Risk Analysis, Vol. 25, No. 3, 2005 DOI: 10.1111/j.1539-6924.2005.00619.x

Perspective

Transmissible Spongiform Encephalopathy Risk Assessment: The UK experience

Eric P. M. Grist*

Transmissible spongiform encephalopathy (TSE) risk assessments undertaken in the United Kingdom have mainly had the objective of determining the risks posed to humans from exposure to the causal agents associated with bovine spongiform encephalopathy (BSE) and variant Creutzfeld-Jakob disease (vCJD). In this article, I examine 19 of these risk assessments published to date and consider how their results might be influenced by underlying model assumptions and methodology. Three separate aspects common to all the assessments are infective load estimation, exposure pathway identification, and risk estimation. These are each discussed in detail.

KEY WORDS: BSE; mad cow disease; model assumptions; quantitative risk assessment; uncertainty; vCJD

1. INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are fatal diseases characterized by spongiform tissue that develops in the brain. Confronted with many uncertainties on TSE transmission, the need for quantitative assessment of the risks associated with exposure to TSE causal agents has achieved global importance.[1] Ultimately, all quantitative TSE risk assessments must translate into an estimated probability of TSE infection by a specified exposure route and thereby provide input into the TSE management decision-making process.

Bovine spongiform encephalopathy (BSE) and vCJD (also known as human BSE) are new TSEs that emerged in the United Kingdom during the mid-1980s and mid-1990s, respectively. BSE has recognized potential to cross from cattle to other mammals and the BSE causal agent in cattle has been causally linked with vCJD in humans.[2,3] This disease has received the overwhelming attention in most of the TSE risk assessments performed to date in the United Kingdom, which have mainly focused on assessment of risks to humans specifically posed by exposure to the BSE causal agent.

BSE is generally thought to have been propagated in the production of meat and bone meal (MBM) derived from cattle after changes in UK rendering practices during the 1970s led to more favorable circumstances for its promotion.[4,5] The MBM was fed to cattle and to a lesser extent probably to sheep, as a protein-rich dietary supplement, throughout the United Kingdom before the ban on ruminant-derived MBM in July 1988. Some of the MBM would have been contaminated with the BSE agent, thus resulting in oral exposure to BSE infectivity of ruminant livestock and the postulated source of the UK BSE epidemic.

With the exception of Sweden, BSE has now been confirmed in cattle populations based in all 25 EU countries, in addition to others as distant as Japan and Canada.[6] In the period since the disease was first recognized in 1986[7] to June 30, 2004, a total of 183,972 BSE cases have been reported in the United Kingdom (http://www.oie.int/eng/info/en_esbru.htm).

* Department of Theoretical Biology, Faculty of Biological Sciences, University of South Bohemia, Branisovska 31, 370 05, Ceske Budejovice, Czech Republic; e.grist@[GRO-C].

This is still the largest global recorded BSE epidemic, which peaked in 1992 with 37,280 reported cases in the United Kingdom that year. The annual number of UK cases has continued to fall since then, reaching its lowest level of 169 reported cases as of June 30, 2004 (http://www.oie.int/eng/info/en_esbru.htm).

Since vCJD was first recognized in the United Kingdom in 1995, the disease has not only been detected in other parts of Europe (vCJD fact sheet, http://www.cdc.gov) but also in the United States[8] as well as Canada.[9] As of July 5, 2004, there have been 147 recorded cases of definite or probable vCJD in the United Kingdom, 142 of whom have died (monthly vCJD disease statistics, http://www.dh.gov.uk/PublicationsAndStatistics). The number of annual reported cases peaked in 2000 in the United Kingdom with 28 cases and has fallen each year since then, with only three cases reported in the United Kingdom in 2004 up to July 5 (monthly vCJD disease statistics, http://www.dh.gov.uk/PublicationsAndStatistics). The duration of the incubation period for vCJD is unknown but has been suggested to be of the order of many years or decades (vCJD fact sheet, http://www.cdc.gov), and hence the current status of the UK outbreak remains uncertain. Ferguson *et al.*[10] estimated that the total number of predicted vCJD deaths from 2001 to 2080 will lie between 40 and 50,000 as a result of bovine exposure, but the upper bound could be as high as 150,000 deaths through inclusion of a worse-case ovine scenario if it turns out that BSE has entered the UK sheep flock.

In this article I consider 19 quantitative TSE risk assessments, henceforth referred to as the UK assessments, published in the United Kingdom since 1997, which specifically focus on the assessment of the risks posed by BSE and vCJD. Fourteen of these were performed by the consultancy Det Norske Veritas (DNV) and are henceforth referred to as the DNV assessments.[11–19] Three other publications, henceforth referred to as the Gale assessments, were by Gale and Stanfield,[20] Gale,[21] and Gale *et al.*[22] with two separate studies by Ferguson *et al.*[10] and the Economic Operational Research Division of the UK Department of Health.[23] A summary of the specific areas together with the key assumptions and modeling approach used in each assessment is shown in Table I.

