BSE AND CJD: SCIENCE, UNCERTAINTY AND RISK

- *The spread of BSE in cattle*
- *Recent concern over possible links to CJD*
- *Assessing the risks and future research*
- *Implications for policy.*

POST **78**
TECHNICAL REPORT **April 1996**

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Recent concerns over the importance of BSE for human health have brought into focus the importance of scientific advice in deciding the public policy response. Yet our scientific knowledge of this disease remains incomplete in both cattle and man. In view of the many uncertainties remaining, there remains scope for disagreement over what measures should be taken, in particular over the extent of any precautionary approach adopted.

This note looks at the key uncertainties, when they may be resolved and the implications for policy in the meantime.

CAUSES OF BSE AND CJD, ETC.

The transmissible spongiform encephalopathies (TSEs) have been known for many years. Their common characteristics are their long incubation period between infection and the onset of disease, and their uniformly fatal outcome through neurodegeneration in the brain, characterised by accumulations of deposits of insoluble proteins (often in the form of plaques) and appearance of 'vacuoles' (holes) which give a 'spongy' appearance (hence the name). As shown in **Table 1**, there are several variants of the disease in different species; in humans, there are transmissible forms (Kuru, transmitted by former cannabilistic practices in Papua New Guinea; CJD, through transplants of infected tissue, etc.), genetically-inherited forms (familial CJD and GSS), and sporadic forms (CJD) whose exact origin is unknown. Although there are differences in incubation period and exact symptoms, the basic process involved in these diseases is thought to be the same.

TABLE 1 DIFFERENT SPONGIFORM ENCEPHALOPATHIES

Species	Disease	First Recorded
Sheep/goats	Scrapie	1732
Man	Creutzfeldt-Jakob Disease (CJD)	1920
	Gerstmann-Straussler -Scheinker disease (GSS)	1936
	Kuru	1900
Mink	Transmissible Mink Encephalopathy	1947
Cattle	Bovine Spongiform Encephalopathy (BSE)	1985
Deer	Chronic Wasting Disease	1967
Antelopes		1980's
Cats	Feline Spongiform Encephalopathy	1990

The **Prion Hypothesis** flowed from much international work over the last 20 years, once it became apparent that it was not possible to implicate any traditional infectious agent containing genetic material (viruses, bacteria, or 'unconventional' viruses) in the TSEs. The predominant (though not yet fully proven) theory is that the key infective agent is the actual protein contained in the deposits present in infected brains. This infectious protein is called a 'prion', and the protein from which it is formed is called prion-related protein (PrP). As described in **Box 1**, normal PrP is produced in brain and other cells (mostly of the nervous system), and the prion itself is an abnormal form of that protein which can catalyse a change in shape (not in basic chemical structure) of the normal PrP. This results in a number of serious consequences. Firstly, the normal function of PrP (Box 1) is clearly compromised. Secondly, the new shape makes the protein 'insoluble' and resistant to breakdown by the natural degrading enzymes (the proteases), leading to aggregates (plaques) building up, thought to disrupt brain function further.

This hypothesis does not yet account for all the characteristics of the disease - some argue that the presence of some co-agent which includes genetic material cannot be ruled out. However, as described in Box 1 the resistance to inactivation of the infective agent and other factors, make this unlikely.

BSE IN CATTLE

Pattern of the disease

The first cases of the disease BSE were diagnosed in 1986 (then, as now, cows have to be diagnosed on the basis of their symptoms confirmed by post-mortem to establish the characteristic 'spongy' brain tissues and associated lesions). The number of UK cases then grew

Box 1 THE NATURE OF THE SPONGIFORM ENCEPHALOPATHIES

Background

The first transmissable spongiform encephalopathy (TSE) was described in 1732 in sheep (scrapie). Subsequently it was discovered that other species suffered analogous diseases (see Table 1 in main text) and that in people there are also rare inherited forms of the disease. All the diseases have common characteristics - a long incubation period, a progressive and invariably fatal course, and common histopathology whereby the brain develops a spongy appearance and amyloid plaques which under an electron microscope reveal abnormal fibrous structures (scrapie-associated fibrils, SAF). These deposits appear to be composed of a modified host membrane protein which is resistant to solvents and enzymes capable of dissolving the normal form.

Brains of diseased animals transmit the infection to other unaffected animals, so must contain the infective agent. Various tests have narrowed it down to a size which is below that of a small virus, and have shown it to be extremely resistant to UV and ionizing radiation, to heat, to chemicals which are effective on conventional microorganisms, and significantly, to enzymes which would attack the genetic material of any conventional virus or bacteria. Although theories have come and gone, the dominant one, which is consistent with most experimental evidence, is that the infectious agent is solely or largely the protein in the deposits in affected brains.

This infectious protein (called a 'prion') has been shown by chemical analysis to be an abnormal form of a protein (called the prion protein - PrP), which is part of normal cells found primarily near the terminals of nerve cells. PrP is thus mostly found in the brain and the rest of the nervous sustem, but also in the spleen, lymph nodes and in lymphoid tissues associated with the gut (the lymphoreticular system). What is different about the abnormal version (PrP^{SE}) is not the primary chemical structure but presumably the three-dimensional shape in which the protein is arranged.

The hypothesis that the abnormal protein itself is the primary infectious agent is supported by the following findings:-

- A protein could exhibit the resistance found, whereas a conventional agent involving genetic material could not.
- Recent in vitro experiments have shown that the presence of PrP^{SE} does trigger the transformation of normal PrP; -once aggregates of the PrP^{SE} are formed, the normal PrP attaches itself to the aggregates adopting the new form.
- Pure PrP^{SE} retains its infectivity.
- Failure to reproducibly identify any nucleic acids specific to scrapie or other TSEs.
- The differences between the two forms of PrP appears to occur after the protein has been produced by the cell, therefore not requiring the involvement of a genetic mechanism.

Proteins, although essentially a linear chain of amino acids, rely on their three-dimensional folded structure for their biological action in the body. The 'bottom line' is thus that the natural protein, after it has been assembled in the brain cells, can fold in more than one way, and it looks as if the normal form is to some extent potentially unstable with an ability to 'switch' to the other form under certain conditions. In the transmitted SEs, it is the presence of the 'seed' in the form of the prion (the abnormal form) which catalyses the switch.

to a maximum of around 4,000 per month in 1993, and have since fallen off to below 1,000 per month (**Figure 1**). Few cows succumb to the disease in less than 3 years, most affected animals displaying symptoms at 4-5 years, while some succumb as old as 18 (the age distribution at the onset of the disease is shown in **Figure 2**).

The source of the disease is now accepted by nearly all scientists as the presence of the infectious prion agent in feed concentrate which included recycled meat and bone meal (MBM). The initial source may have been a particular strain of scrapie from sheep or BSE pre-existing in cattle at a low level[1], which started to survive inactivation in rendering plants when processing conditions were changed in the late 1970s and early 1980s - particularly when solvent extraction processes were discontinued in most plants. At least one SE strain appears to have survived these processes and infected cows, but when parts of cattle with BSE started to be used also in MBM, the 'recycled' BSE prion caused the original infectious agent to be amplified, causing a much more rapid spread of infection.

