

# Rendering practices and inactivation of transmissible spongiform encephalopathy agents

D.M. Taylor <sup>(1)</sup> & S.L. Woodgate <sup>(2)</sup>

(1) SEDECON 2000, 147 Oxbgangs Road North, Edinburgh EH13 9DX, United Kingdom

(2) PDM Group Ltd, Ings Road, Doncaster DN5 9SW, United Kingdom

## Summary

The authors describe the historic form of rendering and provide details on present-day practice. Possible future directions for the rendering industry are considered. The role of rendered meat-and-bone meal (MBM) as a dietary supplement in propagating the United Kingdom (UK) epidemic of bovine spongiform encephalopathy (BSE) is discussed, together with the role of MBM in spreading BSE outside the UK. Evidence that customarily used rendering processes did not substantially inactivate the agents of BSE or scrapie is presented. In addition, the influence that the abandonment of solvent extraction (as an adjunct to rendering) in the UK might have had on BSE infectivity levels in MBM is discussed. The BSE-related safety of tallow and by-products of tallow are considered. Data that associate the BSE agent with a new variant form of Creutzfeldt-Jakob disease in humans, predominantly but not exclusively, in the UK, are also discussed.

## Keywords

Bovine spongiform encephalopathy – Chronic wasting disease – Meat-and-bone meal – Rendering – Scrapie – Solvent extraction – Tallow – Technological developments – Transmissible spongiform encephalopathy – Variant Creutzfeldt-Jakob disease.

## Introduction

Bovine spongiform encephalopathy (BSE) belongs to a group of unusual and fatal neurological diseases of mammals known as the transmissible spongiform encephalopathies (TSEs). These include scrapie in sheep, Creutzfeldt-Jakob disease (CJD) in humans, and chronic wasting disease (CWD) in elk (*Cervus elaphus*) and mule deer (*Odocoileus hemionus*). Transmissible spongiform encephalopathies are caused by unconventional but incompletely characterised infectious agents that have a number of unusual properties, including a relative resistance to chemical and physical inactivation procedures that are effective with conventional micro-organisms (8, 14, 24, 26, 41).

Many of the views presented in this paper are based upon the experience of the United Kingdom (UK) and European Union (EU) with regard to BSE because the disease was recognised originally in England in the mid-1980s (44), and then spread throughout the UK. Somewhat later, the disease appeared in France, the Republic of Ireland, Portugal and Switzerland. In more recent years, it has been detected to varying degrees in all EU countries, except Sweden, largely because of the recent European Commission (EC) requirement to use a rapid test for

BSE on the brains of cattle used for food if they are older than thirty months. Using this procedure, BSE has more recently been detected in some non-EU countries of eastern Europe and in countries such as Israel and Japan that lie outside the European land mass (22). This probably resulted from the importation of BSE-infected products from EU countries within which BSE is endemic.

The first report of BSE in EU countries outside the UK was made in the late 1980s and early 1990s. This finding led to experimental validation studies funded by the EC and designed to examine rendering systems used within the EU to produce meat-and-bone meal (MBM) during the 1980s and early 1990s with regard to their capacity to inactivate BSE and scrapie agents.

The convincing association between BSE and the feeding of ruminant-derived MBM to cattle led to the introduction in 1988 of a ban in the UK on feeding ruminant-derived proteins to ruminants (46). A similar ban was later introduced throughout the EU and in the United States of America (USA). Other regulations introduced in the UK during the late 1980s were designed to prevent 'high-risk' bovine tissues, such as

brain and spinal cord, from being used as human food, and from entering rendering plants that still produced MBM for consumption by non-ruminants. Despite the 1988 ruminant-to-ruminant feed-ban in the UK, the BSE epidemic continued to accelerate until it peaked in 1992 and then started to decline. The delayed downturn occurred because BSE has an average incubation period of approximately five years and the first exposure to MBM in the UK was usually in calthood. Further control measures on the use of MBM were introduced in 1996 when a clear association between BSE and a new variant of Creutzfeldt-Jakob disease (vCJD) in humans was observed in the UK, possibly caused by dietary exposure to the BSE agent (48). This likelihood was enhanced by the demonstration that the BSE and vCJD agents had identical biological properties that were dissimilar to those of other TSE agents (5, 16). By the middle of 2002, 126 cases of vCJD had been observed in the UK. Six cases had been detected in France, and single cases had been observed in Ireland, Italy and the USA (in individuals who had spent most of their life in the UK). The 1996 UK regulation prohibited the feeding of any proteins derived from farmed species back to farmed species. As the number of cases of vCJD started to escalate outside the UK, a similar prohibition was introduced within the EU in 2000.

As will be discussed, the apparent recent geographic spread of CWD within the USA (and present occurrence in Canada) has heightened the degree of concern that this disease might represent a health risk to other animal species or humans.

There is some concern regarding the amount of useful animal protein that is being discarded as a result of the present regulations within the EU. However, these are unlikely to be changed unless there is convincing evidence that systems can convert animal by-products (including high-risk materials) into safe end-products. There are new methods that have been developed for disposing of animal tissues or MBM resulting in the production of heat and energy, or amino acids resulting from protein hydrolysis. Although the latter are nutritionally useful either as feedstuff or as fertiliser, there would have to be changes in EU regulations for amino acids to be used for such purposes. This is a matter that would have to be debated, at least within the EU, when all of the experimental data are available. Whether or not such products could be authorised to be fed back to farmed species within the EU will depend upon the strength of the scientific data, as well as the political perception of acceptability by the general public. Within the EU, there is no apparent willingness to allow ruminant-derived proteins to be fed back to ruminants under any circumstances because ruminants are considered to be naturally vegetarian. However, such proteins might be considered safe for feeding back to non-vegetarian species such as pigs, poultry and fish under ideal circumstances (13).

The number of BSE-related regulations that now exist worldwide, especially within the UK, the EU and Switzerland is

substantial. Consequently, full reference details for such regulations are provided sparingly in this paper. In most cases, these regulations are referred to but are not cited as references.

## Rendering

### Background

Animals slaughtered for human consumption provide inedible by-products. Within the EU, approximately 17 million tonnes of raw animal by-products are produced annually (14.5 million tonnes from animals declared fit for human consumption). The conventional method of stabilising these by-products is with heat, which evaporates the water contained within the tissues and provides a sterilising effect. This process is known as rendering which, in the EU, produces more than 3 million tonnes of processed animal protein and 1.5 million tonnes of rendered fat each year.

Traditionally, rendering was a simple heating process that allowed melted fat (customarily referred to as tallow) to be separated from animal tissues so that it could be used for a variety of purposes. Nowadays, there is a sector of the rendering industry that processes only the large deposits of discrete adipose tissue that can be obtained from animals declared fit for human consumption. These renderers (known more specifically as fat-melters) produce high-quality tallow that is considered to be safe for human consumption if the adipose tissue has been sourced from animals declared fit for human consumption.

There is a common misconception that the rendering process is, and has been, applied only to animal tissues deemed unfit for human consumption. Nowadays, the most commonly rendered tissues are from animal carcasses that have been declared fit for human consumption, and these are subjected to what is known as 'edible rendering.' In contrast, tissues declared unfit for human consumption are subjected to 'inedible rendering.' No differences are necessarily to be found between the rendering methods applied to the two different types of material, but there are differences in the way that these rendered materials would have been used in the past.

