ORDER
for approval of Guideline on the risk minimisation of animal spongiform encephalopathy agents transmission through medicinal products for human use

Taking into account:

- provisions of Law No. 95/2006 on healthcare reform, Title XVII – The medicinal product;

- Government Ordinance No. 125/1998 related to the set up, organisation and functioning of the National Medicines Agency, approved as amended through Law No. 862/2006, as amended ,

based on Government Decision No. 862/2006 on organisation and functioning of the Ministry of Public Health,

on seeing the Approval Report of the Pharmaceutical Directorate No. E.N. 4.709/2006,

the Minister of public health hereby issues the following order:

Article 1. - Guideline on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products for human use is approved in accordance with the Annex, which is integral part of the present order.

Article 2. - On this order coming into force, any other contrary dispositions shall be repealed.

Article 3. - The present order shall be published in the Official Gazette of Romania, Part I.

Minister of public health,
Gheorghe Eugen Nicolăescu

Bucharest, 2 October 2006.
No. 1201.

ANNEX

GUIDELINE
on the risk minimisation of animal spongiform encephalopathies transmission through medicinal products for human use

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 prion protein (PrP). The abnormal PrP (PrP_{sc}) differs from the normal PrP (PrP_c) in that it is highly resistant to protease and to heat denaturation treatments. The abnormal form, PrP_{sc}, is considered by many to be the infectious agent itself.

TSEs in animals include:

- bovine spongiform encephalopathy (BSE) in cattle;
- scrapie in sheep and goats;
- chronic wasting disease in mule deer and elk;
- transmissible mink encephalopathy in farmed mink;
- feline spongiform encephalopathy (FSE) in domestic cat, captive large cats (felidae);
- spongiform encephalopathy in 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. 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.

Several factors act as natural barriers, which limit the interspecies transmission of TSE. Transmission is affected by the species of origin and the prion strain, dose, route of exposure and, in some species, the host allele of the PrP gene. Species barriers can be crossed under appropriate conditions.

BSE was first recognised in the United Kingdom in 1986. A large number of cattle and individual herds have been affected. It is clear that BSE is a food borne disease associated with feeding meat and bone meal derived from TSE affected animals. Other countries have had some 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 Creutzfeldt-Jakob Disease *(vCJD)* is caused by the agent responsible for BSE in cattle. Therefore, due prudence continues to be warranted if biological materials from species naturally affected by these diseases, especially bovine species, are used in the manufacturing process of medicinal products.

Scrapie is a worldwide spread disease and occurs in most European countries. It has the highest incidence in the United Kingdom. Whilst humans have been exposed to naturally occurring scrapie in sheep for over 200 years, there is no epidemiological evidence to establish a direct connection between scrapie and spongiform encephalopathies in humans. However, there remains a theoretical and presently unquantifiable risk that sheep have been fed with some BSE-contaminated protein supplements was introduced into the sheep flock and may be present as. If BSE infection causes a recurrent infection in sheep, it may be diagnosed as scrapie and can be considered a TSE risk for humans. Further, it should also be taken into account that the BSE agent introduced into the small ruminant population via contaminated feed is likely to be recycled and amplified.

1.2. Regulatory compliance

Risk assessment - Given that the use of raw materials and starting materials derived from animals is unavoidable for the production of some medicinal products and that complete removal of risk at source is rarely possible, the measures taken to manage the risk of transmitting animal TSEs via medicinal products represent risk reduction at minimum rather than risk elimination. As a consequence, regulatory compliance is proven by a positive result of the risk assessment, taking into consideration all the pertinent factors as identified in the present Guideline.

Legal Aspects – The present Guideline has been given the force of law by virtue of Annex I to European Parliament and Council Directive 2001/83/EC, replaced by Directive 2003/63/EC, relating to medicinal products for human use. This Directive provides that Marketing Authorisation applicants for medicinal products for human use must demonstrate that medicinal products are manufactured in accordance with the latest version of the present Guideline, published in the Official Journal of the European Communities. 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 does not apply to medicinal products. The use of substances derived from high infectivity tissues must be fully justified following an appropriate risk/benefit evaluation.

The present Guideline should be read in conjunction with the various European Community 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 Proprietary Medicinal Products (CPMP)[1)] and Committee for Veterinary Medicinal Products (CVMP) are applicable for the purpose of regulatory compliance unless otherwise specified in the present Guideline.

A general monograph entitled: "Products with risk of transmitting agents of animal spongiform encephalopathies" has been published in the European Pharmacopoeia *(EP)*. This monograph refers to a general chapter of the EP, which is identical to this present Guideline. This monograph forms the basis for issuing Certificates of Suitability as a procedure for demonstrating TSE regulatory compliance for substances and materials used in the manufacture of human and veterinary medicinal products.

Clarification of Guideline - As the scientific understanding of TSEs, especially the pathogenesis of the disease, is evolving, from time to time CPMP and its Biotechnology

[1)] Meanwhile, this committee has been replaced by the Committee for Human Medicinal Products (CHMP)

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 the present Guideline. The supplementary guidance shall be published by the Commission and on the website of the European Agency for the Evaluation of Medicinal Products (EMEA)[*] and taken into consideration accordingly in the scope of the certification of the European Directorate for the Quality of Medicines (EDQM).

