IV

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Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)

(2011/C 73/01)

This note provides guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products.

This 3rd technical revision of the TSE (Transmissible Spongiform Encephalopathy) Note for Guidance has been undertaken to take into account advancement of science in the area of transmissible spongiform encephalopathies, as well as the evolving situation regarding Bovine Spongiform Encephalopathy (BSE) across the world.

For the classification of countries or regions according to their BSE risk, the revised Note for Guidance will make reference to the rules laid down by the World Organisation for Animal Health (OIE), replacing the previous GBR classification. Nevertheless, for countries that were classified according to the GBR criteria but not yet according to the OIE criteria, the existing GBR classification should apply, provided that there is no evidence of significant change in their BSE risk.

New criteria for the sourcing and processing of gelatin and bovine blood derivatives used in the manufacture of medicinal products for human or veterinary use have been introduced, as well as a new subsection on Peptones.

It replaces the previous revision of the Note for Guidance (EMEA/410/01 Rev. 2 published in the *Official Journal of the European Union* (C 24, 28.1.2004, p. 6)). The proposed date of application of this revised Note for Guidance is 1 July 2011.

1. INTRODUCTION

1.1. *Scientific background*

Transmissible Spongiform Encephalopathies (TSEs) are chronic degenerative nervous diseases characterised by the accumulation of an abnormal isoform of a cellular glycoprotein (known as PrP or prion protein). The abnormal isoform of PrP (PrP^{TSE}) differs from normal PrP (PrPc) in being highly resistant to protease and heat denaturation treatments. PrP^{TSE} is considered to be the infective agent responsible for transmitting TSE disease.

TSE diseases in animals include:

— bovine spongiform encephalopathy (BSE) in cattle,

— scrapie in sheep and goats,

— chronic wasting disease (CWD) in cervids (deer and elk),

— transmissible mink encephalopathy (TME) in farmed mink,

— feline spongiform encephalopathy (FSE) in felids (specifically domestic cats and captive large cats), and

— spongiform encephalopathy of exotic ungulates in zoos.

In humans, spongiform encephalopathies include different forms of Creutzfeldt-Jakob Disease (CJD), Kuru, Gerstmann-Sträussler-Scheinker Syndrome (GSS), and Fatal Familial Insomnia (FFI).

Iatrogenic transmission of spongiform encephalopathies has been reported. In sheep, scrapie has been accidentally transmitted by the use of Louping Ill vaccine prepared from pooled, formaldehyde treated ovine brain and spleen in which material from scrapie-infected sheep had been inadvertently incorporated. Also, transmission of scrapie to sheep and goats occurred following use of a formol-inactivated vaccine against contagious agalactia, prepared with brain and mammary gland homogenates of sheep infected with *Mycoplasma agalactiae*. In man, cases of transmission of CJD have been reported which have been attributed to the parenteral administration of growth hormone and gonadotropin derived from human cadaveric pituitary glands. Cases of CJD have also been attributed to the use of contaminated instruments in brain surgery and with the transplantation of human dura mater and cornea.

Interspecies TSE transmission is restricted by a number of natural barriers, transmissibility being affected by the species of origin, the prion strain, dose, route of exposure and, in some species, the host allele of the PRNP gene. Species barriers can be crossed under appropriate conditions.

BSE was first diagnosed in the United Kingdom in 1986 and a large number of cattle and individual herds have been affected. It is clear that BSE is a food borne disease associated with feed (e.g. meat and bone meal) derived from TSE affected animals. Other countries have experienced cases of BSE, either in animals imported from the United Kingdom or in indigenous animals. There is convincing evidence to show that the variant form of CJD (vCJD) is caused by the agent which is responsible for BSE in cattle. Therefore, a cautious approach continues to be warranted if biological materials from species naturally affected by TSE diseases, especially bovine species, are used for the manufacture of medicinal products.

In the course of active surveillance programs, two previously unrecognized forms of atypical BSE (BSE-L, also named BASE, and BSE-H) have been identified in rare sporadic cases from Europe, North America, and Japan. The 'L' and 'H' identify the higher and lower electrophoretic positions of their protease-resistant PrP^{TSE} isoforms. It is noteworthy that atypical cases have been found in countries that did not experience classical BSE so far, like Sweden, or in which only few classical BSE cases have been found like Canada or USA. The atypical BSE agent has been experimentally transmitted to transgenic mice expressing the human prion protein and to a cynomolgus monkey.

Scrapie occurs worldwide and has been reported in most European countries. It has the highest incidence in Cyprus. While humans have been exposed to naturally occurring scrapie for over 250 years, there is no epidemiological evidence directly linking scrapie to spongiform encephalopathies in humans [1]. However, there remains a theoretical and currently unquantifiable risk that some BSE-contaminated protein supplement may have been fed to sheep. Further, it should also be assumed that any BSE agent introduced into the small ruminant population via contaminated feed is likely to be recycled and amplified [2].

There is interest in infecting cells with TSE agents to develop assays and for basic scientific reasons. Some success has been reported, usually but not always with neural cell lines. The conditions needed to infect a cell are not well understood and the process is difficult requiring particular combinations of agent and cell. It is not considered appropriate to make specific recommendations in terms of cell substrates to be used for production of biological / biotechnology-derived substances. Nevertheless, the possibility of infection of cell lines with TSE agents should be taken into account in risk assessments.

1.2. ***Regulatory Compliance***

Risk assessment – Since the use of animal-derived materials is unavoidable for the production of some medicinal products and that complete elimination of risk at source is rarely possible, the measures taken to manage the risk of transmitting animal TSEs via medicinal products represent risk minimisation rather than risk elimination. Consequently, the basis for regulatory compliance should be based on a risk assessment, taking into consideration all pertinent factors as identified in this Note for Guidance (see below).

Legal basis – This Note for Guidance is published by the European Commission following

— Annex I, part I, module 3, section 3.2: *Content: basic principles and requirements*, point (9) of Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [3], as amended, and

— Annex I, Title I, part 2, section C *Production and control of starting material* of Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products [4], as amended.

[1] This is currently being assessed by EFSA and ECDC. For updated information, please refer to the following link: http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?mandate=M-2009-0221

[2] In January 2005, after confirmation of BSE in a goat in France, additional legislative measures were taken related to monitoring and an increased testing of small ruminants. The increased surveillance did not identify additional cases of BSE in sheep and goats in the EU.

[3] OJ L 311, 28.11.2001, p. 67.

[4] OJ L 311, 28.11.2001, p. 1.

These Directives require that applicants for Marketing Authorisation for human and veterinary medicinal products must demonstrate that medicinal products are manufactured in accordance with the latest version of this Note for Guidance published in the *Official Journal of the European Union*. This is a continuing obligation after the Marketing Authorisation has been granted.

By definition, the principle of Specified Risk Materials as defined in Regulation (EC) No 999/2001 of the European Parliament and of the Council [5] does not apply to medicinal products. However, Regulation (EC) No 1774/2002 of the European Parliament and of the Council [6], which applies since 1st May 2003, lays down health rules concerning animal by-products not intended for human consumption. As a general rule, and unless properly justified, all animal by-products used as starting materials in the manufacture of medicinal products should be 'Category 3 (i.e. safe) materials or equivalent', as defined in Regulation (EC) No 1774/2002. Justification for the use of substances derived from other, high infectivity materials must follow an appropriate benefit/risk evaluation (see further below).

This Note for Guidance should be read in conjunction with the various European EU legal instruments including Commission Decisions progressively implemented since 1991. Where appropriate, references to these Decisions are given in the text. Position statements and explanatory notes made by the Committee for Medicinal Products for Human Use (CHMP) and Committee for Medicinal Products for Veterinary Use (CVMP) are still applicable for the purpose of regulatory compliance unless otherwise superseded by this Note for Guidance.

A general monograph entitled: 'Products with risk of transmitting agents of animal spongiform encephalopathies' is included in the European Pharmacopoeia. This monograph, which refers to a general chapter of the European Pharmacopoeia, is identical to this Note for Guidance. The monograph forms the basis for issuing Certificates of Suitability as a procedure for demonstrating TSE compliance for substances and materials used in the manufacture of human and veterinary medicinal products.