The large majority (18) of the UK assessments focused on the risks posed to humans from exposure to TSE infectivity through environmental pathways or the food chain. As an exception to this, the risk assessment DNV 1998[13] focused on the estimation of the potential risks to UK sheep from exposure to BSE through contaminated MBM as a feed supplement.[13] Only two UK assessments considered iatrogenic risks to humans from vCJD, respectively, from blood transfusion[14] and surgical instruments.[23] Some other risk assessments

Table I. Summary of Key Assumptions and Modeling Approaches used in the UK Assessments

Risk Assessment	TSE Agent	Source Material	Exposure Pathways	Emanating from	Risks to	Risk Estimation Approach
1. DNV (1997a)	BSE	Cattle	Environment	(overview)	Humans	(overview)
2. DNV (1997b)	BSE	Cattle	Environment	Rendering plant	Humans	Probabilistic MC
3. DNV (1997c)	BSE	Cattle	Environment	Burning	Humans	Probabilistic MC
4. DNV (1997d)	BSE	Cattle	Environment	Incinerators	Humans	Probabilistic MC
5. DNV (1997e)	BSE	Cattle	Environment	Landfills	Humans	Probabilistic MC
6. DNV (1997f)	BSE	Cattle DRG	Human food chain	Beef	Humans	Probabilistic MC
7. DNV (1997g)	BSE	Cattle	Human food chain	Meat products	Humans	Probabilistic MC
8. DNV (1998)	BSE	MBM	Sheep food chain	Sheep feed	Sheep	Probabilistic MC
9. DNV (1999)	vCJD	Human blood	Blood transfusion	Human blood	Humans	Probabilistic MC
10. DNV (2001a)	BSE	Cattle	Environment	SRM incinerators	Humans	Probabilistic MC
11. DNV (2001b)	BSE	FMD cattle	Environment	Carcass disposal	Humans	Probabilistic MC
12. DNV (2001c)	BSE*	Sheep	Human food chain	Meat products	Humans	Probabilistic MC
13. DNV (2001d)	BSE*	Sheep	Environment	Carcass disposal	Humans	Probabilistic MC
14. DNV (2002)	BSE*	Sheep intestine	Human food chain	Sausage casings	Humans	Probabilistic MC
15. Gale (1998)	BSE	Cattle	Environment	Groundwater	Humans	Deterministic
16. Gale *et al.* (1998)	BSE	Cattle	Environment	Groundwater	Humans	Deterministic
17. Gale and Stanfield (2001)	BSE	Cattle	Environment	Sewage sludge	Humans	Deterministic
18. Ferguson *et al.* (2002)	BSE*	Sheep	Human food chain	Meat products	Humans	Dynamic model
19. EOR (2001)	vCJD	Human tissue	Surgical instruments	Surgery	Humans	Dynamic model

Key: MC = Monte Carlo simulation.
* = assuming BSE has entered the UK sheep flock.

performed outside the United Kingdom have also considered risks from vCJD through medical routes, such as bovine graft material used for dental application.[24]

Before considering the quantitative approaches that were employed, it is essential to provide a general background on the current knowledge of the nature of TSE causal agents.

2. TSE CAUSAL AGENTS

Although some authors contend that TSEs are viral in nature,[25] TSE causal agents are more widely considered to be malformed proteins (PrP^{Sc}), concisely referred to as "prions," which accumulate in a host by a process of catalytic conversion of normal cellular PrP^{C} protein.[26] Ultimately, this results in the onset of illness and untimely death.

Early clinical symptoms of TSE diseases are, in general, vague or variable, making diagnosis difficult. Additionally, the typically long incubation period—the interval between infection and the onset of disease symptoms—and variation of individual susceptibility has constrained attempts to understand transmissibility both within and across species. In this context a "species barrier" is encountered, which refers to the relative difficulty in transmitting a disease between different species. With TSEs this is typically exhibited as a prolongation of the length of the incubation period when durations between successive infections of different species are compared.[27] The twin concepts of species barrier and incubation period are thus closely related.

In humans there is a large gap in our knowledge of the vCJD incubation period as well as the vCJD transmission risks associated with various human tissue, organ, and blood transfusions.[28] Recent concerns for public health have been heightened by the demonstration that the vCJD causal agent has been found in human tissue taken from the highly vascularized lympho-rectricular system.[29] Although there is currently no definitive evidence that vCJD can be contracted directly by blood transfusion between humans, the findings of BSE studies on exogenous sheep models, which demonstrate that BSE can be transmissible by blood transfusion in sheep,[30,31] have resulted in vCJD risks now being taken into account in human blood safety issues worldwide.[32]

In the context of TSEs, the concept of a species barrier is made opaque by the fact that certain individuals or species may act as causal agent "reservoirs" without displaying any clinical TSE disease symptoms, as demonstrated (for example) by certain types of mice experimentally infected with BSE.[33] The reservoir property could also include situations where the incubation period exceeds an infected individual's natural lifespan. In the case of vCJD, it has been acknowledged that the existence of such subclinical forms of prion infection could have important public health implications, especially in raising the worrying possibility that the typical vCJD incubation period could be very long.[34] When combined together, these aspects ensure that quantitative assessment of TSE risk is an endeavor necessarily fraught with uncertainty.

Data from animal experiments have demonstrated that depending on species and breed, there can be striking variability in TSE disease outcome between different individuals, even when "challenged" with the same initial dose. This is observed especially in the variability of incubation period.[35] However, it has not yet been established how much of the variance is simply due to practical difficulties in ensuring that identical oral doses are ingested by different individuals. Experimental studies with bovines suggest that the level of BSE infectivity peaks around the time when disease symptoms first appear, that is, at the end of the incubation period.[36] This observation provides a retrospective justification for the 1996 UK "Over Thirty Month Scheme" (OTMS) bovine safeguard rule, which directs that only younger cattle, perceived as less infectious, should be allowed to enter the human food chain.

The transmission dynamics of TSEs are poorly understood.[37] However, in the case of BSE it is clear that both transmission and exposure to the causal agent are dependent on several factors, in particular the management of young livestock.[38] Indeed, changes to the diets of young calves (especially dairy) in the United Kingdom during the 1970s resulted in MBM being regularly included.[38] Since bovine susceptibility to BSE infection is more likely at a younger age[40,41] this would have contributed to the exposure to the disease in UK cattle.

