowledge of the incubation period resulting from inoculation of the tissue under investigation.

From the definition of the ID_{50}, it does not mean that if an amount smaller than this is inoculated there will be no deaths. Furthermore, because of the special characteristics of the agent (it is sticky) and because the disease-specific form of the prion protein (PrP^{Sc}) is insoluble and has a strong tendency to aggregate, it means that it is inappropriate to consider that dilution of the infectivity can occur just like salt can be diluted in water.

The examples given above are assays done across a *species barrier* that creates a loss of sensitivity in the bioassay, so the real level of infectivity for the host species is underestimated. If, instead of using mice, cattle were used to assay the infectivity, thus avoiding the species barrier**,** the titre would be greater by a factor of about 500 times (Wells, 2001).

In *assessing risks to human health* from, say, bovine brain, it would be ideal to have a knowledge of the titre of bovine brain measured in humans, but this is clearly impossible. We have therefore no way of measuring the extent of the species barrier between cows and man and it remains probably the greatest uncertainty among the major factors influencing the risk. Thus, estimates have to be made usually over a range of selected figures. The UK SEAC have said that equal probability should be given for the cow to human species barrier being 10, 100, 1000 and >1000 with a 1% chance of it of it being 1.

To make a more accurate measurement of the *cow to human species barrier*, genetically altered mice have been created. These have the murine PrP gene removed ('knockout mice') and have it replaced with the human gene. The transgenic mouse then behaves as if it is a human in its response to challenge with BSE, or at least that is the intention. However, there are several ways to construct such mice and some have not been as efficient as expected. There are currently still no reliable data to make a more realistic estimate for the cow to human barrier for the BSE agent than that given above.

APPENDIX 3. THE EUROPEAN UNION CURRENT LIST OF SPECIFIED RISK MATERIAL (SRM) FOR CATTLE, SHEEP AND GOATS.

During the course of 1989 in the UK and following publication of the Report of the Southwood Working Party on BSE, attention was being given to the issue of protecting the public from exposure to BSE mainly from consumption of food containing bovine materials. As a result, it was decided that, as a cautious approach, certain offals from cattle called the specified bovine offals (SBO), selected on the basis of the infectivity known to be present in tissue of goats and Suffolk sheep with natural scrapie or incubating the disease (Hadlow *et al.,* 1979,1980, Hadlow, Kennedy and Race, 1982), should be removed. These were the brain and spinal cord, the tonsils and thymus and the spleen and intestine from all cattle over 6 months of age. Subsequently, modifications were made to the list in the light of experience and the results of research so that the list was extended to include first the skull (including the eyes) and finally the head. As the head comprised muscle and bone the word 'offals' was changed to 'materials'. The ban then became known as the specified bovine materials (SBM) ban (EC1998a). Other restrictions that were implemented in the UK included removal of visible lymph nodes and nervous tissue before sale of meat to the public and for export. Also, for a time, bone-in meat was excluded from the food chain, though the bones (like the lymph nodes and nerves) were not classed as SBM. As a result of appraising the possible risks of sheep being infected with the BSE agent, on the advice of the UK Spongiform Encephalopathy Advisory Committee (SEAC) and on the intervention of the EC, certain other materials from sheep and goats were also designated risk materials and the resultant legislation called the prohibited tissues from both cattle and small ruminants 'specified risk materials' (SRM), the appropriate ban being the SRM ban. The inclusion of sheep materials was purely a risk reduction procedure should BSE ever be found in sheep or goats in the EU. The list of SRM has evolved over the whole period of the BSE epidemic, essentially starting in the UK in November 1989, to protect public health and being officially adopted in the whole of the EU since 1 October 2000. However, all countries with BSE in native-born cattle adopted some form of SBO, SBM or SRM ban during the period 1990–1 October 2000

The Office International des Epizooties (OIE) has adopted a list of SBM that varies between countries, depending on the category of BSE risk but this applies only to cattle and cattle products that are exported. The list is specified in the current OIE *International Animal Health Code* chapter on BSE (OIE, 2001) and is less extensive than the list provided for in EC legislation. There is no operative *Code* chapter on scrapie and therefore no formal restriction on the worldwide trading of materials from small ruminants.