Clarification of Note for Guidance – As the scientific understanding of TSEs, especially the pathogenesis of the diseases, is evolving, from time to time CHMP and its Biologics Working Party in collaboration with CVMP and its Immunologicals Working Party may be required in the future to develop supplementary guidance in the form of position statements or explanatory notes for the purpose of clarifying this Note for Guidance. The supplementary guidance shall be published by the Commission and on the website of the European Medicines Agency and taken into consideration accordingly in the scope of the certification of the European Directorate for the Quality of Medicines & Health Care (EDQM).

2. **SCOPE**

TSE-relevant animal species – Cattle, sheep, goats and animals that are naturally susceptible to infection with transmissible spongiform encephalopathy agents or susceptible to infection through the oral route other than humans [7] and non-human primates are defined as 'TSE-relevant animal species' [8].

Materials – This Note for Guidance is concerned with materials derived from 'TSE-relevant animal species' that are used for the preparation of:

— active substances,

— excipients and adjuvants, and

— raw and starting materials and reagents used in production (e.g. bovine serum albumin, enzymes, culture media including those used to prepare working cell banks, or new master cell banks for medicinal products which are subject to a new Marketing Authorisation).

This Note for Guidance is also applicable to materials that come into direct contact with the equipment used in manufacture of the medicinal product or that come in contact with the medicinal product and therefore have the potential for contamination.

Materials used in the qualification of plant and equipment, such as culture media used in media fill experiments to validate the aseptic filling process, shall be considered in compliance with this Note for Guidance provided that the constituent or constituents are derived from tissues with no detectable infectivity (category IC tissues), where the risk of cross-contamination with potentially infective tissues has been considered (see section 3.3) and where the materials are sourced from countries with negligible BSE risk or controlled BSE risk (Categories A and B, respectively - see section 3.2). Such information shall be provided in the dossier for a Marketing Authorisation and verified during routine inspection for compliance with Good Manufacturing Practice (GMP).

[5] OJ L 147, 31.5.2001, p. 1.

[6] OJ L 273, 10.10.2002, p. 1. Regulation (EC) 1774/2002 has been repealed by Regulation (EC) 1069/2009 that will apply from 4 March 2011 (OJ L 300, 14.11.2009, p. 1).

[7] Regulatory guidance and position papers have been issued by the Committee for Medicinal Products for Human Use and its Biologics Working Party on human tissue derived medicinal products in relation to CJD and vCJD. Such guidance can be found on http://www.ema.europa.eu.

[8] Pigs and birds, which are animal species of particular interest for the production of medicinal products, are not naturally susceptible to infection via the oral route. Therefore they are not TSE-relevant animal species within the meaning of this Note for Guidance. Also dogs, rabbits and fish are non TSE-relevant animal species within the meaning of this Note for Guidance.

Other materials such as cleaning agents, softeners and lubricants that come into contact with the medicinal product during its routine manufacture or in the finishing stage or in the primary packaging are considered in compliance with this Note for Guidance if they are tallow derivatives prepared using the rigorous physicochemical processes as described in section 6.

Seed lots, cell banks and routine fermentation/production [9] – For the purpose of regulatory compliance, master seeds or master cell banks in Marketing Authorisation applications lodged after 1 July 2000 (for human medicinal products) or 1 October 2000 (for veterinary medicinal products) shall be covered by this Note for Guidance.

Master seeds and master cell banks,

— for vaccine antigens,

— for a biotechnology-derived medicinal product as described in the Annex to Regulation (EC) No 726/2004 of the European Parliament and of the Council [10], and

— for other medicinal products using seed lots or cell banking systems in their manufacture,

that have already been approved for the manufacture of a constituent of an authorised medicinal product shall be considered in compliance with this Note for Guidance even if they are incorporated in Marketing Authorisation applications lodged after 1 July 2000 (for human medicinal products) or 1 October 2000 (for veterinary medicinal products).

Master cell banks and master seeds established before 1 July 2000 (for human medicinal products) or 1 October 2000 (for veterinary medicinal products), but not yet approved as a constituent of an authorised medicinal product shall demonstrate that they fulfil the requirements of this Note for Guidance. If, for some raw or starting materials or reagents used for the establishment of these cell banks or seeds, full documentary evidence is no longer available, the applicant should present a risk assessment as described in Section 4 of this Note for Guidance.

Established working seeds or cell banks used in the manufacture of medicinal products authorised before 1 July 2000 (for human medicinal products) or 1 October 2000 (for veterinary medicinal products), which have been subjected to a properly conducted risk assessment by a Competent Authority of the Member States or the European Medicines Agency and declared to be acceptable, shall also be considered compliant. However, where materials derived from the 'TSE-relevant animal species' are used in fermentation/routine production processes or in the establishment of working seeds and working cell banks, the applicant must demonstrate that they fulfil the requirements of this Note for Guidance.

[9] See also: Position paper on the assessment of the risk of transmission of animal spongiform encephalopathy agents by master seed materials used in the production of veterinary vaccines (EMEA/CVMP/019/01 - February 2001 adopted by the Committee for Veterinary Medicinal products (CVMP) in July 2001 (OJ C 286, 12.10.2001, p. 12)).

[10] OJ L 136, 30.4.2004, p. 1.

3. GENERAL CONSIDERATIONS

3.1. *Scientific principles for minimising risk*

When manufacturers have a choice the use of materials from 'non TSE-relevant animal species' or non-animal origin is preferred. The rationale for using materials derived from 'TSE-relevant animal species' instead of materials from 'non-TSE-relevant species' or of non-animal origin should be given. If materials from 'TSE-relevant animal species' have to be used, consideration should be given to all the necessary measures to minimise the risk of transmission of TSE.

Readily applicable diagnostic tests for TSE infectivity *in vivo* are not yet available. Diagnosis is based on post mortem confirmation of characteristic brain lesions by histopathology and/or detection of PrP^{TSE} by Western Blot or immunoassay. The demonstration of infectivity by the inoculation of suspect tissue into target species or laboratory animals is also used for confirmation. However, due to the long incubation periods of all TSEs, results of *in vivo* tests are available only after months or years.

Several immunochemical tests have been developed for detection of PrP^{TSE} in post-mortem samples and some are now considered to be extremely sensitive. However, their ability to detect an infected animal depends on the timing of sample collection in relation to timing of exposure, the type of tissue collected and infectious dose acquired, together with consequential timing of onset of clinical disease. There is currently insufficient information on how this might be affected by strain variations.

Although screening of source animals by *in vitro* tests may prevent the use of animals at late stages of incubation of the disease and may provide information about the epidemiological status of a given country or region, none of the tests are considered suitable to unambiguously confirm the negative status of an animal.

Minimising the risks of transmission of TSE is based upon three complementary parameters:

— the source animals and their geographical origin,

— nature of animal material used in manufacture and any procedures in place to avoid

— cross-contamination with higher risk materials,

— production process(es) including the quality assurance system in place to ensure product consistency and traceability.

3.2. ***Animal source***

The source materials used for the production of materials for the manufacture of medicinal products shall be derived from animals fit for human consumption following ante- and post mortem inspection in accordance with EU or equivalent (third country) conditions, except for materials derived from live animals, which should be found healthy after clinical examination.

3.2.1. **Geographical sourcing**

3.2.1.1. *Bovine materials*

The World Organisation for Animal Health (OIE) ([11]) lays down the criteria for the assessment of the status of countries in the chapter of the International Animal Health Code on bovine spongiform encephalopathy. Countries or regions are classified as follows:

A. countries or regions with a negligible BSE risk;

B. countries or regions with a controlled BSE risk;

C. countries or regions with an undetermined BSE risk.

As stipulated in Commission Regulation (EC) No 999/2001, as amended ([12]), the classification of countries or regions thereof according to their BSE risk, based on the rules laid down by OIE, is legally binding in the EU since 1 July 2007. Commission Decision 2007/453/EC ([13]) as amended, provides the classification of countries or regions according to their BSE risk.

Previously, the European Commission Scientific Steering Committee (SSC) ([14]) had established a temporary system for classifying the countries according to their geographical BSE risk (GBR) ([15])

For the purposes of this Note for Guidance the BSE classification based on the OIE rules should be used. If a country, which was previously classified in accordance to the SSC GBR criteria, has not been classified yet according the OIE rules, the GBR classification can be used until OIE classification has taken place, provided that there is no evidence of significant change in its BSE risk ([16]).