Although there is no evidence of horizontal transmission of BSE in cattle through contact or pasture,[42] a more recent study has suggested that maternal transmission is limited to the last 6 months of the incubation period of the dam, with a transmission probability of around 0.5%.[43] This is also consistent with placental transmission having been proposed as a likely route for transmission of the long-established TSE disease *scrapie* in sheep.[44]

It is currently unknown whether the BSE pathogen has entered the UK sheep flock either through the feed-borne route or by any other route.[45,46] The main difficulty is caused by the fact that symptoms of BSE in experimentally challenged sheep are not distinguishable from those of scrapie.[47] The latter disease has never been demonstrated to cause disease in humans.[48] Surveillance studies are currently underway to determine whether BSE may therefore be "masked as scrapie" in a percentage of the UK sheep flock. On a wider European scale, active surveillance to determine the prevalence of both BSE and scrapie in all ruminant populations at local and national levels is now ongoing throughout the European Union.[49,50]

Considering BSE in the UK sheep flock as a possible eventuality,[46] some of the UK assessments focused on estimating human exposure risks potentially associated with sheep meat products.[10,17,19] Breed and category of sheep (lowland, upland, hill) would be an important risk determinant in this context because of differences in sheep management. In particular, the diet of lowland sheep is more frequently supplemented with pellets and therefore lowland breeds would be expected to have incurred greater historical exposure to BSE infectivity.[13,45,51]

It is generally presumed in humans that all the known individual cases of vCJD acquired the disease through the oral route through consumption of tissues or meat products that were derived from BSE-infected cattle.[52,53]

Risk refers to the probability or likelihood that something "unpleasant" will happen.[5] There are three parts to any TSE risk assessment, which lead to the quantification of such an outcome (or not). These are, respectively, estimation of infective load, exposure pathway identification, and risk estimation. Each will now be considered separately.

3. INFECTIVE LOAD

The most fundamental part of any TSE risk assessment is the estimation of the amount of TSE infectivity contained in the source under consideration. This quantity is referred to as the *infective load*. The infective load is usually expressed in Infectious Dose 50 (ID_{50}) units where an ID_{50} unit is the estimated mass of infected tissue that each individual in a population would need to ingest for 50% of the population to become infected. Equivalently, an ID_{50} is the estimated quantity of tissue that an average individual would need to ingest to have a 50% probability of becoming infected (the choice of 50% is traditional, but completely arbitrary). Throughout this article, unless otherwise stated, an ID_{50} unit is expressed in terms of an *oral* dosage.

More formally, the estimated infective load L in the bovine or ovine source under consideration can be expressed in human oral ID_{50} units by the equation:

$$L = npti(1/s), \qquad (1)$$

where n is the number of source animals under consideration in the risk assessment [dimensionless], p is the prevalence of infection in the cohort under consideration [dimensionless], t is the mass of infected tissue per animal [g], i is infectivity in bovine (or ovine) oral ID_x units per gram of infected tissue [$ID_x\ g^{-1}$], and s is the bovine- (or ovine)-to-human species barrier factor [dimensionless].

3.1. Prevalence of Infection p

Notable efforts have been made to determine values for prevalence p (the proportion of a population that is TSE infected) both for scrapie and BSE at regional and national levels.[41,43,51,54] All such estimations are heavily reliant on the import of accurate epidemiological data.[10,54,55]

But progress in the quantification of the parameter p has been hindered by several factors typical of TSE disease surveillance. Most obviously these are difficulties in accurate diagnosis of disease symptoms and underreporting of disease incidence.[54] Difficulties in establishing TSE prevalences have been confounded further by problems in developing diagnostic tests to desired levels of specificity and sensitivity.[55] Progress has also been hindered by UK government establishment errors that at times have reached farcical dimensions, as with the notorious 'brains blunder' case involving the UK government Veterinary Laboratories Agency (VLA), where cattle and sheep brains were mixed up in experiments estimated to have cost the UK taxpayer 217,000 pounds sterling[56]. The study aimed at determining whether the BSE causal agent had passed to the UK sheep flock, but results were rendered useless as a direct result of an apparently simple labelling error.[56]

Theoretical modeling studies have been constrained through difficulties in establishing whether a minimum threshold dose exists to ensure the onset of disease in a given species. In addition, TSE disease incubation period is subject to variability both within and across species. Under oral challenge experiments, BSE disease symptoms in cattle usually take 4 to 6 years with a mean incubation period estimated as 5.2 years[57] whereas in sheep they usually

appear earlier, most frequently between 2 and 4 years in age.[58] An in-depth statistical analysis of scrapie titration experiments conducted on mice has shown that while incubation period decreases as dose is increased, individual variability becomes greater with lower doses.[35]

Throughout the DNV and Gale risk assessments, prevalence of BSE in UK cattle was assumed to be around the estimated level of 0.54% calculated for OTMS cattle in 1996 by Anderson *et al.*[40] For sheep, a ceiling prevalence value of 2% was used for scrapie, with scenarios for proportions of scrapie as BSE ranging from a minimum of 0.01% to a maximum of 10% of scrapie in the ovine UK risk assessments.[10,17–19]

3.2. Infective Tissue *t*[*g*]

The UK assessments make conservative assumptions of the potential infectivity contained in tissue. They thus emphasize worse-case scenarios, thereby simplifying the uncertainties connected with incubation period. This is achieved by assuming that a perceived upper bound on the proportion (and sometimes all) of the animal carcasses from which infective tissue originates are *maximally* infective and therefore each contains the infectivity of a fully symptomatic individual.[11]

