

Table 4. Summary of Results of Infectivity Assays in mice of Tissues from sequentially killed cattle exposed orally to the BSE agent. (S.A.C.Hawkins & G.A.H.Wells, unpublished)

Tissue	Infectivity	Estimate of range of titre of infectivity (mouse i.c./i.p. ID ₅₀ /g) relative to incubation period (months) of donor cattle
Neural: Brain: frontal cortex, caudal medulla Pituitary Cerebrospinal fluid Dura Spinal cord: C2-C3, T10-T11, L3-L4 Nodose ganglia Dorsal root ganglia: C3-C6, T5-T8 Trigeminal ganglia Stellate ganglia Sciatic nerve Facial nerve Phrenic nerve Radial nerve Semitendinosus muscle Diaphragmatic muscle Triceps muscle Masseter muscle Sternocephalicus muscle Longissimus dorsi muscle	+ , + - - N.D. + , + , + - + + - - - - N.D. N.D. N.D. - - N.D. - -	[C57BL] ≤ 10 ^{1.0} (32-40m) [C57BL] ≤ 10 ^{1.0} (32-40m) [C57BL] ≤ 10 ^{1.0} (32-40m) [C57BL] ≤ 10 ^{1.0} (32-40m)
Alimentary: Tongue (dorsum, include mucosa) Submandibular salivary gland Parotid salivary gland Cranial esophagus Rumen Omasum Abomasum (pyloric) Duodenum Distal ileum (inc. Peyer's patches) Spiral colon Faeces [‡] Pancreas Liver	- - - N.D. - N.D. - - + - - - -	[R111] < 10 ^{0.5} - 10 ^{1.5} (6-14m), 10 ^{1.2} (18m) [C57BL] < 10 ¹ (36-40m)
Lymphoreticular: Spleen Thymus (cervical) Tonsil Submandibular lymph node Retropharyngeal lymph node Bronchial-mediastinal lymph node Hepatic lymph node Mesenteric lymph node Prescapular lymph node Popliteal lymph node	- - - - - - - - - -	Note: Tonsil was found positive in the cattle bio-assay (SEAC, 2002) – see elsewhere in this report
Other: Kidney Urine* Adrenal Lung (left caudal lobe) Nasal mucosa (midturbinate) Pericardium [‡] Heart (left ventricle/ septum) Mitral valve [‡] Aorta [‡] Blood (buffy coat) Blood (serum) Blood (clot) Bone marrow (sternum) Collagen (Achilles tendon) [‡] Skin [‡] Bone (femoral diaphysis) [‡]	- ‡ N.D. - - ‡ - ‡ ‡ - - N.D. N.D. +* - ‡ - -	[C57BL] < 10 ^{1.0} (38m)

Key to Table 4:

- + positive
- negative (i.e. < 10^{1.4} mouse (i.c. + i.p.) log₁₀ LD₅₀/g)
- N.D. Not Done (collected and reserved for future study)
- ‡ Selected tissue assays in R111 mice conducted at only two kill time-points (18 and 32 months after exposure).
- * Very low level of infectivity detected only at one time point (38 months after exposure) which is within the range of onset of clinical signs (end of incubation period) for cattle exposed in the study (Wells *et al* 1999)

II.5. BSE IN CATTLE: BIOASSAY OF CATTLE TISSUES BY INOCULATION OF CATTLE

In contrast to the widespread infectivity found in lymphoid tissues of cases of scrapie of sheep, the failure of the mouse bioassay to detect infectivity in tissues outwith the central nervous system of cattle naturally affected with BSE raised the issue of the efficiency of this assay system for the BSE agent. A study was therefore initiated (VLA/CSG SE1821) to provide a measure of the underestimation of the titre of infectivity in tissues across a species barrier in mice and to produce an approximate dose-incubation curve for infectivity of brain from BSE affected cattle by simultaneous titration of a primary inoculum in cattle and in mice. In addition, spleen and lymph node collected from natural cases of BSE were assayed in cattle to provide an order of magnitude estimate of concentration of infectivity in these tissues.