Where there is a choice, animals should be sourced from countries with the lowest possible BSE risk (negligible BSE risk countries (Category A)) unless the use of material from countries with a higher BSE risk is justified. Some of the materials identified in Section 6., 'Specific Conditions' can be sourced from countries with controlled BSE risk (Category B) and, in some cases, from countries with undetermined BSE risk (Category C), provided that the controls and requirements as specified in the relevant sections below are applied. Apart from these exceptions, animals must not be sourced from countries with undetermined BSE risk (Category C), and justifications for the use of animals from countries with undetermined BSE risk (Category C) must always be provided.

3.2.1.2. *Sheep and goats (small ruminants)*

Naturally occurring clinical scrapie cases have been reported in a number of countries worldwide. As BSE in sheep and goats could possibly be mistaken for scrapie, as a precautionary measure, sourcing of materials derived from small ruminants shall take into account the prevalence of both BSE and scrapie in the country and the tissues from which the materials are derived.

The principles related to 'BSE negligible risk (closed) bovine herds' (see section 3.2.2) could equally be applied in the context of small ruminants in order to develop a framework to define the TSE status of a flock of small ruminants. For sheep, because of the concern over the possibility of BSE in sheep, the use of a genotype(s) showing resistance to BSE/scrapie infection could be considered in establishing TSE free flocks ([17]). However, the possibility that genotypes resistant to scrapie could be susceptible to BSE (experimental oral exposure) or atypical scrapie (natural cases) should also be taken into account. Goats have not been studied sufficiently with regard to a genotype specific sensitivity.

([11]) http://www.oie.int/eng/Status/BSE/en_BSE_free.htm

([12]) Regulation (EC) No 722/2007 (OJ L 164, 26.6.2007, p. 7).

([13]) OJ L 172, 30.6.2007, p. 84.

([14]) The Scientific Steering Committee established by Commission Decision 97/404/EC (OJ L 169, 27.6.1997, p. 85) shall assist the Commission to obtain the best scientific advice available on matters relating to consumer health. Since May 2003, its task have been taken over by the European Food Safety Authority (EFSA): http://www.efsa.europa.eu

([15]) The European Scientific Steering Committee classification for geographical BSE risk (GBR) gives an indication of the level of likelihood of the presence of one or more cattle clinically or pre-clinically infected with BSE in a given country or region. A definition of the four categories is provided in the Table:

GBR level	Presence of one or more cattle clinically or pre-clinically infected with BSE in a geographical region/country
I	Highly unlikely
II	Unlikely but not excluded
III	Likely but not confirmed or confirmed at a lower level
IV	Confirmed at a higher level (≥ 100 cases/1 Million adult cattle per year)

Reports of the GBR assessment of the countries are available on the SSC website (http://ec.europa.eu/food/fs/sc/ssc/outcome_en.html).

([16]) Experts consider that the GBR classification system is stable enough, so that it can continue to be used, during the interim period, for the demonstration of compliance with this guidance.

([17]) Opinion of the Scientific Panel on Biological Hazards on 'the breeding programme for TSE resistance in sheep': http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775678.htm

Material of small ruminant origin should preferably be sourced from countries with a long history of absence of scrapie. Justification shall be required if the material is sourced from some other origin.

3.2.2. **BSE negligible risk (closed) bovine herds**

The safest sourcing is from countries or regions with a negligible BSE risk (Category A countries). Other countries may have or have had cases of BSE at some point in time and the practical concept of 'Negligible risk (closed) bovine herds' has been developed by the SSC and endorsed by the CHMP and CVMP. Criteria for establishing and maintaining a 'BSE negligible risk (closed) bovine herd' can be found in the SSC opinion of 22-23 July 1999 ([18]).

For the time being it is not possible to quantify the reduction of the geographical BSE risk for cattle from BSE 'Negligible risk (closed) bovine herds'. However, it is expected that this risk reduction is substantial. Therefore, sourcing from such closed bovine herds shall be considered in the risk assessment in conjunction with the OIE classification of the country.

3.3. ***Animal parts, body fluids and secretions as starting material***

In a TSE infected animal, different organs and secretions have different levels of infectivity. If materials from 'TSE-relevant animal species' have to be used, consideration should be given to use materials of the lowest category of risk. The tables in the Annex of this Note for Guidance ([19]) summarise current data about the distribution of infectivity and PrP^{TSE} in cattle with BSE, and in sheep and goats with scrapie ([20]).

The information in the tables is based exclusively upon observations of naturally occurring disease or primary experimental infection by the oral route (in cattle) but does not include data on models using strains of TSE that have been adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those of naturally occurring disease. Because immunohistochemical and/or western blot detection of misfolded host protein (PrP^{TSE}) have proven to be a surrogate marker of infectivity, PrP^{TSE} testing results have been presented in parallel with bioassay data. Tissues are grouped into three major infectivity categories, irrespective of the stage of disease:

Category IA: High-infectivity tissues: central nervous system (CNS) tissues that attain a high titre of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS.

Category IB: Lower-infectivity tissues: peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE.

Category IC: Tissues with no detectable infectivity: tissues that have been examined for infectivity, without any infectivity detected, and/or PrP^{TSE}, with negative results.

Category IA tissues and substances derived from them shall not be used in the manufacture of medicinal products, unless justified (see Section 5).

Although the category of lower-infectivity tissues (category IB tissues) almost certainly includes some (e.g. blood) with a lower risk than others (e.g. lymphoreticular tissues), the data about infectivity levels in these tissues are too limited to subdivide the category into different levels of risk. It is also evident that the placement of a given tissue in one or another category can be disease and species specific, and subject to revision as new data emerge.

For the risk assessment (see section 4), manufacturers and/or Marketing Authorisation Holders/applicants shall take into account the tissue classification tables in the Annex to this Note for Guidance.

The categories in the tables are only indicative and it is important to note the following points.

— In certain situations there could be **cross-contamination** of tissues of different categories of infectivity. The potential risk will be influenced by the circumstances in which tissues were removed, especially by contact of tissues with lower-infectivity or no detectable infectivity (categories IB and IC tissues) with high-infectivity tissues (category IA tissues). Thus, cross-contamination of some tissues may be increased if infected animals are slaughtered by brain stunning (penetrative or non-penetrative) or if the brain and/or spinal cord is sawed. The risk of cross-contamination will be decreased if body fluids are collected with minimal damage to tissue and cellular components are removed, and if foetal blood is collected without contamination from other maternal or foetal tissues including placenta, amniotic and allantoic fluids. For certain tissues, it is very difficult or impossible to prevent cross-contamination with Category IA tissues (e.g. skull). This has to be considered in the risk assessment.

([18]) SSC Scientific Opinion on the conditions related to 'BSE Negligible Risk (Closed) Bovine Herds' adopted at the meeting of 22-23 July 1999. http://ec.europa.eu/food/fs/sc/ssc/out56_en.html

([19]) The tissue classification tables are based upon the most recent WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies (2010) http://www.who.int/bloodproducts/tablestissueinfectivity.pdf

([20]) A Scientific opinion on BSE/TSE infectivity in small ruminant tissues is currently being reviewed by EFSA (Question No EFSA-Q-2010-052). For updated information please follow this link: http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?mandate=M-2010-0041

— For certain classes of substances the **stunning/slaughtering techniques** used may be important in determining the potential risk ([21]) because of the likelihood of disseminating the brain particles into the peripheral organs, particularly to the lungs. Stunning/slaughtering techniques should be described, as well as the procedures to remove high infectivity tissues. The procedures to collect the animal tissues/organs to be used and the measures in place to avoid cross-contamination with a higher risk material must also be described in detail.

— The risk of contamination of tissues and organs with BSE-infectivity potentially harboured in central nervous material as a consequence of the stunning method used for cattle slaughtering depends on the following factors:

  — the amount of BSE-infectivity in the brain of the slaughtered animal,

  — the extent of brain damage,

  — the dissemination of brain particles in the animal body.

These factors must be considered in conjunction with the OIE/GBR classification of the source animals, the age of the animals in the case of cattle and the *post-mortem* testing of the cattle using a validated method.

The underlying principles indicated above would be equally applicable to sheep and goats.