3.2.1. Cattle

Results from experimental studies performed on cattle have demonstrated that BSE infectivity may be detected in the distal ileum as early as 6 months post-oral exposure.[36] However, at the end of the incubation period when clinical disease signs first appear, infectivity is known to accumulate only in the central nervous system (CNS). For this reason, estimation of the total infectivity contained in tissue taken from a symptomatic infected bovine has been assumed to be *directly proportional* to the mass of its brain and connected spinal tissue.[11]

3.2.2. Sheep

For sheep, the situation is more complicated because both BSE and scrapie infectivity have been shown to spread extensively into a variety of tissues during the course of pathogenesis.[58–60] TSE infectivity in sheep tissue is therefore dependent on tissue type and age (as well as sheep genotype), so *t* must somehow be summed over all tissue and age categories that may contain infectivity.

The early pathogenesis investigations by Hadlow[60] demonstrated that scrapie enters several tissues in sheep but especially the lymphatic system soon after infection. In the absence of available information on BSE pathogenesis in sheep, these data sets were incorporated into the respective UK assessments to estimate the potential proportions of BSE infectivity in different ovine tissue types.[10,17,19] An underlying assumption was made that pathogenesis of BSE in sheep is the same as for scrapie and relevant parameters were grouped into three main categories for tissue type (I. brain and spinal cord; II. lymph node, spleen, tonsil; III. stomach, liver, thymus) and four main groups by age at slaughter (lambs under 6 months, lambs 6 to 12 months, boggetts 1 to 2 years, cull ewes older than 2 years).

Experimental studies conducted more recently with both scrapie and BSE on sheep have shown the existence of a wide range of genetic susceptibility to TSE disease.[58,62] This susceptibility can be characterized at the genetic level in terms of 15 main sheep genotypes through the presence or absence of pairs of five specific alleles.[62] In the most resistant genotype (homozygous sheep with ARR/ARR alleles), there is no evidence to date that either scrapie or BSE can be induced by an oral challenge.[63,64] However, it has been shown that the ARR/ARR genotype may become infected by intracerebral challenge,[64] thus raising the possibility that they may still act as potential TSE causal agent carriers. To further complicate matters, different genotypes are not uniformly distributed among the numerous UK sheep breeds.[65] In the face of such complex uncertainties, UK risk assessments have proceeded by simply assuming that sheep carcasses are either composed of all of the most susceptible genotype[17–19] or that a worse-case upper proportion (such as one third of the UK sheep population, as in Ferguson *et al.*[10]) is of that genotype. If less conservative assumptions on susceptibility were made it would imply that the estimated TSE risks would also be less, although depending on the risk model choice, not necessarily linearly reduced.

3.3. Infectivity *i*[$ID_{50}\ g^{-1}$]

Analysis of experimental TSE studies on animals has shown that the likelihood of successfully inducing TSE infection increases with the dose of infectious material.[35,36] However, it has not been established whether or not there is a minimum "threshold" dose that is required to initiate infection in a given species. Applying a worse-case scenario, the UK assessments have generally assumed there is no threshold dose and that infectivity *i* accumulates in an individual in direct proportion to the amount of infected

tissue ingested by an individual over a period of time. This implies that any dose, no matter how small, may cause deaths in an exposed population through individual variability of:

1. susceptibility to infection,
2. infectivity in tissue,
3. infectivity movements through the gut wall.

Following advice by the Spongiform Encephalopathy Advisory Committee (SEAC), a value of 1 bovine oral ID_{50} unit was set at 0.1 grams (g) of BSE-infected bovine CNS tissue in the majority of the DNV assessments. This value was based on available data quoted in Anderson[40] obtained from an ongoing bovine oral challenge experiment that had commenced in 1992 at the Central Veterinary Laboratory, UK. The ID_{50} was calculated using the computer program "QUAD," which employed a logit model fitted to the mortality proportions incurred under the different oral exposure dosage levels (which were 1, 10, 100, 300 g of BSE-infected bovine brain tissue). The "delta method" was used to derive confidence intervals.[66] This generated an estimate of 0.38 g for 1 bovine oral ID_{50} unit with a wide 95% confidence interval of 0.03 g to 5.27 g. The 0.38 g point estimate was rounded down conservatively to 0.1 g after SEAC expert opinion.

However, the fitting of any such dose-response model to these sparse experimental data would be subject to high uncertainty. In particular, it would not be possible to estimate the BSE risks associated with small fractions of an ID_{50} unit with any meaningful confidence. Throughout the DNV risk assessments it was simply assumed that BSE infectivity contained in exposure doses of between 0 g and 0.1 g of BSE-infected tissue would be proportional to the respective fractional quantity of an ID_{50} unit ingested.

Gale *et al.*[22] adopted a different approach to estimate TSE risks associated with small fractions of an ID_{50} unit. This was achieved by considering the number of PrP^{Sc} molecules that might make up 1 ID_{50} unit. Using a species-adapted scrapie model, a value of 10^5 PrP^{Sc} molecules had been previously estimated to make up a bovine intracerebral bovine ID_{50}.[67] On the basis that the oral route is 10^5 times less efficient than the intracerebral challenge,[68] together with the assumptions that the number of PrP^{Sc} molecules in an ID_{50} unit is fixed within a species and that the cow-to-human species barrier is 1,000, the authors estimated that 1 human oral TSE ID_{50} unit would therefore contain $10^5 \times 10^5 \times 1{,}000 = 10^{13}$ PrP^{Sc} molecules.