The inclusion of ruminant-derived protein in feed was banned in July 1988 with the intention of removing the primary source of infection. If that had worked, and there were no subsequent cases of vertical (cow to calf) or horizontal (cow to cow) transmission, as suggested in Figure 2, the incidence of the disease would have peaked 4-5 years after contamination of feed had ceased, and then declined to zero over the ensuing 10-15 years.

While some of the expected pattern has occurred (e.g. the peak in 1992-3 followed by a decline), the rate of decline has been slower because significant numbers of cattle born after the (July 1988) ban have developed the disease. As shown in **Figure 3** over 25,000 'BABs' have contracted BSE, even one animal born in 1993. MAFF epidemiologists maintain that contaminated feed was still the main factor responsible because:

- nothing was done to eliminate the large amount of feed in the distribution 'pipeline', so contaminated feed caused significant amounts of exposure to prion after the ban.
- In the original ban, ruminant protein was prohibited only from ruminant feed and still allowed in feed for pigs and chickens. Subsequently (September 1990), the most potentially infectious parts of cattle (specified bovine offal - SBO) were banned from any animal feed (e.g. for pigs), but sheep and non-SBO cattle residues continued to be used. MAFF also now believe that some SBO material was still

1. While scrapie as the source fits the epidemiological evidence, experiments in the USA to infect cows directly with scrapie (rather than via feed), lead to different types of disease than BSE. Analogous experiments have not been carried out in the UK, so the reasons for these differences are unclear. However, it is also possible that the original source was a form of bovine scrapie - rare cases of 'oxen scrapie'have been reported in the past.

rendered until at least 1995, providing a source of fresh infection. Since the same processing facilities were used for both ruminant and non-ruminant feeds, there was potential for cross-contamination, mislabelling or other operational sources of contamination, between feed containing MBM from different sources. This persisted until March 1996 when mammalian protein was banned from all farm animal (and fish) feed, leaving no route for infectious material to enter the feed. **Table 2** gives the key events and their timing;

But scrapie is endemic in the UK and other countries because ewes infect their lambs by one mechanism or another. Can vertical transmission be ruled out in cattle? Here there is no definitive answer yet. An experiment to establish if such transmission occurs was started under the direction of the Central Veterinary Laboratory (CVL) in 1990. Calves from 315 cows with BSE, and 315 without BSE were held to see if there would be any differences between the incidence of BSE in the two groups. The experiment ends in November 1996 with the slaughter and analysis of remaining animals. So far, 47 calves have succumbed to BSE, but since the experiment is (for scientific reasons) 'blind', it is not known to which category these cases belong. Final results are not expected until 1997.

Meanwhile, MAFF points to statistical analysis of patterns of BSE in individual herds and other information (e.g. calf embryos from BSE cows transplanted into BSE-free cows have so far not developed the disease), as suggesting that maternal transmission, if it occurs at all, is rare. This view is not accepted by all, however, and some scientists argue that the pattern of the epidemic suggests that maternal transmission and other factors than feed contamination may well be involved. Such uncertainties have led **some to call for the CVL experiment to be 'unblinded'**, and its interim results examined, on the grounds that the results are important to inform epidemiological models. MAFF on the other hand argues that to do so could lead to bias and loss of power in the study, and that SEAC monitors the progress of the experiment. Such arguments are not accepted by other statisticians who point to the ability to safeguard quality - e.g. via an independent data safety and monitoring board.

The evidence on horizontal transmission is that cross-infection from one cow to another does not occur - otherwise there would be a different pattern to the disease, with herds affected by BSE suffering more cases rather than the largely sporadic incidence observed.

Are there any other possible routes of infection? In this context, the question of land contamination has been raised, because the endemic status of scrapie may be due in part to the survival of the infectious prion in material (e.g. faeces or placentae) left in the fields, and either eaten by other sheep or transmitted through grazing. Cows, however, do not seem to be picking up the disease in this way. Passage via prion remaining in MBM used as a fertiliser is another theoretical possibility, and for this reason, SBOs were banned from fertilisers in November 1991. Recent measures prohibit the use of any mammalian protein in agricultural fertiliser.

Table 2 CHRONOLOGY RELEVANT TO FEED CONTAMINATION

(a)	*Pre-BSE outbreak*	*(b)*	*Post-BSE outbreak*
1970s	Sheep protein continues to enter ruminant feed	July 1988	Ruminant protein banned from ruminant feed
1970s to 1980s	Processing conditions changed to remove solvent extraction phase in move to continuous processing	Sept 1990	Specified Bovine Offals banned from all animal feed
		March 1996	All mammalian protein banned from all animal feed.

Figure 1 CONFIRMED CASES OF BSE (3-MONTHLY TOTALS)

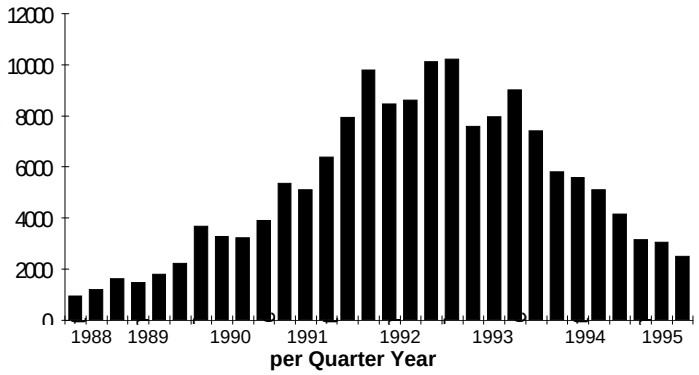


Figure 2 AGES OF BSE-CONFIRMED CASES

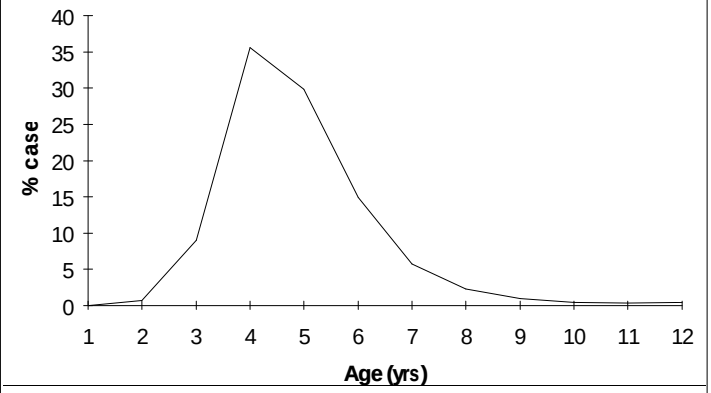
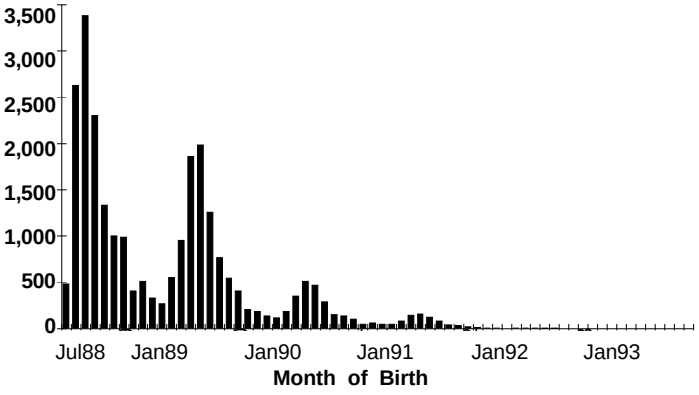


Figure 3 BSE IN ANIMALS BORN AFTER THE BAN (BAB)



Finally, with regard to the possibility of transmission via milk, the WHO has pointed out, not only has infectivity never been detected (even at very large doses) but there is evidence from other TSEs that milk will not transmit these diseases.