Historically, the rendering industry was born when it was realised that tallow could be used to manufacture useful products such as soap. The first written record of tallow being used to make soap appears to date back to the time of Christ. At that time, Pliny the Elder (a Roman soldier and scholar) described the production of soap from goat tallow and wood ash. However, this procedure may have been adopted from the earlier, but undocumented, practices of the Bedouins and Celts who were reported to have produced soapy substances by boiling animal fats and plant ashes together. The manufacture of candles from tallow also goes back at least to the days of the Roman Empire. For two centuries or more, soap and candles produced from tallow were the only recognised by-products

obtained from the rendering process although there are reports from the 1800s of the remaining solids being fed to dogs and ducks. While the historic aspects of rendering are fascinating, they will not be pursued further in this paper. Those who wish to learn more about the historical aspects of this subject may refer to the publication of Burnham (6).

It was determined in the early 1900s that the solid material that remains after tallow has been extracted from rendered animal tissues is rich in protein, and might usefully be fed back to animals as a dietary supplement. Thus, the concept of rendering changed because it involved the production of MBM by pulverising the cooked animal tissue that remained after tallow had been extracted. Traditionally, rendering had been conducted as a batch process. However, a commercial advantage could possibly be derived if tallow and MBM could be obtained in continuous production systems. Such systems were developed and used increasingly in Europe and the USA between the 1970s and the 1990s. It is considered that the pattern of usage of these various systems during this time period was probably comparable within Europe and the USA. However, the only reasonably accurate record of the range of systems being used relates to the Member States of the EU. The intent to conduct BSE- and scrapie-spiked validation studies, funded by the EC, led to a survey on rendering practices that prevailed within the EU in the 1980s and early 1990s. The data were used to provide the generic definitions of the rendering processes described in the publications of Taylor *et al.* (35, 38).

Animal by-products have traditionally made a contribution to the value of animals by finding their way into a wide variety of applications either before or after further processing. For example, MBM has been included in livestock feed, pet foods and fertilisers. Tallow has been transformed into soaps and oleochemicals (fatty acid derivatives) in addition to being used in food, pet food and feed applications.

Until recently, CWD appeared to be confined to a few northern states of the USA. More recently, the disease seems to have spread within the USA and spilled over into parts of Canada. This has heightened concern regarding the safety of elk and deer consumption by humans. Although there is no current evidence that CWD is transmissible to humans, a CJD-like disease was reported in three deer-hunters that were under thirty years of age. The sporadic form of CJD customarily affects individuals at an average age of 68. In contrast, the BSE-associated vCJD that has occurred predominantly in the UK affects individuals at a younger average age of 28. However, the affected hunters had no clinical or pathological features that might have indicated that they were suffering from vCJD (20). Because of the increasing concern regarding CWD and its potential risk to animal and human health, there are reports that renderers in Canada and the USA are imposing restrictions on what types of tissues they will process from elk and deer species. There is a clear need to establish national policies with regard to the disposal of the tissues or carcasses of elk or deer

species known or suspected to be infected with the CWD agent.

Although the EU is a relatively rich area of the world, Member States cannot consider the constant disposal of the huge amounts of animal proteins that are present in naturally discarded animal tissues, together with the specified risk materials (SRMs) that are removed from all cattle and sheep at the abattoir because of TSE-related concerns (15). There are various new procedures applicable to waste animal tissue that could have a significant inactivating effect on BSE-like agents but still provide useful end-products, including heat and energy or the amino acid products of protein hydrolysis. If the latter products were authorised in the future to be fed back to farmed species or used as fertiliser, the definition of rendering would have to change once again to include hydrolysed proteins, together with the production of tallow and MBM.

## Raw materials

Raw material characterisation has altered significantly over the past twenty years, but there are world-wide variations in the terminologies applied. New classifications are being discussed currently in the EU that should lead to clarification of this issue (Regulation of the European Parliament and of the Council laying down health rules concerning animal by-products not intended for human consumption). The classification of raw materials described within these proposed regulations will be used here (Table I). Although this classification system may not be accepted universally, the basic terminology and principles will be understood by the rendering industry throughout the world. The specific application of the EU-recommended autoclaving procedure (12) for certain raw materials referred to in Table I is based on the precept of 'safe sourcing/safe processing/safe application'. Thus, only raw materials from animals slaughtered fit for human consumption are intended to enter the 'feed for food animal' chain. One intention is to restrict the feeding of animal by-products to natural omnivores. There is also a stated policy that there should be no intra-species recycling as well as permanent bans on feeding processed animal proteins to ruminants.

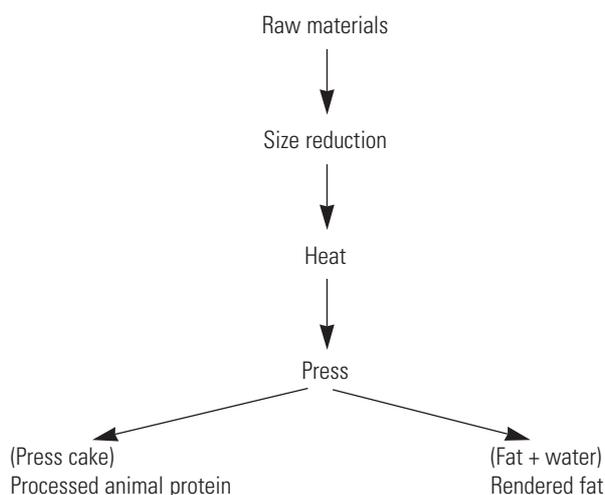
## Rendering processes

In general, rendering most commonly refers to the processing of natural or high-fat raw materials. In simple terms, there are two main methods of rendering that can be described as either dry or wet. The dry procedure is subdivided into processes that involve only natural fat, or those in which additional fat is added. However, this is an oversimplification because many types of processes exist throughout the world, and several have been altered and adapted in accordance with technical advances and legislative changes over the years. A simplified generic process description for rendering of high-fat raw material is shown in Figure 1. With wet rendering, the heat applied (approximately 95°C) is just sufficient to melt the fat, and both the press-cake (the solids) and the fluid fat still

**Table I**  
**Categorisation of raw materials proposed by the European Union**

Meat and meat products for human consumption	Category 1	Animal by-products not intended for human consumption Category 2	Category 3
Includes melted edible fats, and food-grade gelatine obtained from bones from edible carcasses	Animals or parts of animals presenting a TSE risk, including specified risk material  Proposed processing: incineration, or rendering, followed by incineration or burial (with some restrictions)	Animals or parts of animals that are unfit for human consumption because the animals a) were found dead on the farm b) contained veterinary residues, or c) failed ante- or post-mortem health inspection at the abattoir  Proposed processing: as for category 1, or autoclaving by the recommended process at 133°C for 20 min. at 3 bar (12) and then using the products in defined processes. These include the use of tallow to produce oleochemicals, meat-and-bone meal for fertiliser, or composting the end-product to produce biogas and using the residue as fertiliser	By-products from slaughtered animals declared fit for human consumption  Proposed processing: as for category 1, or processing to specified standards and using the end-product to produce oleochemicals, fertiliser and feedstuffs for pets and farmed species used to provide food. Mammalian protein shall be processed by the recommended autoclaving process (12), and shall not be re-cycled within the same species

contain water after the pressing stage of the process. The water is subsequently evaporated and this results in the production of dry tallow and residual animal protein. Animal by-product processors may also utilise raw materials with a low fat content, such as feathers or hair, that are processed by steam hydrolysis and drying to produce hydrolysed feather meal. Blood is either processed by coagulation and drying or by separation of the plasma and haemoglobin fractions followed by spray-drying, to produce plasma and haemoglobin powders.