The authors went on to consider beta-Poisson and negative-exponential distribution models fitted to dose-response data obtained for BSE infectivity in inbred mice[69] and the latter model curve provided a better fit. The mathematical form of the negative-exponential model is $P = 1 - \exp(-rN)$, where P is the probability of infection from N pathogens and r is a species-specific parameter. For low doses this model can be approximated by the linear function $P = rN$. However, it was further claimed that the simpler relationship of 0.5 × (fraction of 1 ID_{50} unit) ingested would hold equally well. Thus it was concluded that the probability of infection from a minute BSE prion aggregate made up of 100,000 PrP^{Sc} molecules would be equal to $0.5 \times 10^5/10^{13} = 0.5 \times 10^{-8}$ and hence would be extremely remote.

3.4. Species Barrier Factor *s*

Perhaps the least certain parameter in all the risk calculations considered here is the cattle (or sheep)-to-human "species barrier factor." Early advice by SEAC stated that the cattle-to-human species barrier factor for BSE could lie anywhere in the range of 1 to 10,000 with 10 as a best estimate.[70] The risk assessment by Gale *et al.*[22] speculated that it could be as high as 1,000. More recent statistical research has indicated that the value is likely to be orders of magnitude higher than 10.[43,71]

Since no evidence has been found to date of BSE in the UK sheep flock, there are no direct data available from which to estimate the sheep-to-human species barrier for BSE. Risk assessments undertaken to address the potential risk of human exposure to BSE infectivity in UK sheep (should it be found) have proceeded with the hypothetical assumption that the species barrier for BSE from sheep-to-human exposure would be the same as for BSE from cattle-to-human exposure.[10,17,19]

With bovine infectivity assumed at 10 bovine ID_{50} units per gram of BSE-infected CNS tissue and a cattle-to-human species barrier of 10, the estimated infectivity to humans translates to 1 human ID_{50} unit per gram of infected bovine CNS tissue (= 10 bovine ID_{50}/10). This value was taken as a best estimate for both cattle and sheep BSE-infected CNS tissue across all the DNV assessments apart from the single assessment that addressed the potential BSE risks from the disposal of sheep.[18] In this preemptive risk assessment, the sheep-to-human species barrier factor was elevated to 50 (again following the contemporary SEAC advice), thereby giving a reduced estimate of

10/50 = 0.2 oral human ID_{50} unit per gram of BSE-infected sheep CNS tissue.

In summary, most of the UK assessments have calculated infective load L as the total mass of infected tissue (npt) contained in the source term under consideration multiplied by the estimated number of ID_{50} units associated with 1 g of infective tissue (i/s). The potential exposure risks from BSE in sheep were addressed by incorporating supplementary assumptions that distribution and accumulation of BSE infectivity in sheep tissue occurs in an identical pattern to that reported for scrapie by Hadlow.[10,13,17–19,60]

4. EXPOSURE PATHWAY IDENTIFICATION

The perceived risks associated with the infective load in any quantitative risk assessment can be concisely described by an "event tree." In the context of TSE risk assessment, an event tree has most often been used to identify pathways by which exposure to TSE infectivity might be expected to occur.[11,20,38,72] In the UK assessments, the proportion of infective load to pass through each branch of the event tree was estimated either as an average value based on data, expert opinion, or a guess. Such an event tree provides a framework for estimating the individual and societal risk associated with each exposure pathway. Event trees underlie all the UK risk assessments and can be broadly classified as either environmental, human food chain (meat products), or other.

4.1. Environmental Pathways

A comprehensive charting of the environmental pathways by which humans living in England and Wales might encounter exposure to BSE infectivity is provided in DNV 1997a.[11] This risk assessment is an overview of the results formulated in the four other environmental risk assessments DNV 1997b–1997e.[11] The general methodology developed in the DNV 1997a–1997e[11] compendium is a continuation of an earlier BSE risk assessment, in which the BSE risks associated with the waste water effluent discharged from the Thruxted Mill rendering plant (http://www.bseinquiry.gov.uk/report/volume6/chapt104.htm) were estimated.[73] In addition to descriptions of transport and eventual fate of BSE infectivity in the environment, estimates of the amounts likely to be present in cattle waste products were also provided. Incineration or burning effects were assumed to act on prions in the same way as on proteins, by reducing the infectivity content by a factor of 10^6 as discussed in DNV 1997c and 1997d.[11] The effect of degradation in the ground was assumed to produce a 98% reduction in prion infectivity, based on sparse experimental data collected by Brown and Gajdusek.[74]

The DNV 1997a–1997e[11] BSE risk assessment compendium served as a basic template for the DNV risk assessments performed thereafter. Although the connection between vCJD in humans and BSE in cattle was unproven at that time (1997), the authors made the key connection that vCJD could be caused by ingestion of a sufficient quantity of BSE infectivity.

BSE exposure risks from environmental pathways were generally estimated to be extremely low. The societal risk for the whole population of England and Wales ranged from as low as 6×10^{-4} human ID_{50} units from emissions from a foot and mouth disease pyre of 100 burning cattle carcasses,[16] to 3 human ID_{50} units across the entire United Kingdom emitted from all environmental sources over the period of the 1996 year.[11] If located in the worst-case scenario, the exposure risk to an individual ranged from less than 10^{-10} human ID_{50} units per year from ash generated from a specified risk material incinerator[15] to below 10^{-6} human ID_{50} units from all environmental sources over the period of the year 1996.[11] The maximum number of expected vCJD cases that would result in a UK population of 60 million people from the total BSE infectivity emitted in 1996 from environmental sources would therefore be estimated at $0.5 \times 60{,}000{,}000 \times 10^{-6} = 30$ cases. However, due to the unknown duration of the vCJD incubation period, no prediction of when these infected individuals would appear as clinical cases is possible. Statistical decomposition between such anticipated future cases and those presumed to have been caused from intake of contaminated food would not be possible.

