

**Annex 1: 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 unamended from Kimberlin 1994)**

Tissues	Titre (mean $\pm$ SEM of (n) samples) <sup>a</sup>				Titre <sup>a</sup>
	Scrapie, sheep		Scrapie, goats		
<b>Category I</b>					
Brain	5.6 $\pm$ 0.2	(51)	6.5 $\pm$ 0.2	(18)	5.3
Spinal cord	5.4 $\pm$ 0.3	(9)	6.1 $\pm$ 0.2	(6)	+ve
<b>Category II</b>					
Ileum	4.7 $\pm$ 0.1	(9)	4.6 $\pm$ 0.3	(3)	<2.0
Lymph nodes	4.2 $\pm$ 0.1	(45)	4.8 $\pm$ 0.1	(3)	<2.0
Proximal colon	4.5 $\pm$ 0.2	(9)	4.7 $\pm$ 0.2	(3)	<2.0
Spleen	4.5 $\pm$ 0.3	(9)	4.5 $\pm$ 0.1	(3)	<2.0
Tonsil	4.2 $\pm$ 0.4	(9)	5.1 $\pm$ 0.1	(3)	<2.0
<b>Category III</b>					
Sciatic nerve	3.1 $\pm$ 0.3	(9)	3.6 $\pm$ 0.3	(3)	<2.0
Distal colon	<2.7 $\pm$ 0.2	(9)	3.3 $\pm$ 0.5	(3)	<2.0
Thymus	2.2 $\pm$ 0.2	(9)	<2.3 $\pm$ 0.2	(3)	not done
Bone marrow	<2.0 $\pm$ 0.1	(9)	<2.0	(3)	<2.0
Liver	<2.0 $\pm$ 0.1	(9)	--		<2.0
Lung	<2.0	(9)	<2.1 $\pm$ 0.1	(2)	<2.0
Pancreas	<2.1 $\pm$ 0.1	(9)	--		<2.0
<b>Category IV</b>					
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*, (Table 5.2 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).

<sup>a</sup>Titres are expressed as arithmetic means of log<sub>10</sub> 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 30  $\mu$ l 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.

**Annex 2:**

**HARVESTING OF CHEEK MEAT OF CATTLE**  
**SPECIFICALLY: LIST OF CRITICAL HYGIENE POINTS IN HARVESTING OF**  
**BOVINE HEADS AND CHEEK MEAT OF CATTLE**

**Report submitted on 25 April 2002 by the German federal Institute for Consumer Health Protection and Veterinary Medicine (BvGG) to the Government of the federal Republic of Germany**

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As instructed, we herewith deliver the following opinion, in agreement with the Federal Institute for Meat Research (BAFF).

In the framework of talks between the Federation and the *Länder* on 15 April 2002 at the BMVEL, we discussed how to proceed with the transposition of Regulation (EC) 270/2002 of 14 February 2002 amending Regulation (EC) 999/2001. The amended Regulation provides that the entire head (without tongue) must be removed in the slaughterhouse as specified risk material, unless a Member State permits the harvesting of cheek meat or tongues in cutting plants specifically authorised for this purpose.

Considerable quantities of meat are currently harvested from bovine heads. According to the Federal Statistical Office, a total of 4 114 093 cattle and 485 43 calves were slaughtered in Germany in 1998. According to estimates furnished by the economic operators concerned, half of the harvested bovine heads come from animals aged over 30 months, mainly cows. Depending on age and sex, up to 6 kg meat can be harvested from a bovine head. This includes approximately 2 kg (internal and external) cheek meat, approximately 2 kg neck fat, approximately 1 kg skull meat (i.e. meat attached to the skull), approximately 200 g temple meat and approximately 1 kg snout meat; at present this meat is mainly used in the feedingstuffs industry (as feedingstuffs for animals not intended for human consumption).

Since the derogation in Regulation (EC) 270/2002 applies only to cheek meat of cattle and the harvesting of neck fat, skull meat, temple meat and snout meat will not be permissible in future even under the derogation, the amount of meat harvestable from bovine heads will be reduced to a maximum of 2 kg per head. However, in individual cases it will be impossible to monitor compliance with the restriction prescribing the harvesting of cheek meat only!

In our report of 10 January 2001 (Ref.: BgVV 5222-04-133846) we already indicated a number of critical points where meat can be contaminated with the BSE agent during slaughter. Bearing in mind the usual cattle slaughtering practices in Germany, contamination with BSE risk material cannot be ruled out, particularly when harvesting and processing bovine heads.

In order to prevent contamination in general and contamination with BSE risk material in particular, we consider that comprehensive precautionary measures are of the essence. Notably, care should be taken to avoid contamination of meat with fluids containing brain tissue residue.