The current list of SRM that must be removed and destroyed in EU Member States is defined in Commission Decision 2001/2/EC (Commission Decision 2001) operative since 1 January 2001 and amends Commission Decision 2000/418/EC (Commission Decision 2000) operative in the context of SRM since 1 October 2000. The latter Decision, however, regulates other issues like bones, mechanically recovered meat (MRM) and some slaughter techniques used on animals destined for human consumption. It also imposes restriction on the importation of SRM and products derived from SRM. Also proposed is a regular review of the scientific evidence and

need for possible changes to the provisions. Annex 1 of Decision 2000/418/EC is now updated as follows:

1 (a) The following tissues shall be designated as SRM in all member States and Regions thereof:

a. the skull including the brains and eyes, the tonsils, the spinal cord of bovine animals aged over 12 months, and the intestines from the duodenum to the rectum of bovine animals of all ages.
b. the skull including the brains and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months or have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages.

(b) In addition to the SRM listed in point 1 (a) the following tissues shall be designated as SRM in the UK and in Portugal with the exception of the Autonomous Region of the Azores:

(i) The entire head excluding the tongue, including the brains, eyes, trigeminal ganglia and tonsils; the thymus; the spleen and spinal cord of bovine animals over six months;
(ii) The vertebral column, including dorsal root ganglia, of bovine animals aged over 30 months.

2. In Annex II of Commission Decision 2000/418/EC, point (e) is added to the list of products subject to restrictions on import into the Community pursuant to Article 6(1):

(e) 'bovine intestines' as referred to in Article 2(b)(v) of Directive 77/99/EEC.

In addition to the Commission Decision a recent Regulation**,** (EC) No. 999/2001 of the European Parliament and of the Council of 22 May 2000 (Regulation 2001), laying down rules for the prevention, control and eradication of certain TSE**,** has entered into force since 1 July 2001. This decision has designated the following tissue as SRM for cattle by all member states from 1 July 2001:
The vertebral column except the vertebrae of the tail and the transverse processes of the lumbar vertebrae but including dorsal root ganglia and spinal cord in cattle over 12 months.

The EC has previously classed countries on the basis of a geographical BSE risk (GBR) assessment into one of five categories. The UK and Portugal are listed in CATEGORY 5 and have to remove and destroy precisely the same tissues as listed in Commission Decision 2000/2/EC. Countries in CATEGORIES 1 and 2 are required to remove nothing and those in CATEGORIES 3 and 4 must remove those listed in Article 1, 1(a) of Decision 2000/2/EC. The only discrepancy is that in the regulation there is a relaxation of the rules in regard to SRM based on the category of country. Nevertheless, initially the rules of the Commission Decision prevail.

