Listing of Specified Risk Materials: a scheme for assessing relative risks to man

Opinion of the Scientific Steering Committee adopted on 9 December 1997

(Re-edited* version adopted by the Scientific Steering Committee during its Third Plenary Session of 22-23 January 1998)

(*) The corresponding sections are printed in italics and are underlined)

Executive summary

The SSC set up a working group to address the question of SRM at its first meeting on 21/11/97. This working group consisted of the members of the SRM working group established by the MDSC/SSC at its last meeting plus the members of the TSE/BSE ad-hoc group of the SSC.

1. The SSC is of the opinion that in an animal (bovine, ovine or caprine) infected with BSE a differentiation of the different tissues/organs with regard to their relative infectivity is possible. The different levels of infectivity reflect a graded phenomenan and it is unwise the BSE agent as either present or absent in any particular tissue of an infected animal. The SSCIt has adopted the following table. Additional justification can be found in the complete document.

Categorising the potential infectivity of different organs in BSE-infected animals.

The assessment of the infectivity is based in part on scrapie titres, on the finding of high infectivity in the brain of BSE-affected cattle, on the differential impact of BSE-infective organs on the infection of mice to intracerebral inoculation and on the presumed CJD infectivity of human dura mater and human pituitary gland based on transplant data and the effects of human growth hormone infection. For practical reasons relating to slaughterhouse contamination, some tissues are categorised at a higher level than warranted by their intrinsic infectivity.

Category		Organs
1. High infectivity	a)	Bovine brain, eyes, bovine spinal cord and bovine dorsal root ganglia, *dura mater*[1,2], pituitary[1,2], skull[2,3] and bovine vertebral column[2] , lungs[5]
	b)	Ovine/caprine brain, eyes and spinal cord, dorsal root ganglia and vertebral columns[2]; ovine and caprine spleens[3], lungs[5]
2. Medium infectivity	a)	Total intestine from duodenum to rectum[6], tonsils
	b)	Bovine spleen, placenta, uterus, fetal tissue[7], adrenal, cerebrospinal fluid, lymph nodes
3. Low infectivity		Liver, pancreas, thymus, bone marrow, other bones[8] nasal mucosa, peripheral nerves
4. No detected infectivity[9]		Skeletal muscle, heart, kidney, colostrum, milk, discrete adipose tissues[10], salivary gland, saliva, thyroid, mammary gland, ovary, testis, seminal testis, cartilaginous tissue, connective tissue, skin, hair, blood clot[11], serum[11], urine, bile, faeces

Notes

Where no species specification is given then the tissues refer to bovine, ovine and caprine species.
1. These tissues are included because iatrogenic CJD in humans has been associated with tissues or extracts from humans which were contaminated with CJD agent.
2. These tissues have been moved up 1 to 3 categories because of the possibility of contamination by tissues of higher infectivity during slaughter and their inclusion of dorsal root ganglia. Ovine/caprine spinal cord, dorsal root ganglia and vertebral column are put in this sub-category because they could be infected or contaminated if sheep/goats have in practice become "back infected" with BSE from their feeding on infective bovine products.
3. Definition of Skull: Entire head excluding the tongue.
4. Ovine spleens are included because of the finding of the BSE agent in the spleens of sheep challenged experimentally with large doses of BSE. Caprine have not been tested for infectivity with BSE but showed infectivity for Scrapie. Bovine spleen has been tested and showed no infectivity in the mice test.
5. Lung should be considered in the category if the slaughtering method induces through the stunning/pithing method a transfer of brain through the blood stream into the lung.
6. This applies to cattle only unless sheep and goats are considered to be infected by BSE, in which case there would be a need to remove lymph nodes and thymus also.
7. These may best be considered in the same category as placenta because of the high probability of contamination when removing the placenta at slaughter.
8. The likely presence of bone marrow in long bones now means that these bones, on the basis of potential infectivity in older animals, should be placed in the same category as bone marrow.
9. All materials listed under category 4 have been tested in mice with samples reflecting 0.01-0.1 g of original infective tissue. In such samples infectivity titres 1000 fold lower than in brain cannot be detected by this method. Further improvements in the sensitivity can be expected. This may require the revision of the table of relative infectivity given above.
10. This new term is used to describe those reserves of fat which can be removed readily during slaughter in the abattoir or at meat-cutting plants. It does not refer to lipid extracted from mechanically recovered meat or from many other tissues, or at a later stage in the production process. It presupposed the removal of the key associated lymph nodes.
11. There is some albeit in-conclusive evidence that experimentally circulating peripheral blood mononuclear cells may transmitt nvCJD.

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2. Definition of SRM

Hitherto there has been a tendency to consider the specified risk materials (SRM) as simply relating to the tissue itself However, it is now clear that SRMs should not be defined simply on the basis of the grades of infectivity as documented by challenge tests of different tissue extracts. This conclusion reinforces the concepts underlying Table 2, i.e. that the different levels of infectivity do reflect a graded phenomenon and that it is unwise to consider the BSE agent as either present or absent in particular tissues. In addition the following have to be considered before a true risk assessment can be made: animal age, species and geographical origin. Table 6 specifies the SRMs for different species but excludes the detailed geographical analysis. The actual risk is also affected by whether or not the material is processed before consumption and its final use.