At approximately 4 months of age groups of calves were injected intracerebrally (i.c.) each with a single dilution of inoculum prepared from pooled brain stems from BSE affected cattle using a ten fold dilution range of 10^{-3} to 10^{-8} . Two additional groups of calves were similarly inoculated with a 10^{-1} dilution of a pool of spleen or lymph nodes. All calves were monitored clinically and retained until definite signs of clinical disease developed when they were killed and the brain examined to confirm the morphological phenotype of BSE and the presence of disease specific PrP by immunohistochemistry. A parallel titration in sinc⁸⁷ (RIII) mice was conducted according to standard mouse end point titration protocols over a dilution range of 10^{-1} to 10^{-6} . Mice were inoculated by the i.c. and intraperitoneal (i.p.) routes simultaneously to maximise the efficiency of the assay.

Brain titres of $10^{3.3}$ mouse (i.c. + i.p.) ID₅₀/g and $10^{6.0}$ cattle (i.c.) ID₅₀/g were established. The resultant value of the underestimation of the infectivity titre of BSE tissue when titrated across a species barrier in mice is therefore a factor of 500 fold (G.A.H.Wells and S.A.C.Hawkins, unpublished data). Expressed as relative titres, 10^0 mouse (i.c./i.p.) LD₅₀/g is equivalent to $10^{2.7}$ cattle (i.c.) LD₅₀/g, or the limit of detection of the mouse bioassay (at approximately $10^{1.4}$ mouse [i.c./i.p.] LD₅₀/g) is equivalent to $10^{4.1}$ cattle [i.c.] LD₅₀/g. Additional assays of selected tissues from the original pathogenesis study by intracerebral inoculation of cattle initially confirmed infectivity only in certain tissues which were already found to be positive by the mouse bioassay. However, recent results (SEAC, 2002) show the presence of low levels of infectivity in bovine tonsil from cattle 10 months after experimental oral exposure to BSE infection. So far only 1 of the group of 5 intracerebrally inoculated recipient cattle has developed disease. SEAC (2002), considered that this finding was unlikely to be an experimental artefact or due to residual infectivity in inoculum. An overall review of the results of additional assays of selected tissues, from the pathogenesis study, by intracerebral inoculation of cattle (as of August 2002) is presented in Table 5 (G.A.H.Wells and S.A.C.Hawkins, unpublished data).

That the relative degree of insensitivity of the mouse bioassay could explain the apparent absence of widespread LRS infectivity in BSE is not supported by the

results of assays by intracerebral inoculation of cattle with pooled lymph nodes (retropharyngeal, mesenteric and popliteal) or pooled spleens from five terminal clinical cases of BSE. In this study survival data suggested that, if present, the concentration of infectivity in these tissues was, at least, less than one, and possibly less than 0.1 cattle (i.c) LD₅₀/g.

Table 5: Bioassay of tissues from cattle exposed orally to BSE agent (Pathogenesis Study) by intracerebral inoculation of cattle (5 per inoculum group): details of inocula, according to sequential kill point of source cattle, inocula and inoculation dates.

Inoculum (months p.i.)	Date of inoculation	Survival time ⁴ (months) up to 29/8/02
Skeletal muscle ¹ (18m p.i.)	18.10.96	71
Liver (18m p.i.)	4.11.96	71
Kidney (18m p.i.)	6.11.96	71
Distal ileum (18m p.i.)	7.11.96	Mean incubation period 24 (5/5)
Skeletal muscle ¹ (32m p.i.)	11.11.96	70
Liver (32m p.i.)	13.11.96	70
Kidney (32m p.i.)	14.11.96	70
Peripheral nerve ² (32m p.i.)	9.12.96	69
Buffy coat (32m p.i.)	12.12.96	69
Caudal medulla/spinal cord (32m p.i.)	23.2.98	Mean incubation period 23 (5/5)
Distal ileum (32m p.i.)	25.2.98	55
Caudal medulla/spinal cord (22 m p.i.)	27.2.98	55
Thymus (6m p.i.)	6.4.98	53
Distal ileum (10m p.i.)	8.4.98	Mean incubation period 22 (5/5)
Skin (32m p.i.)	24.4.98	53
Caudal medulla (10m p.i.)	27.4.98	53
Caudal medulla/spinal cord (26m p.i.)	30.4.98	53
Spinal cord (10m p.i.)	28.5.98	52
Spleen (10m p.i.)	9.7.98	50
Tonsil (10m p.i.)	27.8.98	45† (1/5)
Thymus (10m p.i.)	1.9.98	49
Kidney (6m p.i.)	4.9.98	49
Liver (6m p.i.)	21.9.98	48
Skeletal muscle (6m p.i.)	22.9.98	48
Regional lymph nodes ³ (6m p.i.)	24.11.98	46
Peripheral nerve ² (6m p.i.)	26.11.98	46
Buffy coat (6m p.i.)	30.11.98	45
Spleen (6m p.i.)	2.12.98	45
Tonsil (6m p.i.)	3.12.98	45
Distal ileum (6m p.i.)	22.12.98	Mean incubation period 27 (5/5)
Mesenteric lymph nodes (6m p.i.)	23.12.98	45
Caudal medulla (6m p.i.)	5.1.99	44
Spinal cord (6m p.i.)	7.1.99	44
Peripheral nerve ² (18m p.i.)	11.1.99	44
Buffy coat (18m p.i.)	12.1.99	44
Regional lymph nodes (18m p.i.)	13.1.99	44
Salivary gland (18m p.i.)	19.1.99	44
Skin (18m p.i.)	21.1.99	44
Mesenteric lymph nodes (18m p.i.)	26.1.99	44
Spleen (18m p.i.)	28.1.99	43
Tonsil (18m p.i.)	2.2.99	43
Caudal medulla (18m p.i.)	9.2.99	43
Spinal cord (18m p.i.)	10.2.99	43
Skeletal muscle ¹ (26m p.i.)	11.2.99	43
Regional lymph nodes (26m p.i.)	12.2.99	43
Liver (26m p.i.)	16.2.99	43