APPENDIX 4. SLAUGHTERING METHODS USED IN AUSTRALIAN ABATTOIRS

General hygiene procedures

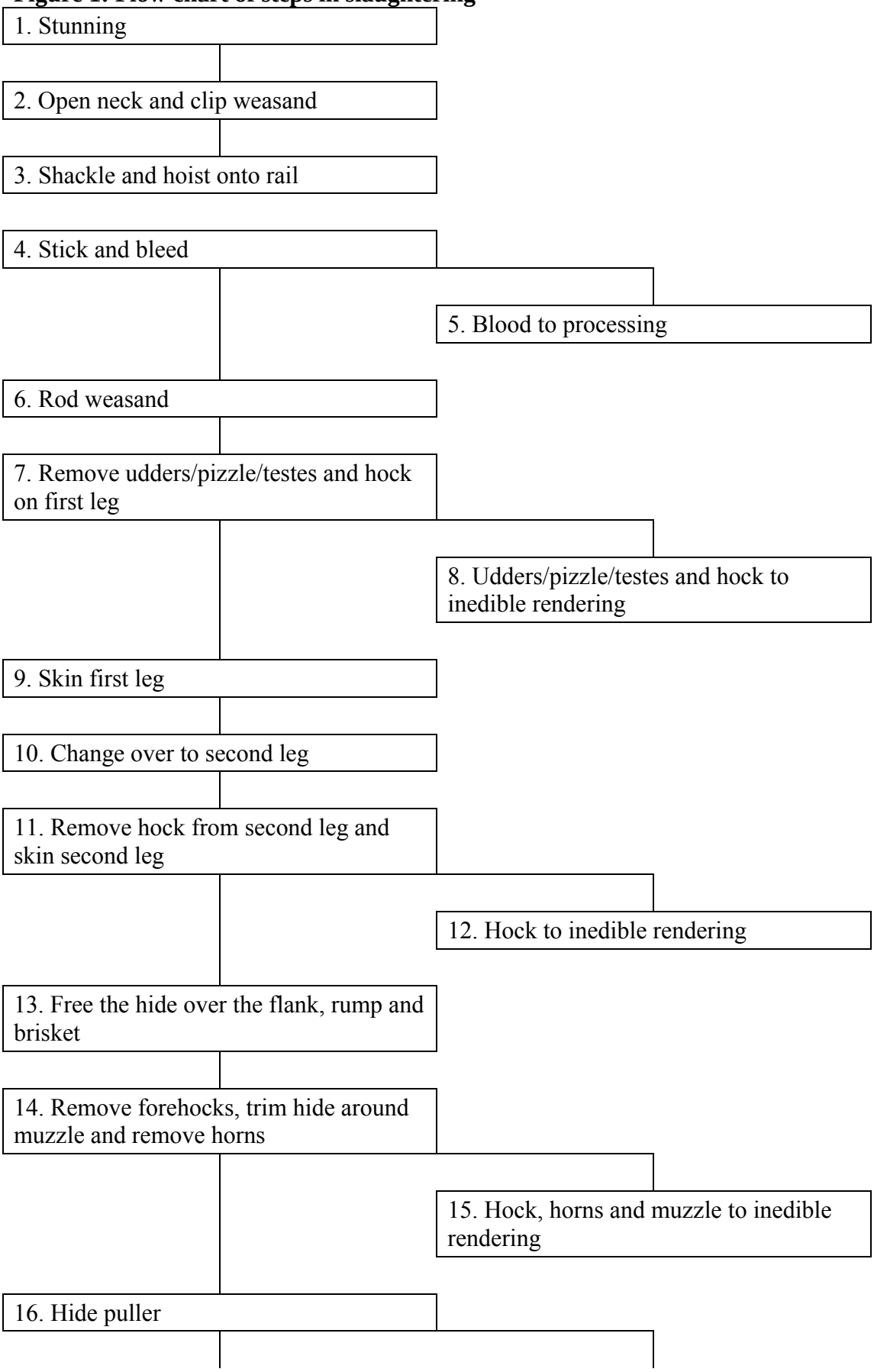
Domestic and export standards include mechanisms to prevent cross-contamination from one carcase to another during dressing procedures. The export requirements contain more specific carcase separation distances before and after evisceration and after splitting. Requirements to prevent cross-contamination via equipment include hot water sanitation for knives, saws and other implements that come in contact with carcases.

Requirements to prevent cross-contamination between carcases apply until the point of final inspection on the slaughter floor, after which carcases are deemed to be fit for human consumption

The slaughter process

The steps in the slaughtering process are summarised in the flow chart in Figure 1. The steps involved in processing heads are shown in Figure 2.