Latest Prognosis for BSE in the UK

The most credible scenario is thus that feed took much longer than expected to become non-infective because of the 'hangover' of stocks of contaminated feed, the partial nature of the 1989 ban, and inadequate implementation allowing for cross-contamination of ruminant feed to occur. Since it is now illegal to include any mammalian protein in any animal feed, it is possible to apply a simple test for **any** animal protein, which now allows compliance with the feed restrictions to be tested.

If current measures, when fully implemented, finally remove the source of fresh infections, and there is no significant vertical or horizontal transmission, the disease should die out of its own accord. Predicting the exact rate of decline is however still problematic because of uncertainty over the exact degree of exposure before and after the feed ban. Those calves ingesting large doses would be expected to succumb to BSE in 4-5 years, but those exposed to only low levels of infection could take much longer. Together with the remaining uncertainties over maternal transmission occurring at low levels, there could be a long 'tail' to the epidemic.

Had the initial feed ban been fully effective in avoiding further cases of infection, the disease profile would have been as in **Figure 4**, where it can be seen that the latest figures are only 10% of those at the peak of the epidemic in 1993, and the disease could have declined to very low levels in a few more years. In reality, therefore, **the 'born after the bans' have significantly extended the epidemic**. Assumptions made by MAFF in forecasting the ultimate fate of the epidemic have not been published, nor have MAFF released any 'scenarios' based on different assumptions made on the degree of exposure after the ban. Statisticians point out that the unavailability of such data impedes modelling and prediction of BSE and of CJD in humans.

Recently, attention has been given to options for faster eradication - for instance slaughter of cows over a certain age, slaughter of all cows in any herd affected by BSE etc. The difficulty is that because of the uncertainty over exposure, there is no way of identifying the relatively small proportion of cows which have become infected. As a result, any slaughter policy would either have to be draconian (e.g. eliminating all cattle more than 3 years old), or still carry a risk of leaving cattle with a continued risk of succumbing to BSE.

Options include using the previous BSE record as a surrogate indicator that contaminated feed was used at some stage on a farm, and which might therefore harbour infected animals. Had there been a clear 'cut-off' in infectivity at the 1989 feed ban, only those cattle born before then would need to be considered at risk, totalling some 800,000 animals. However, this is not a safe assumption and cases of BSE in previously-unaffected herds and in animals born after the ban continue. MAFF points out that the disease is fading out in older animals, but there are still significant numbers of BAB cases which are succumbing after the typical 4-5 years incubation period suggesting exposure to contaminated feed around 1990-2. It is thus proposed to trace back (using the birth records system introduced in September 1990) all animals born in these affected herds from Sept 1990-3. The numbers in these categories would be nearer 40,000 (**Table 3**), and their removal might reduce the incidence of BSE by 15-30% over the rest of this year. The effects of other slaughter options on the forecast outcome of the epidemic and, critically, on the date by which BSE could be expected to be eliminated, have not been published.

Figure 4 CASES OF BSE WITHOUT BABs

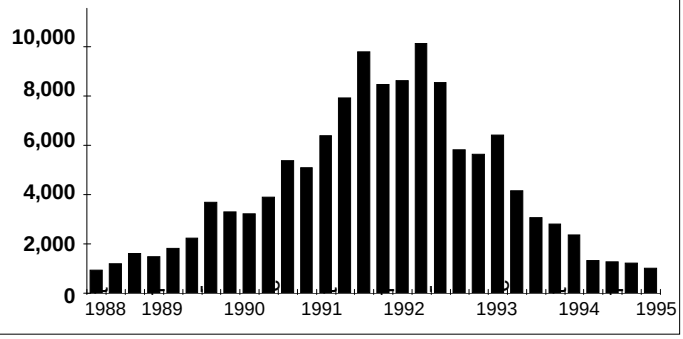


Table 3 NUMBERS OF CATTLE IN VARIOUS CATEGORIES

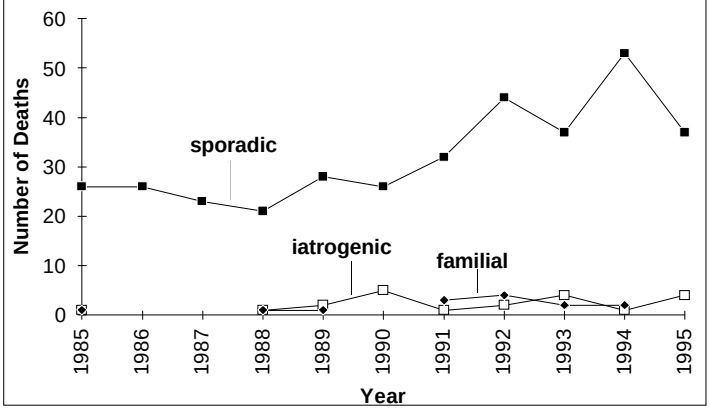
Category	No. Animals (M)
Whole UK herd	12
All herds affected by BSE (59% of dairy and 15% of beef herds)	5
All animals over 30 months	4.5
Animals over 7 only (i.e. born before feed ban) from BSE-affected herds	0.8
Animals from herds with BAB cases born after September 1990	0.04

BSE Abroad

There have been many claims that BSE exists in other countries, but is not recognised as such. Such data as exist suggest that cases outside the UK are not numerous (MAFF quotes 205 for Switzerland, 13 for France, 123 for Ireland, 31 for Portugal, as well as cases in imported cattle in Denmark, Germany and Italy). Compared to the UK's over 150,000 cases, even major under-reporting would not bring other countries into the same league. Overall, the UK BSE epidemic required at least four contributing factors, and no other country is believed to have had all these operating at the same time:-

- Sheep with endemic scrapie;
- Sheep offals reprocessed into ruminant feed;
- Rendering processes which allowed the scrapie and

Figure 5 CASES OF CJD 1985-1995



BSE agents to survive;

- Amplification of the initial cross-species transmission via recycling cattle residues into cattle feed.