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Table 5: Bioassay of tissues from cattle exposed orally to BSE agent (Pathogenesis Study) by intracerebral inoculation of cattle (5 per inoculum group): details of inocula, according to sequential kill point of source cattle, inocula and inoculation dates (continued)

Inoculum (months p.i.)	Date of inoculation	Survival time ⁴ (months) up to 29/8/01
Kidney (26m p.i.)	18.2.99	43
Distal ileum (26m p.i.)	19.2.99	43
Peripheral nerve ² (26m p.i.)	22.2.99	43
Buffy coat (26m p.i.)	23.2.99	43
Salivary gland (26m p.i.)	25.2.99	43
Skin (26m p.i.)	1.3.99	42
Mesenteric lymph nodes (26m p.i.)	2.3.99	42
Spleen (26m p.i.)	10.3.99	42
Tonsil (26m .i.)	11.3.99	42
Caudal medulla (26m p.i.)	15.3.99	42
Spinal cord (26m p.i.)	16.3.99	42
Bone marrow (32m p.i.)	18.3.99	42
Bone marrow (22m p.i.)	24.3.99	42
Bone marrow (36m p.i.)	29.3.99	41
Bone marrow (26m p.i.)	31.3.99	41
Urine (18m p.i.)	17.8.99	37
Nictitating membrane (field case material)	13.3.00	30

¹ Pool of semitendinosus, longissimus dorsi and masseter muscles

² Pool of sciatic and radial nerves

³ Pool of prescapular and popliteal lymph nodes

⁴ Survival time of animals remaining in the experiment rounded to nearest whole month (see text)

⁵ No. cattle developing clinical disease/no. inoculated

† Incubation period of single affected recipient. Survival period of remaining 4 recipients in group (at August 29/8/02) is 49 months

From a titration of a pool of BSE affected bovine brain tissue by intracerebral inoculation of cattle a dose/incubation curve has been produced from which it may be possible to obtain an approximation of the titre of an inoculum by reference to incubation period data for that tissue. From the available data to date on the bioassay of Pathogenesis study tissues in cattle, tissues containing infectivity are: distal ileum, 6 m.p.i., 10 m.p.i. and 18 m.p.i. and brain stem/spinal cord, 32 m.p.i. The mean incubation periods for the tissues at these time points, when estimated from the dose/incubation curve for the cattle titration suggest titres of 10^1 - 10^2 (6.m.p.i.), 10^3 (10 m.p.i.), 10^2 - 10^3 (18 m.p.i.) and 10^2 - 10^3 (32 m.p.i.) respectively. This corresponds very approximately to RIII mouse incubation period data in as much as by the mouse bioassay a rising titre (reducing mean incubation) was indicated by the results of distal ileum assay from cattle 6 months and 10 months after exposure and a plateau of incubation period in mice inoculated with distal ileum from cattle 18 months after exposure. The estimated values in certain instances do however show up to a 1 log₁₀ discrepancy between the cattle and mouse infectivity data when estimations are compared

between those calculated on the basis of the cattle i.c. titration dose-response regression curve and those derived from extrapolation from an anticipated 500 fold increased sensitivity over the mouse bioassay. Discrepancies between infectivity estimates by cattle ic titration and extrapolation from the mouse assays fall within the range of experimental error and the values can only be regarded as indicative of general trends.