Figure 1: Flow chart of steps in slaughtering



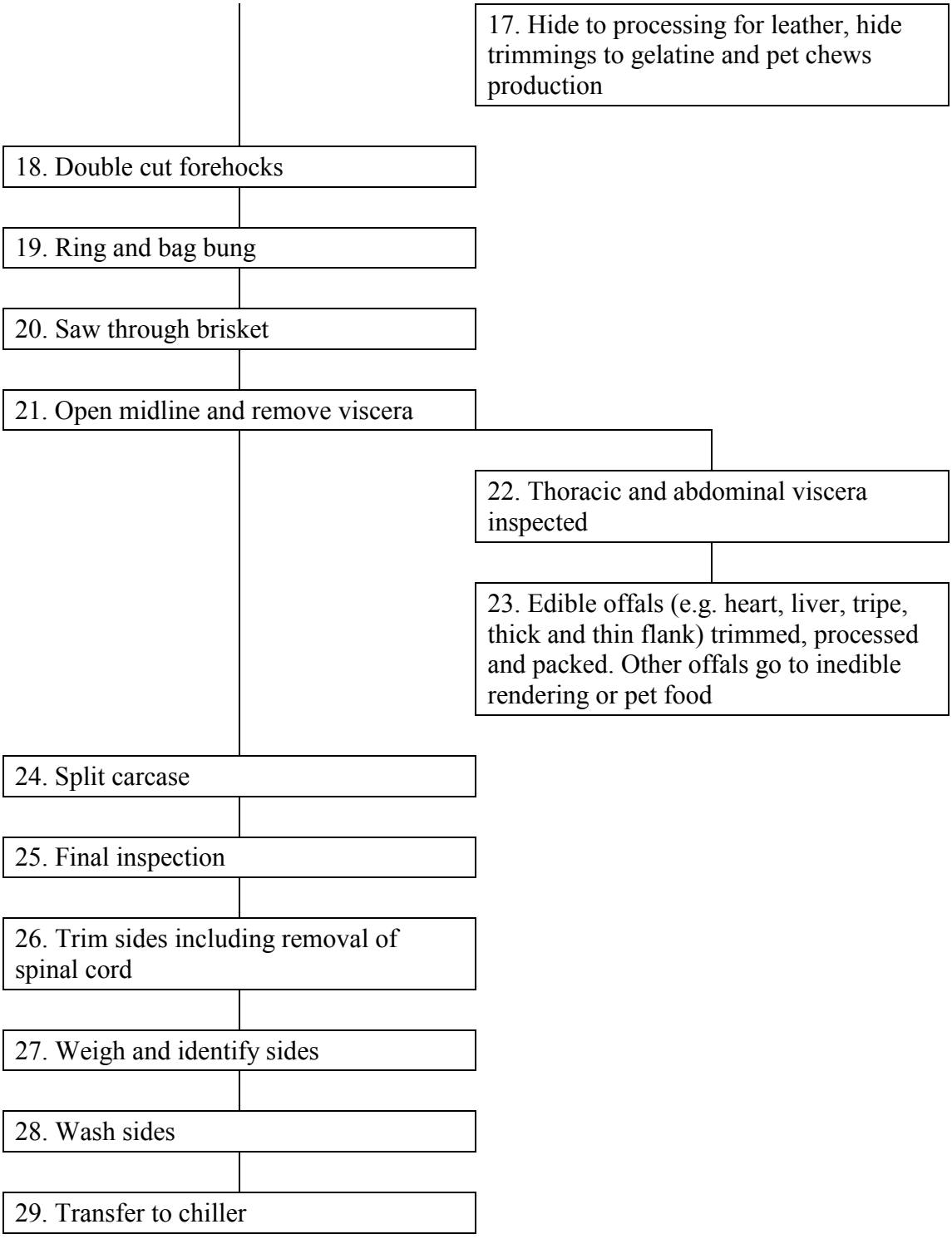
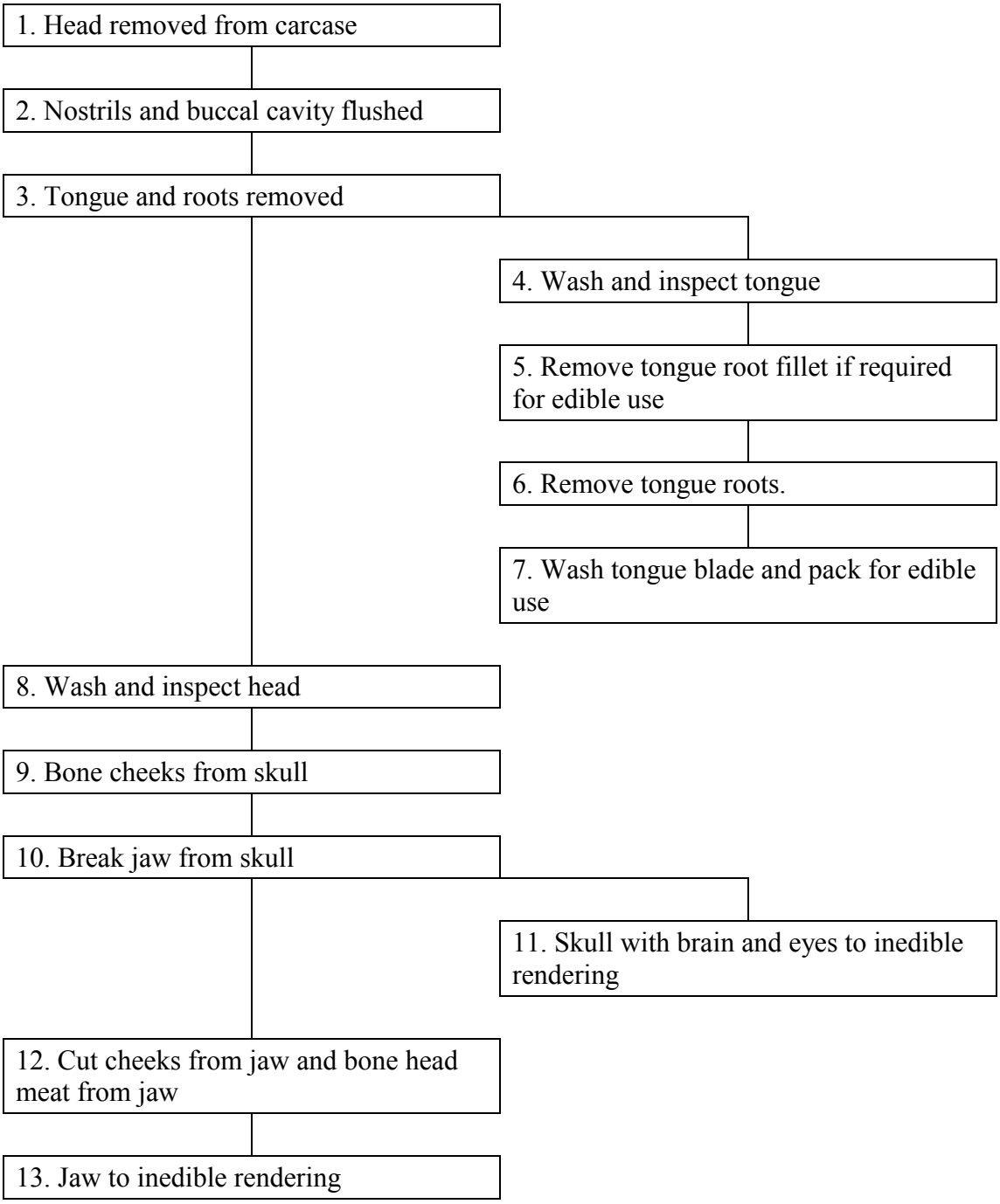