TRANSMISSION TO HUMANS

The 'new' Cases of CJD

CJD occurs throughout the world at low levels. Cases fall into one of three main groups: -

- **an inherited form** (familial);
- **iatrogenic cases** where the agent has been transmitted through transplants, contaminated instruments, or materials (such as human growth hormone prepared from human pituitary glands) which have included material from people infected by CJD;
- **sporadic cases** whose exact cause is unknown.

Following a recommendation of the 'Southwood Report' in 1989, the CJD Surveillance Unit at Edinburgh was set up to monitor the incidence of the disease. The trends in overall detection of the disease are shown in **Figure 5**, from which it can be seen that cases of sporadic CJD identified have increased, with 1994 numbers double levels typical of the latter half of the 1980s. Even these higher levels are not out of line with other countries' overall rates, and the likelihood that a dedicated notifications and surveillance scheme would reveal more cases, meant that such variations were not indicative in themselves of a link with BSE.

However, within these overall trends, there are 12 cases in the last 2-3 years which differ from the normal types of CJD in a number of ways, and are more like Kuru viz:

- they occur in much younger patients - the average age of the first ten cases was 26-27, ranging from late teens to 42;
- the progression of the disease is slower than with CJD, with different symptoms (the new cases started with anxiety and depression rather than just forgetfulness and uncharacteristic behaviour);
- the patterns of lesions in the brain are similar to each other, but different from 'normal' CJD, particularly in the amount of abnormal protein deposits (Kuru -like plaques).

It is possible that these forms of 'new' CJD do represent a form present but not recognised before, but the Spongiform Encephalopathy Advisory Committee (SEAC) concluded this was unlikely and was unable to identify any explanation for these cases from patient history, genetic analysis and other possible causes, and "*concluded that the most likely explanation is that these cases are linked to exposure to BSE before the introduction of the specified offal ban in 1989*".

Because mice succumbing to TSEs display characteristic incubation periods and lesion types, they may indicate the type of TSE involved. At the Institute for Animal Health's Neuropathogenesis Unit at Edinburgh, brain samples are being screened against five strains of mice whose response to different TSEs is known. The results will show if 'new' cases of CJD resemble normal CJD or share characteristics with BSE. If the origin is BSE, it is likely that one of the mice strains will develop the disease in a year; if it is normal CJD, it is likely to be longer. In the latter case, the possibility would still exist that BSE was involved but had changed its characteristics upon its passage through the human host.

A second approach is to use transgenic mice (see next section) which possess human PrP and are thus more susceptible to CJD, with an incubation period of ~200 days. Transmission of BSE to mice produces a characteristic disease signature, and so it may be possible to distinguish human cases that have resulted from BSE exposure from normal CJD. However in order to interpret such findings, it will also be necessary to see if 'normal' CJD has more than one strain. Large-scale studies are now underway at Imperial College School of Medicine at St Mary's, to investigate these issues.

Both approaches may lead to conclusive results, but there is a chance that the results will be open to multiple interpretations, extending the uncertainty further.

THE ROLE OF GENETICS IN TSEs

As described in **Box 2**, under the prion hypothesis, inherited forms of SE (familial CJD and GSS disorder) derive entirely from mutations in the gene responsible for making PrP, and do not require the person involved to be infected to contract the disease. In the case of GSS, just one mutation in the 230 amino acid-long 'backbone' of the PrP appears to render the protein liable to change spontaneously over time. The GSS variant is, however, very rare, and the question arises whether there are different susceptibilities to TSEs among people with other more common variants (polymorphisms) in the PrP gene.

The most common PrP gene polymorphism is in the amino acid at position 129, where methionine or valine can be present. Since everyone inherits one copy of

Box 2 GENETIC FACTORS

The Role of Genetic Variations

The PrP gene contains the 'blueprint' to manufacture the PrP protein, whose 'backbone' is a chain of 230 amino acids. In most genes, there are natural variations (called polymorphisms) in the exact composition (and therefore the protein it produces), both within the same species and between species. With the PrP gene, the exact sequence differs between mouse, hamster and humans in around 10% of the chain, with more differences between the sheep and bovine equivalents. Much work has thus gone into defining how these subtle changes affect the infectivity and behaviour of the infection.

In humans, the most extensively understood mutation is that associated with GSS disorder. Here, a single mutation in the protein sequence (GSS is a mutation whereby leucine is substituted for proline at position 102 of the human PrP gene) renders the PrP susceptible to spontaneous change and leads over time to the onset of the disease. That this is sufficient has been demonstrated by inserting the GSS gene variant into mice, which causes them to develop the disease.

Other genetic variants interact with the transmitted SEs - either increasing or decreasing susceptibility to them. The picture is far from clear at this stage, but some findings from genetic work are:

- because the PrP protein is common to many species, it is assumed to have an important role in cellular function. However, one mouse strain with a disrupted PrP gene which therefore does not produce the protein, develops normally but has disrupted sleep patterns. Also because it lacks the PrP protein, it has complete resistance to the TSEs.
- Some TSEs have many different 'strains' (e.g. scrapie has over 20), which differ in their incubation period and the detailed structure of the lesions in affected brains. These strains may reflect different variants of the PrP gene in different breeds of sheep.
- The differences in ease of infectivity between species (the 'species barrier') is also probably due to the fact that prion from one species differs in from the PrP in the species being infected. Thus BSE infects mice with much greater difficulty than with the same species (calves). CJD infects humans more readily than mice etc. Such difference are significant - e.g. mice are at least 1,000 times less susceptible than calves to the BSE prion.
- When the prion from a different species infects another, it is often with difficulty, as evidenced by a long incubation period. However, when the infected brain is then used again in the same species, the new prion is more infective. This suggests that on 'passage' through a different species, the prion protein becomes closer to the host PrP, ultimately 'evolving' to a form indistinguishable from the species' own prion.

Transgenic Animal Experiments

Because of the potential gravity of transmission of TSEs between species (as has already happened between sheep and cows, and also into deer, cats, etc.), further work on understanding the nature of the 'species barrier' is important. One approach uses transgenic mice, which, by incorporating brain material from other species in the mouse, can offer a 'test bed' for the selectivity and preferences of different forms of prion (BSE, Scrapie, CJD, etc.). In one set of experiments, mice have been provided with both hamster and normal mouse PrP genes, another model (the one employed at St Mary's Hospital Medical School) has developed mice with human PrP genes, to allow the relative responses of these proteins to be compared to incoming infective agents.

Relevant results are that:-

- These tests confirm that the prions originating from a different species have greater difficulty infecting another species. Thus a mouse prion will have much greater difficulty converting the hamster PrP than mouse PrP.
- The mouse/human protein combination has been challenged with BSE and so far this has resulted in conversion only of the mouse PrP, not the human one, suggesting that the BSE prion is closer to the mouse PrP than human PrP. However, this does not mean that it is not capable of converting human PrP, merely that it is more difficult to do so.

