

SUMMARY OF SCIENTIFIC EVIDENCE REGARDING THE POSSIBILITY OF TSE INFECTIVITY IN EXCRETORY PRODUCTS FROM RUMINANTS, AND THE POTENTIAL PERSISTENCE OF SUCH INFECTIVITY IN THE ENVIRONMENT

GENERAL CONSIDERATIONS ON THE SAFETY OF ORGANIC FERTILISERS

1. In their 2001 updated opinion on the safety of organic fertilisers derived from ruminant animals, the Scientific Steering Committee (SSC) considered that the level of BSE-infectivity in organic fertilisers (not including manure) produced from BSE infected animal tissues or organs depends on the manufacturing technology and the initial level of infectivity in the source material. Once such fertilisers are used on crops and/or soils, the following risks emerge:

- The risk of ingestion of infectious residue by humans or animals. The Committee observed that human consumption of crops would normally follow washing which could remove any surface contamination, whereas animal consumption of crop surface contamination could be more substantial.
- The risk of soil and water contamination, including potential accumulation. Evidence shows that the TSE agent is resistant to many treatments and can survive burial. The Committee observed that although no information is available as to the behaviour (including accumulation) of the BSE agent in soil, ground or surface water, it is not possible to exclude the possibility of recycling of infectivity via these media.
- The risk of human or animal exposure during application or handling of organic fertilisers.

POTENTIAL SOURCES OF THE BSE AGENT FROM LIVESTOCK AT DRAYTON & VLA WEYBRIDGE

2. Potential sources of the BSE agent from cattle and sheep at Drayton and VLA Weybridge include faeces, urine, placentae and placental fluids, saliva and milk (including colostrum).

3. On the basis of available scientific evidence, manure and gut contents are defined as Category 2 material under Regulation (EC) No.1774/2002 which lays down health rules for animal by-products not intended for human consumption (OJ, 2002). The definition of manure includes any excrement and/or urine of farmed animals with or without litter. With certain exceptions (e.g. TSE suspects) placentae are also considered as Category 2 material. Milk may be considered as Category 2 material. Category 2 material includes animal by-products which present a risk of contamination with diseases other than TSEs. Generally Category 2 material must be disposed of by processing (rendering) and/or incineration. However, in view of their perceived lower risk status, manure, digestive tract content and milk may, where the competent authority does not consider there to be a risk of spreading any serious transmissible disease, be used “raw” in biogas or composting, or applied directly to land. Category 2 materials contaminated with Category 1 materials (which carry a TSE risk) must be disposed of as the higher risk category, in most cases by processing (rendering) and/or incineration.
4. The European Food Safety Authority (EFSA) concluded that the application of untreated manure to land, within the same epidemiological geographical area where the manure has been produced and in association with good agricultural practices, poses little problem to human and animal health (EFSA, 2005¹). The Codes of Good Agricultural Practice for the Protection of Soil and Water provide guidance on good agricultural practice for the application of manure and slurry to land (MAFF, 1998¹ and MAFF, 1998²).
5. The bulk of manure consists of faeces and bedding with other animal by-products as potential contaminants. Liquid wastes drain into liquid waste handling systems. At Drayton, with the exception of urine, the liquid excretions referred to would be extremely low volumes if any. As far as practicable, placentae, if any would ultimately be incinerated (unless a specific research project required otherwise).
6. Evidence for the likelihood of the BSE agent in these animal by-products is outlined below.

BSE Infectivity in Cattle Faeces

7. Available evidence suggests that if faeces from cattle infected with BSE is infectious for BSE either constantly or periodically, the infectivity titres are extremely low.
8. VLA's BSE Pathogenesis Study has identified infectivity in the wall of the distal ileum throughout most of the incubation period following an oral dose of infected brain material. (Wells *et al.*, 2005; Wells *et al.*, 1998). If the infectious agent were to enter the gut lumen, the faeces could theoretically contain infectivity during part or all of the period of infection. There is no evidence that this occurs in BSE in cattle.
9. Faeces collected from cattle at 18 and 32 months after exposure to an oral dose of BSE was considered negative for infectivity by RIII mouse bioassay ($<10^{1.4}$ mouse (i.c + i.p) \log_{10} LD₅₀/g). (Hawkins & Wells (unpublished) in SSC, 2002)
10. There is limited understanding of the pathogenesis of BSE in cattle following intracerebral inoculation, and one cannot assume that there is no spread to the lymphoreticular system and then to the intestinal lumen. In laboratory models, intracerebral inoculation does not guarantee sequestration of infectivity in the brain.
11. Epidemiological evidence including the effect of the specified risk material and feed controls on the epidemic suggests that horizontal transmission of BSE (e.g. animal to animal via infected faeces) is not a significant route of infection. (Hill, 2005)

BSE Infectivity in Sheep Faeces

12. If the distribution of BSE infectivity in sheep is assumed to reflect that of scrapie, the infection will be distributed throughout the intestines in the pre-clinical and clinical stages of disease (EFSA, 2005²). If the infectious agent were to enter the gut lumen, the faeces could theoretically contain infectivity during part or all of the period of infection.
13. Scrapie infectivity in sheep faeces is considered undetectable (EFSA, 2005²).
14. Scrapie is known to transmit between sheep under natural and experimental conditions. There is evidence that natural transmission of BSE in sheep can occur although it is unclear

whether this is vertical or horizontal transmission. It is also unclear how the infectious agent is transmitted if it is horizontal transmission. The incubation period of the infected lambs suggests that infection occurs at or just after birth (Bellworthy *et al.*, 2005¹).

