EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions

UPDATE OF THE OPINION ON

TSE INFECTIVITY DISTRIBUTION IN RUMINANT TISSUES

INITIALLY ADOPTED BY

THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 10-11 JANUARY 2002

AND AMENDED AT ITS MEETING OF 7-8 NOVEMBER 2002

following the submission of (1) a risk assessment by the German Federal Ministry of Consumer Protection, food and Agriculture and (2) new scientific evidence regarding BSE infectivity distribution in tonsils

OPINION

At the end of 2001, the Scientific Steering Committee (SSC) was invited:

(1) To update, on the basis of the most recent scientific data, the sheep tissue infectivity titre table presented in the SSC opinion of 22-23 July 1999 on The Policy of Breeding and Genotyping of Sheep;

(2) To create a similar table for cattle on the basis of all available scientific evidence;

(3) To consider whether any new evidence exists since the adoption of its opinion of 9 December 1997 on the listing of Specified Risk Materials which would indicate that the entire head of cattle, sheep and goats, including skeletal muscle, tongue and associated innervation should be considered as specified risk material.

The SSC invited the TSE/BSE *ad hoc* Group to prepare a scientific report that could serve as the basis for preparing an answer to the above question. A first report was finalised by the TSE/BSE *ad hoc* Group at its meeting of 13 December 2001 it served as the basis for an opinion adopted by the SSC at its meeting of 10-11 January 2002.

On 10 may 2002, the Government of the Federal Republic of Germany requested the European Commission to elicit the opinion of the Scientific Steering Committee on a report of 25 April 2002 prepared by the Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), which evaluates the possible risks related to harvesting cheek meat and more specifically lists the critical hygiene points in harvesting of bovine heads and cheek meat of cattle. Whilst preparing this opinion, the SSC was informed about a recent outcome of the ongoing UK research, into the pathogenesis of BSE in cattle showing low levels of infectivity in bovine tonsil. In the light of these new data, the TSE/BSE *ad hoc* Group updated its report of 13 December 2001. This update report is attached and it served as the basis for the current update of the SSC opinion on TSE infectivity distribution in ruminant tissues. An English translation of the German report is also attached, for ease of reference (**see Annex 2** of the attached report).

The SSC adopts the following answers to the above questions:

(1) **Tissue infectivity tables applicable for small ruminants.**

Scrapie in small ruminants. There is no new evidence that became available since February 2001 and the SSC's therefore considers that the table attached to its pre-emptive risk assessment of 8-9 February 2001 remains valid. It is annexed as **Table 1** for ease of reference.

BSE in small ruminants. The SSC considers that, pending more experimental data becoming available, it would be prudent on the latest available evidence to adopt tabulations given at **Table 1** as being probably as representative of BSE as scrapie with regard to distribution and level of infectivity in tissues. However, 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.

(2) **Tissue infectivity tables related to BSE in cattle.** Available 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. The early results of the re-evaluation of such tissues by bioassay in cattle compliment the mouse data, but such assays will not be completed for at least a further five years. Nevertheless, any further positive results would become available in that period. A tentative summary of available infectivity data for cattle with naturally acquired BSE is given at **Table 2** (Tissues with no infectivity from confirmed cases) and **Table 3** (Preliminary estimates of tissue infectivity after experimental and natural exposure).

(3) **Possible consideration as specified risk material of the entire head of cattle, sheep and goats, including skeletal muscle, tongue and associated innervation.**

Regarding ***cattle*** affected by or incubating BSE, the SSC considers that there is new evidence from tissue infectivity studies showing that certain head tissues (in

addition to: brain, eyes, dura mater, pituitary gland and skull) could possibly be regarded as SRM at least under certain circumstances. So far results of infectivity bioassays in cattle have supported the view that in the clinical disease stage of BSE, regional lymph nodes, including those of the head have no detectable infectivity. Furthermore, assay results of trigeminal ganglion suggest a low titre of infectivity only in the clinical disease stage, probably secondary to CNS involvement. However, whereas completed results of mouse bioassays of pituitary, cerebro-spinal fluid (CSF), the cranial cervical ganglion, facial nerve, tongue, salivary glands and several lymph nodes of the head from preclinical and clinical stages of experimental BSE in cattle have not revealed infectivity, there is now evidence from cattle-to-cattle transmission studies that the palatine tonsil may contain low levels of infectivity at an early stage of the incubation period and that this may affect the safe consumption of tongue if there is a risk of contamination of this tissue.