17. Hide to processing for leather, hide trimmings to gelatine and pet chews production
18. Double cut forehocks
19. Ring and bag bung
20. Saw through brisket
21. Open midline and remove viscera
22. Thoracic and abdominal viscera inspected
23. Edible offals (e.g. heart, liver, tripe, thick and thin flank) trimmed, processed and packed. Other offals go to inedible rendering or pet food
24. Split carcase
25. Final inspection
26. Trim sides including removal of spinal cord
27. Weigh and identify sides
28. Wash sides
29. Transfer to chiller

Figure 2 Steps in head process



APPENDIX 5. DESCRIPTION OF THE STANDARD BEEF CARCASE

A standard beef carcase is the body of a slaughtered bovine animal after:

- Bleeding
- Skinning
- Removal of all internal digestive, respiratory, excretory, reproductive and circulatory organs
- Minimum trimming as required by the meat inspection service for the carcase to be passed fit for human consumption
- Trimming of the neck region may be extended to ensure compliance with Zero Tolerance for ingesta contamination, especially where Halal slaughter has been performed. This extension to the Standard Carcase Trim is limited to a hygiene trim and must be controlled by the Company MSQA program

And the removal of the:

- Head between the skull (occipital bone) and the first cervical vertebrae, by a square cut transversely across the neck muscles,
- Feet between the knee joint (carpus and metacarpus) and the hock (tarsus and metatarsus)
- Tail at the junction between the sacral and coccygeal vertebrae
- Skirts (thick and thin) by separating the connective tissues as close as possible from the abdominal and thoracic walls. (Thick and thin skirts are the diaphragm).
- Kidneys, kidney fat and fat from within the pelvic channel
- Udder, testes, penis and external fat on the ventral abdomen including precrural (flank) fat, udder and cod fats
- Fat on the channel rim (anal fold) from the tuber ischii to the sacro-coccygeal junction so that the sacro-sciatic ligament is just visible
- Excess fat on the topside rim up to 1 cm from the underlying muscles
- Xiphoid cartilage and intra-thoracic fat
- Excess external brisket fat up to 1 cm from the underlying muscle at the midline, using a knife held 90 degrees to the midline sawn surface.

