Table 6: **Tentative summary of preliminary estimations[1] on classification of tissues of cattle according to infectivity after experimental oral or natural exposure to the agent of BSE.**

Infectivity titre[2] (approx. range)		Experimental				Natural (Clinical)
		Preclinical (months after exposure)			Clinical (months after exposure)	
Mouse	Cattle[3]	(6-14)	(18)	(32)	(36-40	
High ($10^{3.0}$-$10^{5.0}$)	High ($10^{5.7}$-$10^{7.7}$)					**Brain** Spinal cord ?Retina (data not published)
Medium ($10^{1.5}$-$10^{3.0}$)	Medium $10^{3.3}$-$10^{5.6}$)	**Distal ileum (10 months)**		**Brain**		
Low ($\leq 10^{1.5}$)	Low ($\leq 10^{3.2}$)	Distal ileum	**Distal ileum**	Brain Spinal cord Dorsal root ganglia	Brain Spinal cord Dorsal root ganglia Trigeminal ganglion Distal ileum Bone marrow (38 months)	
Undetectable						
?($<10^{1.0}$)	?($<10^{0}$)	For list of tissues see **Tables 1, 5 & Annex**				**Retropharyngeal LN** **Mesenteric LN** **Popliteal LN** For remaining tissues tested see **Table 2** and associated references

1. Refer to **Tables 1, 5** and **Annex** for further detail
2. The classification used is preliminary and arbitrary because of a skewed range of infectivity in cattle with BSE compared to sheep with scrapie. It does not correspond to the Groups or Categories used in **Table 1 and Annex**.
3. Values in bold in the table are based on bioassay in cattle.

III. THE SAFETY OF RUMINANT HEADS

Note: Particular note is drawn to previous definitions used in Opinions and Reports for the head and its anatomical parts. For the purpose of the current report, "head" and "entire head" are considered the same and include the whole head, including the tongue. The term "skull" in the bovine context is the head excluding cheek meat (Masseter muscle) and tongue. In small ruminants the term "skull" is the head, excluding skin and tongue.

III.1. INFECTIVITY IN RELATION TO INCUBATION PERIOD

III.1.1. Bovine

In relation to the head in cattle with BSE, infectivity is consistently detected in the central nervous system (CNS) in the clinical disease, both in natural and experimental cases. In the experimental disease in cattle infectivity is detected in the CNS prior to the onset of clinical signs. But, the Pathogenesis Study does not provide interpretable data on the relationship between the earliest detectable infectivity in CNS (or any other tissue) and incubation period after experimental oral infection of cattle with the agent of BSE. In naturally occurring BSE, the age at which brain material may contain infectivity is unknown and it is not possible to predict when a case of BSE will show infectivity in the CNS. In the experimental study of BSE in cattle after oral exposure, in which the lower limit of the incubation period range was 35 months, evidence of infectivity [by conventional mouse bioassay] in the CNS was detected at 32 months, but not at 26 months after dosing (Wells *et al.*, 1998). However, these two observations, of clinical onset and tissue infectivity, cannot be compared directly since (given the sequential kill protocol of the study) the incubation period range of all animals in the study cannot be determined. A preliminary estimate from dose response data of cattle infected orally with a dose of BSE infectivity closely similar to that administered to induce disease in the Pathogenesis Study (G. A. H. Wells, unpublished data) suggests a mean incubation of almost 45 months (range 33-55 months). Because there is no direct experimental data relating infectivity of tissues to incubation period in BSE there is no equation that might be applicable to calculate initial detectability of tissue infectivity in relation to incubation of the natural disease. However, in certain experimental mouse models of scrapie, after peripheral routes of exposure, a constant relationship can be shown between the initial detection of infectivity in CNS and incubation. Within the range of models examined, infectivity was detectable at approximately 54% of the incubation period (Kimberlin and Walker 1988; Kimberlin and Walker 1989). It is not known if such a constant relationship might be applicable to BSE of cattle, but data from naturally occurring sheep scrapie where the approximate incubation period is apparent a similar value of 50% has been suggested (Opinion on SRM of Small Ruminants Adopted 13-14 April 2000). Based therefore, on the overall knowledge gained from natural incidents of TSEs in animals, and on available data, it seems not unreasonable to accept that infectivity may be first *detectable* in the CNS in natural BSE well in advance of clinical onset. This might be as little as 3 months before clinical signs, by conventional mouse bioassay, but theoretically

at least, it could be 30 months, in an animal with an average estimated field case incubation of 60 months. BSE infectivity has been assayed in mice and cattle, providing evidence for a cattle-to-mouse species barrier of about 500 fold ($10^{2.7}$)(G. A. H. Wells, unpublished data) As the cattle-to-human species barrier is yet unknown (E.C., 1999), no calculation of infectivity risk for man from an estimated onset of detectable infectivity in cattle CNS can be made.