The risk posed by cross-contamination will be dependent on several complementary factors including:

— measures adopted to avoid contamination during collection of tissues (see above),

— level of contamination (amount of the contaminating tissue),

— amount and type of materials collected at the same time.

Manufacturers or the marketing authorisation holders/applicants should take into account the risk with respect to cross-contamination.

3.4. *Age of animals*

As the TSE infectivity accumulates in bovine animals over an incubation period of several years, it is prudent to source from young animals.

Presence of infectious material has essentially been reported in the central nervous system and related tissues, as well as in the lymphoreticular system, depending on the TSE agent (BSE in cattle or scrapie in sheep and goat). The exact time course of infectivity in the respective body parts and tissues, from the date of infection, is not known in both species and, as such, it is difficult to give clear guidance on the age above which the various tissues may be infected and should not be collected. The initial recommendation to collect tissues in the youngest age is still valid. In addition, it is noteworthy that the age criteria depend also on the geographical origin. Age is a more important parameter for materials from countries where the risk is higher (Category B and C countries), than from countries with a negligible BSE risk (Category A countries).

3.5. *Manufacturing process*

The assessment of the overall TSE risk reduction of a medicinal product shall take into account the control measures instituted with respect to:

— sourcing of the raw/starting materials, and

— the manufacturing process.

Controlled sourcing is a very important criterion in achieving acceptable safety of the product, due to the documented resistance of TSE agents to most inactivation procedures.

A quality assurance system, such as ISO 9000 certification, HACCP ([22]) or GMP, must be put in place for monitoring the production process and for batch delineation (i.e. definition of batch, separation of batches, cleaning between batches). Procedures shall be put in place to ensure traceability as well as self-auditing and to auditing suppliers of raw/starting materials.

Certain production procedures may contribute considerably to the reduction of the risk of TSE contamination, e.g. procedures used in the manufacture of tallow derivatives (see section 6). As such rigorous processing cannot be applied to many products, processes involving physical removal, such as precipitation and filtration to remove prion-rich material, are likely to be more appropriate than chemical treatments. A description of the manufacturing process, including in-process controls applied, shall be presented and the steps that might contribute to reduction or elimination of TSE contamination should be discussed. Whenever different manufacturing sites are involved, the steps performed at each site shall be clearly identified. The measures in place in order to ensure traceability of every production batch to the source material should be described.

([21]) SSC opinion on stunning methods and BSE risk (The risk of dissemination of brain particles into the blood and carcass when applying certain stunning methods), adopted at the meeting of 10-11 January 2002 http://ec.europa.eu/food/fs/sc/ssc/out245_en.pdf.
Report of the EFSA Working Group on BSE risk from dissemination of brain particles in blood and carcass.
Question No EFSA-Q-2003-122, adopted on 21 October 2004, http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620777397.htm

([22]) Hazard Analysis Critical Control Point.

Cleaning process – Cleaning of process equipment may be difficult to validate for the elimination of TSE agents. It is reported that after exposure to high titre preparations of TSE agent, detectable infectivity can remain bound to the surface of stainless steel. The removal of all adsorbed protein by the use of 1 M sodium hydroxide or chlorine releasing disinfectants (e.g. 20,000 ppm. chlorine for 1 hour) have been considered acceptable approaches where equipment that cannot be replaced has been exposed to potentially contaminated material. Milder treatments with limited concentrations of alkali or stabilized bleach, when properly formulated with detergents and used at specified temperatures, have been shown to exhibit similar efficiency for removing prions as did classical NaOH or chlorine treatments. A system based on vaporized hydrogen peroxide also appeared to be efficient for inactivating TSE agents. These new treatments are more compatible with delicate materials and may be suitable for practical use ([23]).

If risk materials are used in the manufacture of a product, cleaning procedures, including control measures, shall be put in place in order to minimise the risk of cross-contamination between production batches. This is especially important if materials from different risk categories are handled in the same plant with the same equipment. In the case of using category IA materials in the manufacture of a product, dedicated equipment shall be used, unless otherwise justified.

Further research is needed to develop and validate new decontamination procedures to lower the risk of cross–contamination for material and devices which are not compatible with WHO-recommended procedures.

Removal/Inactivation validation – Validation studies of removal/inactivation procedures for TSEs can be difficult to interpret. It is necessary to take into consideration the nature of the spiked material and its relevance to the natural situation, the design of the study (including scaling-down of processes) and the method of detection of the agent (*in vitro* or *in vivo* assay). Further research is needed to develop an understanding of the most appropriate 'spike preparation' for validation studies. Therefore, validation studies are currently not generally required. However, if claims are made for the safety of the product with respect to TSEs based on the ability of manufacturing processes to remove or inactivate TSE agents, they must be substantiated by appropriate investigational studies ([24]).

In addition to appropriate sourcing, manufacturers are encouraged to continue their investigations into removal and inactivation methods to identify steps/processes that would have benefit in assuring the removal or inactivation of TSE agents. In any event, a production process wherever possible shall be designed taking account of available information on methods which are thought to inactivate or remove TSE agents.

([23]) WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies (2006) http://www.who.int/bloodproducts/tse/WHO%20TSE%20Guidelines%20FINAL-22%20JuneupdatedNL.pdf

([24]) Guideline on the investigation of manufacturing process for plasma-derived medicinal products with regard to vCJD risk CPMP/BWP/5136/03

For certain types of products (see section 6.3 Bovine blood derivatives), where validated removal/inactivation is not readily applicable, process evaluation might be required. This should be based on the starting material and any published data on TSE risk.

4. RISK ASSESSMENT OF MATERIALS OR SUBSTANCES USED IN THE MANUFACTURE AND PREPARATION OF A MEDICINAL PRODUCT IN THE CONTEXT OF REGULATORY COMPLIANCE

The assessment of the risk associated with TSE needs careful consideration of all of the parameters as outlined in section 3.1 (Scientific Principles for Minimising Risk).

As indicated in the introduction to this Note for Guidance, regulatory compliance is based on a favourable outcome from a risk assessment. The risk assessments, conducted by the manufacturers and/or the Marketing Authorisation holders or applicants for the different materials or substances from 'TSE-relevant animal species' used in the manufacture of a medicinal product shall show that all TSE risk factors have been taken into account and, where possible, risk has been minimised by application of the principles described in this Note for Guidance. TSE Certificates of suitability issued by the EDQM may be used by the Marketing Authorisation holders or applicants as the basis of the risk assessments.

An overall risk assessment for the medicinal product, conducted by the Marketing Authorisation holders or applicants, shall take into account the risk assessments for all the different materials from 'TSE-relevant animal species' and, where appropriate, TSE reduction or inactivation by the manufacturing steps of the active substance and/or finished product.

The final determination of regulatory compliance rests with the Competent Authority.

It is incumbent upon the manufacturers and/or the Marketing Authorisation holders or applicants for both human and veterinary medicinal products to select and justify the control measures for a given 'TSE-relevant animal species' derivative, taking into account the latest scientific and technical progress.

5. BENEFIT/RISK EVALUATION

In addition to the parameters as mentioned in sections 3. (that may be covered by a TSE Certificate of Suitability issued by EDQM) and 4., the acceptability of a particular medicinal product containing materials derived from a 'TSE-relevant animal species', or which as a result of manufacture could contain these materials, shall also take into account the following factors:

— route of administration of the medicinal product,

— quantity of animal material used in the medicinal product,

— maximum therapeutic dosage (daily dose and duration of treatment),

— intended use of the medicinal product and its clinical benefit,

— presence of a species barrier.

High-infectivity tissues (Category IA tissues) and substances derived thereof shall not be used in manufacture of medicinal products, their starting materials and intermediate products (including active substances, excipients and reagents), unless justified. A justification why no other materials can be used shall be provided. In these exceptional and justified circumstances, the use of high-infectivity tissues could be envisaged for the manufacture of active substances, when, after performing the risk assessment as described in Section 4. of this Note for Guidance, and taking into account the intended clinical use, a positive benefit/risk assessment can be presented by the marketing authorisation applicant. Substances from Category IA materials, if their use is justified, must be produced from animals of countries with negligible BSE risk (Category A).