If one considers the currently available survival times for inoculated cattle in this cattle assay (**Table 5**) it becomes clear that, should there be any infectivity in the remaining tissue groups it would already (as of August 2002) be $<10^1$ cattle i.c. ID_{50}/g for most and considerably lower for some groups. A preliminary summary of infectivity classification for cattle tissues is given in **Table 6**.

II.6. BSE IN SHEEP: BIOASSAYS OF SHEEP TISSUES AFTER ORAL EXPOSURE TO THE AGENT OF BSE BY INOCULATION OF MICE.

II.6.1. The report attached to the *Opinion of the SSC on Specified Risk Materials of Small Ruminants, adopted 13-14 April 2000* (EC 2000) states that from early results of the transmission of BSE to sheep studies (Sheep BSE pathogenesis experiment, carried out by the UK Institute for Animal Health -IAH) some ARQ/ARQ infected sheep have widespread PrP^{Sc} demonstrable in the lymphoreticular system tissues from 16 months after exposure, but there are, as yet, no corresponding bioassay results for infectivity. The report also stresses that this does not exclude finding infectivity or PrP^{Sc} at other (including younger) ages. Additional evidence, not cited in that report (Somerville *et al.*, 1997) demonstrated PrP^{Sc} in spleens of some QQ₁₇₁ Cheviot sheep infected with BSE.

The IAH sheep BSE pathogenesis experiment is ongoing. Immunocytochemical studies of tissues animals succumbing to BSE have been published (Foster *et al.*, 2001). The 7 animals that succumbed to BSE (6 are still alive) all showed PrP^{Sc} immunostaining in CNS and LRS tissues but not elsewhere. While the published results provide information only on clinical cases of experimental BSE in ARQ/ARQ Cheviot sheep (mean incubation period approximately 25 months after exposure to 5g oral dose) it is important to note that tissues from most major organs, including heart, lung, liver or thymus, showed no PrP^{Sc} immunostaining. Minimal staining was seen in glomeruli of the kidney. No evidence of PrP^{Sc} was found in any of the skeletal muscles tested, nor in reproductive tissues or skin.

It is of interest also that of the peripheral nerves examined (vagus, radial, sciatic) only the vagus, which has been proposed by many as implicated in the pathogenesis of scrapie after oral exposures, and not the somatic peripheral nerves, showed PrP^{Sc} immunostaining. Infectivity assays on a range of tissues from these animals are in progress. Studies on animals killed at intermediate times throughout the incubation period are not complete. Preliminary data support the findings of Jeffrey *et al* (2001) which suggest that in some animals evidence of the presence of TSE infectivity (e.g. PrP^{Sc} immunostaining) can be detected in some lymphoid tissues from early on after infection.

II.6.2. Interim updated results of studies by the VLA, UK of the tissue distribution of PrP^{Sc} (Jeffrey *et al*, 2001) and/or infectivity (mouse bioassay) in Romney (ARQ/ARQ) and Suffolk (ARQ/ARQ) sheep orally exposed to the BSE agent (5g affected brain homogenate) (S. Bellworthy, unpublished data) have established the earliest evidence of the presence of agent in tissues as follows:

Romneys (current data on incubation period range: 20-37 months)

- Retropharyngeal lymph nodes (LN)	4 months after exposure
- Peyer's patch	4 months after exposure
- Spleen	10 months after exposure
- Mesenteric LN	16 months after exposure
- Ileocaecal LN	16 months after exposure
- Mediastinal LN	16 months after exposure
- Tonsil	16 months after exposure
- Submandibular LN	16 months after exposure
- Distal ileum (excluding Peyer's patches)	16 months after exposure
- Mesenteric LN	16 months after exposure
- Prescapular LN	16 months after exposure
- Broncho-mediastinal LN's	16 months after exposure
- Brain and spinal cord	16 months after exposure
- Liver (low level of infectivity)	16 months after exposure
- Intestine	16 months after exposure
- Vagus nerve	16 months after exposure
- Forestomachs	22 months after exposure
- Abomasum	22 months after exposure
- Coeliaco-mesenteric ganglion (sympathetic)	22 months after exposure