Such experiments also shed light on what is happening when one form of prion enters another species and then goes on to infect others. For instance, when mice are infected initially with a hamster prion, the incubation period is long (400 days), indicating that the hamster prion is having difficulty converting the mouse protein. However, when the infected brain is used to provide prion to infect other mice, this barrier is reduced, suggesting that the prion has changed to one closer to the mouse version. This is why even though scrapie started the BSE problem, by the time the BSE had passaged into the cow and been recycled it had become a different agent, moreover one that was substantially more infectious to cows because it had become adapted to that host's brain proteins.

every gene from each parent, about 10% of people have both copies containing valine (VV), 50% have one of each (MV), and 40% both methionine (MM). The 8 'new' CJD cases on which measurements have been made are all the MM variant, while previous work has shown that those most susceptible to catching CJD from infected transplants tend to be VV. It appears likely therefore that the heterogeneous combination (i.e. MV) has some level of protection relative to those with two copies of the same gene for this protein. It is possible that there are, however, other common genetic factors at work, since the gene includes several sequences which do not code fro PrP protein but which may still be important to regulating how much, where and when PrP is produced. Each of these factors could be involved in susceptibility to these diseases, and work to explore this is currently under discussion.

DIAGNOSIS AND TREATMENT

The unusual nature of the infective agent makes its detection very difficult, whether in cattle or man. Since the 'rogue' protein is the same basic chemical structure as the normal one and no genetic material has been found to be involved, there are no simple tests for its presence in feed, in cattle, or in humans. Even now, the only way of diagnosing an affected animal (or person in the case of CJD) was by the symptoms of the disease, confirmed by examining the brain after death. The only way of testing for the presence of the infective agent in

tissue, animal feed, etc. is to inject a sample into the brains of experimental animals (generally mice) and wait through the incubation period to see if the animals develop the disease. In the case of BSE, this takes two years to demonstrate a likely negative result, though strong infections would be detected earlier (200-400 days).

The lack of a test to give immediate results has hampered much of the basic and applied research which has been carried out, and has precluded some important research because of the logistics, time and expense of the bioassay. A more applicable test has thus been on the research agenda of the SEAC from the beginning of the BSE outbreak, and MAFF and BBSRC have supported work in this area.

Any test for PrP must detect the shape, not the composition of the PrP, or it must discover some second-order but specific consequence of the abnormal protein's presence (e.g. a different metabolite), or symptoms such as early neuro-physiological changes resulting from the disease. Some progress has been made in faster screening methods for assessing brain lesions at post mortem (e.g. at CVL), but this still cannot be applied to living cases. It is possible to distinguish between normal and abnormal PrP on the basis of their relative resistance to proteinase digestion, followed by detection of remaining PrP with antibodies. Unfortunately, it is not easy to detect PrP^{BSE} in accessible tissues or fluids. Attempts to find some 'tell-tale' in urine, cerebro-spinal fluid (CSF) or blood have also been underway. The CVL is patenting a urine test which may aid diagnosis of BSE at the end phase of the disease, though its use as a predictive tool has yet to be validated. Other approaches (e.g. to look for signs of scrapie-associated fibrils) may hold promise. Very recent work in the USA also claims that there are 'indicator' proteins in the CSF of CJD sufferers which can help differentiate CJD from other forms of dementia, but it is not yet clear how much notice this can give ahead of the onset of clinical symptoms, and the work would require independent validation.

Another consequence of the prion causation theory is that many 'traditional' cures will not work, since the infective agent is not recognised as foreign by the body's immune system, and is not vulnerable to antibiotics, antivirals etc. The question of a cure for cattle has never attracted a high priority, but now that the possibility exists that BSE may affect humans, the issue of treatment is attracting more attention[2]. It is possible to conceive of a number of approaches - for instance, there is some evidence that the abnormal protein only works on the natural form after aggregates have been formed, so something that prevented the initial aggregation of abnormal PrP could offer protection; equally if PrP^{BSE} differs in the sugar molecules attached to the amino-acid 'backbone', enzymes which interfere with these might have an effect. Chemical substances which can differentiate between the two forms of PrP and interfere with the change of PrP to PrP^{BSE} could work. It appears from mice that the agent travels up nerves, so substances which interfere with neural transport could also delay the prion or prevent it reaching the brain. Trials in mice have also shown that some agents (e.g. strong antifungals, congo red dye) can delay infection. Growing PrP in cell culture also offers a means of screening for active substances, and some (e.g. pentosan or heparin sulphates) can inhibit the prion conversion *in vitro*. However, this and other lines of research depend on long-term basic research to develop a better understanding of the mechanism of disease transmission and of susceptibility to and progression of disease.

ISSUES

Assessing Risk to the Population

Principles

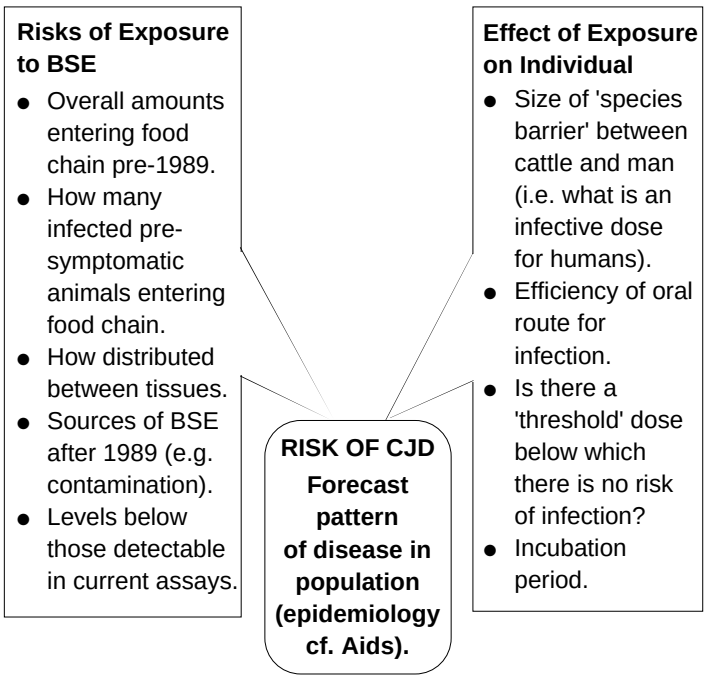
The Southwood Report and subsequent scientific advice, while finding no evidence that BSE was being transmitted to humans, could not rule out the possibility that infection was occurring or would occur in the future. Now the presumption has been reversed, with the most likely explanation of the new case types of CJD being that they had a BSE origin.

In the public and political debate, there has been a tendency to equate the absence of any evidence of risk with a conclusion that there is no (or minimal) risk. The political need to rely on qualitative terms such as 'safe', 'extremely low risk', etc. derives from the fact that precise and reliable quantitative assessments of the risk are not available. In other fields - particularly those of workplace safety and environmental protection, quantitative risk assessment (QRA) is a well-established process and allows first the risk to be properly evaluated, secondly the costs of avoiding or reducing it to be calculated, and thirdly these costs compared with the value of the resulting benefits (cost-benefit analysis). Such data can be used to inform policy on what amount of society's finite resources should be devoted to avoiding that particular risk, or whether resources should be focused on other areas bringing greater rewards.