**Fig. 1**  
**European Union rendering process overview**

Although a large number of different rendering processes have been used over the years for the processing of non-mammalian and mammalian materials, there are now five generic methods described and accepted by the authorities and the industry within the EU, as shown in Table II.

## End-product applications

The final application for products from the rendering industry depends on the nature, quality and freshness of the raw material, together with the type of processing applied (including aspects of post-processing such as filtration). For example, the production of fats for calf milk replacer involves the use of starting material obtained from animals declared fit for human consumption. These fatty tissues would customarily be processed under conditions in which the temperature would not exceed 90°C at atmospheric pressure. However, the fat produced would have a very low protein content (typically <0.02%) because of the raw material specification, the processing conditions and the filtration method employed. At the other extreme, Category 1 raw material is required to be destroyed to prevent any exposure of animals, humans or the environment to TSE-related risks.

## Rendering standards

The EU application of the precautionary principle to human and animal health considerations has impinged on MBM and

**Table II**  
**Generic animal by-product rendering processes**

Process*	Description	Continuous (C) or batch (B)	Pre or post method 1
1	133°C/3 bar/20 min.	C or B	N/A
2	Atmospheric/natural fat	B	Pre and post
3	Atmospheric/natural fat	C (B)	Pre and post
4	Atmospheric/added fat	C (B)	Pre and post
5	Atmospheric/defatted	C (B)	Post

N/A: not applicable  
\*ABP regulation 2000/0259 (COD)

tallow that are the traditional products of rendering. Although the USA has adopted some of the measures introduced by the EU to prevent the spread of BSE, there is a reservation about adopting all of the precautionary measures that are implemented within the EU. There is clearly a feeling that many of these steps are unnecessary because BSE has not been detected in the USA. One aspect of rendering that remains unchanged for many years is the use in the USA and many other parts of the world of processes that operate at atmospheric pressure. It is expected that processes 2 and 5 (Table II) will continue to apply in these geographical areas for the manufacture of feed-grade MBM because it is considered that control measures such as ruminant-to-ruminant feed bans will provide a sufficient degree of risk reduction as far as the development of BSE is concerned. In contrast, the steam-under-pressure system was the only one authorised to produce MBM for feeding farmed species within the EU until a complete ban was introduced in 2000 (12).

A simple message that has emerged from the BSE crisis in the UK is that the introduction of regulations is not effective unless adequate inspection systems ensure that compliance is being observed. For example, the introduction of the UK ban in the late 1980s on the use of bovine SRM for any food or feed purpose should have immediately ended this practice. Nevertheless, it was later acknowledged that such materials were subsequently entering rendering plants producing MBM for monogastric species such as pigs and poultry (1). In theory, this should not have presented any problems because pigs and poultry are not susceptible to BSE by dietary challenge. However, it was later recognised that feed for pigs, poultry and cattle were commonly prepared in the same feedmills, and that cattle feed was consequently becoming cross-contaminated with the BSE agent (47).

## The role of rendering in the propagation of bovine spongiform encephalopathy-like diseases

### Epidemiological evidence

When a scrapie-like disease was detected in a small number of cattle in England in the early 1980s (44), there was no suspicion that it would escalate into the enormous epidemic of BSE that has now affected more than 182,000 British cattle. Furthermore, at that time, it was not suspected that it would eventually infect cattle not only within other European countries but also in Israel and Japan. The early suspicion that BSE might have a dietary origin (10, 21) was confirmed by extensive epidemiological studies that implicated the feeding of MBM to cattle as the source of the infection (46). These studies indicated that the UK epidemic had been fuelled by feeding

cattle with BSE-infected MBM produced by the rendering industry. This did not imply that the rendering industry had been irresponsible in the way that it had rendered these BSE-infected materials. It was more a reflection of the fact that BSE-like agents are extremely resistant to inactivation by procedures that readily inactivate conventional micro-organisms (4, 9, 19, 23, 30, 31, 32, 34). The rendering industry had neither previously considered the question of the potential survival of TSE agents after exposure to rendering processes that substantially inactivate conventional micro-organisms, nor had it been required to do so by any governmental or trade organisations. Wilesmith *et al.* (46) could not determine whether BSE was originally caused by a sheep-derived scrapie agent, or a previously-unrecognised bovine TSE agent. Archival studies on the brains of cattle suffering from neurological diseases before the advent of BSE did not reveal any evidence of a pre-existing BSE-like disease (42) and the origin of BSE became a matter of later controversy. The UK BSE Inquiry concluded that the disease was bovine derived (1) but the Horn Committee determined that it was more likely to have originated in sheep (17). Thus, the true origin of BSE may never be known. As the epidemic expanded, so did the amount of potentially BSE-infected MBM being fed to cattle, until the feeding of ruminant-derived proteins to ruminants was prohibited in the UK in 1988. However, because MBM could still be fed legally to non-ruminant species within the UK at that time, the product was still permitted to be exported. Although it would have been inadvisable for those who imported this product to have used it to feed ruminants, there were no regulatory constraints that would have prevented this.

The 1988 UK MBM feed ban resulted in a downturn in the incidence of BSE in 1992. The reason for the delay was because the first exposure to MBM was usually in calthood, and the average age at which cattle display clinical signs of BSE is around five years. Although there have been significant year-to-year reductions in the incidence of BSE since 1992 within the UK, it has been acknowledged that the rate of decline would have been greater had there been a greater degree of supervision of the control measures. For example, it was recognised that, even after the 1988 MBM feed ban, cattle feed was still becoming contaminated with the BSE agent in feedmills that were using the same plant to legally produce feed for non-ruminant species (47). Until 1996, it was permissible in the UK to feed ruminant-derived MBM to non-ruminant species. The MBM produced for feeding non-ruminants should, in theory, have contained no BSE infectivity because regulations required the segregation and destruction of high-risk bovine tissues such as brain and spinal cord. The problem was that these SRMs had not been reliably excluded from the MBM manufacturing process (1). Although this weakness was later rectified, it did result in the occurrence of a significant number of cases of BSE in cattle born after the 1988 MBM feed ban. However, these did not prevent the overall decline in the incidence of BSE that has been experienced within the UK since 1992. During the early 1990s, BSE became endemic in a number of other European

countries, and was most likely to have been precipitated by the importation of MBM from the UK (7). Although the incidence of BSE outside the UK has been relatively modest compared with the UK experience, there has not yet been a sustained year-to-year decline in the incidence of BSE in some of these countries (22). This issue is obviously clouded by the number of cases that are now being detected because of the relatively recent EU requirement that the brain tissue of cattle older than 30 months has to be subjected to a rapid test for BSE; it remains to be seen what the eventual scales of these epidemics will be.

The recognised association between BSE and the feeding of MBM raised questions regarding the capability of rendering systems that had been used within the UK to inactivate the BSE agent. This question could not be answered immediately because the rendering industry world-wide had never been asked to consider whether manufacturing systems had the capacity to inactivate BSE-like agents. Nevertheless, from what was known about the time/temperature conditions used in rendering, it was considered that most rendering processes could be suspect, given the capacity of some TSE agents to survive even extended periods of autoclaving (4, 9, 11, 19, 23, 30, 31, 32). Until the emergence of BSE, the only TSE-infected tissues that renderers might have received in any significant quantity would have been from scrapie-infected sheep, but there is no evidence that MBM produced from potentially scrapie-infected tissues had previously caused any problems by feeding it to ruminants. Scrapie has never been demonstrated to be transmissible to humans through dietary or occupational exposure (3). Nevertheless, with the hindsight afforded by the BSE epidemic, it became important to know if the feeding of MBM to sheep might have resulted in BSE being introduced into the sheep population. This would be potentially difficult to determine because experimental studies have indicated that the clinical signs of experimentally induced BSE in sheep are similar to those of natural scrapie (15). One might suspect that BSE could be involved in instances where a scrapie-like disease has suddenly appeared in sheep flocks that had apparently been previously free from scrapie. However, this picture would be clouded if new breeding stock had been introduced into these flocks because the prion protein (*PrP*) genotype of sheep clearly influences their susceptibility to scrapie (18). At present, there are extensive studies being conducted within the UK to determine whether or not BSE has infected the sheep population. This includes full strain typing in mice of the agents recovered from suspect sheep. Fuller details of the UK research effort in this area are described by Taylor (33).