3. Conclusion

On the basis of the detailed analysis of the different factors influencing the definition of SRMs the SSC suggests a system for specifying SRMs on the basis of relative tissue infectivity, species and age (Table 2). The SSC proposes that the tissues included in table two should be excluded temporarily from the human food and animal feed chain depending on the geographical source.

Table 2: Suggested list of specified risk materials to be excluded from human and animal consumption except when derived from a BSE free country *with a negligible risk*.

Tissue	Species	Age limit (months)	Basis
Brain	Bovine	>12	Infectivity
	Ovine*,	>12	"
	Caprine*	>12	"
Eyes	Bovine	>12	Infectivity
	Ovine*	>12	"
	Caprine*	>12	"
Dura mater	Bovine	>12	Contamination[1]
	Ovine*	>12	"
	Caprine*	>12	"
Pituitary	Bovine	>12	Contamination[1]
	Ovine*	>12	"
	Caprine*	>12	"
Skull	Bovine	>12	Contamination
	Ovine*	>12	"
	Caprine*	>12	"
Spinal cord	Bovine	>12	*Infectivity*
	Ovine	>12	*Theoretical back infection*
	Caprine	>12	*" " "*
Dorsal root ganglia	Bovine	>12	*Infectivity*
	Ovine	>12	*Theoretical back infection*
	Caprine	>12	*" " "*
Vertebral column	Bovine	>12	*Contamination and low infectivity*
	Ovine	>12	*Contamination if back infected*
	Caprine	>12	*Contamination if back infected*
Spleen	Ovine	All	*Infectivity*[2]
	Caprine	"	*"*
Intestine	Bovine	All	*Infectivity / Contamination*[3]
	Ovine	"	*" "*
	Caprine	"	*" "*
Tonsils	Bovine	>12	*Infectivity*[4]
	Ovine	>12	*"*
	Caprine	>12	*"*
Lung	Bovine	>12	*Contamination from brain via blood*
	Ovine,	>12	*when animals are killed by pithing or*
	Caprine	>12	*stunning.*

* Practicalities may well dictate the removal of the heads of sheep and goats at all ages.

Note: In countries specified as at high risk of BSE it may be considered appropriate to reduce further the age limit for these tissues from 12 to 6 months. The risks from bovine tissues need to be considered separately from those of ovine and caprine origin. In high risk countries, all tissues from cattle over 30 month age may be considered as at greater risk and therefore be considered as SRM. Long bones from cattle below 30 months showing no clinical signs of BSE may be considered at present acceptable for human consumption.

Footnotes to table 2:

1. Known CJD-transmission with human tissue
2. BSE infectivity found in spleen of BSE challenged sheep; assumption that goats would have displayed the same response if tested.
3. Ileal infectivity in calves; colonic infectivity in sheep scrapie and potential for contamination of adjacent colon as small intestine removed in slaughter houses.
4. PrP^{Sc} shown in man with nvCJD; also infectivity in scrapie sheep (see Table 2).

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Report

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Introduction:

The EU July 1997 Directive set out the Specified Risk Materials (SRM) which needed removing to limit the risk of BSE-contaminated tissues or their products being eaten. These tissues and the EU qualifications to the listing are set out in Table 1.

Table 1: Commission decision of 30 July 1997 on the prohibition of the use of material presenting risks as regards transmissible spongiform encephalopathies

Article 1:	Specified Risk Material (SRM) (a) The skull, including the brain and eyes, tonsils and spinal cord of:- - bovine animals aged over 12 months, - ovine and caprine animals which are aged over 12 months or have a permanent incisor tooth erupted through the gum. (b) The spleens of ovine and caprine animals.
Article 2:	The use of specified risk material for any purpose shall be prohibited.
Article 3:	Prohibits the use of the vertebral column of bovine, ovine and caprine animals for the production of mechanically recovered meat.
Article 4:	Deals with the disposal of SRM but with special powers for the use of material for teaching, research, etc.
Article 5:	Deals with monitoring of slaughterhouses, storage and disposable facilities, etc.
Article 6:	Specifies that all imports from whatever origin should comply with these requirements.

Decision 97/534/EC in the Official Journal No. L216, 08/08/1997 P.0095

The SSC has now identified some changes needed on the basis of new published and unpublished data. A re-evaluation of the experimental and epidemiological evidence relating to the infectivity of tissues from different species also affects the listing.

1. Currently designated SRMs

1.1 The tissues specified in Article 1(a) of the Directive are appropriate. The brain of BSE-infected cattle has been shown to be highly infective for a wide range of species. The infectivity of the spinal cord is also not in doubt. The inclusion of the eyes conforms with previous analyses (*e.g.* of WHO, see Table 2) and advice. The inclusion of the skull is also important.