6. SPECIFIC CONSIDERATIONS

The following materials prepared from 'TSE-relevant animal species' are considered in compliance with this Note for Guidance provided that they meet at least the conditions specified below. The relevant information or a certificate of suitability granted by the EDQM shall be provided by the Marketing Authorisation applicant/holder.

6.1. *Collagen*

Collagen is a fibrous protein component of mammalian connective tissue.

For collagen, documentation to demonstrate compliance with this Note for Guidance needs to be provided taking into account the provisions listed in sections 3 to 5. In addition, consideration should be given to the following.

— For collagen produced from bones, the conditions specified for gelatin are applicable (see below). Lower inactivation capacity is expected from the collagen manufacturing process than from that of gelatin. Therefore, sourcing becomes a more critical aspect to consider.

— Collagen produced from tissues such as hides, skins, tendons and sinews do not usually present a measurable TSE risk provided that contamination with potentially infected materials, for example spillage of blood and/or central nervous tissues, is avoided during procurement. Therefore, hides represent a safer raw material for human implants derived from collagen. However, cross-contamination with brain material released during the slaughtering process that may have dried on the surface of hides would be difficult to eliminate. This is another aspect to consider in the evaluation of the safety of this source material.

The collagen manufacturing process can have some steps in common with the manufacture of gelatin such as alkaline and sodium sulphate treatment, calcium hydroxide and sodium hydroxide treatments or enzyme treatment. However, even these common steps can differ in duration and pH condition which can result in significant differences in their inactivation capacity. Manufacturers should at least conduct a process evaluation based on the similarities of the collagen processing steps, as compared to known inactivation steps in the manufacture of gelatin, in order to support the safety of the product. In addition to processing, differences also exist in the final use of the material and, consequently, in their risk assessment, while gelatin is widely used for oral administration, many collagen applications are in the form of surgical implants. This aspect should also be considered in the final risk assessment.

6.2. *Gelatin*

Gelatin is a natural, soluble protein, gelling or non-gelling, obtained by the partial hydrolysis of collagen produced from bones, hides and skins of animals.

For gelatin, documentation to demonstrate compliance with this Note for Guidance needs to be provided taking into account the provisions listed in sections 3. to 5. In addition, consideration should be given to the following ([25]):

(i) **The source material used**

Gelatin used in medicinal products can be manufactured from bones or hides.

— *Hides as the starting material* - On the basis of current knowledge, hides used for gelatin production represent a safer source material as compared to bones. However, it is highly recommended that measures should be put in place to avoid cross-contamination with potentially infected materials during procurement.

— *Bones as the starting material* - Where bones are used to manufacture gelatin, the quality of the starting materials needs to be controlled as an additional parameter to ensure the safety of the final product. Therefore, the following should be applied.

1. Skulls and spinal cord shall be removed from the collected bones (raw/starting material) independent of the age or the country of origin of the cattle.

2. Vertebrae shall be removed from the raw/starting materials from cattle over 30 months from countries with a controlled or an undetermined BSE risk (Categories B or C).

([25]) Based on the Opinion of the Scientific Panel on Biological Hazards of the European Food Safety Authority on the 'Quantitative assessment of the human BSE risk posed by gelatine with respect to residual BSE risk', The EFSA Journal, 312, (1-28). http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620776107.htm
The requirements for source material selection and manufacture are appropriate for oral or parenteral gelatin for use in human and veterinary medicinal products.

3. Gelatin for parenteral use should only be manufactured from bones coming from countries with a negligible or a controlled BSE risk (Category A and B, respectively). Gelatin for oral use can be manufactured from bones from countries with a negligible, a controlled or an undetermined BSE risk (Category A, B and C, respectively).

4. Gelatin shall be manufactured using one of the manufacturing methods described below.

(ii) **Manufacturing methods**

— *Hides* - No specific measures with regard to the processing conditions are required for gelatin produced from hides provided that control measures are put in place to avoid cross-contamination both during the procurement of the hides and during the manufacturing process.

— *Bones* - Where bones are used as the starting material, the mode of manufacture will be the second parameter that will ensure the safety of gelatin.

— Gelatin can be manufactured from bones from countries with a negligible, a controlled or an undetermined BSE risk (Categories A, B or C) sourced in accordance with the conditions described in section 6.2. (i), using the acid, alkaline or heat/pressure manufacturing process.

— The manufacturing process shall be taken into consideration when performing the risk assessment as described in Section 4 of this Note for Guidance. Both the acid and the alkaline manufacturing methods have shown similar overall inactivation/removal of TSE infectivity in the gelatin validation experiments. Studies have shown that an additional alkaline treatment (pH 13, 2 hours) of the bones/ossein further increases the TSE inactivation/removal capacity of the manufacturing process. Other processing steps such as filtration, ion exchange chromatography and UHT sterilisation also contribute to the safety of gelatin.

— For a typical alkaline manufacturing process, bones are finely crushed, degreased with hot water and demineralised with diluted hydrochloric acid (at a minimum of 4 % and pH < 1,5) over a period of at least two days to produce the ossein. This is followed by an alkaline treatment with saturated lime solution (pH at least 12,5) for a period of at least 20 days.

— Bovine bones may also be treated by an acid process. The liming step is then replaced by an acid pretreatment where the ossein is treated at pH < 3,5 for a minimum of 10 hours.

— A 'flash' heat treatment (sterilisation) step at 138 °C minimum for 4 seconds at least is applied to both acid and alkaline manufacturing process.

— In the heat/pressure process, the dried degreased crushed bones are autoclaved with saturated steam at a pressure greater than 3 bar and a minimum temperature of 133 °C, for at least 20 minutes, followed by extraction of the protein with hot water.

The finishing steps are similar for the alkaline, acid and heat/pressure process and include extraction of the gelatin, washing, filtration and concentration.

6.3. ***Bovine blood and blood derivatives***

Foetal bovine serum is commonly used in cell cultures. Foetal bovine serum should be obtained from foetuses harvested in abattoirs from healthy dams fit for human consumption and the womb should be completely removed and the foetal blood harvested in dedicated space or area by cardiac puncture into a closed collection system using aseptic technique.

Newborn calf serum is obtained from calves under 20 days old and calf serum from animals under the age of 12 months. In the case of donor bovine serum, given that it may be derived from animals less than 36 months old, the TSE negative status of the donor herd shall be well defined and documented. In all cases, serum shall be collected according to specified protocols by personnel trained in these procedures to avoid cross-contamination with higher risk tissues.

For bovine blood and blood derivatives, documentation to demonstrate compliance with this Note for Guidance needs to be provided taking into account the provisions listed in sections 3 to 5. In addition, consideration should be given to the following:

(i) **Traceability**

Traceability to the slaughterhouse must be assured for each batch of serum or plasma. Slaughterhouses must have available lists of farms from which the animals are originated. If serum is produced from living animals, records must be available for each serum batch which assures the traceability to the farms.

(ii) **Geographical origin**

Whilst tissue infectivity of BSE in cattle is more restricted than scrapie, as a precautionary measure bovine blood should be sourced from Category A countries. Bovine blood from Category B countries is also acceptable provided that there is no risk for cross contamination of blood with brain material from the slaughter of animals over 21 months ([26]) of age.

([26]) Opinion of the Scientific Panel on Biological Hazards on the assessment of the age limit in cattle for the removal of certain Specified Risk Materials (SRM). Question No EFSA-Q-2004-146, adopted on 28 April 2005

(iii) **Stunning methods**

If it is sampled from slaughtered animals, the method of slaughter is of importance to assure the safety of the material. It has been demonstrated that stunning by captive bolt stunner with or without pithing as well as by pneumatic stunner, especially if it injects air, can destroy the brain and disseminate brain material into the blood stream. Non-penetrative stunning is no more considered as an alternative to penetrative stunning because contamination of blood with brain material has been demonstrated ([27]). Negligible risk can be expected from electro-narcosis ([28]), but this even does not provide strict safety because, when unsuccessful, animals may have to be additionally stunned. The stunning methods must therefore be described for the bovine blood collection process.

Whenever a risk of cross-contamination of blood with brain cannot be avoided at routine slaughtering in countries with a controlled BSE risk (Category B), safety measures such as restriction of the age of cattle and/or reduction of infectious agents during manufacture have to be applied.