Where a risk cannot be quantified, bounds of uncertainty can be constructed to allow different scenarios to be developed to inform policy-making. For instance, if a risk is known to lie between a lower and upper value, scenarios could be developed to see what the consequences (e.g. to human health) would be of making the

2. The Association of British Pharmaceutical Industries, Zeneca and the MRC are organsising a workshop to discuss possible treatment and research strategies.

Figure 6 RISK ANALYSIS FOR BSE / CJD



assumption that the risk was low when in reality it was high. By comparing the health costs of such a scenario against the costs of preventing it, a judgement could be better reached of whether the precautionary principle (i.e. assuming the 'worst case' scenario) should be justified in that particular case. How might such an approach work with BSE in the two main areas where a more quantitative approach might apply?

Preventing all sources of new infection to cattle

With the benefit of hind-sight, there were several areas where a comprehensive RA could have helped avoid or contain the problem of feed contamination by prions. A risk assessment at the time of process changes by the renderers could have laid the burden of proof on the industry to demonstrate complete deactivation of all potentially infective material. But having decided in 1988 the principle that ruminant protein should not be fed to ruminants, a more formal RA would have identified old stocks as a significant loophole which would extend the period of infection and delay the eradication of the disease. A risk analysis should also have revealed scope for continued secondary contamination through continuing to allow ruminant protein in non-ruminant animal feed. The limitations of enforcement could have also featured in the assessment, and the 'safety net' advantages of simpler but enforceable bans (as applied since March 1996) weighed against the costs of the additional measures.

A risk analysis conducted now might well conclude that all remaining routes of feed contamination had been closed, and that the means now exists (through the mammalian protein test) to enforce it. **The question of what to do with old stocks however remains**. Moreover, key questions on maternal transmission are still unanswered, complicating further the calculations of the rate of decline in the disease. Another possibility which could extend the period during which BSE remains active is if the BSE agent were to infect sheep - either because of earlier contact with contaminated feed or through other means. In this case, the BSE prion might survive longer due to the ability of sheep to pass on scrapie to their offspring.

Risks to Humans from BSE Exposure

Assessing the risk to humans is more complex and requires information on the extent of exposure and the effects of ingesting different amounts of BSE; some of the main uncertainties are summarised in **Figure 6**. As SEAC pointed out in its report to Ministers on March 24, 1996, a precise measure of risk is currently not possible because much of the information required to carry out a formal risk assessment does not exist. Key uncertainties are summarised below.

Exposure. Since the disease was first recognised, a number of measures have been introduced to minimise human exposure (see **Table 4**). Before the 1989 offals ban, the main route of human exposure would have been from the most highly infective tissues (brain etc.) in infected animals which had not been diagnosed as having BSE (and thus entirely removed from the food chain). Unfortunately there are no data on which to base an assessment of the numbers of presymptomatic infected animals reaching the abattoirs. Such a survey was recommended by the Tyrrell Committee in 1989, but was not carried out, at least partly because of the logistical difficulties associated with the mouse bioassay[3]. The best prospect of shedding light on the foods posing greatest risk at the time, would be an 'audit trail' of the most infectious material, based on knowledge of the food processing industry.

After all suspect BSE cases were destroyed (from August 1988) and after the November 1989 offals ban, most accept that the risk and levels of human exposure will have declined substantially. However, residual infection could still enter human food if low levels of infectivity were present in the tissues from infected animals still used. In this context, some of the tissues subsequently found to contain infectivity (e.g. retina, intestine in calves under 6 months old) were not banned straight away and could have provided a route for small levels of infectivity. Other tissues still reaching the food supply have all shown zero infectivity (see **Box 3**) in the mouse bioassay, but this has limited sensitivity because of the species barrier to infection, so that, low levels of PrP^{BSE} would not be detected. The possibility that non-SBO tissues contain some levels of the prion can thus not be evaluated.

3. This proposal was awarded only a 'low priority' ranking; in the event, other projects awarded 'medium priorities, including a large-scale study of the effects of rendering conditions on infectivity requiring large numbers of animals, were supported by public funds.

Table 4 SUMMARY OF MEASURES TO MINIMISE EXPOSURE TO BSE

Aug 1988	**All suspected cases destroyed**
Nov 1989	**Specified Bovine Offals from cattle over 6 months of age banned from human food**
Nov 1994	**SBOs extended to include thymus and intestine from calves of any age**
Aug 1995	**SBO extended to include eyes**
Dec 1995	**Ban on mechanically recovered meat**

New 1996 Measures

- **Whole head (apart from tongue) and lymph glands classed as SBO.**
- **All animals over 30 months to be deboned in special plants (until plants ready, animals not to enter human food).**
- **Additional inspections of abattoirs etc.**

A further potential route of infection is via contamination of meat destined for human consumption in the abattoir. Here inspections by the State Veterinary Service and Meat Hygiene Service during 1995 revealed that practice fell short of the standard required in a significant proportion of cases, and the potential existed for cross-contamination (e.g. from inadequate separation of SBO material, remnants of spinal cord attached to carcases, leaking of brain onto meat through the stun gun hole). The degree of exposure to BSE from these routes is likely to be sporadic, but a risk analysis could determine if it is also likely to be rare - again the risk will be related to the number of infected cattle reaching the abattoir.

Knowing how many infected animals enter the system is thus central to any estimate of exposure, and underlay the Tyrrell Committee's recommendation in 1989 that appropriate data be collected. Options could have included separate scrutiny of random samples of brains of cattle sent for slaughter to find out if any had recognisable effects of BSE. More sensitivity would have been achieved by mouse bioassay (as envisaged by Tyrrell). Statisticians point out that such data would assist not only in estimating possible human exposure, but also in providing age profiling on affected animals to inform projections of the future course of the epidemic. Had infectivity tests shown that PrP^{BSE} was rarely present in cattle reaching the abattoir, this could also have affected the degree of public concern in recent weeks over the risks of eating beef.

While eating infected cattle products remains the prime candidate for exposure, occupational exposure - whether to infected feed or animals- is a potential risk factor for some. Since MBM has continued until now to be included in feed for pigs and chickens, a risk analysis could also consider whether there is any mechanism whereby these could provide a pathway for significant human exposure. Another theoretical possibility is that BSE could be 'recycled' into sheep, thereby opening up a new route of transmission through sheep offals.

When it comes to estimating the effects of exposure to BSE on human health, the key questions are what is an infective dose for humans, and how does the disease progress once infection has taken place? As already described, the TSEs are characterised by a species barrier whereby a prion from one species has greater difficulty in infecting another species than its own. Much work has been done on this 'gap' for BSE, and laboratory work has been reassuring in suggesting that BSE does not readily infect primates and man. For example, BSE is consistently more transmissible to cattle than ordinary mice, which are in turn more susceptible to BSE than mice containing human PrP genes (Box 2).