1.2 The tonsils have now been shown in a human case of nvCJD to have evidence of the prion affecting the tissue (Collinge ref.), and the tonsils in sheep with scrapie have also been found to be highly infective (Table 3). Therefore the inclusion of tonsils in this category is appropriate.

Table 2: The WHO categorisation of infectivity in bovine tissues and body fluids (Based on relative scrapie infectivity of tissues and body fluids from naturally infected Suffolk sheep and goats with clinical scrapie)

Category I:	**High infectivity** Brain, spinal cord, (eye)*****
Category II:	**Medium infectivity** Spleen, tonsil, lymph nodes, ileum, proximal colon, cerebrospinal fluid, pituitary gland, adrenal gland, (dura mater, pineal gland, placenta, distal colon)
Category III:	**Low infectivity** Peripheral nerves, nasal mucosa, thymus, bone marrow, liver, lung, pancreas
Category IV:	**No detectable infectivity** Skeletal muscle, heart, mammary gland, milk, blood clot, serum, faeces, kidney, thyroid, salivary gland, saliva, ovary, uterus, testis, seminal testis, foetal tissue, (colostrum, bile, bone, cartilaginous tissue, connective tissue, hair, skin, urine).

*****Tissues in brackets were not titrated in the original studies (1, 2) but relative infectivity is indicated by other data on spongiform encephalopathies. Table 3 provides a compilation of the original data on the levels of infectivity in different tissues of sheep clinically infected with scrapie.

References:

1. Hadlow, W.J., Kennedy, R.C., Race, R.E. (1982) *Journal of Infectious Diseases* **146**: 657-664.
2. Hadlow, W.J., Kennedy, R.C., Race, R.E., Eklund, C.M. (1980) Veterinary pathology 17: 187-199.

Note: This is an exact duplication of the Table as presented by WHO in its document relating to a Consultation on Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies held in Geneva, Switzerland on 24-26 March, 1997. This meeting had the participation of The Office International des Epizooties (OIE).

Table 3: Infectivity titres (bioassayed 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

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)	?.?
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)	?.?
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), these proceedings as Kimberlin (below). The classification of tissues is according to the CPMP Guidelines (EC, 1991).

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

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.

+ve = transmission positive but not titrated
?.?,-- = not done or not available

Taken from *SEAC* 1994, Table 5.2

2. Spleens of ovine and caprine species

2.1 The SSC agrees that these tissues should also be included in view of clear experimental evidence that the spleen does become very infective after the induction of BSE in sheep and goats fed or injected with BSE-affected brain extracts from cattle (Foster *et al.*, 1993).

2.2 The issues raised by the other articles in the Directive are dealt with later, but in addition there are questions relating to other tissue questions of age limits and the geographical origin of the material. These will be dealt with first.

3. Intestine

3.1 The EU Directive does not include intestinal tissue since this evaluation was primarily based on the WHO assessment of the relative infectivity of different tissues of sheep with clinical scrapie (Table 2). The titre of infectivity was judged in the US studies by Hadlow *et al.* (1980, 1982) on the basis of the dilutions of the donor tissue required to display scrapie infectivity in mice injected intracerebrally with extracts of these tissues (see Table 3). On this basis the intestine ranked slightly lower in infectivity terms than the central nervous system (CNS) because these animals were clinically affected by scrapie and with a highly infective CNS. It is on this basis that the WHO June classification was also developed. The EU decision accordingly concentrated on the central nervous system as the highest risk tissue.

3.2 The concepts underlying the development of BSE presume that infection has usually come from the ingestion of contaminated feed. Therefore, as a precautionary measure, the intestine should be considered as a primary route of infection.

3.3 Studies in cattle, sheep and goats with experimentally-induced infections of BSE through the deliberate feeding of untreated brain from BSE clinically-affected cattle **(Wells et al., 1996)** have shown that in calves the ileum of the intestine becomes infective at 6 months after dosing with 100 g of infective brain. This is judged by the intracerebral injection of mice with extracts of the ileum of these cattle. The localisation of the infective material in the ileum was not matched by any demonstrable infectivity of the duodenum, jejunum, colon or rectum. These studies were undertaken as part of the UK MAFF research programme and are still underway: mice are monitored having been injected intracerebrally with 46 different tissues the majority of which were collected during the course of the incubation of the disease. These tissues were obtained from 30 animals in 11 batches each of three cattle inoculated with oral BSE infective brain at 4 months of age and killed at 6 months and then at approximately 4 month intervals thereafter (SEAC, 1994). Ten undosed control animals were also included. It is noteworthy that in the infected

calves the ileum demonstrated persisting infectivity in the ileal area from 6 months post-dosing up to 22 months and then also at 38 and 40 months post-challenge. Clinical disease was first recognised at 35 months post infection. The latest sampling time still needs a further 2 years for the mice to respond to any BSE infection.