BSE Infectivity in Cattle Urine

15. Urine collected from cattle at 18 and 32 months after exposure to an oral dose of BSE was considered negative for infectivity by RIII mouse bioassay ($<10^{1.4}$ mouse (i.c + i.p) $\log_{10}LD_{50}/g$). (Hawkins & Wells (unpublished) in SSC 2002)
16. VLA's BSE Pathogenesis Study indicates that cattle intracerebrally inoculated with urine taken from cattle killed eighteen months after oral inoculation with BSE infected material, survived for at least 65 months without developing clinical signs of BSE (Wells *et al.*, 2005).
17. Epidemiological evidence suggests that horizontal transmission of BSE is not a significant route of infection.

BSE Infectivity in Sheep Urine

18. BSE infectivity has been detected by mouse bioassay in the kidney of ARQ/ARQ Romney sheep orally infected with BSE (Bellworthy *et al.*, 2005²).
19. Chronic inflammatory kidney disease in mice infected with scrapie has been shown to result in the excretion of infectivity in urine. Urinary proteins from affected mice caused disease when inoculated into non-infected healthy mice (Seeger *et al.*, 2005).

BSE Infectivity in Cattle Placentae and Placental Fluids

20. No infectivity was detected in uterine caruncles, placental cotyledons, and placental fluids by bioassay in mice injected intracerebrally and intraperitoneally (Kimberlin, 1996)
21. Epidemiological evidence suggests that horizontal transmission of BSE is not a significant route of infection.
22. The probability of maternal transmission of BSE in cattle is less than 1% in the last six months of maternal incubation (Donnelly *et al.*, 2002).

BSE Infectivity in Sheep Placentae and Placental Fluids

23. Scrapie infectivity in sheep uterus is considered undetectable (EFSA 2005²).
24. Scrapie is known to transmit between sheep under natural and experimental conditions. There is evidence that natural transmission of BSE in sheep can occur although it is unclear whether this is vertical or horizontal transmission. It is also unclear how the infectious agent is transmitted if it is horizontal transmission. The incubation period of the infected lambs suggests that infection occurs at or just after birth (Bellworthy *et al.*, 2005¹).

BSE Infectivity in Cattle Saliva

25. VLA's BSE Pathogenesis Study indicates that cattle intracerebrally inoculated with parotid/submandibular salivary glands taken from cattle killed eighteen and twenty-six months after oral inoculation with BSE infected material, survived for at least 71 months without developing clinical signs of BSE (Wells *et al.*, 2005).

BSE Infectivity in Sheep Saliva

26. Scrapie infectivity in sheep saliva and salivary gland is considered undetectable (EFSA, 2005²).

BSE Infectivity in Cattle Milk

27. In June 2005, SEAC concluded that the results of a study presented to them, together with the findings of previous epidemiological and experimental research (particularly no specific persistence of BSE in suckler herds), provided no evidence for the presence of PrP^{BSE} in, or for transmission of BSE via, bovine milk (SEAC, 2005¹).

BSE Infectivity in Sheep Milk

28. In 2004, EFSA advised that TSE infectivity in the milk from small ruminants could not be totally excluded. The presence of mastitis increased the risk of potentially infected blood crossing the blood-milk barrier. EFSA concluded that on the basis of limited knowledge, milk and milk products from small ruminants are unlikely to provide any risk of TSE contamination provided that milk is sourced from clinically healthy animals (EFSA, 2004¹).

29. PrP^{SC} has been detected in mammary gland tissue of sheep co-infected with scrapie and maedi-visna virus and exhibiting lymphofollicular mastitis (Ligos *et al.*, 2005)

DILUTION

30. The presence of any BSE infectivity in animal excretions, if it occurs, would be subject to several dilution factors. These dilution factors include:
- the volume of faeces (affected by the diet and the total number of animals);
 - the volume of inert materials in faeces (affected by diet);
 - the volume of other excretions (e.g. urine);
 - the volume of inert materials added (e.g. bedding);
 - the degree of mixing during storage;
 - the rate of application to land;
 - rainfall; and
 - soil permeability.
31. Beef cows produce up to 32 litres of excrement per animal per day with a moisture content of 90%. Housed cows require about 0.7kg to 2.9kg bedding per day (depending upon the housing system and bedding material used). No figures are available for sheep as these are mainly kept extensively on commercial farms. The land area required for spreading livestock waste to meet the recommended maximum loading of 250kg per hectare per year of total nitrogen in applied organic manure, is 0.09 ha per 400kg beef bullock housed for 6 months (Source: Water Code, MAFF 1998²). The maximum nitrogen application rate is significantly reduced in Nitrate Vulnerable Zones.