New Zealand Suffolk (current data on incubation period of initial clinical cases: 24 months)

- CNS (including spinal cord)	}	10m
- Retropharyngeal LN	}	
- Submandibular LN	}	
- Prescapular LN	}	
- Spleen	}	
- Mesenteric LN	}	
- Peyer's patch	}	
- Ileo-caecal LN	}	
- Tonsil	}	
- Brain		16m

It must be stressed that there is marked variation in PrP detection results between animals and infectivity bioassay has been conducted on tissue pools from multiple animals. In particular there is no constant pattern of LRS involvement.

This work has also demonstrated PrP^{Sc} immunostaining of neurons in the enteric nervous system (ENS) throughout the alimentary tract (least in forestomachs) in some Romney sheep, but not in sheep that lacked immunostaining in Peyer's patches.

No immunostaining has been detected thus far in thymus, even in clinical cases, nor in somatic peripheral nerve trunks (sciatic, phrenic) or nerve roots of the spinal cord.

There are no new data from this study with regard to possible skeletal muscle infectivity.

Similarly dosed ARQ/ARR (heterozygous for BSE/scrapie susceptibility) Romney sheep are currently approximately four years after dosing and remain healthy.

Sequentially killed animals from this component of the study have not, as yet, shown PrP^{Sc} in any tissues suggesting, at least, that infectivity is extremely low in tissues, certainly up to two years after challenge.

These data suggest that unlike the situation in cattle experimentally infected by the oral route with a relatively large exposure dose of BSE agent, the results in sheep indicate a potentially widespread involvement of lymphoid tissues early in the incubation period at least in ARQ/ARQ scrapie/BSE susceptible sheep. New data are consistent with the previously expressed view that BSE in sheep after oral exposure is pathogenetically closely similar to scrapie, particularly with respect to the tissue distribution of infectivity and/or PrP^{Sc}.

II.6.3. The data published by Houston *et al* (2000) and Hunter *et al* (2002) and show that a high volume blood transfusion from sheep to sheep can transmit a BSE or scrapie illness within the same species and that infectivity can be transmitted from blood taken during the asymptomatic incubation period of the disease of the donor sheep. Little in terms of infectivity data can be drawn from these results and there is insufficient information on the relative efficiencies of routes of infection with BSE in sheep, but one interpretation might be taken from the generally accepted differences between efficiency of routes of inoculation in experimental models. The difference between the efficiency of the oral route and the intracerebral route in cattle is in the range 10^5 to 10^6 (G.A.H. Wells and S.A.C. Hawkins, unpublished). A similar value is frequently cited for the difference in efficiency between such routes in mice. If we assume that the intravenous route is almost as efficient as the intracerebral route, and that this could apply equally to sheep, than in the study cited previously (Jeffrey *et al*, 2001) the oral dose of $10^{4.0} \times 5$ which gave a minimum incubation period of 20 months, the total infectivity contained in 400 ml of blood, producing a similar incubation period, could be as low as 1-10 mouse ID₅₀ units. Notwithstanding discrepancies in making such calculations across sheep breeds this would certainly be undetectable by mouse bioassay.

II.6.4. Although no endpoint titration was conducted, incubation period data from primary transmission of infection from brain and spleen of sheep (Cheviot ARQ) infected intracerebrally or orally with BSE agent showed comparable incubation periods in each tissue (Foster *et al.*, 1996). These incubation periods were shorter than those obtained from the original primary transmissions of cattle BSE agent to mice (Fraser *et al.*, 1992) which gave endpoint titration results of at least $10^{5.1}$ (i.c.) LD₅₀/g. Experiments to compare the effects of i.c. and i.p. routes or their combination on incubation period in RIII mice (Bruce *et al.*, 1994) have shown slightly increased efficiency of detection of BSE infection (from cattle) with the combined route. It might be concluded, therefore, that the titre of infectivity in

the BSE affected sheep brain and spleen tested by Foster *et al* (1996) was of the order of 10^5 i.c./i.p. LD₅₀/g. Caution has been urged with regard to interpretation of incubation period assays in different tissues/organs since it has been shown that on a single pass of 263K hamster scrapie there was modification of the dose-response relationship for spleen compared to brain (Robinson *et al.*, 1990).