3.4 This ileal concentration suggests that the BSE agent is probably affecting the lymphoreticular tissue. The high infectivity may reasonably be ascribed to the concentration of Peyer's patches and other lymphatic tissue within the ileum. However, since there is about a 1000-fold diminished sensitivity when calf BSE agent material is tested in mice brains rather than directly in calf brains, one cannot therefore infer that only the ileal part of the intestine would be infective if tested in calves. In Hadlow's scrapie experiments the proximal colon was also infective, albeit at a lower level than ileum, when expressed on a weight basis. British cattle almost certainly were infected by BSE-contaminated feed which means that the agent passed first into the anaerobic rumen where fermentation is intense before infecting the host. In the calf and sheep experimental feeding studies the infective material was also passed into the rumen and therefore also passed through the rumen. The subsequent infection of the host therefore shows the capacity of the agent to resist the fermentative activity of the large and complex anaerobic microflora in the rumen. If undigested by the proteolytic enzymes of the abomasum and small intestine, then the agent will pass into the colon where it can also be expected to resist anaerobic fermentation. The BSE agent might therefore be capable of being taken up by the colonic mucosa.

3.5 Sheep and goats experimentally infected with BSE have not been tested to see whether their intestine becomes infective. However, sheep, unlike cattle**,** show a surprisingly high infective titre of BSE in their spleens. This demonstrates that the lymphoreticular system can become**,** infected so, given the fact that half the body's lymphoreticular system is associated with the intestine, it is prudent to assume that sheep and goats, if infected with BSE, also have infective intestines and, as in scrapie, their lymph glands, possibly thymus and bone marrow.

3.6 The SSC considers that slaughterhouse contamination of other intestinal tissue can hardly be avoided. Therefore, on a simple practical basis, it is prudent to remove the whole of the small and large intestine of cattle. If sheep and goats are classified as infected by BSE (see below) then their intestine should also be considered infective.

4. Vertebral columns

4.1 Three issues relate to whether or not vertebral columns can be used:

4.1.1 the potential contamination of the vertebral columns by spinal cord during the course of its removal;

4.1.2 the presence of coexisting neuronal material, *e.g.* dorsal root ganglia, with the same infectivity as other neuronal tissue;

4.1.3 any potential infectivity from bone marrow itself.

4.2 These three issues will be dealt with separately.

4.2.1 ***Contamination***. This can be expected under most practical slaughterhouse circumstances, so without any clear understanding of what the minimal dose of BSE is for infecting man, the SSC advises the removal of the vertebral columns from all older animals even when the presumed infective spinal cord has been removed. Thus the age limit for vertebral column removal should be the same as that relating to the central nervous system.

4.2.2 ***Dorsal root ganglia***. New (unpublished) evidence shows that the dorsal root ganglia - sited within the general structure of the vertebral column - should be considered as having an infectivity for BSE equivalent to that of the spinal cord. The dorsal root ganglia proved infective at the same time after infection as the spinal cord, i.e. 32 months. The trigeminal ganglia where also infective, but so far no autonomic nervous system tissue has been found to be infective. The dorsal root ganglia cannot be removed without extreme difficulty. This therefore means that a precautionary proposal relating to the removal of the whole vertebral column (other than the coccyx) is now appropriate. Care needs to be taken to ensure that the removal of vertebral column incorporates the lateral aspect of the vertebral bodies. This dissection may sometimes be difficult in practice unless the musculature is selectively removed from the vertebral bones for selling as bone-free meat.

4.2.3 ***Bone marrow***.

4.2.3.1 Early studies with mice intracerebrally injected with bone marrow from cattle with spontaneous clinical BSE cases had not demonstrated any infectivity (SEAC, 1994). However, studies on calves, experimentally infected by feeding 100g of BSE infected brain tissue, have now shown bone marrow infectivity in cattle studied at 38 months after feeding the BSE infected brain. These animals were clinically affected by BSE. (MAFF, unpublished evidence 3.12.1997). This has wide-ranging implications because it implies that long bones as well as vertebral columns must be considered potentially infective. The concerns on contamination and the dorsal ganglia mean that on these grounds alone the vertebral columns of older animals should be included in the specified risk material.

4.2.3.2 Several issues now emerge from the new report on bone marrow infectivity. first the apparent infectivity of bone marrow might need to be redefined. Bone marrow in Table 3 (on the basis of scrapie studies) was placed in Category III, i.e. as showing low infectivity. In previous bone marrow studies on clinical cases of BSE infected cattle, no infectivity was detected so in theory that might have suggested that the WHO classification (Table 2) was inappropriate in persisting with a Category III, rather than a Category IV, rating, i.e. with no demonstrable infectivity. However, new evidence shows 2 of 18 mice developing late clinical disease having been injected with marrow from cattle of 38 months post infection. Now another 3 mice show immuno cytological evidence of the presence of PrP^{Sc}, having been injected with the same bone marrow extract. Given the late development of the demonstrable infectivity in cattle bone marrow despite the substantial infective dose (100 g untreated BSE infective brain) it seems now appropriate to consider that the WHO classification is maintained for BSE as well as scrapie. This then signifies that as

evidence slowly accumulates, BSE is increasingly being revealed as having a tissue based infectivity effect which seems similar to that of scrapie.