(iv) **Age**

For countries with a controlled BSE risk (Category B), a precautionary age limit of 21 months shall apply for bovine blood or blood derivatives where no significant reduction of TSE agents can be assumed from manufacture. An age limit of 30 months is considered sufficient for blood derivatives where significant reduction of TSE agents can be demonstrated as described below.

(v) **Reduction of TSE agents during manufacture**

For blood derivatives, the capacity of the manufacturing process to reduce/eliminate TSE agents should be estimated from investigational studies. The estimation may be based on published data or in house data whenever it can be shown that such data is relevant to the specific manufacturing process. If it cannot be concluded that the reduction capacity is comparable, it is recommended that manufacturers undertake product-specific investigational studies. Investigations using biochemical assay may be sufficient if there is scientific evidence that this assay correlates with infectivity data. General guidance for investigational studies on reduction of TSE agents has been outlined ([29]). Brain-derived spike preparations are appropriate for studies investigating the risk from brain-contaminated blood.

Table 1

Concept for acceptance of bovine blood/sera and derivatives

Product	Foetal bovine serum	Donor calf serum	Adult bovine donor serum	Calf serum	Adult bovine serum / plasma	Adult bovine serum / plasma / serum derivative	Adult bovine serum derivative	Adult bovine serum derivative
Geographical origin of cattle	Cat. A and B	Cat. A and B	Cat. A and B ([1])	Cat. A and B	Cat. A	Cat. B	Cat. A	Cat. B
Age of cattle	unborn	< 1 year	< 36 months	< 1 year	No limit	< 21 months ([2])	No limit	< 30 months
Slaughtering/cross contamination of blood with CNS material	No risk of cross contamination			Risk of cross contamination				
Demonstration of Prion reduction during manufacture	No			No				Yes ([3])

([1]) When sourced in Category B countries, cattle should be from well-defined and documented herds.
([2]) A higher age may be allowed if cross contamination of blood with CNS material can be clearly ruled out (e.g. halal slaughter).
([3]) Demonstration of prion reduction may not be required if cross contamination of blood with CNS material can be clearly ruled out (e.g. halal slaughter).

6.4. ***Tallow derivatives***

Tallow is fat obtained from tissues including subcutaneous, abdominal and inter-muscular areas and bones. Tallow used as the starting material for the manufacture of tallow derivatives shall be 'Category 3 material or equivalent', as defined in Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption.

Tallow derivatives, such as glycerol and fatty acids, manufactured from tallow by rigorous processes are thought unlikely to be infectious and they have been the subject of specific consideration by CPMP and CVMP. For this reason, such materials manufactured under the conditions at least as

([27]) The tissue classification tables are based upon the most recent WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies (2010) http://www.who.int/bloodproducts/tablestissueinfectivity.pdf

([28]) Report of the EFSA Working Group on BSE risk from dissemination of brain particles in blood and carcass. Question No EFSA-Q-2003-122, adopted on 21 October 2004, http://www.efsa.europa.eu/en/science/biohaz/biohaz_opinions/opinion_annexes/733.html

([29]) Guideline on the investigation of manufacturing process for plasma-derived medicinal products with regard to vCJD risk CPMP/BWP/5136/03.

rigorous as those given below shall be considered in compliance for this Note for Guidance, irrespective of the geographical origin and the nature of the tissues from which tallow derivatives are derived. Examples of rigorous processes are:

— trans-esterification or hydrolysis at not less than 200 °C for not less than 20 minutes under pressure (glycerol, fatty acids and fatty acid esters production),

— saponification with NaOH 12 M (glycerol and soap production)

  — batch process: at not less than 95 °C for not less than 3 hours,

  — continuous process: at not less than 140 °C, under pressure for not less than 8 minutes, or equivalent,

— distillation at 200 °C.

Tallow derivatives manufactured according to these conditions are unlikely to present any TSE risk and shall therefore be considered compliant with this Note for Guidance.

Tallow derivatives produced using other conditions must demonstrate compliance with this Note for Guidance.

6.5. ***Animal Charcoal***

Animal charcoal is prepared by carbonisation of animal tissues, such as bones, using temperatures higher than 800 °C. Unless otherwise justified, the starting material for the manufacture of animal charcoal shall be Category 3 material or equivalent, as defined in Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Irrespective of the geographical origin and the nature of the tissue, for the purpose of regulatory compliance, animal charcoal shall be considered in compliance with this Note for Guidance.

Charcoal manufactured according to these conditions is unlikely to present any TSE risk and shall therefore be considered compliant with this Note for Guidance. Charcoal produced using other conditions must demonstrate compliance with this Note for Guidance.

6.6. ***Milk and milk derivatives***

In the light of the current scientific knowledge and irrespective of the geographical origin, bovine milk is unlikely to present any risk of TSE contamination ([30]).

Certain materials, including lactose, are extracted from whey, the spent liquid from cheese production following coagulation. Coagulation can involve the use of calf rennet, an extract from abomasum, or rennet derived from other ruminants. The CHMP/CVMP have performed a risk assessment for lactose and other whey derivatives produced using calf rennet and concluded that the TSE risk is negligible if the calf rennet is produced in accordance with the process described in the risk assessment report ([31]). The conclusion was endorsed by the SSC ([32]), which has also performed an assessment of the TSE risk of rennet in general ([33]).

Bovine milk derivatives manufactured according to the conditions described below are unlikely to present any TSE risk and shall therefore be considered compliant with this Note for Guidance:

— the milk is sourced from healthy animals in the same conditions as milk collected for human consumption, and

— no other ruminant materials, with the exception of calf rennet, are used in the preparation of such derivatives (e.g. pancreatic enzyme digests of casein).

Milk derivatives produced using other processes or rennet derived from other ruminant species must demonstrate compliance with this Note for Guidance.

6.7. ***Wool derivatives***

Derivatives of wool and hair of ruminants, such as lanolin and wool alcohols derived from hair shall be considered in compliance with this Note for Guidance, provided the wool and hair are sourced from live animals.

([30]) For milk and milk derivatives from small ruminants, please see EFSA opinion on Question No EFSA-Q-2008-310, adopted on 22 October 2008, http://www.efsa.europa.eu/en/scdocs/scdoc/849.htm

([31]) Committee for Medicinal Products for Human Use and its Biologics Working Party conducted a risk and regulatory assessment of lactose prepared using calf rennet. The risk assessment included the source of the animals, the excision of the abomasums and the availability of well-defined quality assurance procedures. The quality of any milk replaces used as feed for the animals from which abomasums are obtained is particularly important. The report can be found on http://www.ema.europa.eu/pdfs/human/press/pus/057102.pdf

([32]) Provisional statement on the safety of calf-derived rennet for the manufacture of lactose, adopted by the SSC at its meeting of 4-5 April 2002 (http://ec.europa.eu/food/fs/sc/ssc/out255_en.pdf).

([33]) The SSC issued an opinion on the safety of animal rennet in regard to risks from animal TSE and BSE in particular, adopted at its meeting of 16 May 2002 (http://ec.europa.eu/food/fs/sc/ssc/out265_en.pdf).

Wool derivatives produced from wool, which is sourced from slaughtered animals declared 'fit for human consumption' and the manufacturing process in relation to pH, temperature and duration of treatment meets at least one of the stipulated processing conditions listed below are unlikely to present any TSE risk and shall therefore be considered compliant with this Note for Guidance.

— Treatment at pH ≥ 13 (initial, corresponding to a NaOH concentration of at least 0,1 M NaOH) at 60 °C for at least 1 hour. This normally occurs during the reflux stage of the organic-alkaline treatment.

— Molecular distillation at ≥ 220 °C under reduced pressure.

Wool derivatives produced using other conditions must demonstrate compliance with this Note for Guidance.

6.8. ***Amino acids***

Amino acids can be obtained by hydrolysis of materials from various sources.

Unless otherwise justified, the starting material for the manufacture of amino acids shall be 'Category 3 material or equivalent', as defined in Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption.

Amino acids prepared using the following processing conditions are unlikely to present any TSE risk and shall be considered compliant with this Note for Guidance:

— amino acids produced from hides and skins by a process which involves exposure of the material to a pH of 1 to 2, followed by a pH of > 11, followed by heat treatment at 140 °C for 30 minutes at 3 bar,

— the resulting amino acids or peptides are filtered after production, and

— analysis is performed using a validated and sensitive method to control any residual intact macromolecules, with an appropriate limit set.