APPENDIX 6. ABATTOIR PRACTICES IN EUROPE

The following legislation describes the present regulatory framework for abattoir practices in Europe and is included to allow for the impact of control measures on the exposure of consumers to the BSE agent. A summary of BSE control measures adopted by the European Commission is detailed in Appendix 2.

All EU member states must according to Regulation (EU) No. 999/2001 of the European Parliament and of the Council, latest amended by Commissions Regulation No. 1248/2001 of 22 June 2001 carry out an annual BSE monitoring programme via tests of certain groups of cattle that are at risk. The test must be carried out in accordance with one of the rapid tests approved by the EU.

As a minimum the BSE monitoring program carried out must comprise tests of:

1. all cattle more than 24 months of age that are subject to emergency slaughter;
2. a random sample of cattle above 24 months that have died at the farm or during transport, but which have not been slaughtered for consumption; and
3. all cattle above 30 months that are slaughtered for consumption.

By way of derogations from the third of these points, Finland, Sweden and Austria may decide to examine only a random sample of cattle that are born, reared and slaughtered on their territory, however this would include at least 10,000 cattle per year.

In addition, the member states may carry out monitoring targeted at animals at risk, comprising:

1. animals originating from countries with BSE;
2. animals that have been fed with potentially contaminated feed; and
3. animals that are offspring of BSE-infected female animals.

According to Commission Regulation No. 1248/2001 the measures following testing of cattle slaughtered for consumption have been changed from 1 July 2001 as follows:

- The health mark provided by the Fresh Meat Directive (64/433/EU) shall not be carried out on the carcase until a negative result to the rapid test has been obtained (member states may, however, derogate from this provision).
- All parts of the body of a tested animal including the hide shall be retained until a negative test has been obtained, unless they are destroyed.
- All parts of the body of an animal tested positive to the rapid test including the hide shall be destroyed.
- At least the carcase immediately preceding the test-positive carcase and two carcases immediately following the test-positive carcase on the same slaughter line shall be destroyed (member states may, however, derogate from this provision).

Specified risk material

This has been described in Section 7.2.

In all member states (except the UK and Portugal) only the skull is considered SRM, but not the tongue, external and internal masseter muscles and mandible. In the UK and Portugal (except the Azores) the entire head including the eyes, ears, horns, skin, salivary glands, external and internal masseter muscles and the mandible—except the tongue—is considered SRM.

The EU may allow the use of the vertebral column and dorsal root ganglia from cattle:

- born, reared and slaughtered in member states in which a scientific evaluation has established that the occurrence of BSE in cattle of national origin is highly unlikely, or unlikely but not excluded (at present Sweden, Finland and Austria);

- born after the date of effective enforcement of prohibition on the feeding of mammalian protein to ruminants in member states with reported BSE in native cattle or in which a scientific evaluation has established that the occurrence of BSE in native cattle is likely (at present the UK and Portugal).

The UK, Portugal, Sweden, Finland and Austria may benefit from this derogation, however allowance to derogate from the rules will not be granted for the use of the vertebral column and dorsal root ganglia from cattle aged over 30 months from the UK and Portugal (except the Azores).

Member states allowed derogations from the rules must in addition to the requirements for the monitoring program test all cattle aged over 30 months that:

- have died at the farm or during transport, but which have not been slaughtered for human consumption; and
- were subject to normal slaughter for consumption.

According to information available, the vertebral column is removed from cattle in all EU member states, and none of the above five countries have filed an application for allowance to derogate from the rules.

Mechanically recovered meat

The production of MRM has been described previously. Selected bones from beef, pig, sheep or chicken are used for MRM production in the preparation of products such as sausages, burgers, pies, pasties, canned products, infant food and ready-made meals (Food Standards Agency, 2000; Will, 2001). The UK Food Standards Agency has initiated a study to determine which meat products in the 1970s and 1980s contained MRM derived from cattle, to estimate the possible exposure of people to BSE at that time. According to the Regulation (EU) No. 999/2001 all EU member states must ensure that bones of cattle, sheep and goats are not used in the production of recovered meat.

Stages in the slaughter process that present a low risk of cross-contamination of carcases with SRM.

Meat inspection
During inspection of the thoracic and abdominal cavity with incision in the medial iliac lymph nodes, there is a risk of additional spreading of risk material from the carcase splitting. There is also a risk of spreading risk material from the tonsils when removing the tonsils for incisions in external and internal masseter muscles.