There is still no new infectivity data for cattle to suggest that skeletal muscle, tongue or associated nerves should be considered SRM at any age. However, the risk assessment carried out on behalf of the German authorities show that the SSC's initial statement that the "*Exclusion from SRM of bovine tongue and cheek meat remains justified providing contamination by CNS, introduced during slaughter, can be avoided*" may not necessarily be appropriate. That conclusion was reached considering the long list of critical points in the process of slaughtering the animal, the removal, storage and transport of the head and of the harvest of cheek meat.

On the basis of what precedes the SSC considers that:

(1) the tonsil of a bovine animal of any age should be regarded as posing a risk.

(2) the tongue of animals certified safe for human consumption does not pose a risk if contamination with CNS and tonsil material is avoided for animals of any age. This may imply that the harvested section of the tongue is shortened [to the "short tongue"], to avoid, by a cautious margin, removal with the tongue of that part of the root of the tongue containing lingual tonsil.

(3) cheek meat of animals certified safe for human consumption, which is collected as part of a different process (**Annex 2**), does not pose a risk if a

wide range of precautions to avoid cross-contamination is taken. The feasibility of implementation of these precautions under field conditions may however be questioned and would in any case require to be previously verified.

With respect to ***sheep***, there is involvement of lymphoid tissue of the head at an 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 carcass 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.

Furthermore, the practicalities in slaughtering of small ruminants may 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 be present in sheep, the whole or entire head, including the tongue, of all ages of sheep should be included in the list of SRMs irrespective of slaughterhouse practices, until evidence to the contrary becomes available.

Very limited data are available for goats. The conclusions for sheep are therefore considered to be a reasonable approximation also for goats.

Table 1: Natural scrapie in sheep and goats: classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats[1] (Re-edited but unammended from Annex: Opinion on The Policy of Breeding and Genotyping of Sheep, 22-23 July 1999) (EC 1999)

Infectivity titres*: A = high ($\geq 10^{4.0}$)
B = medium ($10^{3.2} - 10^{4.0}$)
C = low ($\leq 10^{3.2}$ or unknown)
D = undetectable

	PRE-CLINICAL				CLINICAL	
Age (months)	**≤ 8**	**10-14**[2]	**25**	**> 25**	**34-37**	**38-39**
Numbers positive / examined	**0/16**	**8/15**	**1/13**	**1/6**	**9/9**	**3/3**
Brain					A	A
Brain (medulla)		D	C			
Brain (medulla / di-encephalon)			C			
Brain (cortex mid-brain)			D			
Pituitary					C	B
Spinal cord			D		A	A
Cerebro-spinal fluid					C	C
Sciatic nerve					C	C
Thymus	D		D		C**	C**
Thyroid					D	
Spleen	D	B	C		B	B
Tonsil	D	C	B		B	
Lymph node (RP/MP)	D	B	B		B	B
Lymph node (BM)		D	C		B	B
Lymph node (PS/PF)	D	C	C			
Lymph node (PF, 1/9 negative)					B	
Lymph node (PS, 2/9 negative)					B	
Lymph node (supra-mammary)			D		C	B
Colon-proximal		B	B		B	B
Colon-distal		D	D		C	C
Ileum	D					
Ileum-distal		B	B		B	
Ileum-proximal						B
Rectum-distal					B+	B
Pancreas					C**	
Adrenal			D		C	C
Nasal mucosa			D		C	C

1 After Hadlow et al. (1979, 1980, 1982), Pattison *et al.* (1964, 1972), Groschup et al. (1996). Regarding DRG: see Report.

2 Techniques for the determination of infectivity become more and more sensitive. The age range may go below 10 months. In individual cases, tonsil infectivity has been detected in lambs of 16 weeks. Placenta has been placed in Group C, but titres are unknown.