### **Experimental studies on traditional European Union rendering practices**

The BSE epidemic in the UK demonstrated that UK rendering procedures used in the 1980s to manufacture MBM had not inactivated the BSE agent. However, it was unknown whether this was related to one procedure or several, or what degree of inactivation might be achieved by any given process.

Nevertheless, it was suspected that the principal reason might have been the wide-scale introduction in the 1970s of a continuous system (compared with the traditional batch process) that operated at relatively low temperatures under vacuum but produced high-quality tallow. As will be discussed below, this system was eventually found to be the least inactivating with regard to TSE agents. Recognising the potential European dimension of the BSE problem, the EC supported an EU-wide rendering validation study that was conducted in two phases. In the first phase, BSE-spiked abattoir waste was exposed to the range of rendering processes that existed within the EU, and output samples were tested for BSE infectivity by mouse bioassay. In phase two, the same experiments were performed using scrapie-spiked abattoir waste. Before conducting these experiments, the range of rendering conditions used throughout the EU was determined through surveys carried out by the European Renderers Association, the UK Ministry of Agriculture, Fisheries and Food, and the United Kingdom Renderers Association. These revealed that the types of equipment being used by renderers in the late 1980s and early 1990s was relatively limited but that they were used in many different ways. It was therefore necessary to generically define the processes and then identify the minimal and average time/temperature combinations for each generic process. As the full details of these studies have been published (35, 38), the results will only be briefly considered here. The experiments involving BSE-spiked abattoir waste clearly demonstrated that the most recently introduced rendering system in the UK had little capacity to inactivate the BSE agent. This was the low-temperature vacuum system that has already been discussed, and which produced MBM with almost as much infectivity as in the untreated, spiked raw-material (35). This system involved cooking the raw materials under vacuum for 10 or 40 min. in pre-heated tallow; the temperatures at the end of these processes were 112°C and 122°C, respectively. Some of the other systems were also shown to permit the survival of lower levels of BSE infectivity. More systems would probably have been shown to be unreliable if the level of BSE infectivity in the spiked raw materials had been higher. It was recognised retrospectively that, for technical reasons, the challenge level of the BSE agent in the spiked raw materials was sub-optimal (35). The challenge level in the BSE-spiked tissues was only  $10^{1.7}$  mouse intracerebral infectious dose (ID)<sub>50</sub>/g; in the scrapie-spiked studies, this value was  $10^{3.1}$ . In the scrapie-spiked experiments, infectivity was detected after exposure to all the rendering procedures apart from one that involved autoclaving at 133°C under a pressure of 3 bar for 20 min. (38). This process appeared to be effective with both the BSE and scrapie agents, and produced up to a 1,000-fold reduction in infectivity levels. The apparent greater efficiency of inactivating BSE-spiked compared with scrapie-spiked raw materials is likely to be attributable to the  $10^{1.4}$  higher titre of the scrapie-spiked starting material, rather than an inherent higher resistance of the scrapie agent to inactivation.

Although the EU subsequently adopted the 133°C autoclaving procedure as the only appropriate method for producing MBM for inclusion into animal feed (12), this process might not be robust under worst-case conditions (29). This proved to be the case when Schreuder *et al.* (25) reported the survival of some BSE infectivity when spiked raw materials containing a ten-fold higher level of infectivity were exposed to the same process. This correlates with the known general inability of autoclaving at temperatures ranging from 121°C-138°C to reliably inactivate high titres of BSE-like agents, even when the exposure times are extended. Nevertheless, the collective data of Taylor *et al.* (35, 38) and Schreuder *et al.* (25) suggest that the hyperbaric 133°C rendering process is likely to be effective if high-risk tissues such as bovine brain and spinal cord are reliably excluded from the rendering process.

### Solvent extraction

It is unclear why BSE first occurred in England during the 1980s, given that MBM had been fed to cattle for at least 70 years previously. Several hypotheses were proposed relating to the following:

- a) an increasing incidence of scrapie in UK sheep
- b) an increasing UK sheep population
- c) changes in rendering practices in the UK (28, 46).

An additional argument was based on the observation that, in the past, rendered tissues in the UK had commonly been subjected to solvent extraction which enhanced the yield of tallow and produced a low-fat MBM that had attracted premium prices. However, solvent extraction was largely discontinued throughout the 1970s and 1980s. This process involved the exposure of rendered materials to hot solvents and then to dry and wet heat procedures to remove any remaining solvent. Given that the average incubation period of BSE is approximately five years, and that the disease was first observed in the mid-1980s, it was suggested that the emergence of BSE might have been associated with the discontinuation of the use of solvent extraction by the rendering industry. In 1975, approximately 65% of the MBM manufactured in the UK had been subjected to solvent extraction, but by 1982 the proportion had dropped to 10% (47). In the solvent extraction process, greaves (the solids that remain after rendering) were exposed to hot solvents to increase the yield of tallow that could be extracted from them. It was considered that the application of solvent extraction to already rendered tissues might have provided sufficient additional inactivation of BSE or scrapie infectivity to prevent these agents being present in MBM at levels that would constitute an effective oral dose for cattle (46). The TSE agents are much more resistant to inactivation than conventional micro-organisms, and organic solvents do not generally have any significant inactivating activity on these in crude tissue preparations (31). However, nothing was known about the inactivating potential of the solvent extraction processes used by the rendering industry on TSE agents (27).

These processes had involved the exposure of greaves to hot solvents such as benzene, hexane, heptane, perchlorethylene, petroleum spirit or trichlorethylene, followed usually by exposure to dry heat and steam at 100°C to remove residual solvent from the processed greaves.

Experiments were performed in which BSE- and scrapie-infected tissues were exposed to hot solvents (heptane, hexane, perchlorethylene and petroleum spirit), followed by exposure to dry heat and steam. As the UK rendering industry had largely abandoned the use of solvent extraction by the mid- to late 1970s, it was difficult to plan these experiments because discussions with renderers showed that there was no clear recollection of the technical details of some of the processes that had been used in the past. The experimental processes involving petroleum spirit and perchlorethylene were therefore not necessarily precise facsimiles of the commercial processes. However, those involving heptane and hexane were based on reliable data provided by the last two UK renderers to have used solvent extraction until the 1990s. The laboratory-scale solvent extraction procedures are not claimed to be precise reproductions of the industrial processes, but were intended to provide a yardstick by which it could be judged whether there was any substance to the hypothesis that the abandonment of solvent extraction processes by UK renderers facilitated, or contributed to, the emergence of BSE.