There is potential for spreading of SRM (via spinal cord and sawdust from the splitting saw) to other parts of the carcase through physical contact to the abdominal and thoracic cavity. As the pharynx and tonsils must be inspected, there is a risk of the inspector spreading SRM (tonsils) with his hands to the rest of the head/jaw meat. This risk would be minimized by visual inspection of the abdominal and thoracic cavity, with an incision in the medial iliac lymph nodes only if indicated.

BSE test sampling

During this procedure, there is a potential risk of cross-contamination to the next carcase via the knife as a consequence of accumulated risk material on the knife or the operator's hands.

No potential risk of contamination from SRM was found from evaluation of the following process steps:

- cutting off fore- and hind legs
- dehiding
- opening of the abdominal and thoracic cavity
- removal of the intestines
- removal of pluck trimming of carcase
- weighing

Table 2 summarises the processes involving a risk of contamination with SRM.

Table 2. Schematic outline of the slaughter process, focusing on processes involving a risk of contamination with SRM

Process	SRM material	Risk of Contamination of Products
Transport		
Lairage		
Stunning	**Cerebral tissue**	**(X)**
Electrical stimulation/immobilisation	**Cerebral tissue**	**(X)**
Shackling and sticking	**Cerebral tissue**	
Removal of head	**Head Tonsils**	**X**
Cutting off fore- and hind legs		
Dehiding		
Opening the abdominal and thoracic cavity		
Removal of intestines	**Intestines Spleen (UK+PT)**	**(X)**
Removal of pluck	**Thymus (UK + PT)**	**X**
Carcase splitting	**Vertebral column (sawdust)**	**X**
Meat Inspection	**Spinal cord Tonsils**	**(X)**
Removal of spinal cord	**Spinal cord (sawdust)**	**X**
Trimming of carcase		
Taking samples for BSE test	**Cerebral tissue**	**(X)**
Weighing		
Chilling		
Handling of heads	**Tonsils**	**X**

X = risk is present

(X) = risk is possible

APPENDIX 7. STATUS OF PROCESS AND TECHNOLOGY DEVELOPMENT THAT MAY MINIMISE SRM CROSS-CONTAMINATION

As a result of the increasing number of BSE incidents and the new BSE-legislation both research and commercial institutions have initiated developments in areas with the aim to reduce SRM cross-contamination. In this section, an overview of recent developments available to the public is given. It varies somewhat from country to country depending on the degree of publication in the respective countries. In a recent issue of the German meat industry magazine *Fleischwirtsschaft*, Vol. 4 (2001), is a description of some of these developments, whereas other information has come from the International Meat Industry Equipment Exhibition of IFFA held in Frankfurt on 19–24 May 2001.

Stunning

Elimination of captive bolt stunning would reduce both environmental, operator and product cross contamination.

Alternatives

Electric stunning is a commercial ly available method used for more than a decade in certain countries. The drawbacks of the method are that it is more costly than bolt stunning and a reputation, although poorly documented, of meat quality problems in the form of minor blood splash in the muscles. The cost of the system would change the industry structure overnight should it become a legal requirement.

Carbon dioxide (CO_2) stunning of cattle is a method so far unproven and undocumented. At present a confidential pilot test is under way within the EU and tests have been made on sheep previously. As yet, no results have been published. CO_2 systems will be even less cost effective than electric stunning and have even wider industry implications should they prove a realistic alternative. The development, documentation, legislative approval and implementation phases will take a number of years.

Elimination of carcase vertebral column splitting

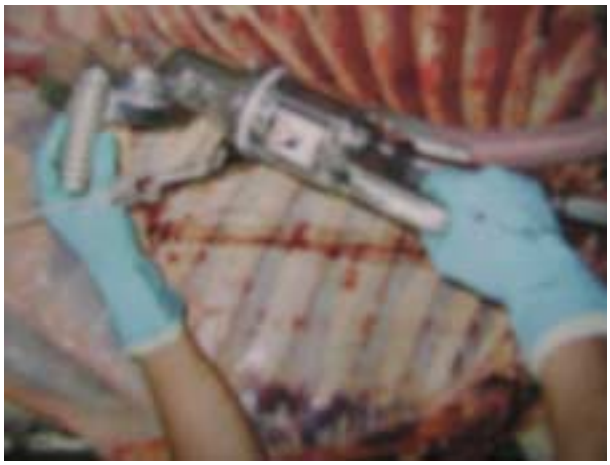
Mechanical damage of the spinal cord during splitting of the vertebral column is a concern for cross-contamination as far as the product, operator and environment are concerned.