Since many scientists believe that the species gap is related to the differences between the infecting PrP and that of the host, the degree of similarity (or difference) between the Host PrP gene and that of the species from which the prion emerged is relevant. In this respect, the differences between the human and bovine PrP gene are known at the molecular level, but at present levels

Box 3 PROGRESS OF THE DISEASE AND RELATIVE INFECTIVITY OF CATTLE TISSUES

Much work has been carried out into how the infectious prion moves from the gut after ingestion and, much (often years) later, reaches the brain and affects its fatal changes on the host's PrP. In mice, the pathway is reasonably well understood :-

- initial infection in the gut leads to replication in lymphoid tissue within the gut wall,
- further spread and replication to the spleen and lymph nodes,
- movement from the spleen to the spinal cord via the splanchic nerve,
- movement up the spinal cord to the brain.

In cattle, less detail is known, but infected calves do display infectivity 6 months after eating BSE-infected brain in the distal ileum (the end of the short intestine). This infectivity may fade and none be detected until later stages of the disease when the BSE prion is present in the brain and cervical spinal cord. It may be reasonable to suppose however, that similar pathways occur, and that the BSE agent may also replicate in the lymphoid tissues, spleen and parts of the nervous system, but that the mouse bioassay test is too insensitive to detect the presence of low levels of prion. As yet incomplete bio-assays in cattle have not yet detected BSE infectivity in spleen or lymph nodes.

The outcome of a large number of screening tests on various cattle tissues is that infectivity can be detected in the mouse tests in the following tissues:-

(a) In clinically affected cattle:
- brain
- cervical and terminal spinal chord
- retina

(b) In calves infected orally (in experiments):
- the distal ileum of calves (the lower short intestine)

No infectivity has been detected in the following:-

- milk (at very high dosage rates)
- blood
- bone-marrow
- gastro-intestinal tract
- heart
- tonsil
- kidney
- liver
- lung
- muscle
- lymph nodes
- nerves
- reproductive organs
- skin
- spleen
- trachea

of knowledge, this does not allow us to predict what degree of barrier to infection this poses[4].

Another 'safety margin' comes from the fact that most infection is transmitted in test animals by direct administration into the brain, and intracerebral injection is clearly going to be much more efficient than other routes. Tests show that the efficiency of transmission declines in the following order:-

- intravenous injection
- injection into the abdominal cavity
- injection under the skin
- oral ingestion by eating infective material.

The latter route (ingestion) is some 10,000 times less efficient than intracerebral inoculation, although this does not necessarily apply to all strains, particularly to those which have developed via the oral ingestion route.

Such findings, though reassuring, are not conclusive and there are other questions to be asked for a QRA: -

- What is the minimum infective dose?
- Can it be 'fractionated' - i.e. delivered in smaller packets, and if so, does the time interval between doses matter?
- Are there genetic susceptibilities in the population (see Box 2).
- What is the incubation period likely to be relative to CJD?
- Do secondary factors affect the vulnerability of individuals to infection via the gut (e.g. alcohol, medicines affecting the permeability of the intestine, illness).

Were data appropriate for a risk analysis to be available, these could allow epidemiological models to be developed to predict the numbers of cases and their pattern of distribution. This is not possible, but much could be deduced if the incubation period of the 'new' cases of CJD (assuming they are of BSE origin) were known[5]. The clinical onset of the 'new' CJD cases was in 1994 and 1995 so that if the incubation period is 6-8 years, it would suggest the cases reflect exposure during the 'peak exposure' years of 1985-9, which might suggest that current cases represent the maximum number likely to be encountered. If on the other hand, the incubation period is more like 10-15 years, the people concerned could have been infected at the beginning of the epidemic when levels of infectivity were only beginning to rise; in this event, more cases would be expected in fuure. Epidemiological statisticians point out that much more detail needs to be made available on the individual characteristics of the 12 key cases to try and establish epidemiological models to fit the emerging patterns, and narrow the future uncertainties over the likely course of the disease. For instance, could some of the cases have come from direct exposure to contaminated feed or animals rather than eating beef? In this respect, several scientists have pointed to the experience gained from AIDS epidemiology, which is also characterised by a long incubation period, and have recommended that **MAFF/DH should urgently involve experts from this field**.

A RA would also have to consider the consequences of CJD entering the human population in significant numbers and the measures which would be necessary to safeguard transplant and transfusion work[6].

Uncertainties and the Precautionary Principle

From the discussion above, it is clear that there have been substantial uncertainties over the significance of BSE to human health from the outset, and that many of these remain. This has led to questions over how policy formulation should deal with such uncertainty.

In the environment field, scientific evidence of a possible link between human activity and an environmental impact is often merely suggestive and not conclusive at early stages of investigation - examples being the initial suggestions of a role of DDT and similar pesticides in the decline of birds of prey, of the role of CFCs in ozone depletion and of sulphur and nitrogen oxides in lake acidification. The science in such cases proceeds through initial suggestions generating a case for further investigation, hypotheses of cause and effect, challenge by governments and industry, and growing scientific consensus ultimately leading to national or international controls to remedy a problem taken as scientifically proven. Because of this experience and the wish to avoid the danger that serious environmental damage might occur while 'proof' was obtained, international organisations, including the EU have developed the Precautionary Principle - generally accepted to mean that where there are threats of serious or irreversible damage, lack of full scientific certainty should not be used as a reason for postponing cost-effective measures to prevent environmental degradation.

The evolving evidence on BSE is an area where the PP can be seen to apply in view of the large areas of uncertainty involved. Thus scientific evidence has never ruled out the possibility that BSE could be transmitted to humans. Equally, even now it has not been positively proven that BSE does afflict people. It has

4. Very recent work shows some sequences of the bovine and human PrP gene share some common features not shared by sheep PrP genes, leading to speculation that humans could be more susceptible to infection by BSE than scrapie.

5. Current data on CJD incubation periods only come from iatrogenic transfer which may not be a good guide for ingestion route, but which ranges from 18 months to 25 years, while the incubation period for Kuru is mostly 5 to 15 years, but can be as long as 30 years.

6. In this respect, of the 2,000 people who received human growth hormone extracted from human pituitary glands until 1985, and which may have conained glands from people with CJD, 17 have contracted CJD so far.

thus been necessary for policy-makers to decide how far beyond established scientific proof it is prudent to act. Some policy has included a precautionary element - e.g. the offals ban was recommended by the Southwood Committee originally only for baby food but applied by MAFF to all human consumption. However in other areas such as regulation of feed, measures have followed a step-wise approach, acting when scientific evidence pointed to problems already existing.

The existence of such large areas of uncertainty requiring policy judgements has caused some to question the responsibilities allocated to SEAC and its role in the policy-making process. As an expert committee, it is constituted to analyse objectively matters on which there are objective data, but as seen above, BSE and its implications for human health are areas where quantitative risk assessment has not been able to be applied, so that it can be difficult to draw the line between sound scientific conclusions and informed judgement. Moreover, the scientific assessment of risk is only one factor. The consequences for the beef industry of an over-cautious approach, and the acceptability of risks to human health of inadequate measures are questions which involve more than scientific judgement. Thus some have argued that there should be a clear separation between scientific advice and policy-making. In this way, the scientific uncertainties would be more explicit and the many other public interests involved could also contribute to the decision-making process, and scientific statements such "there is no evidence so far" would not be equated with a pronouncement of "zero" or "inconceivably low risk".