The original intention had been to use TSE-infected mouse brain as the test material, but it was recognised that the fat content of mouse brain would be significantly higher than that of the greaves customarily subjected to solvent extraction. The efficiency of heat inactivation of conventional micro-organisms is impaired by the presence of fat. The only other organ of any appreciable size that becomes infected to a significant degree in mice infected with TSE agents is the spleen, and this was selected as the preferred tissue for these studies because of its relatively low fat content. The use of spleen tissue was also considered to represent a worst-case scenario as far as inactivation is concerned because (unlike the spleens of cattle with BSE) the spleens of sheep with scrapie and experimentally induced BSE (15) become infected, and spleen is a relatively solid tissue. In contrast, the other principal 'high-risk' tissues (brain and spinal cord) are much softer, and would tend to become smeared over the surface of other more solid particles during rendering and solvent extraction.

For the experiments, halved spleens of mice infected with the 22A strain of mouse-passaged scrapie agent that has been shown to be more thermostable than other strains of scrapie agent (9, 19), and the 301V strain of mouse-passaged BSE agent that is now known to be the most heat-resistant mouse-passaged agent tested to date (41) were used as the starting material. These were exposed to the hot solvents described above at temperatures and times that were appropriate according to whatever information had been gleaned from the rendering industry. The remaining solids were then exposed to

dry heat at 100°C for 30 min. followed by exposure to steam at 100°C for 30 min. The results of these experiments are presented by Taylor *et al.* (39). These show that, on average, the solvent extraction systems achieved only approximately a ten-fold reduction in the titre of the TSE agents tested. The average infectivity titres in the untreated spleen material was approximately  $10^7$  intracerebral ID<sub>50</sub>/g.

From the time of the earliest probable dietary exposure of cattle to the agent that causes BSE, the only two surviving solvent extraction facilities that existed within the UK were in Scotland, but these ceased to operate in the early 1990s. These plants are known to have manufactured a large proportion of the MBM used, at least in 1988, in Scotland (47). Given the low incidence of BSE in Scotland, and the fact that the reported incidence has been increased through the known acquisition of subclinically BSE-affected cattle from England, it has been suggested that the solvent process may have had the capacity to inactivate, or significantly reduce the titres of infectivity of BSE or scrapie agents (46). However, the small overall losses of titre observed after solvent processing in the laboratory study suggest that the capacity of these procedures to reduce BSE or scrapie infectivity was extremely limited. The data indicate that the hot solvents per se had little effect on infectivity, and that the slight degree of inactivation achieved was mainly due to the application of heat to remove residual solvent. In a study in which scrapie-infected tissues were subjected to solvent extraction with heptane in an actual rendering plant, there was no detectable loss of infectivity (38). The process involved the exposure of scrapie-infected greaves to heptane at 80°C for 10 min., followed by sequential exposures to steam at 100°C for 10 and then 20 min. In that study, the spiked raw materials had been rendered before subjecting them to solvent extraction. The failure of the solvent extraction process to further reduce the titre of scrapie infectivity was possibly due to the survival of a thermostable subpopulation of scrapie agent after rendering. The existence of thermostable subpopulations has been demonstrated by Taylor and Fernie (36) and raises the possibility that the reduction in infectivity achieved in commercial solvent extraction plants may have been even less than those achieved in the solvent extraction processes tested in the laboratory. However, the procedures used for exposing the infected materials to hot solvents in the laboratory was not truly representative of the industrial processes. The principal difference was that in the industrial processes, fat-containing solvent was continuously removed and replaced by redistilled solvent, whereas in these experimental studies the solvent was not recycled. Thus, it might be argued that if significant amounts of infectivity partitioned with the fat fraction during commercial solvent extraction, the infectivity could have been 'siphoned off' in the solvent/tallow fraction, and have resulted in an appreciable reduction of infectivity in the resulting MBM. However, no infectivity has been detected in tallow produced under 'worst-case' conditions during experimental rendering studies involving BSE- and scrapie-spiked raw materials (35, 38). Furthermore, there was no detectable difference between the

titres of scrapie agent in spiked materials before and after exposure to a real solvent extraction process with heptane (38). Collectively, these studies indicate that there is no predilection for scrapie-like agents to associate with tallow, and the fact that solvents were not recycled in the studies described is unlikely to have affected the results significantly. In another respect, the experimental methods are considered to have provided rather more rigorous conditions than the normal commercial processes. The average diameter of the individual spleen fragments was approximately 4 mm, whereas the average particle diameter of the greaves processed in commercial solvent extraction plants was approximately 15 mm. In the experimental procedure, the solvents therefore had a greater potential to percolate into the fragments of spleen than would have been the case for the fragments of greaves in the commercial process.

The data suggest that the abandonment of solvent extraction processes by the UK rendering industry was not the single key factor that permitted the emergence of BSE. However, if, as seems likely, a number of factors conspired to allow the disease to emerge, then the accelerating abandonment of the solvent extraction process throughout the late 1970s may have played a part despite its relatively modest role in reducing infectivity titres.

## The bovine spongiform encephalopathy-related safety of tallow and tallow-derived products

The feeding of MBM to cattle has been clearly identified as the most likely route by which BSE epidemics have been initiated and expanded in most of the affected countries (7). In contrast, tallow has been considered generally to be relatively free from BSE-related risks for the following reasons:

- a) epidemiological studies failed to find any association between the occurrence of BSE and the consumption of tallow by cattle (46)
- b) in the BSE-spiked rendering studies, no infectivity was detectable in crude, unfiltered tallow produced by a rendering procedure that produced MBM with almost as much infectivity as was present in the untreated, BSE-spiked raw materials (35).

Nevertheless, it would be unrealistic to consider that tallow could never become contaminated with the BSE agent despite the evidence that this agent does not preferentially migrate into tallow during the manufacturing process. Tallow derived from the discrete masses of adipose tissue that are removed in dedicated lines before the carcass is split is likely to be free from BSE contamination, provided there has been no damage to the

intestine, because experimental studies have shown that the distal ileum of cattle challenged experimentally with the BSE agent by the oral route becomes infected at various times post-exposure (45). It is considered more realistic to assume that low levels of contamination could occur from time to time despite the regulations requiring the removal and destruction of SRM. It is recognised that relatively crude tallow can contain up to 0.5% of suspended solids, and it is now a requirement that this level be reduced to <0.15% (by centrifugation, filtration, precipitation, etc.) before the product is marketed. By-products produced from the 'splitting' of tallow have been consistently considered to be free from any BSE-related risk because of the severity of the splitting processes. For example, pharmaceutical grade magnesium stearate is obtained by splitting tallow at 250°C at a pressure of 40 bars for 2 h, which produces fatty acids and glycerol. The fatty acids are then distilled at 200°C to produce pure fatty acids.

Approximately 95% of the tallow obtained by melting carefully collected discrete adipose tissues is used as animal feed (e.g., in pet food or milk replacer for calves), the remainder being used in food (e.g., for baking and frying). However, in contrast to the crude tallow discussed above, such tallow is required to have an impurity level of <0.02%. The daily intake of tallow in calves consuming milk replacers averages 120 g, usually for a period of approximately sixty days; the calves are then supplemented with calf, and later cattle, concentrates. The use of tallow in milk replacer has been incriminated as the cause of BSE in some countries (e.g., Japan) but the evidence remains circumstantial and uncertain.