4.2.3.3 This conclusion reinforces the concepts underlying Table 2, i.e. that the different levels of infectivity do reflect a graded phenomenon and that it is unwise to consider the BSE agent as either present or absent in particular tissues.

4.2.3.4 The bone marrow findings also raise the issue of whether bones from older animals, e.g. >30 months, should be removed from the human food chain.

5. Other tissues

5.1 The categorization of other tissues can only be based at present on the infectivity data obtained by Hadlow *et al.* and results of on-going studies.

6. Conclusions

6.1 Table 4 now presents**,** modifications of the WHO classification. This still means, however, that the SSC considers the WHO classification as appropriate in terms of the relative risks of different tissues. The most recent findings on the infectivity of dorsal root ganglia are comparable with the infectivity of the CNS and spinal cord and emphasise the practical issue of how best to remove these tissues in those animals deemed potentially infective. Given the range of potentially infective tissues - albeit theoretical since it is based predominantly on scrapie data - this allows a graded approach to the use of tissues for direct consumption or for use after suitable processing[1].

6.2 These conclusions presuppose that any animal with clinical BSE or Scrapie, should be handled as totally infective. None of its tissues should therefore enter the food chain and special arrangements should be made to dispose of the animal tissues safely.

Table 4:Categorising the potential infectivity of different organs in BSE-infected animals.

The assessment of the infectivity is based in part on scrapie titres, on the finding of high infectivity in the brain of BSE-affected cattle, on the differential impact of BSE-infective organs on the infection of mice to intracerebral inoculation and on the presumed CJD infectivity of human dura mater and human pituitary gland based on transplant data and the effects of human growth hormone infection. For practical reasons relating to slaughterhouse contamination, some tissues are categorised at a higher level than warranted by their intrinsic infectivity.

Category	Organs
1. High infectivity	Bovine brain, eyes, bovine spinal cord and bovine dorsal root ganglia, *dura mater* [1,2], pituitary [1,2], skull [2,3] and bovine vertebral column[2], lungs[5] Ovine/caprine brain, eyes and spinal cord, dorsal root ganglia and

[1] See table 4, footnote 9

Category	Organs
2. Medium infectivity	vertebral columns[2]; ovine and caprine spleens[3], lungs[5] Total intestine from duodenum to rectum[6], tonsils Bovine spleen, placenta, uterus, fetal tissue[7], adrenal, cerebrospinal fluid, lymph nodes
3. Low infectivity	Liver, pancreas, thymus, bone marrow, other bones[8] nasal mucosa, peripheral nerves
4. No detec*ted* infectivity[9]	Skeletal muscle, heart, kidney, colostrum, milk, discrete adipose tissues[10], salivary gland, saliva, thyroid, mammary gland, ovary, testis, seminal testis, cartilaginous tissue, connective tissue, skin, hair, blood clot[11], serum[11], urine, bile, faeces

Notes

Where no species specification is given then the tissues refer to bovine, ovine and caprine species.

1. These tissues are included because iatrogenic CJD in humans has been associated with tissues or extracts from humans which were contaminated with CJD agent.
2. These tissues have been moved up 1 to 3 categories because of the possibility of contamination by tissues of higher infectivity during slaughter and their inclusion of dorsal root ganglia. Ovine/caprine spinal cord, dorsal root ganglia and vertebral column are put in this sub-category because they could be infected or contaminated if sheep/goats have in practice become "back infected" with BSE from their feeding on infective bovine products.
3. Definition of Skull: Entire head excluding the tongue.
4. Ovine spleens are included because of the finding of the BSE agent in the spleens of sheep challenged experimentally with large doses of BSE. Caprine have not been tested for infectivity with BSE but showed infectivity for Scrapie. Bovine spleen has been tested and showed no infectivity in the mice test.
5. Lung should be considered in the category if the slaughtering method induces through the stunning/pithing method a transfer of brain through the blood stream into the lung.
6. This applies to cattle only unless sheep and goats are considered to be infected by BSE, in which case there would be a need to remove lymph nodes and thymus also.
7. These may best be considered in the same category as placenta because of the high probability of contamination when removing the placenta at slaughter.
8. The likely presence of bone marrow in long bones now means that these bones, on the basis of potential infectivity in older animals, should be placed in the same category as bone marrow.
9. All materials listed under category 4 have been tested in mice with samples reflecting 0.01-0.1 g of original infective tissue. In such samples infectivity titres 1000 fold lower than in brain cannot be detected by this method. Further improvements in the sensitivity can be expected. This may require the revision of the table of relative infectivity given above.
10. This new term is used to describe those reserves of fat which can be removed readily during slaughter in the abattoir or at meat-cutting plants. It does not refer to lipid extracted from mechanically recovered meat or from many other tissues, or at a later stage in the production process. It presupposed the removal of the key associated lymph nodes.
11. There is some albeit in-conclusive evidence that experimentally circulating peripheral blood mononuclear cells may transmitt nvCJD.