As indicated earlier, infectivity in trigeminal ganglia (anatomically located within the base of the skull) in experimentally induced BSE has been detected only in the clinical disease stage and is probably secondary to replication of agent in CNS.

III.1.2. Sheep

There is little new information as yet, but from the VLA's experimental study of BSE in sheep (exposed to a relatively large dose of 5g of infective brain tissue), it appears that after this dose, involvement of the lymph nodes of the head (retropharyngeal), can be as early as 17% (4 months in the specific study) of the incubation period, and CNS involvement may occur from 40-66% (10-16 months in the specific study) of the incubation period. Clearly, with a range of much lower exposures in field situations that might be anticipated in endemic BSE in sheep and possibly different susceptible PrP genotypes in sheep, there may well be proportionally longer incubation periods and correspondingly later involvement of the CNS. However, it must be considered that dissemination of agent to widespread lymphoid sites may be a relatively constant early event in incubation of scrapie and BSE in sheep but could be influenced by their genotype.

III. 2. FACTORS ASSOCIATED WITH AGE

Age-cut-off limits for the skull, central nervous system, eyes and tonsils for bovine, ovine and caprine animals below which age the named tissue is not considered a risk need to be determined on a case-by-case basis which takes into account the criteria of animal species, infectivity in relation to incubation period, factors associated with slaughter protocols and geographical risk level of the source country or region.

There are no new data on the age specific incidence of BSE which would suggest any change in the risk in relation to head tissues of cattle. It has been previously established that the incidence of clinical disease occurrence in cattle below 30 months of age is approximately 0.05%. Experimental data also suggests that after oral exposure of calves to BSE infection, doses of the order of 100g of high titre brain material are required to give an incubation period range with a minimum of approximately 30 months (G. A. H. Wells and S. A. C. Hawkins, unpublished data). There are no further data on tissue infectivity of cattle relative to age which would impact on previous recommendations on listing of SRM pertaining to the head.

The absence of evidence of naturally occurring cases of BSE in sheep or goats and the preliminary nature of information on the pathogenesis of experimentally

induced BSE in sheep prevent clear inferences regarding age factors and the relative infectivity of head tissues. It must be acknowledged that natural exposures to BSE agent via feed or through endemic infection of sheep would probably result in a mean incubation period much like that of naturally occurring scrapie and greater than those resulting from the experimental oral exposures to BSE infection for which there is some data (Foster et al., 1993, and above Bellworthy, personal communication). However, the interactions of dose and host genetics, constituting the variables of effective exposure, do not as yet allow the sort of assessments that have been made in the case of cattle with BSE. Because of this uncertainty and the potential for the involvement of lymphoid tissues of the head at an early stage of incubation in sheep with BSE, there is no basis on which to recommend an age cut-off for the small ruminant head SRM's were BSE to be confirmed in small ruminants. Clearly, this needs also to be considered in relation to the geographical risk of BSE occurring in sheep and, dependent on possible grading of risk, an age cut-off could be applied, as suggested previously [*Opinion and Report from the Working Group: Specified Risk Materials of Small Ruminants, Opinion adopted 13-14 April 2000*] (EC 2000), particularly with respect to certain unprocessed meat products, such as MRM and/or offals (presumed tongue) derived from the head.

III.3. FACTORS ASSOCIATED WITH SLAUGHTER PROTOCOLS

This aspect is discussed in detail in the *Scientific Opinion and Report on Stunning methods and TSE risks* adopted by the SSC on 10-11 January 2002 (E.C., 2002).

The definition of bovine skull (entire head less cheek meat and the tongue) and the related non categorisation of bovine tongue as SRM (see above Table 2*)* may remain appropriate in relation to certain slaughter procedures. The regulations currently allow removal of tongue provided it is not contaminated (and can be removed within the confines of the abattoir and before contact with heads from other animals might occur). While this remains a reasonable and practical procedure the tongue could nevertheless be at risk from cross contamination with CNS material as a result of leakage from the foramen magnum and notably from the stun hole if a penetrative method of stunning is used.