There are no titration data on tissues from sheep experimentally infected with BSE agent.

Mouse bioassay of tissues from the VLA study of oral exposure of Romney and Suffolk sheep to BSE agent (Jeffrey *et al* 2001) are incomplete but for some tissues of exposed Romney (ARQ/ARQ sheep) there is sufficient data on incubation period (S.Bellworthy, personal communication) to attempt approximations of titres of infectivity from RIII mouse dose response curves.

By 16 months after exposure (5g dose of $10^{4.0}$ mouse (i.c. + i.p.) ID₅₀/g) it appears that spleen is approaching a titre of approximately $10^{2.8}$ mouse (i.c. + i.p.) ID₅₀/g, lower at 10 months after exposure and increasing thereafter (data incomplete). Other lymphoid tissues at 16 months after exposure are probably $10^{1.0}$ but increasing thereafter and at 22 months after exposure (still preclinical) central nervous system infectivity is $\geq 10^3$.

No data are available as yet from clinically affected sheep (incubation periods 20-28 months (Jeffrey *et al* 2001).

The Annex 3 of the Report: Pre-emptive Risk Assessment Should BSE in Small Ruminants be found under domestic conditions, adopted 8-9 February 2001 (EC 2001a), which is based on results of this study is, therefore, still applicable with regard to classification of tissue infectivity for Romney (ARQ/ARQ) sheep experimentally exposed to the BSE agent (Table 3).

II.6.5. In view of this apparent close similarity in the distribution of infection in tissue between experimental BSE in sheep and natural scrapie it would seem that further guidance on the probability and possible levels of infectivity in different tissues should be drawn from previous tabulations of scrapie infectivity in tissues of small ruminants (see Table 1 and Annex 1).

II.7. CONCLUSIONS

II.7.1. TSES IN SHEEP (AND GOATS)

Scrapie in small (sheep) ruminants

There are no new data from which to update the Table 1 and the Annex 1 for infectivity of tissues of sheep for scrapie. These tables remain therefore valid as far as scrapie infectivity distribution is concerned.

BSE in small (sheep) ruminants

Recent data which would enable updating of sheep tissue infectivity titre tables for infection with the scrapie agent and for infection with the BSE agent are extremely limited. With respect to sheep experimentally exposed to the BSE agent interpretation of data set out above would suggest that infectivity titres in brain and spleen during the clinical disease phase may be comparable. Thus for BSE the possibility has to be considered that spleen (and possibly other lymphoreticular system tissues) may have to be regarded, together with CNS tissues, as containing a High level of infectivity. This is in contrast to previous data (Tables 1 and 2) in which spleen of sheep with scrapie has been assigned Medium infectivity. This clearly has implications for consideration of SRM for sheep where there is a probability of occurrence of BSE in sheep. This accepted, there are no new data from which to update the Tables 1 and 2 for infectivity of tissues of sheep for scrapie or BSE.

With respect to BSE in sheep, it would be prudent on the latest available evidence to adopt tabulations given at **Table 1** and the **Annex 1** as being probably as representative of BSE as scrapie with regard to distribution and level of infectivity in tissues. The single and important exception is that lymphoreticular tissues in BSE in sheep should provisionally at least, be considered comparable in their level of infectivity with central nervous system tissues.

II.7.2. BSE IN CATTLE:

A basis for producing cattle tissue infectivity tables for infection with BSE is emerging but the data are incomplete and much of the information emanates from a single study of the distribution of infectivity after experimental oral exposure. Available incubation period assay values from the few tissues containing infectivity in experimentally exposed cattle suggests that in most of the infected tissues infectivity is close to the limit of detection of the assay, even in central nervous system (**Table 4**). The current results of the re-evaluation of such tissues by bioassay in cattle (**Table 5**) compliment the mouse data. They show that low levels of infectivity (below those detectable by the mouse bioassay⁶) may be present in the palatine tonsil, but, to date not in any of the other tissues which gave negative results on mouse bioassay. The currently still ongoing such assays will not be completed for at least a further four years. Nevertheless, any further positive results would become available in that period. A tentative summary of available infectivity data for cattle with BSE is given at **Table 6**.

⁶ The cattle/mouse species barrier is estimated between 500 to 1000; a lack of detection of infectivity by the mouse bioassay may leave infectivity close to 1 oral infectious dose.