Table 1 (continued): Natural scrapie in sheep and goats: classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats [1] (Re-edited but unammended from Annex: Opinion on The Policy of Breeding and Genotyping of Sheep, 22-23 July 1999) (EC 1999)

Infectivity titres*: A = high ($\geq 10^{4.0}$)
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C = low ($\leq 10^{3.2}$ or unknown)
D = undetectable

	PRE-CLINICAL				CLINICAL	
Age (months)	**≤ 8**	**10-14[3]**	**25**	**> 25**	**34-37**	**38-39**
Numbers positive / examined	**0/16**	**8/15**	**1/13**	**1/6**	**9/9**	**3/3**
Bone marrow					C**	D
Liver					C**	
Blood clot		D			D	D
Serum		D				D
Salivary glands			D		D	D
Saliva					D	
Muscle- skeletal					D	D
Heart					D	
Kidney					D	D
Lung					D	
Ovary					D	D
Uterus					D	D
Placenta					C**°	
Fetus					D	
Mammary gland					D	D
Colostrum				D		
Milk						D
Semen vesicle					D	
Testis					D	
Faeces		D				D

* = Log_{10} mouse intracerebral LD/50 per 30 mg tissues; (titres given as approximate ranges)
** = trace or exceptional
\+ = Not assayed but high content of lymphoreticular tissue
° = negative in other studies
MP = Mesenteric/portal
PF = Prefemoral
CSF = Cerebro-spinalfluid
PS = Prescapular
LN = Lymph node
RP = Retropharyngeal
BM = Bronchomediastinal

[3] Techniques for the determination of infectivity become more and more sensitive. The age range may go below 10 months. In individual cases, tonsil infectivity has been detected in lambs of 16 weeks. Placenta has been placed in Group C, but titres are unknown.

Table 2: Tissues from confirmed cases of BSE in cattle in which no infectivity was detected by bioassay in mice injected both intracerebrally and intraperitoneally (Taken from Kimberlin, 1996)

Nervous tissues	***Lymphoreticular tissues****
Cerebrospinal fluid	Spleen
Cauda equina	Tonsil
Peripheral nerves :	Lymph nodes
- sciaticus	- prefemoral
- tibialis	- mesenteric
- splanchnic	- retropharyngeal
Alimentary tract	***Reproductive tissues***
Oesophagus	Testis
Reticulum	Prostate
Rumen (pillar)	Epididymis
Rumen (oesophageal groove)	Seminal vesicle
Omasum	Semen
Abomasum	Ovary
Proximal small intestine	Uterine caruncle
Distal small intestine	Placental cotyledon
Proximal colon	Placental fluids :
Distal colon	- amniotic fluid
Rectum	- allantoic fluid
	Udder
	Milk
Other tissues	
Blood :	Liver
- buffy coat	Lung
- clotted	Muscle
- foetal calf	- semintendinous
- serum	- diaphragma
Bone marrow	- longissimus
Fat (midrum)	- masseter
Heart	Pancreas
Kidney	Skin
	Trachea

* Tonsil was found positive in the cattle bio-assay – see opinion and attached report.

Table 3: Tentative summary of preliminary estimations* on classification of tissues of cattle according to infectivity after experimental oral or natural exposure to the agent of BSE.

Infectivity titres**:

A = high:	$10^{3.0} - 10^{5.0}$ in mouse;	$10^{5.7} - 10^{7.7}$ in cattle ***
B = medium	$10^{1.5} - 10^{3.0}$ in mouse;	$10^{3.3} - 10^{5.6}$ in cattle ***
C = low	$\leq 10^{1.5}$ in mouse;	$\leq 10^{3.2}$ in cattle ***
D = undetectable		
? = data not published		

	EXPERIMENTAL				NATURAL
				Clinical	clinical
months after exposure	**6-14**	**18**	**32**	**36-40**	-
Brain			B / C	C	A
Retina					?
Spinal cord			C	C	A
Dorsal root ganglia			C	C	C
Trigeminal ganglion				C	
Ileum-distal	B / C	C		C	
Palatine tonsil	C†				
Lymph node (Retropharyngeal)					D
Lymph node (Mesenteric)					D
Lymph node (Popliteal)					D
For the list of tissues in which no detectable infectivity was found: see tables 1 and 2 of this opinion and table 5 and the Annex of the attached report.					

* Refer to the report for further detail

** 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**.

*** Values in bold in the table are based on bioassay in cattle.

† Preliminary evidence of estimated titre of $< 10^1$ in cattle