The subject of the BSE-related safety of tallow was re-examined when it was reported that the effectiveness of inactivating prion rods by autoclaving was significantly impaired when these were suspended in different concentrations of lipid (2). The study reported that autoclaving processes at temperatures of up to 170°C became less effective as the concentration of lipid within which the prion rods were suspended was increased. The authors suggested that BSE infectivity would have a tendency to associate with tallow, rather than MBM, during the rendering process because of the hydrophobic nature of the disease-associated scrapie-associated prion protein (PrP<sup>Sc</sup>) molecules. They also hinted at the possibility that tallow-derived products obtained by splitting tallow under high temperature and other extreme conditions might be unsafe with regard to BSE. However, in contrast to what is suggested by Appel *et al.* (2), there is evidence that BSE infectivity does not preferentially migrate into the tallow fraction under actual rendering conditions but tends to remain in the MBM fraction (35). In the latter study, there was no detectable infectivity in the tallow resulting from a process that produced MBM containing almost as much infectivity as there was in the unprocessed raw materials. In addition, Appel *et al.* (2) used prion rods (analogous to scrapie-associated fibrils which are large fibrillar polymers of PrP<sup>Sc</sup>) as the source of infectivity. These were obtained by detergent extraction from hamster-brain infected

with the 263K strain of scrapie agent. However, these types of large fibrillar structures are not actually present in either 263K-infected hamster-brain or BSE-infected cattle-brain; they are unnatural agglomerates of PrP<sup>Sc</sup> that are produced by the detergent extraction process. Thus, the experiments were conducted in such a fashion that they did not realistically represent the conditions that prevail in everyday rendering, because the source of infectivity was quite unrepresentative of the way in which BSE infectivity exists in bovine brain tissue. In addition, bioassays were not performed to determine whether or not infectivity had survived. The assessments were based entirely upon Western blotting techniques that detect proteinase K-resistant forms of PrP that are commonly associated with, but do not always correlate with, infectivity, especially after exposure to partially inactivating procedures.

## The future for rendering: emerging technologies

Increasingly stringent EU regulations apply to the disposal of fallen and dead stock, and to tissues rejected by abattoirs because of consumer preferences or for public health reasons. This has meant the loss of traditional markets for MBM and tallow for the rendering industry that was compounded by the EU-wide ban in 2000 on feeding proteins derived from farmed species to farmed species. However, there is an expectation in the EU that there will be new applications for rendered products after the likely adoption of new EC regulations in 2003 relating to animal by-products not intended for human consumption. Although any new legislation will be based on the precautionary principle, there is likely to be an emphasis on the adverse environmental consequences of maintaining the current European blanket ban on including any processed animal proteins into the feed of animal species that are farmed to produce food. This concept has been gathering strength within the EC (13) and has led to the conclusion that some products might be safely fed to animals if production is accompanied by the application of hazard analysis and critical control points (HACCP). There is also a recognition that even the most hazardous starting material might be used to produce safe and useful by-products. These include biogas resulting from anaerobic digestion of such materials, and the production of biodiesel for use in internal combustion engines produced by the trans-esterification of fatty acid methyl esters contained in rendered products.

In some EU countries, especially the UK, there are large amounts of MBM and tallow being stored until they can be disposed of or utilised in a safe and suitable fashion. These materials are potential sources of heat and/or energy, and combustion technologies have been considered as suitable disposal methods. In addition, if safe methods could be developed for processing the stored MBM and tallow or the fresh raw materials that are being constantly generated, it is

conceivable that the end-products might once again be permitted to be used as feed or fertiliser in the future. Ideally, such systems should be designed to minimise any environmental impact. Candidate systems are described below.

### Bubbling fluidised bed combustion

The principle upon which this process operates is that a bed of a granular substance such as silica sand is heated by gas or oil until it reaches 850°C. Air for combustion is then blown upwards through the heated bed which becomes fluid, and bubbles. The waste materials are then delivered onto the fluidised bed at a controlled rate. Due to the fluid motion, the particles of waste are evenly distributed over the surface of the bed and are rapidly combusted during the dwell period that is in excess of two seconds. An innovative technology was developed, allowing the waste to also become the fuel after the initial heating-up stage. This is achieved by mixing aqueous waste and sludges with dry materials such as MBM (in appropriate ratios, and at particular particle sizes) in highly controlled ratios to achieve a consistent calorific value. This ensures that combustion in the fluidised bed is autothermic, i.e. the combustion process is maintained at the appropriate temperature without the need for any further addition of fossil fuel. The non-combustible element (ash) of the fuel-product is continuously removed from the combustion bed to ensure that its depth remains constant. The process has been found to provide a very high degree of protein destruction; the residual level is significantly less than the 5 mg of amino acid nitrogen per 100 g of ash limit laid down by the UK Government. The system has been designed so that the hot gas generated in the combustion chamber is drawn into a combined heat and power generation module. Within this module, high-pressure steam is generated by the heating of water in a system of tubes. The steam can be used for on-site purposes, or sold to adjacent third parties. In addition, the electrical power generated can be used for the following:

- a) in-house
- b) by neighbouring organisations
- c) by general consumers connected to the national network.

### Alkaline protein hydrolysis

Tissue digestors for the disposal of carcasses of small and large animal species have been available for almost ten years. The process exposes tissues or carcasses for a minimum of 3 h to a solution of 1M potassium or sodium hydroxide at 150°C in stainless-steel vessels at a steam-pressure of around 4 bars. The digestion system is sufficiently aggressive to allow the processing of intact carcasses of species as large as horses. To achieve maximum exposure of the tissue to the hydrolysing agent, the alkali solution is continuously circulated through the vessel. Unlike traditional rendering systems, alkaline hydrolysis does not produce MBM. In contrast, the tissues and carcasses are dissolved and then progressively degraded to yield a sterile solution that contains amino acids, peptides, sugars and soaps.

Laboratory studies have shown that the processed solution contains no intact proteins. The remaining solids consist entirely of bone that has lost its organic content, and these 'bone shadows' crumble between finger and thumb. The process has been shown to destroy representative index agents for all conventional infectious agents as well as chemotherapeutic agents, protein-fixing aldehydes and the biological agents and toxins listed in the United States Department of Health and Human Services (USDHHS) Final Rule (43). The amino acid-rich solution that results from the process is potentially a useful additive to animal diets, but this is prohibited by current EC regulations. The system at present for obtaining a useful by-product is to subject the solution to anaerobic digestion. This produces copious amounts of methane that can be used as a profitable source of energy. The alkaline hydrolysis system and the anaerobic digestion processes are both self-contained, and produce no pollution to land, air or water. At the end of these combined processes there is a small amount of residual sludge or powder that can be safely land-filled. Regarding the 'bone shadow' solids that remain after hydrolysis, these could be used as a fertiliser if prepared using potassium hydroxide, because this would result in a product rich in potassium phosphate. Alternatively, these can be fed into the anaerobic digestion process.

Although autoclaving at 121°C-138°C or exposure to sodium hydroxide at room temperature are not completely effective *per se* for inactivating TSE agents, inactivation can be achieved by combining these procedures (32, 37). Indeed, inactivation has been achieved by boiling in sodium hydroxide for 1 min. (40), and the World Health Organization now recommends hot alkali processes as a means of inactivating the CJD agent (49). Validation studies on the commercial alkaline system with regard to its capacity to inactivate BSE-like agents were funded by the UK Department for Environment, Food and Rural Affairs (DEFRA). These studies have shown that the process is effective in this respect.