7. Age issues

7.1 The proposed age structure in the Commission decision of July deals with the SRM on a common age basis. This age relationship cannot now be applied to the intestinal category of SRM: no age limit should be specified for the intestine in cattle. The intestine is the presumed route of infection. Its infectivity should therefore be

presumed to apply to animals immediately after they have been first fed with infective material. The only logical course is then to assume that intestinal tissue is infective at all ages despite the infectivity of the intestine in sheep scrapie only becoming first apparent at 10 months of age.

7.2 The Directive (Table 1) specifies that the skull, central nervous system, eyes and tonsils should have a 1-year cut-off limit with all bovine, ovine and caprine animals below 1 year of age not being considered as at risk. Whether this decision needs to be maintained should be considered on a species-by-species basis.

Age

7.3 There are indications that the relative infectivity of the different tissues differs depending on the stage of the infection (period since first effective exposure). Assuming transmission from mother to calf, the age becomes a synonym for this period, *i.e.* infection is taken to have occurred at birth or shortly after.

7.4 The central nervous system of cattle, experimentally orally challenged with 100 g of untreated brain from natural cases of BSE, has been shown to be infective only after 32 months, even if shorter incubation periods have been observed. This CNS infectivity was accompanied by infectivity of only the associated CNS-ganglia and the intestine, the latter from 6 months post-challenge.

7.5 In Suffolk sheep with natural scrapie, infectivity is first detected in the central nervous system at about 24 months of age, but very rare clinical cases of scrapie have been described, *e.g.* in a 4-month old lamb. An unusually early case of BSE has also been described in a 20-month old cow and there are small numbers of others under 30 months of age (see below).

7.6 Epidemiological analysis of maternal transmission in cases of BSE is relevant to an assessment of the age at which tissues become infected. On the basis of a statistical evaluation it is that an offspring may become infected by its mother if born within 2 years of the onset of clinical disease in the mother.

7.7 On analysis of the risk of infection from mothers, it is evident that the capacity to induce fetal infection becomes apparent in the majority of cattle at the mean incubation period for BSE of about 60 months of age. Thus the occurrence of BSE in animals from maternal transmission has approximately the same incubation period as in other cases of BSE.

7.8 In experimental BSE in sheep, the clinical signs emerged at a late age (Foster et al.). Whether there is a large individual (and perhaps genetically based) variability in the susceptibility to BSE in sheep exposed to infection is uncertain but likely, so it is theoretically possible to have shorter incubation times for BSE in sheep.

7.9 The SSC concludes that the intestine of young animals should be seen as a risk, *i.e.* by the oral route from first ingesting BSE-contaminated feed. The central nervous system of cattle is, however, extremely unlikely to be detectably infected below an age of 30 months even in cattle exposed to infection as calves. However, the exceptional animal of 20 months with clinical signs of BSE supports a cautious approach. On this

basis, an extremely cautious limit for the CNS as a highly infective tissue could be set at 12 months and provide considerable reassurance of non-infectivity. In cattle greater reassurance would be derived by limiting the use of the CNS to <6 months. This might only be deemed necessary if animals are derived from high risk geographical areas.

7.10 Although the infective agent by definition transfers from the intestine to the CNS, no BSE other than CNS-associated ganglia infectivity of other organs has been documented in cattle during the first 30 months incubation period. This must reflect the very low and/or transient dose of the agent in the intermediate tissues, *e.g.* nerves. Thus age classification of the animal does not allow a differentiation to be made between other tissues with theoretical, but unobserved, infectivity until the CNS, dorsal **root** ganglia and then eventually the bone marrow become infective from about 30 months post infection (minimum) onwards (see below).

7.11 Experimental BSE in sheep seems to have a similar incubation period to that of scrapie in sheep. So the removal of CNS in sheep and goats over 1 year of age will substantially limit the risk of BSE for humans and animals. In order to improve still further the protection, an even younger age could be chosen, but the additional protection afforded would be very small considering the rarity of scrapie in sheep and goats under 1 year of age and that the studies of Hadlow *et al.* (1979, 1982) did not detect infectivity in CNS until 24 months of age.

Older age limits

7.12 Previously the EU has not considered the additional safety which could be gained by putting an upper limit on the age of an animal. Yet if the principal concern is to exclude BSE-infected cattle, then the age distribution for the clinical onset of BSE may provide valuable information. Table 5 summarises the numbers and proportion of BSE-affected animals observed within the UK at different ages. Clearly the total 1986-1997 period includes the early years when susceptible animals might show the clinical disease at younger ages. Nevertheless it would seem that the choice of an upper age limit of 3 years might exclude over 99.5% of clinically-affected cattle from entering the food and animal feed chain. About 1 in 2000 of the clinical presentations might occur in cattle of 30 months or younger.

Table 5: Confirmed BSE cases in cattle by age at onset in the UK between 1986 and 1997 together with recent data (suspected cases excluded).