The Scientific Steering Committee (SSC) came to the same conclusion and stated in its opinion of 9 December 1997 that the entire head excluding the tongue is to be considered as "organs" with high BSE-infectivity. This is because of the possible risk of contamination with the TSE agent when harvesting heads and subsequent handling. During usual slaughtering practice, contamination of the meat with tissue which may have a very high degree of BSE-infectivity in the case of infected animals is unavoidable. When the head is removed fluid spills out through the foramen magnum and the spinal cord channel; besides, spinal cord tissue is cut. In addition, in the case of captive bolt stunning, blood containing brain material may spill through hole in the forehead, contaminate the skin of the scalp and enter the meat during subsequent handling of the head.

However, depending on the stunning practice and slaughtering technique, the extent of this external contamination with blood, fluid and brain material can vary. In individual cases, brain material may even gush out of the opening as a result of the way stunning is performed. During subsequent skinning of the head this contamination inevitably reaches the meat and may subsequently be spread over the entire surface of the head.

The extent of the potential hazard for consumers as a result of the TSE agent entering the meat following captive bolt stunning depends on the content of infectious prion proteins in the brain and hence on (a) the probability that an animal has been infected and (b) the incubation stage. Reliable estimates of infection probability are not possible given the current number of BSE cases in Germany. However, since most cattle were obviously infected while still calves (Heynkes, 2001: Most BSE cattle were infected as calves, <http://www.heyne.de/peaks.htm>) and the diffusion of the pathogens from the intestines to the brain along the nerve tract takes a certain time, the risk also depends on the age of the slaughtered animal. This was taken into account by specifying the age limit for the removal of the CNS as SRM (cattle aged over 12 months).

According to the SSC (The risk of dissemination of brain particles into the blood and carcass when applying certain stunning methods; SSC report of 10-11 January 2002, Part II, VII.4), several risk (GBR) levels can be distinguished as regards contamination of meat with the TSE agent following captive bolt stunning. For GBR level III countries (including Germany), the SSC has adopted an age limit of 30 months for cattle. The SSC recognises that the TSE risk is less for cattle aged under 30 months, and higher in the case of cattle aged over 30 months. This estimate is based on the result of infectivity tests in which infection was identified in the brain of cattle infected as calves at the earliest 32 months subsequent to infection (Wells, G.A.H., Hawkins, S.A.C., Green, R.B., Austin A.R., Dexter, I., Spencer, Y.I., Chaplin, M.J., Stack, M.J. and Dawson, M., 1998: Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet. Rec.* 142, S. 103-106) and the results of BSE tests of routinely slaughtered cattle. However, the calves referred to by Wells et al. were not infected until they were four months old. Hence these tests suggest that the brain can be infected already after a 28 month incubation period. However, under practical conditions

infection is possible already in first month of life (cf. Heynkes, 2001). Besides, BSE cases with clinical symptoms were also found in Germany even in animals aged under 30 months. The latest BSE case in Great Britain was even younger. In Great Britain during 1986-2002 there were at least 18 cases of BSE in animals aged under 30 months; the youngest animal was only 20 months old (<http://www.defra.gov.uk/animalh/bse/index.html>). Besides, the currently available BSE tests detect infection only at an advanced stage of incubation, and at the earliest six months before outbreak of the clinical disease. Currently one cannot reliably say how long after a BSE infection BSE pathogens will be detected in the brains of infected animals.

In a further opinion of 13 December 2001 (Report on TSE infectivity distribution in ruminant tissues - state of knowledge, December 2001), the SSC indicates (Section III.3 - Factors associated with slaughter process) in particular the possibility of contamination during the transport of bovine heads.

Besides the possibility of contamination with brain material released through the frontal hole in the skull caused by bolt stunning there is a very high likelihood of the meat being contaminated with a BSE-agent containing material released through the foramen magnum. The current practice of suspending the severed head by the nasal septum in such a way that contamination of the meat with blood, fluid and brain material from the foramen magnum is prevented during skinning is not in our opinion satisfactory. This also applied to heads which have been skinned while still attached to the carcass and which are suspended from the lower jaw symphysis. Since the shot hole and the foramen magnum are not on the same plane but rather at right angles to one another on the head, there is no way of positioning the head in such a way that the spillage or leakage of fluid or CNS tissue fragments can be avoided. The situation is aggravated because the shot hole equalises the pressure so that it is easier for the fluid to escape, whereas if there is only one aperture a certain quantity will first be released but then further release is prevented as a result of the increase in underpressure.

One way of preventing contamination of meat with the BSE agent, which several parties have proposed, is to use a seal (fleece, stopper, plastic foam, etc.) to close both the frontal hole (caused by perforation of the skull during bolt stunning) and the foramen magnum. This procedure might considerably reduce the contamination of cheek meat with brain material. However, the stopper would not only have to be impermeable immediately after insertion into the hole of the unskinned head but also survive the skinning process unscathed. While full external sealing of the hole with a stopper would be effective if bolt stunning is correctly performed - because of the relatively small and circular aperture (the presence of hair on the forehead more or less ruling out other seals) - there is no guarantee that stunning will indeed be performed properly.