### High temperature, high pressure and protein hydrolysis

Efficient protein hydrolysis can be achieved by methods other than hot alkaline processes. Over the past twelve years, a system has been developed that has been shown in the laboratory to inactivate conventional micro-organisms and achieve efficient protein hydrolysis in the absence of alkali when applied to organic waste-products such as animal carcasses, food waste etc. The process involves exposure of these materials to steam generated from the raw materials. The operating conditions consist of exposure to steam at a temperature of 180°C and a pressure of 12 bar for 40 min., during which the materials are continuously agitated and denatured; this is followed by a dehydration stage. It has been found that the addition of organic fibrous material eliminates odour-related problems that might otherwise be associated with the end-product. Also, the optional addition of oxidising

nitrate to the raw materials enhances the value of the end-product if used as a fertiliser. To accommodate the requirements of EC regulations, the traditional end-product would be subjected to anaerobic bacterial digestion that would release methane as a useful fuel by-product, and result in a small final volume of residual material that would need to be land-filled. The company envisions its high-pressure steam process as being important in transforming the entire organic waste stream of society into safe and valuable nutrient products through innovative processing, thereby reducing or eliminating the historical polluting practices of incineration or large-scale land-filling. In the past, steam sterilisation studies on TSE

agents have maximally involved a temperature of 138°C. Although complete inactivation was not achieved in these experiments, there was a reduction factor of up to ten million-fold (34). Given that even at 138°C, complete inactivation was almost achieved, it seems likely that the 180°C process can achieve complete inactivation, especially since it has been shown to effectively break proteins down into amino acids and peptides. Validation studies funded by DEFRA are currently being conducted to confirm this.

## Les procédés d'équarrissage et l'inactivation des agents de l'encéphalopathie subaiguë spongiforme transmissible

D.M. Taylor & S.L. Woodgate

### Résumé

Les auteurs replacent l'équarrissage dans son contexte historique et donnent une description détaillée des techniques actuelles. Ils envisagent plusieurs possibilités d'évolution de l'industrie d'équarrissage. Ils examinent le rôle joué par la farine de viande et d'os (FVO), issue de l'équarrissage et distribuée comme complément alimentaire, dans la propagation de l'épizootie d'encéphalopathie spongiforme bovine (ESB) au Royaume-Uni, ainsi que son rôle dans la propagation de la maladie en dehors du territoire britannique. Les auteurs présentent des preuves attestant de l'incapacité des procédés habituels d'équarrissage à inactiver efficacement les agents de l'ESB ou de la tremblante. Ils s'interrogent en outre sur l'impact éventuel de l'abandon, au Royaume-Uni, de l'extraction par solvant (en tant que technique d'appoint de l'équarrissage) sur les niveaux d'infectivité de la FVO par l'ESB. L'innocuité du suif et de ses produits dérivés est examinée en ce qui concerne l'ESB. Les données impliquant l'agent de l'ESB dans une nouvelle variante de la maladie de Creutzfeldt-Jacob, notamment mais non exclusivement au Royaume-Uni, sont également discutées.

### Mots-clés

Encéphalopathie spongiforme bovine – Équarrissage – Encéphalopathie subaiguë spongiforme transmissible – Extraction par solvant – Farine de viande et d'os – Maladie du dépérissement chronique – Progrès technologique – Suif – Tremblante – Variante de la maladie de Creutzfeldt-Jacob.

# Procesamiento de cadáveres y desechos animales e inactivación de los agentes etiológicos de las encefalopatías espongiformes transmisibles

D.M. Taylor & S.L. Woodgate

## Resumen

Los autores describen el procesamiento técnico utilizado en el pasado para asegurar la esterilización de cadáveres animales y desechos de origen animal y exponen en detalle el proceder utilizado actualmente. También consideran las posibles direcciones en que pueden evolucionar las industrias transformadoras. Examinan asimismo el papel de las harinas de carne y huesos utilizadas como complemento alimentario, en la propagación de la epizootia de encefalopatía espongiforme bovina (EEB) que asoló el Reino Unido, así como la influencia de esas harinas en la posterior extensión de la EEB fuera del Reino Unido. Presentan pruebas de que los procesos habituales de transformación utilizados no inactivaban de forma apreciable los agentes de la EEB o del prurigo lumbar. Estudian además la influencia que pudo haber tenido el abandono de la extracción por solventes (como proceso complementario del procesamiento) en el Reino Unido sobre los niveles de infectividad de las harinas de carne y huesos. También consideran el nivel de inocuidad (en relación con la EEB) que ofrecen el sebo y sus derivados. Examinan por último los datos que parecen relacionar al agente de la EEB con una nueva variante de la enfermedad de Creutzfeldt-Jakob en el hombre, que se manifiesta sobre todo, aunque no exclusivamente, en el Reino Unido.

## Palabras clave

Avance tecnológico – Caquexia crónica – Encefalopatía espongiforme bovina – Encefalopatía espongiforme transmisible – Extracción por solventes – Harina de carne y huesos – Procesamiento de cadáveres y desechos animales – Prurigo lumbar – Sebo – Variante de la enfermedad de Creutzfeldt-Jakob.



## References

1. Anon. (2000). – The BSE inquiry. Report, evidence and reporting papers of the inquiry into the emergence and identification of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) and the action taken in response to it up to 20 March 1996, sixteen volumes. Her Majesty's Stationery Office, London.
2. Appel T.R., Wolff M., von Rheinbaden F., Heinzl M. & Riesner D. (2001). – Heat stability of prion rods and recombinant prion protein in water, lipid and lipid-water mixtures. *J. gen. Virol.*, **82**, 465-473.
3. Brown P. (1980). – An epidemiological critique of Creutzfeldt-Jakob disease. *Epidemiol. Rev.*, **2**, 113-135.
4. Brown P., Rohwer R.G. & Gajdusek D.C. (1986). – Newer data on the inactivation of scrapie virus or Creutzfeldt-Jakob disease virus in brain tissue. *J. infect. Dis.*, **153**, 1145-1148.
5. Bruce M.E., Wil R.G., Ironside J.W., McConnell I., Drummond D., Suttie A., McCardle L., Chree A., Hope J., Birkett C., Cousens S., Fraser H. & Bostock C.J. (1997). – Transmissions in mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature*, **389**, 498-501.
6. Burnham F.A. (1996). – The rendering industry – a historical perspective. In *The Original recyclers* (D.A. Franco & W. Swanson, eds). The Animal Protein Producers Industry, The Fats and Proteins Research Foundation and The National Renderers Association, Washington, DC, 1-15.
7. Butler D. (1996). – Statistics suggest BSE now 'Europe-wide.' *Nature*, **382**, 4.
8. Chesebro B. (1998). – BSE and prions; uncertainties about the agent. *Science*, **279**, 42-43.