However, if a minimum threshold level of prions is required to initiate infection, the relevance of such minute average values could be seriously questioned.[21,22] It is suggested in Gale[21] that information based on the subtle effect of hydrogeological and other physical environmental barriers would likely be more critical in environmental BSE risk assessments than the magnitude of the cattle-to-man species barrier.[21]

4.2. Human Food Chain

The pathways by which human oral exposure to BSE infectivity might occur through the food chain

were in general thought to be less complex than those emanating from an environmental source. But BSE exposure risks associated with the human food chain were estimated to be generally higher than those through environmental pathways. Societal risks to the whole UK population calculated by Monte Carlo simulation ranged from 0.05 human ID_{50} units for eating dorsal root ganglia in beef[12] to 2,000,000 human ID_{50} units from beef products consumed specifically in 1989. The lower and upper median estimates for individual risks ranged from 9×10^{-10} human ID_{50} units for dorsal root ganglia consumed in beef[12] to 0.007 human ID_{50} units from eating a single meal consisting of 250 g of sausages made with sheep intestinal casings taken from BSE-infected sheep.[19]

Equivalently, the latter figure translates to an approximate risk of infection of 1 in 300 ($\sim 0.007 \times 0.5$), which, in the event that each person in the UK population of 60 million consumed such a sausage meal, would alarmingly imply the expected number of vCJD infections to be 200,000 ($\sim 60{,}000{,}000 \times 0.007 \times 0.5$). Since only 147 vCJD clinical cases have been reported in the United Kingdom to date, this hypothetical calculation would therefore, appear to produce a gross overestimate. However, the calculation rests on two underlying assumptions: (1) that BSE is present in the UK sheep flock and (2) the prevalence level would be high enough to permit 60 million worse-case meals to be produced and consumed. Despite considerable active surveillance, the first assumption continues to remain unproven and (therefore) it also follows that the second would be extremely unlikely. Hence this rough estimate of 200,000 for the number of vCJD infections seems likely to be highly inflated, but nevertheless cannot be fully discounted because of prevailing uncertainties of BSE in the UK sheep flock together with unknown duration of the vCJD incubation period. In fact, the estimate is not inconsistent with results of Ferguson *et al.*,[10] who concluded that the number of future UK vCJD cases potentially could reach 150,000 through inclusion of a worse-case scenario for BSE in the UK sheep flock (should it be found).

Together with the pessimistic assumption that epidemiology of BSE in sheep resembles that of scrapie, the risks to humans posed by meat and products derived from sheep were estimated to potentially be greater than those derived from cattle.[10] In this respect, the UK Food Standards Authority has evidently acted by recommending that sheep intestine be added to the list of specified risk materials, but paradoxically, falls short of extrapolating a similar risk to the more abundant lymphatic tissues. This may be because of intractable practical difficulties that would inevitably occur in ensuring their complete removal from a sheep carcass.

Risks to humans from either bovine or ovine milk, or milk products, in relation to BSE were considered negligible, based on the opinion of the European Commission Veterinary Committee.[75] Confirmation of the presence of infectivity in other body fluids remains inconclusive.[76]

4.3. Other Pathways

Neither of the two UK vCJD risk assessments[14,23] were able to generate absolute values for the estimated risks to humans from blood transfusion or surgical instruments, respectively.

The human blood exposure risk assessment DNV 1999[14] provided an estimation of the relative risk of secondary infection, given that the source material contained infected blood taken directly from a donor with vCJD. However, prevailing uncertainties were considered too great to permit any estimation of the absolute vCJD risk from human-to-human blood transfusion.

The attempt of the EOR[23] risk assessment to determine exposure risks to vCJD infectivity from surgical instruments was similarly constrained by major deficiencies in information. The risk model therefore incorporated parameter inputs that were based on scrapie in sheep to pessimistically assume that vCJD infectivity would achieve a wide distribution throughout the human body. It concluded that although surgical transmission of vCJD could not be ruled out, a prediction of the potential number of future cases would not be feasible. Importantly, however, this risk assessment identified high variability in contemporary surgical decontamination procedures. Assuming a pessimistic (but not worst-case) surgical instrument decontamination scenario, the authors suggested that surgical instruments could act as a vCJD causal agent vector with a transmission success of between 5% and 10% of the number of individuals that were infected in a primary vCJD outbreak.

The sheep feed-borne BSE risk assessment DNV 1998[13] evaluated the flow of infected material from an infected bovine through to its ultimate consumption in infected feed by sheep. The total amount of BSE infectivity consumed between 1980 and 1995 was calculated from the amount of infected material estimated to have been fed to the entire UK population of sheep and lambs. This was respectively put at 37,500 and 700 bovine BSE ID_{50} units. The authors suggested that consumption of the infected material was most

likely to have occurred in the period between 1980 and 1988 before the ban on ruminant-derived MBM protein in feed was introduced. Sheep fed on compound feed (factory-prepared pellets) would be expected to be exposed to the highest levels of infected material. The greatest exposure to infected material was estimated to have been most likely during 1988, when the quantity of BSE-infected material consumed per ewe was calculated to be 9.7×10^{-4} bovine ID_{50} units.