Besides, because of inter-animal variations in the anatomical conditions of the foramen magnum it may be quite difficult to position an impermeable and durable stopper. Another problem with bolt stunning is the opening of the frontal sinus, which can connect with the nasal cavity. As mentioned above, the stopper must be able to withstand difficult transport conditions and it must be removable so that BSE samples can be taken in cases when sampling is not performed directly after severing the head.

The attached table provides details on individual critical hygiene points in connection with slaughter, transport, BSE sampling and meat harvesting of bovine heads. The risks associated with current practices are set out and consequences and possible remedies proposed.

**Summing up**, the procedures presented for reducing contamination of cheek meat by the BSE agent are of a provisional nature and their effectiveness will have to be put to the test. Besides, even with the aid of the proposed measures and the restriction of cheek meat extraction to cattle of less than 30 months of age, contamination of meat cannot be fully ruled out.

In our view, the following procedures for reducing contamination of cheek meat of cattle are appropriate as an initial approach:

- harvest only the masseters;
- harvest only meat from animals less than 30 months of age;
- stunning should not be performed with a penetrating bolt (this is probably the most important measure, since opening the cranial cavity not only creates another aperture through which CNS material can escape but also equalises pressure in the cranial cavity, facilitating the release of CNS fragments and fluid);
- removal of head: sever neck musculature and spinal marrow using separate knives (“green”, “red”), store/clean and disinfect the utensils in separate sterilisation basins;
- remove horns without opening the cranial cavity;
- mechanically remove the skin over the head;
- directly move the head to a “screened” cleaning chamber, suspended (nose upwards) without contact with wall;
- take BSE sample immediately after removal of head followed by sealing of the foramen magnum;
- transport and further processing in slaughterhouse with head suspended from the snout or corner of lower jaw;
- handle only in splash-proof head chamber ;
- clean only inside of head (nose, oral cavity) with underpressure;
- avoid all other treatment, including external washing;
- avoid use of water under high pressure when cleaning;
- do not remove eyes;
- avoid all unnecessary contact with head;
- use separate knives (“green”, “red”) when cutting the tongue and removing the tonsillar ring;

- always use fresh knife when inspecting the meat. First apply incisions to the external masseters, then remove tonsils;
- in the case of animals to be tested for BSE: do not use the so-called water or pressurised air method; take samples from head in suspended position; as regards sampling utensils (spoon, "cork borer") ensure that effective disinfection equipment is present (such as sterilisation basin with hypochlorite solution);
- when storing and transporting heads ensure that they are not suspended over one another and that they do not touch one another; ideally heads should not be transported at all;
- transport heads to cutting plant suspended side by side without touching one another.

In the interests of preventive consumer protection, if these measures should fail to prevent contamination of cheek meat with CNS tissue the derogation should not be availed of and, in the case of cattle aged over 12 months, the entire head without tongue, but with the brain, eyes, trigeminal ganglia and tonsils, should be classified as specified risk material.

**Table: Critical stages in the harvesting and processing of bovine heads during slaughter, transport and harvesting of meat**

Critical hygiene point	Risks	Consequences/possible remedies
1) <u>Captive bolt stunning</u>	Contamination of adjacent skin and slaughter area Contamination of the environs of the shot hole following removal of skin → Cross contamination hazard	- Avoid penetrative stunning
2) <u>Removal of horns</u>	Opening of the cranial cavity → Cross contamination hazard	- Horn pincer should not be applied lower than the base of the horn; avoid opening cranial cavity - If cranial cavity is opened : no further handling of the head
3) <u>Removal of head</u> Spinal cord is severed with a knife; the same knife is also used on the carcass	Severing of the spinal cord channel with release of fluid and contamination of the exposed areas of meat on the neck with the released fluid and the knives contaminated with spinal cord tissue → Cross contamination hazard	- Sever the vertebral canal as final stage in removal of head after severing the soft parts - Use fresh knife for every carcass and ensure BSE disinfection of the knives used before reuse (ensure that there is a suitable number of single-use knives for the batch) - Remove contaminated meat areas after sealing the foramen magnum (also with single-use knives) - Use separate knife to remove head and for further handling of head - Use separate sterilisation basin for this knife only - Seal perforated cranium (bolt shot hole and foramen magnum) to reduce contamination