In the light of recent events, it can be expected that some will argue that the PP should have been applied more consistently to protect human health interests at an earlier stage. However irrespective of whether this is a valid observation, current measures under debate concerning the slaughter of cows involve different degrees of the PP in so far that these measures do not follow directly from a scientific assessment of the **current** risk to human health. If such measures are to be seen as other than 'cosmetic' with the consequent danger that they will be ineffective, a debate between the scientific community, farmers and public health interests may be needed to reach a consensus on the objectives of such a policy, how to achieve them and how to measure their achievement. By defining such objectives, options other than slaughter could also be considered. For instance, if the objective is to reassure the public there is no PrP^{BSE} reaching the slaughterhouse, this might be more convincingly demonstrated by analysis than by removal of certain categories of animal from the food chain. If the objective were to eliminate BSE from the UK cattle population, then an intensive survey of the incidence of PrP^{BSE} in animals of different age groups could restrict the measures to those animals with a significant risk of carrying the infection, but this awaits a reliable test.

Research Needs

One of the main functions of SEAC since its establishment has been to advise on research needs, and the overall public spend on BSE-related research is listed in **Table 5**. The inherent difficulties in working in this area have meant that, despite much research since the start of the epidemic, questions still outnumber answers in many key areas. Indeed, while uncertainties still exist as to the exact nature of the infective agent and how it exerts its effects, more applied work is bound to be handicapped.

Table 5 CURRENT LEVELS OF UK RESEARCH FUNDING (£M1996)

Funding Source	Amount	Comment
MAFF	6.4	CVL, NPU, IAH and others
BBSRC	2..4	NPU, IAH
MRC	0.68	Neuropathogenesis Unit (NPU)
DH	0.22	CJD surveillance, NPU

Bodies such as the MRC and BBSRC stress the need for long-term fundamental and applied research, building on existing research excellence, while encouraging new collaborations between UK centres of excellence and other teams able to bring new approaches to bear on the increasingly tractable problems. Given the scale of work on understanding the nature of prions abroad (e.g. by Professor Prusiner and co-workers in the USA, and Professor Weissmann in Zurich), increasing openness and collaboration between the groups rather than competition is required. In this respect, the Royal Statistical Society has called for a more open debate on the appropriate methods to be used in projecting the possible course of both BSE and CJD; this will also require more of the basic data to be made available to allow independent statistical evaluation.

Other generic points on research are that because of the very long time taken for much of the applied work (e.g. arising out of the bioassay methods), flexibility has to be shown in meshing the necessary research work to the normal 3-year contract offered by MAFF or DH. In this respect, the BBSRC and MRC institutes with their longer term horizons can have an institutional advantage. These maintained a capability in the prion diseases when they were not seen as priorities by departments, and the Royal Society points out that this should be borne in mind in the current review of such institutes for privatisation (see POSTNote 74), since it doubts whether the private sector would have supported such long-term work before the short-term need in the form of the BSE outbreak became apparent.

Returning to research priorities, the SEAC in 1995 identified a number of areas as including continued

unanswered questions:

- a test for infectivity was still an urgent priority;
- research to better understand the nature of the agent;
- how the PrP turns into PrP^{BSE};
- is PrP^{BSE} sufficient to cause disease;
- what is the role of normal PrP;
- resolve remaining uncertainties on transmission;
- origin of cases of sporadic CJD;
- future fate of BSE epidemic;
- lessons from overseas cases of BSE;
- what accounts for distribution of scrapie;
- inactivation in rendering processes.

Priorities have also been identified at recent BBSRC, MRC and IAH reviews, including:

- further understanding of the prion proteins, (e.g. exploiting recombinant systems and in vitro conversion of PrP to its pathogenic form;
- elucidating the normal function of PrP in the brain, the LRS and elsewhere;
- influences of genetics on susceptibility, disease progression and neuropathology;
- use of transgenic mice to understand mechanisms of transmission and pathogenesis;
- the biological relationship between CJD and BSE;
- rapid and robust diagnostic tools - both for animals and humans suspected of infection. This is particularly important because of the possible need for a screening test arising out of concern over possible transmission through blood transfusions and transplants[7].
- patterns of CJD infection and infectivity before onset of clinical symptoms;
- understanding the causes of sporadic CJD, perhaps using mouse models of the disease;
- BSE development in sheep, its transmission and infectivity of tissues.

The BBSRC has a coordinated programme since 1990 (the Biological Spongiform Encephalopathy Programme (BSEP) (BSEP 1 ran from 1990 to 1994, and BSEP 2 from 1995 to 1999). The Medical Research Council (MRC) has issued a call for proposals to research into TSEs.

Since the concern raised by the new cases of CJD, the Government has asked the DH Director of R&D to develop a coordinated research plan involving DH, MAFF, BBSRC and MRC. All four are developing complementary plans for new research, based on their particular missions and expertise. Research towards in-life diagnosis, treatment and prevention are likely to be emphasised, with the DH and MRC focusing on the fundamental and applied research of particular relevance to human health. The DH has provisionally allocated an additional sum of £5M over and above the existing combined £9M p.a. already allocated (Table 5). This plan is not yet published, but a more formal risk assessment would assist in identifying those uncertainties most critical to our understanding of the risks, and thus to guide research priorities.

7. Some have suggested that while the Edinburgh Unit should remain the sole centre for CJD epidemiology, other centres might be engaged in the clinical assessment of cases, and there is a proposal that the St Mary's Hospital Unit of Imperial College might form the focus for studies of possible diagnostic or treatment approaches involving suspected CJD cases in the Southern half of Britain.

GLOSSARY OF ACRONYMS

BABs	Born after the Ban (July 1988)
BBSRC	Biotechnology and Biological Sciences Research Council
BSE	Bovine Spongiform Encephalopathy
BSEP	Biological Spongiform Encephalopathy Programme
CSF	Cerebro-spinal fluid
CJD	Creutzfeld Jakob Disease
CVL	Central Veterinary Laboratory
DH	Department of Health
EU	European Union
GSS	Gerstmann-Straussler-Scheinker disease
IAH	Institute for Animal Health
LRS	Lymphoreticular System
MAFF	Ministry of Agriculture, Fisheries and Food
MBM	Meat and Bone Meal
MRC	Medical Research Council
NPU	Neuropathogenesis Unit
PrP	Prion-related protein
PP	Precautionary Principle
QRA	Quantitative Risk Assessment
RA	Risk Assessment
SBO	Specified Bovine Offals
SE	Spongiform Encephalopathy
SEAC	Spongiform Encephalopathy Advisory Committee
TSE	Transmissible Spongiform Encephalopathy
WHO	World Health Organisation