5. RISK ESTIMATION

The modeling approach by which risk is estimated in any risk assessment is subject to a wide choice of method and level of complexity. In most of the UK assessments Monte Carlo simulation was employed to derive interval estimates for risk. Some BSE assessments performed outside the United Kingdom[77,78] have used more complex multitiered simulations in which each tier (or module) is itself a dynamic system that may either be stochastic or deterministic. All such assessments have attempted to extrapolate potential BSE exposure risk into situations where few epidemiological data are available. These studies derive risk estimates by considering strategic "what if" scenarios rather than through statistical inference. The first task of the risk assessor therefore is to select an estimation approach considered to be the most appropriate for the situation being assessed.

5.1. Risk Estimation Approach

Assessment of risk is most simply achieved from the derivation of point estimates for an assumed risk scenario. This approach results in a straightforward manner in *a deterministic risk assessment* because there is no attempt to incorporate uncertainty. In recognition of this shortcoming, the approaches of *interval analysis* (virtually neglected in all of the UK assessments) and *probabilistic risk assessment* attempt to include uncertainty by allowing risk model parameters to take any value within a range perceived as possible. It is important to observe that the interval estimate generated by each of the two approaches will in general be different.

Instead of a single calculation from a set of fixed parameter estimates, probabilistic risk was most commonly derived by the computationally intense technique of Monte Carlo (MC) simulation. This technique relies on repeated calculations in which input parameter values are drawn randomly from probability distributions. Output distributions are thereby generated for all exposure pathways that are then used to derive interval (rather than point) risk estimates. However, any MC interval risk estimate must be specified by the risk assessor to an arbitrary level of confidence (typically 95%).

In Monte Carlo simulation, the statistical distributions assigned to each input parameter must be specified in terms of type and shape. These choices determine both the variability and uncertainty imputed onto each model parameter. This influences the form of the output distributions for each exposure pathway and hence also the span of the confidence intervals derived for each MC interval risk estimate.

Given the ubiquitous TSE informational deficiency common to all the UK assessments, it is surprising that the mathematical technique of interval analysis,[79] which does not rely on any probabilistic distributional assumptions, was never used.

Interval analysis is computationally much simpler than Monte Carlo simulation. It employs input parameter intervals $[x_1, x_2]$, which are respectively defined by lower and upper limits x_1 and x_2 to represent the conceivable range that a parameter may take. No value within the interval is considered more likely than any other, so each interval effectively represents a gap in knowledge (this is different from assigning a uniform distribution to a parameter in Monte Carlo simulation because all values within that distribution interval are then assumed to be equally likely). The net effect of all such input parameter uncertainties is then evaluated within the risk model to obtain nonprobabilistic interval risk estimates for all the event tree exposure pathways.

5.2. Specific Approaches

In the DNV assessments, point risk estimates were accompanied by interval risk estimates derived by first-order Monte Carlo simulation. By contrast, the Gale assessments relied on simple calculations in which estimates for all parameters and output risks were obtained from simple deterministic calculation. Justification for this economic approach is provided largely in philosophical terms. The latter authors essentially argued that the combination of very high uncertainty (through environmental pathways) with very low levels of TSE infectivity (through minute amounts of prions) can imply no gain from increased computational complexity in the context of TSE environmental risk assessment. They point out that uncertainty in the animal-to-human species barrier would be more likely to be an overriding factor in these circumstances. They suggest that the central question to

be addressed in environmental TSE risk assessment should not be what the risk of exposure to infectivity is but, rather, whether it is possible that a person could be infected by a given exposure route.

In direct contrast to this computational expedience, the EOR risk assessment[23] and that by Ferguson *et al.*[10] incorporate complex dynamic models to drive epidemiological simulations that then generate interval estimates for associated TSE risks. However, in both these later studies, descriptions of the model and methods by which the (very wide) confidence intervals were projected for the future number of vCJD cases turned out to be mainly intractable.

In the DNV assessments, there is little or no support for the choice of parameter probability distributions used in the Monte Carlo analyses. This is acutely evidenced by the choice of distribution used to represent uncertainty in the cattle-to-human species barrier factor. The uncertainty is represented by a 5-point probability distribution that is claimed to reflect the contemporary SEAC advice that the cattle-to-human species barrier factor might lie anywhere between 1 and 10,000, with 10 as a "best estimate" (ECSSC, 2000).[52] The chosen probability distribution assigns a probability of 0.01 to the value of 1 and 0.2475 each to 10, 100, 1,000, and 10,000. Alarmingly, this implies there is a probability of zero that the cattle-to-human species barrier factor might take any other value (for example, 15, or 101, etc). A more natural choice would be to assign a triangular distribution ranging from 1 to 10,000 with a peak at 10 (as used in Cummins *et al.*[80]). Similarly, for uncertainty in the infectivity of CNS tissue, no grounds are provided for the choice of a log-normal in preference to (say) a log-logistic dose-response model curve. In both cases, distribution choice would be likely to influence the percentile confidence interval derived for each exposure risk estimate. All other parameter distributions employed in the Monte Carlo simulations were normal, often truncated at specific lower and upper limits without explanation.

Such truncations of MC input parameter distributions will, in general, reduce the span of the output distributions derived for the exposure risk estimates. This, in turn, implies the width of the percentile confidence intervals for the MC risk estimates will be reduced. Parameter distribution truncation applied in Monte Carlo simulation is hence necessarily a process that *must* be supported in order for MC results to be scientifically credible, but this crucial point appears to have been largely overlooked in the DNV assessments.