Critical hygiene point	Risks	Consequences/possible remedies
4) Suspension of head	Contamination of the exposed areas of meat on the neck as a result of released fluid and spinal cord particles	- Contamination-free transport and suspension of head
5) Processing of head	When working on tables contamination of surfaces with released CNS/fluid is unavoidable  → Cross contamination hazard	- Work only on suspended head - Avoid all handling which is not essential for harvesting the cheek meat (meat inspection)
6) Cleaning of head (external washing)  The skinned head is washed off externally and internally with a view to cleaning, partly in head-cleaning chambers, partly without screening beside the slaughter line (near the skinned carcasses)  After cleaning, the head is placed on the organ conveyor belt (transport to meat inspection point at end of slaughter line)	Dissemination of CNS material from the shot hole to the surface of the head during external washing  Splashing water, aerosol formation → contamination of neighbouring carcass surfaces. Cleansing liquid (with blood/brain/spinal cord particles) is not collected separately and removed, but flows over floor → spread of contamination by staff, when high-pressure cleaners are used walls and ceilings are contaminated; during transport on conveyor belt liquid drips off and spills onto the floor  During work on tables, contamination of table surfaces with released CNS/fluid unavoidable  → Cross contamination hazard	- Avoid external washing (this will require hygienic removal and skinning of head) - Use fully sealed head-cleaning chamber (in EU-approved plants, this has largely been realised); meat inspection of head (incisions to external masseters, etc.) at this place



Critical hygiene point	Risks	Consequences/possible remedies
<p><b>7) Cleaning of head (internal washing)</b></p>	<p>When head is shifted, contamination hazard as a result of the release of fluid/CNS material from bolt shot hole and/or foramen magnum</p> <p>Brain particles may flow into the nasal cavity through bolt shot hole (the nasal cavity being connected to the brain via the cranial cavity)</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> <li>- Avoid penetrative stunning</li> <li>- Avoid re-positioning of head</li> </ul>
<p><b>8) Meat inspection</b></p>	<p>Surface contamination via deep cuts in musculature</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> <li>- Use a fresh knife for each animal: first apply incisions to external masseters, then remove tonsils, fresh knife for each animal</li> </ul>
<p><b>9) BSE-sampling in the slaughterhouse</b></p> <p>Sampling is performed using sampling instruments (special spoons) or by applying pressure through the hole in the front of the cranium caused by bolt stunning, as a result of which the caudal brain regions are forced out through the foramen magnum</p>	<p>Placing of head on table surface which has been contaminated with spinal cord tissue and fluid following sampling</p> <p>Brain and fluid are deposited on the meat; this applies in particular in the cases of removal techniques in which the brain is forced out of the cranium</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> <li>- Take BSE sample immediately after severing the head and before sealing the foramen magnum</li> <li>- BSE sampling and position should be so designed as to rule out contamination of the meat with brain material</li> <li>- Avoid sampling techniques in which the brain is forced through the cranium</li> <li>- Take BSE sample from suspended head, if possible before final severing of the head</li> </ul>

Critical hygiene point	Risks	Consequences/possible remedies
<p><b>10) Transport of head from slaughterhouse to cutting plant</b></p> <p>Transport of bovine heads on so-called Christmas trees or in several layers suspended from a hook or in cages</p>	<p>Release of CNS and fluid with brain particles as a result of shaking during transport</p> <p>Contamination of lower heads by upper heads</p> <p>Spinal cord protein is released together with the fluid from the frontal bolt shot hole and from the foramen magnum</p> <p>Incomplete sealing of the frontal bore shot hole and the foramen magnum</p> <p>Transport of heads suspended on so-called Christmas trees or in several layers on a hook or transport in cages (the heads are stacked in several layers) leads to the release of brain material</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> <li>- Check inter alia that the frontal bolt shot hole and the foramen magnum are thoroughly sealed</li> <li>- Remove visibly contaminated bovine heads from the batch</li> <li>- Avoid use of so-called Christmas trees and cages/do not transport heads in several layers suspended from hooks</li> <li>- Transport heads suspended side by side but not touching one another</li> <li>- Place collection basin under the heads</li> </ul>
<p><b>11) Removal of eyes, removal of mouth etc..</b></p>	<p>Exposure of optic nerves and hence creation of new apertures in cranium</p> <p>Contamination hazard when head is shifted</p> <p>Contamination hazard through contaminated hands</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> <li>- Avoid removal of eyes and all further handling</li> </ul>
<p><b>12) Cutting of head to harvest cheek meat</b></p> <p>Fixing of head and manual removal of meat from head</p>	<p>Spinal cord protein (possibly containing BSE prions) sticks to the knife; hot water in the sterilisation basin denaturises the protein but does not inactivate the BSE pathogen</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> <li>- Thorough (mechanical) cleaning of knife before hot-water treatment</li> <li>- Treatment of heads in batches by age classes (&lt;12 months; 12 to 24 months; &gt; 24 months).</li> </ul>