Furthermore, head meat under hygiene regulations must be removed in a cutting plant designed for the purpose. The movement of large numbers of heads which are often in contact with each other, from an abattoir to the plant increases the risk of cross contamination of the surface of the meat with CNS material. The risk is increased when any penetrative stunning method is used (in the same order of risk as is specified in the report) but is not zero if penetrative stunning is not used because CNS material can still leak from the foramen magnum. It is noted also that all visible nervous and lymphatic tissue must be removed before sale to the consumer and that these tissues (lymph nodes and peripheral nerves) have not revealed detectable infectivity in cattle with natural or experimental BSE.

Thus, there are circumstances where it could be prudent to include the tongue (the entire head) from cattle as SRM. This could be subject to exclusions on the basis

of the use of a non-penetrative stunning method, on an age basis and in relation to the status of the BSE epidemic of a particular country. That is, where evidence can be provided of a declining epidemic and all the necessary measures are consistently enforced (see below), because the incidence of disease (and thereby infection) is low and becoming lower with time in younger animals.

Under normal abattoir procedures there is no contact between gut tissues (the only other tissue known to contain infectivity during the incubation period of experimentally induced BSE) and the head.

The classification of skull as SRM in small ruminants (the head excluding skin and tongue) also necessarily excludes the tongue from the SRM list but because of practicalities of slaughtering it has been suggested that the entire head of small ruminants may be required to be included as SRM at all ages. This would be particularly so in a situation where BSE has been confirmed or is considerely likely to have occurred in a sheep population.

Cross contamination of tongue with CNS from penetrative stunning or from the foramen magnum decapitation is more likely in sheep than in cattle because of skinning of the head. Furthermore, if the CNS is infective then it is highly likely that all lymph nodes of the head, tonsils and possibly peripheral nerves will also contain infectivity.

Without penetrative stunning, the contamination risk is only marginally reduced.

III.4. CONCLUSIONS

There is no new evidence from tissue infectivity studies of cattle affected by or incubating BSE that any additional tissues of the head, other than those already designated, should be regarded as SRM. On the contrary, results of infectivity bioassays in cattle support the view that in the clinical disease stage of BSE, regional lymph nodes, including those of the head have no detectable infectivity at least by mouse bioassay. Completed results of mouse bioassays of pituitary, CSF, the cranial cervical ganglion, facial nerve, tongue, salivary glands and lymph nodes of the head have not revealed infectivity. Furthermore, assay results, of trigeminal ganglia suggest a low titre of infectivity only in the clinical disease stage, probably secondary to CNS involvement.

Results of assays in cattle of certain tissues from cattle taken during the incubation period of BSE after oral exposure, are awaited, but to date have confirmed infectivity only in those tissues in which infectivity had been detected by the mouse bioassay. Thus there is no new infectivity data for cattle to suggest that skeletal muscle, tongue or associated nerves should be considered SRM at any age.

Exclusion from SRM of bovine tongue and cheek meat remain justified providing contamination by CNS, introduced during slaughter, can be avoided.

The head SRMs if a BSE risk exists remain appropriate for bovines.

With respect to sheep, there is involvement of lymphoid tissue of the head at a relatively early stage of incubation in experimental BSE in sheep, consistent with the view that BSE in sheep has a pathogenesis with respect to tissue distribution of infectivity comparable with natural scrapie. Somatic peripheral nerve trunk infectivity, although categorised as "low" in scrapie, may be widespread in the carcase by the clinical disease stage. If, as seems likely, this results from "centrifugal" spread from the CNS and infectivity can be detected in the CNS in experimental BSE of sheep approximately 40-50% through the incubation period, infectivity may be present in somatic peripheral nerve fibres from this stage. These observations make it difficult to recommend an appropriate lower age limit for the exclusion of any head tissues of sheep if BSE were confirmed or considered likely in a given population also because of a possible influence on incubation and tissue distribution by the genotype of the sheep. Furthermore, as stated previously, the practicalities in slaughtering of small ruminants may also necessitate removal of the entire head as SRM at all ages.

Also, the risk of cross-contamination of tongue with tissues with likely infectivity from early in the incubation of BSE, with or without penetrative stunning, in small ruminants, is considered high.