Amino acids prepared using other conditions must demonstrate compliance with this Note for Guidance.

6.9. ***Peptones***

Peptones are partial hydrolysates of protein, achieved by enzymic or acid digestion. They are used in microbiological culture media to support the nutritional requirements of micro-organisms, which might be used as seed stocks or in industrial scale fermentations for the production of human and veterinary medicinal products, including vaccines. There is considerable interest in the use of vegetable protein as an alternative to animal sourced protein. However:

— where gelatin is used as the protein source material, reference is made to Section 6.2, Gelatin, of this guideline,

— where casein is used as the protein source material, reference is made to Section 6.6, Milk and Milk Derivatives, of this guideline,

— where tissue of TSE-relevant animal species is the protein source material, the tissue must be sourced from animals fit for consumption (see Section 3.2, Source Animals, of this guideline) with a maximum age of 30 months old for cattle from countries with a controlled BSE risk (Category B). The age of animals is of minimal concern for animals from countries with a negligible BSE risk (Category A).

ANNEX

Major categories of infectivity

The tables below are taken from the WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies (2010).

Data entries are shown as follows:

\+ Presence of infectivity or PrP^{TSE}

– Absence of detectable infectivity or PrP^{TSE}

NT Not tested

? Controversial or uncertain results

Category IA: High-infectivity tissues

Tissue	Cattle		Sheep & goats		Elk & deer	
	BSE		Scrapie		CWD	
	Infectivity (1)	PrP^{TSE}	Infectivity (1)	PrP^{TSE}	Infectivity (1)	PrP^{TSE}
Brain	+	+	+	+	+	+
Spinal cord	+	+	+	+	NT	+
Retina	+	NT	NT	+	NT	+
Optic nerve (2)	+	NT	NT	+	NT	+
Spinal ganglia	+	+	+	+	NT	+
Trigeminal ganglia	+	+	NT	+	NT	–
Pituitary gland (3)	–	NT	+	+	NT	+
Dura mater (3)	NT	NT	NT	NT	NT	NT

Category IB: Lower-infectivity tissues

Tissue	Cattle		Sheep & goats		Elk & deer	
	BSE		Scrapie		CWD	
	Infectivity (1)	PrP^{TSE}	Infectivity (1)	PrP^{TSE}	Infectivity (1)	PrP^{TSE}
Peripheral nervous system						
Peripheral nerves	|+|	+	+	+	NT	+
Autonomic ganglia (4)	NT	+	NT	+	NT	+
LymphoretIculart issues						
Spleen	–	–	+	+	NT	+
Lymph nodes	–	–	+	+	NT	+

Tissue	Cattle		Sheep & goats		Elk & deer	
	BSE		Scrapie		CWD	
	Infectivity [1]	PrP^{TSE}	Infectivity [1]	PrP^{TSE}	Infectivity [1]	PrP^{TSE}
Tonsil	+	–	+	+	NT	+
Nictitating membrane	+	–	[+]	+	NT	+
Thymus	–	NT	+	+	NT	–
Alimentary tract [5]						
Oesophagus	–	NT	[+]	+	NT	+
Fore-stomach [6] (ruminants only)	–	NT	[+]	+	NT	+
Stomach/abomasum	–	NT	[+]	+	NT	+
Duodenum	–	–	[+]	+	NT	+
Jejunum [7]	–	+	[+]	+	NT	NT
Ileum [7]	+	+	+	+	NT	+
Appendix	NA	NA	NA	NA	NA	NA
Colon/caecum [7]	–	–	+	+	NT	+
Rectum	NT	NT	NT	+	NT	+
Reproductive tissues						
Placenta [8]	–	NT	+	+	NT	–
Ovary [3]	–	NT	–	–	NT	–
Uterus [3]	–	NT	–	–	NT	–
Other tissues						
Mammary gland/udder [9]	–	NT	–	+	NT	NT
Skin [3], [10]	–	NT	–	+	[+]	[+]
Adipose tissue	–	NT	NT	NT	[+]	NT
Heart/pericardium	–	NT	–	NT	NT	+
Lung	–	NT	–	–	NT	+
Liver [3]	–	NT	+	–	NT	–
Kidney [3], [11]	–	–	[+]	+	NT	+
Adrenal	[+]	+	+	–	NT	+
Pancreas [3]	–	NT	+	NT	NT	+

Tissue	Cattle		Sheep & goats		Elk & deer	
	BSE		Scrapie		CWD	
	Infectivity [1]	PrP^{TSE}	Infectivity [1]	PrP^{TSE}	Infectivity [1]	PrP^{TSE}
Bone marrow [12]	(+)	NT	+	NT	NT	–
Skeletal Muscle [13]	[+]	NT	[+]	+	[+]	–
Tongue [14]	–	NT	[+]	+	NT	–
Blood vessels	–	NT	NT	+	NT	–
Nasal mucosa [15]	–	NT	+	+	NT	+
Salivary gland	–	NT	+	NT	–	–
Cornea [16]	NT	NT	NT	NT	NT	NT
Body fluids, secretion and excretions						
CSF	–	NT	+	–	NT	NT
Blood [17]	–	?	+	?	+	?
Saliva	NT	NT	–	NT	+	[–]
Milk [18]	–	–	+	[+]	NT	NT
Urine [19]	–	NT	–	–	–[+]	[+]
Feces [19]	–	NT	–	NT	–[+]	NT

Category IB: Lower-infectivity tissues

Tissue	Cattle		Sheep & goats		Elk & deer	
	BSE		Scrapie		CWD	
	Infectivity [1]	PrP^{TSE}	Infectivity [1]	PrP^{TSE}	Infectivity [1]	PrP^{TSE}
Reproductive tissues						
Testis	–	NT	–	–	NT	–
Prostate/Epididymis/ Seminal vesicle	–	NT	–	–	NT	–
Semen	–	NT	–	–	NT	NT
Placenta fluids	–	NT	NT	NT	NT	NT
Foetus [20]	–	NT	–	–	NT	(–)
Embryos [20]	–	NT	?	NT	NT	NT

Tissue	Cattle		Sheep & goats		Elk & deer	
	BSE		Scrapie		CWD	
	Infectivity ([1])	PrP^{TSE}	Infectivity ([1])	PrP^{TSE}	Infectivity ([1])	PrP^{TSE}
Musculo–skeletalt issues						
Bone	–	NT	NT	NT	NT	NT
Tendon	–	NT	NT	NT	NT	NT
Other tissues						
Gingival tissues	NT	NT	NT	NT	NT	NT
Dental pulp	NT	NT	NT	NT	NT	NT
Trachea	–	NT	NT	NT	NT	–
Thyroid gland	NT	NT	–	NT	NT	–
Body fluids, secretion and excretions						
Colostrum ([21])	(–)	–	(?)	NT	NT	NT
Cord blood ([21])	–	NT	NT	NT	NT	NT
Sweat	NT	NT	NT	NT	NT	NT
Tears	NT	NT	NT	NT	NT	NT
Nasal mucus	NT	NT	NT	NT	NT	NT
Bile	NT	NT	NT	NT	NT	NT

([1]) Infectivity bioassays of human tissues have been conducted in either primates or mice (or both), bioassays of cattle tissues have been conducted in either cattle or mice (or both), and most bioassays of sheep and/or goat tissues have been conducted only in mice. In regard to sheep and goats not all results are consistent for both species, for example, two goats (but no sheep) have contracted BSE naturally [Eurosurveillance, 2005, Jeffrey et al., 2006]. Similarly, most of the results described for CWD were derived from studies in deer, and findings may not be identical in elk or other cervids.

([2]) In experimental models of TSE, the optic nerve has been shown to be a route of neuroinvasion, and contains high titres of infectivity.

([3]) No experimental data about infectivity in pituitary gland or dura mater in humans with all forms of human TSE have been reported, but cadaveric dura mater patches, and growth hormone derived from cadaveric pituitaries have transmitted disease to hundreds of people and therefore must be included in the category of high-risk tissues. PrPTSE was detected by immunoblot in the dura mater of a vCJD patient who died in the US after an unusually long incubation period (see also Table IB for other positive tissues: skin, kidney, liver, pancreas, ovary and uterus) [Notari et al., 2010]. It must be mentioned that earlier studies of numerous cases examined in the UK reported all of these tissues to be negative [Ironside et al., 2002, Head et al., 2004].