The Gale assessments do not incorporate probabilistic risk estimation approaches. However, these authors also appeal to key points in their arguments that are unsupported. In particular, there is no explanation to support the claim that the probability P of infection equates to the formula $P = 0.5 \times$ fraction of 1 ID_{50} unit at the lower part of their negative-exponential dose-response curve fitted to the mice data of Taylor *et al.*[69] Nor is it explained why a negative exponential relationship should necessarily hold for a conglomerate of prions, which, having been described earlier (in the same text) as a novel pathogen, is unlikely to be a typical water-borne pathogen. Whereas the case for a simpler approach to environmental BSE risk assessment is put logically by these latter authors, it is hence not made fully transparent.

6. DISCUSSION

In any risk assessment, it has been cautioned that merely concluding a risk is "possible" cannot be justifiable.[81] It has been argued further that the language in the field of TSE research may have advanced further than the scientific understanding.[25] To serve as a "decision tool," risk assessments must at least deliver an estimation of risk in terms of an ascertainable likelihood.[82]

Some of the difficulties associated with quantitative TSE risk assessment have been recently highlighted. Gravenor and Kao[83] emphasized that overspecification of exposure pathways may result in underestimation of true exposure risk. As a case in point, the authors cite the Canada BSE risk assessment by Morley *et al.*[38] where the estimated likelihood of infectious material being fed to indigenous cattle was considered to be "negligible." In the same calculation, the probability of importing BSE-infected cattle into Canada was estimated to be high (at 0.007), but this was mitigated through probable dissipation of infectivity via a high number of exposure routes. With BSE having now been identified in Canadian cattle, it would be incorrect to infer that the results of this risk assessment were refuted. Negligible risk should never be equated with a probability of zero.

However, the absence of complete information on mechanisms by which TSEs may be transmitted must inevitably be brought into the TSE risk assessment process. Species population ratios and densities of livestock, which vary in different countries, are likely to be (as yet) uncharted contributory factors

to disease transmission and persistence. As a case in point, this is illustrated by the fact that BSE has continued to appear, albeit in declining numbers, in UK cattle born after the mammalian-derived MBM (MMBM) ban of March 1996. It has been suggested this may be connected with continued importation of contaminated feed from abroad after the MMBM ban was implemented,[84] but a quantification of those risks has yet to be made. Poignantly, it has recently been emphasized that further risk assessment on a global scale is required to evaluate the true BSE distribution worldwide.[1]

In the United Kingdom, BSE risk assessments have played a role in identifying major risk reduction measures. Since the UK MBM ban of July 1988, there has been a continuous tightening of UK controls on protein feed to ruminants. The specified bovine offals (SBO) ban in 1989 was followed by a specified risk materials (SRM) ban in 1990. The SRM ban was extended to include the intestines and thymus of young calves in 1994 and then followed by the complete ban of MMBM in March 1996. In recognition of the marked effectiveness of these controls as a risk reduction measure in the United Kingdom, most countries in Europe have now introduced a total feed ban on all types of MBM to farm animals.[85]

There is considerable scope for future diversification of BSE risk assessment into a variety of uncharted exposure risk scenarios as well as utilization of quantitative methods that have been adopted widely in risk analysis. Most notably, Bayesian estimation methods, increasingly being applied elsewhere in epidemiology and risk estimation,[86,87] have been largely neglected to date.

An aspect deserving more investigation is the significance of variability in the size of infective tissue particulates. This aspect has not been fully explored in any BSE risk assessment. Especially important in this respect are the studies by Anil *et al.*,[88] which have recently confirmed that standard techniques used to stun cattle before slaughter can spread minute parts of brain tissue around their bodies.

In such complex scenarios, where a large number of possible exposure pathways from the source term exist, the average amount of BSE infectivity expected to pass through each pathway would be low. However, if there is high variability in the size of the particulates, this simple calculation would easily underestimate the true exposure risk because the impulsive effect of larger particulates would not be taken into account. This would be especially pertinent if the existence of a minimum quantal (as opposed to cumulative) threshold prion dose is subsequently established. The subject connects with recent practical innovations such as development of a loop saw that cuts out the spinal cord in the spine before the carcass is split at the abattoir.[89] In particular, Helps *et al.*[90] demonstrated statistically significant lower risk of contamination of meat when both sheep and cattle carcasses are split with the new loop saw as compared with a standard design of saw.

In summary, the generic uncertainties encountered in TSE risk assessment are:

1. Prevalence levels of TSE-infected individuals in a specified population.
2. Whether or not there is a minimum (threshold) dose of prions required to initiate infection.
3. Whether or not prions ingested by an individual accumulate in that individual over a period of time.
4. The magnitude of the cattle-to-human or sheep-to-human "species barrier" factor.
5. The nature of prion transportation and destruction through environmental pathways.

In all the UK assessments discussed here, prion infectivity has been consistently assumed to accumulate in direct proportion to the amount ingested by the host. This implies a hidden assumption: that ingested infectivity does not replicate over that *same* time period. For TSE diseases, whose incubation period has been demonstrably shown to be dependent on oral dosage,[35] this simplifying assumption can hardly be justified. In future, it will be necessary to address the question of how time dependence can be meaningfully brought into TSE risk assessment calculations.

ACKNOWLEDGMENTS

I gratefully thank Vlastimil Krivan, Vincent Jansen, and Roland Kao for scientific discussions on the nature of prions and epidemiological representations of their dynamics using mathematical models. I especially thank Colin and Alice Wolff for providing helpful comments on the draft manuscript. This article was supported by Grant MSMT (MSM 123100004).

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