Alternative

Operator skills are essential for exact sawing of the vertebral column that will allow easy and complete removal of the spinal cord. Automatic sawing gear is available but normally not better than a well-trained operator. Australian research on an ultra-sound controlled saw reports of very precise sawing as the ultrasound detects the midline very precisely and this may ease and improve spinal cord removal.

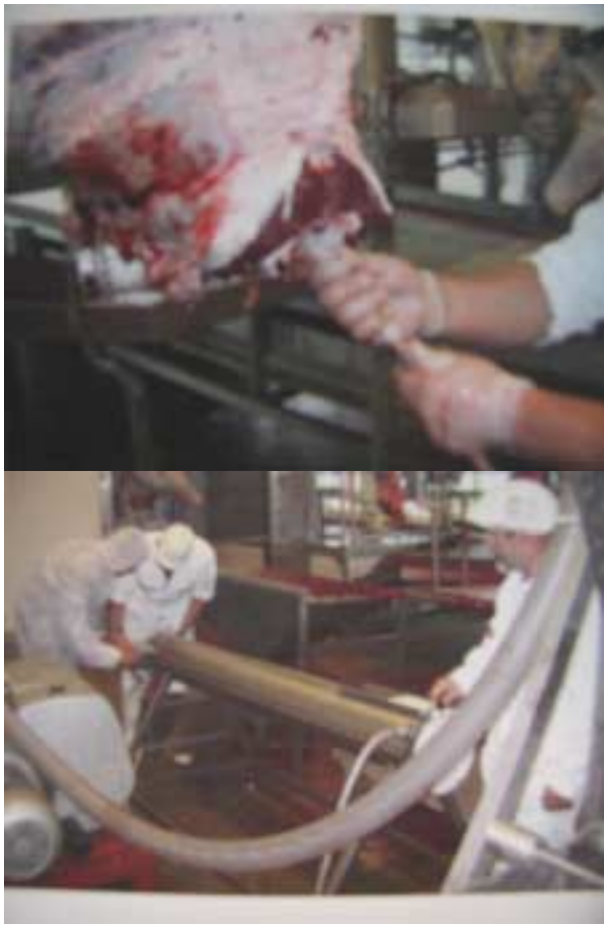
Removal of spinal cord

Manually operated, vacuum-suction systems are commercially available for removal of spinal cord and fragments from the split surface. An example of this system is the JARVIS saw. The adoption rate of this technology is variable but may have positive side effects with respect to product life. Other equipment available, includes a rotating cutter tooth, see picture overleaf, but which more time consuming to use.



Rotating cutter and vacuum suction system from JARVIS

A development in Germany is reported by JARVIS of experimental vacuum suction equipment for removal of the spinal cord of the unsplit carcase. The principle is to have a guidance tube enter the spinal canal upon which a flexible vacuum suction pipe is entered at full length of the vertebral column sucking up the cord. A cleaning system for reuse of the pipes is being tested. The method requires a reasonable conveyer height to be operational.



Entry of suction pipe **Cleaning of pipes**

Extraction off the vertebral column without splitting

Several developments of alternative saws (processes) that do not split the vertebral column have been reported:

- a double saw (an old system);
- freeing and sawing on each side of the vertebral column;
- rotating circular saws around the vertebral column (concept of Andy Knight, Silsoe); and
- other circular saws.

The general impression of the status of these saws is that none of them are commercially available and all appear to have drawbacks such as being slow or reducing the carcase meat yield. More development is necessary as they will not meet requirements from the larger slaughter plants.

Boning out the unsplit, unquartered carcase

Some methodologies that would avoid splitting of the carcases has been tried under experimental conditions. It is indeed possible to bone out an unsplit carcase with intact vertebral column, by several different approaches. However, the processes are all very time consuming and constitute no solution for the large slaughter plants. Furthermore, the slaughter line and chilling conveyors of modern plant do not match unsplit carcases and would need modification. Also it is a EU veterinary demand that the split surface of the carcase must be inspected.