([4]) In cattle, PrPTSE is reported to be inconsistently present in the enteric plexus in the distal ileum, but immunohistochemical examination of tissues from a single 'fallen stock' case of BSE in Japan suggested (albeit equivocally) involvement of myenteric plexuses throughout the small and large intestine [Kimura and Haritani, 2008].

([5]) In vCJD, PrPTSE is limited to gut-associated lymphoid and nervous tissue (mucosa, muscle, and serosa are negative).

([6]) Ruminant fore stomachs (reticulum, rumen, and omasum) are widely consumed, as is the true stomach (abomasum). The abomasum of cattle (and sometimes sheep) is also a source of rennet.

([7]) When a large BSE oral dose was used to infect cattle experimentally, infectivity was detected in the jejunum and the ileo-caecum junction in Tg mice overexpressing PrP [courtesy of Dr. M Groschup]. PrPTSE was detected at low incidence in lymphoid tissue of ileum [Terry et al., 2003] and has been detected at an even lower frequency in jejunal lymphoid tissue of cattle similarly infected by the oral route [EFSA, 2009].

([8]) A single report of transmission of sporadic CJD infectivity from human placenta has never been confirmed and is considered improbable.

([9]) PrPTSE has been detected in scrapie-infected sheep with chronic mastitis, but not from infected sheep without mastitis [Ligios et al., 2005].

([10]) Studies in hamsters orally infected with scrapie revealed that PrPTSE deposition in skin was primarily located within small nerve fibres. Also, apical skin 'velvet' from the antlers of CWD-infected deer is reported to contain PrPTSE and infectivity [Angers et al., 2009].

([11]) PrPTSE detected by immunocytochemistry in the renal pelvis of scrapie-infected sheep [Siso et al., 2006], and in lymphoid follicles within connective tissue adjacent to the renal pelvis in CWD-infected mule deer [Fox et al., 2006].

(12) A single positive marrow in multiple transmission attempts from cattle orally dosed with BSE-infected brain [Wells et al., 1999, Wells et al., 2005, Sohn et al., 2009].

(13) Muscle homogenates have not transmitted disease to primates from humans with sporadic CJD, or to cattle from cattle with BSE. However, intra-cerebral inoculation of a semitendinosus muscle homogenate (including nervous and lymphatic elements) from a single cow with clinical BSE has transmitted disease to transgenic mice that overexpress PrP at a rate indicative of trace levels of infectivity [Buschmann and Groschup, 2005]. Also, recent published and unpublished studies have reported the presence of PrPTSE in skeletal muscle in experimental rodent models of scrapie and vCJD [Beekes et al., 2005], in experimental and natural scrapie infections of sheep and goats [Andreoletti et al., 2004], in sheep orally dosed with BSE [Andreoletti, unpublished data], and in humans with sporadic, iatrogenic, and variant forms of CJD [Glatzel et al., 2003, Kovacs et al., 2004, Peden et al., 2006]. Bioassays of muscle in transgenic mice expressing cervid PrP have documented infectivity in CWD-infected mule deer [Angers et al, 2006], and experiments are underway to determine whether detectable PrPTSE in other forms of TSE is also associated with infectivity.

(14) In cattle, bioassay of infectivity in the tongue was negative, but the presence of infectivity in palatine tonsil has raised concern about possible infectivity in lingual tonsillar tissue at the base of the tongue that may not be removed at slaughter [Wells et al., 2005, EFSA, 2008]. In sheep naturally infected with scrapie, 7 of 10 animals had detectable PrPTSE in the tongue [Casalone et al., 2005, Corona et al., 2006].

(15) Limited chiefly to regions involved in olfactory sensory reception.

(16) Because only one case of iatrogenic CJD has been certainly attributed to a corneal transplant among hundreds of thousands of recipients (one additional case is considered probable, and another case only possible), cornea has been categorized as a lower-risk tissue, other anterior chamber tissues (lens, aqueous humour, iris, conjunctiva) have been tested with a negative result both in vCJD and other human TSEs, and there is no epidemiological evidence that they have been associated with iatrogenic disease transmission.

(17) A wealth of data from studies of blood infectivity in experimental rodent models of TSE have been extended by recent studies documenting infectivity in the blood of sheep with naturally occurring scrapie and in sheep transfused with blood from BSE-infected cattle [Houston et al., 2008], of deer with naturally occurring CWD [Mathiason et al., 2006], and (from epidemiological observations) in the red cell fraction (which includes significant amounts of both plasma and leukocytes) of four blood donors in the pre-clinical phase of vCJD infections [reviewed in Brown, 2006, Hewitt et al., 2006]. Plasma Factor VIII administration has also been potentially implicated in a subclinical case of vCJD in a haemophilia patient [Peden et al., 2010]. Blood has not been shown to transmit disease from humans with any form of 'classical' TSE [Dorsey et al., 2009], or from cattle with BSE (including fetal calf blood). A number of laboratories using new, highly sensitive methods to detect PrPTSE are reporting success in a variety of animal and human TSEs. However, several have experienced difficulty obtaining reproducible results in plasma, and it is not yet clear that positive results imply a potential for disease transmissibility, either because of false positives, or of 'true' positives that are due to sub-transmissible concentrations of PrPTSE. Because of these considerations (and the fact that no data are yet available on blinded testing of specimens from naturally infected humans or animals) the expert group felt that it was still too early to evaluate the validity of these tests with sufficient confidence to permit either a negative or positive conclusion.

(18) Evidence that infectivity is not present in milk from BSE-infected bovines includes temporo-spatial epidemiologic observations failing to detect maternal transmission to calves suckled for long periods, clinical observations of over a hundred calves suckled by infected cows that have not developed BSE, and experimental observations that milk from infected cows reared to an age exceeding the minimum incubation period has not transmitted disease when administered intra-cerebrally or orally to mice [Middleton and Barlow, 1993, Taylor et al., 1995]. Also, PrPTSE has not been detected in milk from cattle incubating BSE following experimental oral challenge [SEAC, 2005]. However, low levels (μg to ng/L) of normal PrP have been detected in milk from both animals and humans [Franscini et al., 2006]. PrPTSE has been detected in the mammary glands of scrapie-infected sheep with chronic mastitis [Ligios et al., 2005], and very recently it has been reported that milk (which in some cases also contained colostrum) from scrapie-infected sheep transmitted disease to healthy animals [Konold et al., 2008, Lacroux et al., 2008].

(19) A mixed inoculum of urine and faeces from naturally infected CWD deer did not transmit disease during an 18-month observation period after inoculation of healthy deer with a heterozygous (96 G/S) PRNP genotype [Mathiason et al., 2006]. However, recent bioassays in Tg mice have transmitted disease from both urine [Haley et al., 2009] and faeces [Tamgüney et al., 2009]. In addition, mice with lymphocytic nephritis that were experimentally infected with scrapie shed both PrPTSE and infectivity in urine, when bioassayed in Tg mice [Seeger et al., 2005]. Very low levels of infectivity have also been detected in the urine (and histologically normal kidneys) of hamsters experimentally infected with scrapie [Gregori and Rohwer, 2007, Gonzalez-Romero et al., 2008]. Finally, in an experimental scrapie-hamster model, oral dosing resulted in infectious faeces when bioassayed in Tg mice over-expressing PrP [Safar et al., 2008].

(20) Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made on fetal calf tissues other than blood (negative mouse bioassay) [Fraser and Foster, 1994]. Calves born of dams that received embryos from BSE-affected cattle have survived for observations periods of up to seven years, and examination of the brains of both the unaffected dams and their offspring revealed no spongiform encephalopathy or PrPTSE [Wrathall et al., 2002].

(21) Early reports of transmission of sporadic CJD infectivity from human cord blood and colostrum have never been confirmed and are considered improbable. A bioassay from a cow with BSE in transgenic mice over-expressing bovine PrP gave a negative result [Buschmann and Groschup, 2005], and PrPTSE has not been detected in colostrum from cattle incubating BSE following experimental oral challenge [SEAC, 2005].