	1986-1989(incl.)	1990-1993 (incl.)	1994-1997 (incl.)	1997	1986-1997 (incl.)

Age of onset Months	Total no cases (%)	Total no of cases (%)	Total no cases (%)	Total no cases (%)	Total no cases (%)
<31	26 (0.23)	51 (0.05)	5 (0.01)	0 (0)	82 (0.05)
<34	54 (0.47)	111 (0.1)	13 (0.03)	0 (0)	178 (0.11)
<38	174 (1.5)	538 (0.5)	61 (0.1)	4 (0)	773 (0.46)
<41	321 (2.8)	1,530 (1.4)	213 (0.4)	21 (0.3)	2,064 (1.2)
<45	753 (6.6)	4,539 (4.1)	701 (1.4)	65 (1.5)	5,997 (3.5)
Total*	11,390 (100)	111,390 (100)	47,561 (100)	2564 (100)	170,341 (100)

* Total includes BSE cases of all ages., 1997 data included up to October, 31.

Note: The figures in table 5 were provided to the Commission by the UK Ministry of Agriculture, Fisheries and Food (MAFF). 1997 figures have been updated by special advice from the UK Spongiform Encephalopathy Advisory Committee (SEAC).

7.13 The clinical manifestation of BSE in experimentally-fed cattle occurs at 32 months of age, but this early age of onset occurred in animals receiving very high doses of BSE infective material. The CNS was only proven to be infective 3 months before the clinical onset of the disease and no infectivity was observed 9 months before the onset of clinical disease. Therefore it might be considered reasonable to consider neuronal and their associated tissues as of minute risk if obtained <2 years of age and negligible risk if obtained <1 year of age.

7.14 This risk can also be tackled by reference to other safety measures such as the origin of the animal and whether or not processing of the products is applied. For example, to allow 1-2 year old tissue to be used, might require additional safeguards in terms of the geographical origin. The industrial processing of the potentially infective tissues prior to their use for any purpose may also be important before it is made available for human consumption. This is of particular concern if there is the need to consider allowing into the European Union tissues from animals derived from areas where there is no absolute assurance that BSE does not exist at all (see below). The risk of including BSE-infective tissues in cattle from a country where BSE might theoretically be present could be reduced by including the specific age limits as well as precautionary processing of the products if they are destined for human consumption.

8. Species

8.1 *The potential infectivity with BSE of sheep and goats*

8.1.1. The possibility that the BSE agent has infected sheep and goats raises the issue of how best to exclude this possibility when, from experimental infection studies, the

pathogenesis of the BSE-infected animals seems similar to those of animals with scrapie. Thus, in theory, BSE-affected animals may be being misdiagnosed as having scrapie. This possibility cannot be excluded by simple pathological analyses and has normally required 2-year studies with BSE-susceptible mice injected intracerebrally with the tissues from infected sheep and goats before a definitive answer can be achieved. In the absence of these data, a new sub-category of potentially infective organs from sheep and goats has been added to Table 4.

8.1.2 Account should be taken of the risk of transmission of BSE to sheep and goats. This will affect the analysis in terms of the tissues, the origin of the animals and their ages in environments where the risk of BSE infection of sheep and goats is considered to be possible.

8.1.3 Until the issue is clear, it is prudent to take precautions on the assumption that sheep and goats in some countries may have BSE. The susceptible countries are those where concentrate feeding of sheep and goats has occurred and where the concentrate could have contained BSE-infected MBM. Additional risks could occur if countries have imported (a) live breeding sheep or goats from countries with scrapie and where potentially BSE infected feed was used, (b) imported MBM for feeding to these species, (c) imported fatstock for slaughter, (d) imported bone-in carcass meat, offals or residues from which MBM was converted for ruminant feed. To this end, the SSC concurs with the recent advice to remove the whole heads of sheep and goats from the food chain and now advocates the additional precaution of ensuring the safe removal of the spinal cord in animals aged 1 year or more. This may be undertaken in the slaughterhouse itself, or under suitable conditions, as part of a secondary stage of meat preparation but before sale to the consumer. The SSC also advocates that the intestine and spleens of these animals be considered as potentially infective, **taking account of their geographical origin.**

8.2 ***Pigs, poultry and other species serving as food for man***

8.2.1 In all countries of the EU, *except the United Kingdom*, and almost everywhere else, mammalian meat and bone meal (MBM) is fed to pigs and poultry. The risk of infection from pigs, especially old breeding pigs, fed MBM in countries with or at risk from BSE has been considered. There is no evidence for the occurrence of clinical disease or spongiform encephalopathy following oral exposure to BSE (MAFF, 1997, unpublished data) (or of clinical disease so far in incomplete experiments using scrapie agent via the oral route, nor of the occurrence of a clinically naturally-occurring form of TSE in pigs anywhere in the world).