Consequently, if BSE is considered to occur in sheep, the whole or entire head, including the tongue, of all ages of sheep might have to be included in SRM irrespective of slaughterhouse practices. Possible exception to this would require additional risk assessment specifically for the occurrence of endemic BSE in sheep and the application of a geographic BSE (sheep) risk assessment.

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ANNEX: **Infectivity titres (bio-assayed in mice) in tissues from up to 9 Suffolk sheep (34-57 months old) and up to 3 goats (38-49 months old), at the clinical stage of natural scrapie compared with the titres in tissues from 1 or more confirmed cases of BSE** (Re-edited but unammended from Kimberlin 1994)

Tissues	Titre (mean SEM of (n) samples)[a]				Titre[a]
	Scrapie, sheep		Scrapie, goats		BSE, cattle
Category I					
Brain	5.6 ± 0.2	(51)	6.5 ± 0.2	(18)	5.3
Spinal cord	5.4 = 0.3	(9)	6.1 ± 0.2	(6)	+ve
Category II					
Ileum	4.7 ± 0.1	(9)	4.6 ± 0.3	(3)	<2.0
Lymph nodes	4.2 ± 0.1	(45)	4.8 ± 0.1	(3)	<2.0
Proximal colon	4.5 ± 0.2	(9)	4.7 ± 0.2	(3)	<2.0
Spleen	4.5 ± 0.3	(9)	4.5 ± 0.1	(3)	<2.0
Tonsil	4.2 ± 0.4	(9	5.1 ± 0.1	(3)	<2.0
Category III					
Sciatic nerve	3.1± 0.3	(9)	3.6 ± 0.3	(3)	<2.0
Distal colon	<2.7± 0.2	(9)	3.3 ± 0.5	(3)	<2.0
Thymus	2.2 ± 0.2	(9)	<2.3 ± 0.2	(3)	not done
Bone marrow	<2.0 ± 0.1	(9)	<2.0	(3)	<2.0
Liver	<2.0 ± 0.1	(9)	--		<2.0
Lung	<2.0	(9)	<2.1 ± 0.1	(2)	<2.0
Pancreas	<2.1 ± 0.1	(9)	--		<2.0
Category IV					
Blood clot	<1.0	(9)	<1.0	(3)	<1.0
Heart muscle	<2.0	(9)	--		<2.0
Kidney	<2.0	(9)	<2.0	(3)	<2.0
Mammary gland	<2.0	(7)	<2.0	(3)	<2.0
Milk*	--		<1.0	(3)	not done*
Serum	--		<1.0	(3)	<1.0
Skeletal muscle	<2.0	(9)	<2.0	(1)	<2.0
Testis	<2.0	(1)	--		<2.0

The data are taken from the following sources: sheep scrapie, Hadlow *et al* (1982); goat scrapie, Hadlow *et al* (1980); BSE, Fraser *et al* (1992); Fraser & Foster (1994), and Kimberlin (1994). The classification of tissues is according to the CPMP Guidelines (EC, 1991). The Table is from Kimberlin (1994) and has been reproduced previously as Table 3 in the SSC Opinion of 9 December 1997 providing a *Listing of Specified Risk Materials (re-edited 23 January 1998) and in SEAC Report 1994*, (Table5.2 Amended). The only positive bovine tissue (brain), for which a titre is quoted, is from Fraser *et al* (1992). The remaining tabulation for negative tissues of cattle provides the cut off of sensitivity of the assay according to standard calculation of the minimum detectable titre taking into consideration volume of inoculum used. The <1 and <2 entries quoted in the table are in the original paper. The <1 values may relate to the possibility that inoculum used for blood clot and serum was undiluted, but this is not stated in the source paper of the bioassay of tissues from clinical cases of BSE (Fraser and Foster 1994), or (Kimberlin 1994).

[a]Titres are expressed as arithmetic means of log 10 mouse i/c. LD 50/g or ml of tissue (+ve > 2.0).

+ve = transmission positive but not titrated

NOTE: None of the bovine tissues in categories II and III and no tissues in Category IV had any detectable infectivity. The values shown are maxima based on the limits of detectability of the bioassay in mince (calculated for 30µl of inoculum injected intracerebrally).

* Data on the negative results of bioassay of milk from cattle with BSE were not available in Kimberlin (1994). Subsequently, negative results of bioassay in mice were published and cited by Kimberlin (1996), see Table 2 of Report.