PERSISTENCE OF THE BSE AGENT IN THE ENVIRONMENT

32. The TSE agent has been shown to be resistant to a number of very drastic treatments (Taylor, 2000).
33. The failure of several scrapie control programmes in Iceland may be attributable to persistence of the scrapie agent in the environment (Sigurdarson, 1991).
34. A high-titre scrapie agent from hamster brain retained infectivity after experimental burial for three years in soil inside perforated petri dishes. Between 2 and 3 log units (equivalent to only 0.3-1.7%) of the input infectivity of nearly 5 log units survived. There

was little leaching of the infectious agent into deeper soil layers (Brown & Gajdusek, 1991). The burial did not necessarily represent “real” conditions such as the effect of ploughing. The failure to recover the remaining 98.3-99.7% of the input scrapie infectivity cannot necessarily be attributed to decay (Gale and Stanfield 2001 cited by Gale 2004).

35. In their 2004 assessment of the safety of the application of organic fertilizers and soil improvers to pastureland, the EFSA working group concluded that it remains accepted that TSE agents (or infectivity) are degraded very slowly in the environment, however a definite period after which TSE agents can be considered to have been completely cleared from the environment, based on scientific evidence cannot be established (EFSA 2004²).
36. There is evidence that Chronic Wasting Disease (CWD) can be indirectly transmitted to susceptible mule deer from environments contaminated by excreta or decomposed carcasses. Under experimental conditions, mule deer became infected in two out of three paddocks containing naturally infected deer, in two out of three paddocks where infected deer carcasses had decomposed in situ \approx 1.8 years earlier, and in one of three paddocks where infected deer had last resided 2.2 years earlier (Miller *et al.*, 2004).
37. Epidemiological evidence suggests that an infectious dose of the BSE agent in contaminated feed can persist in feed stores for at least four years (SEAC 2005³).

UPTAKE OF INFECTIVITY INTO PLANTS

38. Plants absorb nitrates, ammonia and amino acids from soil for protein synthesis. It is unlikely that plants absorb proteins the TSE agent (SSC 2001).

INFECTION REDUCTION

39. Brown and Gajdusek’s work published in 1991, suggests that there may be a natural reduction in TSE infectivity in the environment over time but it is impossible to determine the precise effects of composting or other factors. Unless composting is carried out under strict conditions (e.g. turning, temperature monitoring) it is unlikely that all parts of the compost windrow will experience identical conditions (e.g. temperature, microbial activity). Therefore the resultant compost cannot be considered homogeneous.

40. A worst-case risk assessment of the use of composting and biogas treatment to dispose of catering waste containing meat assumes that composting has no effect on the BSE agent and that there is no decay in the soil. On this basis and using worst-case assumptions for the contamination of the raw material with the BSE agent and known application rates, the assessment concludes that the risk of such compost to grazing cattle is remote due to the effects of dilution. The assessment cites that, on average, humans ingest 0.384kg root crops per person per day (European Union System for the Evaluation of Substances (EUSES), 1997 cited by Gale, 2002). It has been estimated that as much as 2% (w/w) of root crops may be soil. Therefore, assuming that there is no subsequent washing or peeling of vegetables prior to consumption, a human may ingest a maximum of 2.8kg soil per person per year as a worst-case. Using the same worst-case parameters and assuming a tenfold cattle to human species barrier, the assessment concludes that the risk of applying such compost to crops consumed by humans is remote and is further reduced by washing the soil off the crops prior to consumption (Gale, 2002). Peeling vegetables would also reduce the risk of consumption of surface contamination. Although this risk assessment cannot be applied directly to the situation at Drayton, it demonstrates a worst-case scenario in which raw material containing known BSE infectivity can be diluted, composted and applied to land with negligible risk to human or animal health.

SPECIES BARRIER

41. It is likely that a species barrier limits transmission of BSE to humans. The species barrier between cattle and humans for BSE is unknown.
42. A preliminary estimation of cattle to human species barrier by the oral route has been proposed as 7-20 fold, but this is based on an extremely small amount of data from oral challenges of two macaques with infectious cattle brain (Lasmézas *et al.*, 2005 cited by EFSA 2005²). The worst-case composting risk assessment assumes a tenfold species barrier to BSE transmission between cattle and humans (bovine oral ID₅₀ =0.1g; human oral ID₅₀=1.0g) (Gale 2002).
43. In September 2005, SEAC stated (SEAC, 2005²) that “there are barriers to transmission (of TSEs) from one host species to another and there appears to be an appreciable barrier to transmission

between cattle and humans. It is not currently possible to predict the ability, or likelihood, of transmission between species based on current understanding of strain characteristics.”

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