8.2.2 If MBM fed to pigs or poultry is infected with scrapie or BSE agents, these agents will be present in the abattoir premises. Under the normal hygiene rules in the EU this is not a problem as such potentially infective feed will be contained. Any risk that there may be would thus be confined to:

(a) the stomach and intestine (due to any residual feed being incompletely removed rather than from inherent infectivity in the tissues themselves) if used for human food or animal feed or for any medicinal purpose;

(b) the contents of the alimentary tract.

8.2.3 A long-term strategy on within-species recycling of animal tissue should be assessed carefully. The SSC concluded that at present there is no indication of BSE infecting pigs or poultry.

8.2.4 Farmed fish should not be fed potentially infected MBM, particularly if fish-to-fish recycling takes place.

9. Geographical origin of tissues or products

A classification will be developed, bearing in mind the previous work conducted by OIE in its document "guidelines for continous surveillance and monitoring of BSE". Once countries are classified then this will affect the risks of different tissues derived from animals from these countries. This in turn will affect the listing of specified risk materials given below.

10. Integrating information on tissue infectivity, the species, age of animal and geographical origin

The designation of Specified Risk Materials

Hitherto there has been a tendency to consider the specified risk materials (SRM) as simply relating to the tissue itself However, it is now clear that SRMs should not be defined simply on the basis of the grades of infectivity as documented by challenge tests of different tissue extracts. This conclusion reinforces the concepts underlying Table 2, i.e. that the different levels of infectivity do reflect a graded phenomenon and that it is unwise to consider the BSE agent as either present or absent in particular tissues. In addition the following have to be considered before a true risk assessment can be made: animal age, species and geographical origin. Table 6 specifies the SRMs for different species but excludes the detailed geographical analysis. The actual risk is also affected by whether or not the material is processed before consumption and its final use.

11. Future Research Needs

11.1 The rapidly evolving data on the characteristics of the particular agent responsible for BSE in cattle, new TSEs in several other animal species and nvCJD in man means that there is an urgent need for the EU to promote research to identify clearly the agent(s) involved. This is particularly important in relation to the use of new antibodies for specifying the tissue location of the pathogenic form PrP^{Sc}.

11.2 It should also be possible to develop a formal risk assessment scheme which looks at the differential impact on risk of the different assumptions proposed in the current analysis.

11.3 It is important to establish as soon as possible whether any BSE infection of sheep or goats has occurred. Account has to be tacken of the genotype of the animals.

Table 6: Suggested list of specified risk materials to be excluded from the food and feed chains except when derived from a BSE free country with a negligible risk.

Tissue	Species	Age limit	Basis
Brain	Bovine	>12 months	Infectivity

	Ovine*	>12 months	"
	Caprine*	>12 months	"
Eyes	Bovine	>12 months	Infectivity
	Ovine*	>12 months	"
	Caprine*	>12 months	"
Dura	Bovine	>12 months	Contamination[1]
mater	Ovine*	>12 months	"
	Caprine*	>12 months	"
Pituitary	Bovine	>12 months	Contamination[1]
	Ovine*	>12 months	"
	Caprine*	>12 months	"
Skull	Bovine	>12 months	Contamination
	Ovine*	>12 months	"
	Caprine*	>12 months	"
Spinal	Bovine	>12 months	Infectivity
cord	Ovine	>12 months	Theoretical back infection
	Caprine	>12 months	" " "
Dorsal root	Bovine	>12 months	*Infectivity*
ganglia	Ovine	>12 months	*Theoretical back infection*
	Caprine	>12 months	*" " "*
Vertebral	Bovine	>12 months	*Contamination and low infectivity*
column	Ovine	>12 months	*Contamination if back infected*
	Caprine	>12 months	*Contamination if back infected*
Spleen	Ovine	All	*Infectivity*[2]
	Caprine	"	*"*
Intestine	Bovine	All	*Infectivity / Contamination*[3]
	Ovine	"	*" "*
	Caprine	"	*" "*
Tonsils	Bovine	>12 months	*Infectivity*[4]
	Ovine	>12 months	*"*
	Caprine	>12 months	*"*
Lung	Bovine	>12 months	*Contamination from brain via blood when animals are killed by pithing or stunning.*
	Ovine,	>12 months	
	Caprine	>12 months	

* Practicalities may well dictate the removal of the heads of sheep and goats at all ages.

Note: In countries specified as at high risk of BSE it may be considered appropriate to reduce further the age limit for these tissues from 12 to 6 months.The risks from bovine tissues need to be considered separately from those of ovine and caprine origin. In high risk countries, all tissues from cattle over 30 month age may be considered as at greater risk and therefore be considered as SRM. Long bones from cattle below 30 months showing no clinical signs of BSE may be considered at present acceptable for human consumption.

1. Known CJD-transmission with human tissue
2. BSE infectivity found in spleen of BSE challenged sheep; assumption that goats would have displayed the same response if tested.
3. Ileal infectivity in calves; colonic infectivity in sheep scrapie and potential for contamination of adjacent colon as small intestine removed in slaughter houses.
4. PrP^{Sc} shown in man with nvCJD; also infectivity in scrapie sheep (see Table 2).

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