9. Dickinson A.G. & Taylor D.M. (1978). – Resistance of scrapie agent to decontamination. *N. Engl. J. Med.*, **229**, 1413-1414.
10. Dickinson A.G. & Taylor D.M. (1988). – Options for the control of scrapie in sheep and its counterpart in cattle. *In Proc. Third World Congress on sheep and beef cattle breeding*, Vol. 1, 19-23 June, Paris. Institut National de la Recherche Agronomique, Paris, 553-564.
11. Ernst D.R. & Race R.E. (1993). – Comparative analysis of scrapie agent inactivation. *J. virol. Meth.*, **41**, 193-202.
12. European Economic Community (EC) (1996). – On the approval of alternative heat treatment systems for processing animal waste with a view to the inactivation of spongiform encephalopathy agents, Commission Decision 96/449/EC. *Off. J. Eur. Communities*, **L 184**, 43-46.
13. European Union (EU) (2002). – Questions and answers on animal by-products (rapid press release). Memo/02/54, 13 March, Brussels. EU, Brussels, 4 pp. (europa.eu.int/rapid/start/cgi/guesten.ksh?p\_action.gettxt=gt&doc=MEMO/02/54|0|AGED&lg=EN&display= accessed on 27 January 2003).
14. Farquhar C.F., Somerville R.A. & Bruce M.E. (1998). – Straining the prion hypothesis. *Nature*, **391**, 345-346.
15. Foster J.D., Bruce M.E., McConnell I., Chree A. & Fraser H. (1996). – Detection of BSE infectivity in brain and spleen of experimentally infected sheep. *Vet. Rec.*, **138**, 546-548.
16. Hill A.F., Desbruslais M., Joiner S., Sidle K.C.L., Gowland I. & Collinge J. (1997). – The same prion strain causes vCJD and BSE. *Nature*, **389**, 448-450.
17. Horn G. (2001). – Review of the origin of BSE. Department for the Environment, Food and Rural Affairs, London, 66 pp.
18. Hunter N., Foster J.D., Goldmann W., Stear M.J., Hope J. & Bostock C.J. (1996). – Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Arch. Virol.*, **141**, 809-824.
19. Kimberlin R.H., Walker C.A., Millson G.C., Taylor D.M., Robertson P.A., Tomlinson A.H. & Dickinson A.G. (1983). – Disinfection studies with two strains of mouse passaged scrapie agent. *J. neurol. Sci.*, **59**, 355-369.
20. McCombie B. (2002). – Who is to blame for mad deer? *Progressive*, **66** (8), 22-25.
21. Morgan K.L. (1988). – Bovine spongiform encephalopathy: time to take scrapie seriously. *Vet. Rec.*, **122**, 445-446.
22. Office International des Epizooties (OIE) (2002). – Bovine spongiform encephalopathy: geographical distribution of countries that reported BSE confirmed cases from 1989 to 2002. OIE, Paris ([www.oie.int/eng/info/en\\_esb.htm](http://www.oie.int/eng/info/en_esb.htm) accessed on 11 February 2003).
23. Peano S., Reiner G., Carbonatto M., Bodenbender L., Boland P. & Abel K.-J. (2000). – Determination of the clearance factor for transmissible spongiform encephalopathy agents during the manufacture of polygeline. *Intensive Care Med.*, **26**, 608-612.
24. Prusiner S.B. (1996). – Prion biology and diseases. *In Transmissible subacute spongiform encephalopathies: prion diseases* (L. Court & B. Dodet, eds). Elsevier, Paris, 209-237.
25. Schreuder B.E.C., Geerstma R.E., van Keulen L.J.M., van Asten J.A.A.M., Enthoven P., Oberthur R.C., de Koeijer A.A. & Osterhaus A.D.M.E. (1998). – Studies on the efficacy of hyperbaric rendering procedures in inactivating bovine spongiform encephalopathy (BSE) and scrapie agents. *Vet. Rec.*, **142**, 474-480.
26. Somerville R.A. (2000). – Is there more to TSE diseases than PrP? *Trends Microbiol.*, **8**, 157-158.
27. Taylor D.M. (1989). – Bovine spongiform encephalopathy and human health. *Vet. Rec.*, **125**, 413-415.
28. Taylor D.M. (1993). – Bovine spongiform encephalopathy and its association with the feeding of ruminant derived protein. *Dev. Biol. Stand.*, **80**, 215-224.
29. Taylor D.M. (1997). – Research on the safety of MBM with regard to bovine spongiform encephalopathy and scrapie agents. *In Transcripts of an International Conference on meat and bone meal*, 1-2 July, Brussels. European Commission, Brussels, 37-40, 42-44, 63.
30. Taylor D.M. (1999). – Inactivation of prions by physical and chemical means. *J. Hosp. Infect.*, **43** (Suppl.), S69-S76.
31. Taylor D.M. (1999). – Transmissible degenerative encephalopathies. Inactivation of the causal agents. *In Principles and practice of disinfection preservation and sterilisation* (A.D. Russell, W.B. Hugo & G.A.J. Ayliffe, eds). Blackwell Scientific Publications, Oxford, 222-236.
32. Taylor D.M. (2000). – Inactivation of transmissible degenerative encephalopathy agents: a review. *Vet. J.*, **159**, 10-17.
33. Taylor D.M. (2002). – Current perspectives on bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. *Clin. Microbiol. Infect.*, **8**, 332-339.
34. Taylor D.M., Fraser H., McConnell I., Brown D.A., Brown K.L., Lamza K.A. & Smith G.R.A. (1994). – Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Arch. Virol.*, **139**, 313-326.
35. Taylor D.M., Woodgate S.L. & Atkinson M. (1995). – Inactivation of the bovine spongiform encephalopathy agent by rendering procedures. *Vet. Rec.*, **137**, 605-610.
36. Taylor D.M. & Fernie K. (1996). – Exposure to autoclaving or sodium hydroxide extends the dose-response curve of the 263K strain of scrapie agent in hamsters. *J. gen. Virol.*, **77**, 811-813.
37. Taylor D.M., Fernie K. & McConnell I. (1997). – Inactivation of the 22A strain of scrapie agent by autoclaving in sodium hydroxide. *Vet. Microbiol.*, **58**, 87-91.
38. Taylor D.M., Woodgate S.L., Fleetwood A.J. & Cawthorne R.J.G. (1997). – Effect of rendering procedures on scrapie agent. *Vet. Rec.*, **141**, 643-649.
39. Taylor D.M., Fernie K., McConnell I., Ferguson C.E. & Steele P.J. (1998). – Solvent extraction as an adjunct to rendering: the effect on BSE and scrapie agents of hot solvents, followed by dry heat and steam. *Vet. Rec.*, **143**, 6-9.

40. Taylor D.M., Fernie K. & Steele P. (1999). – Boiling in sodium hydroxide inactivates mouse-passaged BSE agent. In Abstracts of a Meeting of the Association of Veterinary Teachers and Research Workers, 29-31 March, Scarborough. Association of Veterinary Teachers and Research Workers, Edinburgh, 22.
41. Taylor D.M., Fernie K., Steele P.J., McConnell I. & Somerville R.A.S. (2002). – Thermostability of mouse-passed BSE and scrapie is independent of the host PrP genotype: implications for the nature of the causal agents. *J. gen. Virol.*, **83**, 3199-3204.
42. Taylor K. (1995). – Origin of BSE. *Vet. Rec.*, **137**, 674-675.
43. United States Department of Health and Human Services (USDHHS) (1996). – Federal Register, 24 October, Vol. 61. USDHHS, Washington, DC, No. 207, 31 pp.
44. Wells G.A.H., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., Dawson M. & Bradley R. (1987). – A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.*, **121**, 419-420.
45. Wells G.A.H., Hawkins S.A.C., Green R.B., Austin A.R., Dexter I., Spencer Y.I., Chaplin M.J., Stack M.J. & Dawson M. (1998). – Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet. Rec.*, **142**, 103-106.
46. Wilesmith J.W., Wells G.A.H., Cranwell M.P. & Ryan J.B.M. (1988). – Bovine spongiform encephalopathy: epidemiological studies. *Vet. Rec.*, **123**, 638-644.
47. Wilesmith J.W., Ryan J.B.M. & Atkinson M.J. (1991). – Bovine spongiform encephalopathy: epidemiological studies on the origin. *Vet. Rec.*, **128**, 199-203.
48. Will R.G., Ironside J.W., Zeidler M., Cousens S.N., Estibeiro K., Alperovitch A., Poser S., Pocchiari M. & Hofman A. (1996). – A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet*, **347**, 921-925.
49. World Health Organization (WHO) (2000). – WHO Infection control guidelines for TSE. Report of a WHO consultation. WHO, Geneva, 38 pp. ([www.who.int/emc-documents/tse/whocdscsgraph2003c.html](http://www.who.int/emc-documents/tse/whocdscsgraph2003c.html) accessed on 27 January 2003).