Implementation of the revised Guideline. – Regarding all medicinal products authorised by the EU conformity of the Guideline concerning the diminution of the animal spongiform encephalopathy infectious agents transmission risk, due to administering medicinal products of human and veterinary use (EMEA/410/01- Rev.1), in accordance with the juridical provisions in Annex I of Directive 2001/83/EC, modified by Directive 2003/63/EC (medicinal products for human use). The revised Guideline must be prospectively applied, in other words, to all medicinal products which will receive authorisation or whose marketing authorisation will be renewed after the date of the coming into force of the revised present Guideline.

2. Scope of the Guideline

TSE relevant animal species. - Cattle, sheep and goats that are naturally susceptible to infection with transmissible spongiform encephalopathy agents or susceptible to infection through the oral route other than human[2)] and non-human primates, are called "TSE relevant animal species"[3)].

Materials – The present Guideline is concerned with materials derived from the "TSE relevant animal species", which are used for the preparation of:

- active substances,
- excipients and adjuvants,
- raw and starting materials of reagents used in the manufacturing process (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 Guideline is also applicable to materials that come into direct contact with the equipment used in manufacture of the medicinal product, and therefore have the potential for contamination, and to materials used in the production of the primary packaging.

Test media used in the qualification of production units and equipment, such as culture media used in fill simulation tests, having in view the validation of the aseptic filling process, shall be considered in compliance with this Guideline provided that the constituents of the media are derived from tissues with no detectable infectivity (category C tissues) and where the risk of cross contamination with potentially infective tissues has been considered (see section 3.3) sourced from a GBR I/II country (see section 3.2). Such

---

[*] Meanwhile, this name has been replaced with the European Medicines Agency, while the EMEA acronym has been preserved.

[2)] Regulatory Guidance or position papers have been issued by the Committee for Proprietary Medicinal Products and its Biotechnology Working Party on human tissue derived medicinal products in relation to CJD and vCJD. Such Guidance can be found on http://www.emea.eu.int

[3)] 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 Guideline. All the same, dogs, rabbits and fish are not TSE relevant animals in the context of the present Guideline.

information shall be provided in the Marketing Authorisation dossier and verified during routine inspections relating to the Good Manufacturing Practise (GMP).

Other materials such as cleaning agents, softeners and lubricants that come into contact with the medicinal product during its manufacturing process or in the finishing stage or in the primary packaging are considered in compliance with the present Guideline if they are derived from tallow under the conditions described in section 6.

Seed lots, cell banks and routine fermentation/production. – As regards the regulatory compliance, master seeds or master cell banks for Marketing Authorisation applications lodged after 1 July 2000 (for human medicinal products) are covered by the present Guideline.

Master seeds and master cell banks:

- for vaccine antigens;

- for a biotechnology-derived medicinal product within the meaning of Part A of the Annex to Council Regulation (EEC) No. 2309/93[**)];

- for other medicinal products using seed lots or cell banking systems in their manufacture, that have already been approved as a constituent of an authorised medicinal product shall be considered in compliance with this Guideline, even if they are incorporated in Marketing Authorisation Applications lodged after 1 July 2000 (for human medicinal products).

As regards the master cell banks and the master seeds established before 1 July 2000 (concerning medicinal products for human use), but still unauthorised as constituent of any medicinal product, it has to be proven that they meet the demands of the present Guideline. If complete documented proofs are not available for certain (starting or raw materials) or other reagents used in the determination of these seeds or cell banks, the applicant must bring up the risk assessment described in section 4.

Established working seeds or cell banks used in the manufacture of medicinal products for human use authorized before 1 July 2000 which have been subjected to a properly conducted risk assessment carried out by the Competent Authority of the European Community or by EMEA 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 and in the establishment of working seeds and working cell banks, the applicant must demonstrate that they fulfil the requirements of the present Guideline.

3. General considerations

3.1. Scientific principles for minimizing risk

When given the freedom of choice, it is preferable that manufacturers use materials derived from "TSE relevant animal species" or from non-animal materials. The justification of the use of materials derived from "TSE relevant animal species" rather than materials derived from "TSE non-relevant animal species" or from a non-animal origin will have to be provided. If "TSE relevant animal species" materials must be used, all measures must be taken into consideration in order to minimize the TSE transmission risk.

[**)] Meanwhile, this Regulation has been replaced with the European Parliament and Council (CE) Regulation No. 726/2004.

Readily applicable diagnostic tests for TSE infectivity *in vivo* are not yet available. Diagnosis is based on clinical signs with *post mortem* confirmation of characteristic brain lesions by histopathology and/or detection of PrP^{sc} via the Western
Blot technique or immunodosage. 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, *in vivo* test results are only available after months or years.

Several *in vitro* diagnostic tests capable of detecting PrP^{sc} in brain samples from infected animals have been approved for use but in the main they are less sensitive than *in vivo* infectivity assays. Nonetheless, 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.

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;
- manufacturing process/processes and the quality assurance system in place to ensure product consistency and traceability.

3.2 **Source animals**

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 the *ante-* and *post-mortem* inspection, according to the Community conditions or to equivalent conditions (third countries), except materials derived from living animals which were diagnosed as healthy after the clinical examination.

3.2.1**. *Geographical sourcing***

3.2.1.1 *Bovine Materials*

There are currently two organisations involved with the assessment of the BSE status of a specified country or zone. Firstly, The International Organisation of Epizooties (Organisation Internationale des Epizooties=*OIE*)[4)] lays down in the Chapter of the International Animal Health Code on BSE the criteria for the assessment of the status of countries. OIE also provides a list of notified BSE cases worldwide. Secondly, the European Commission Scientific Steering Committee (*SSC*)[5)] has established a system for classifying the countries according to their geographical BSE-risk (GBR).

Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible products, medical devices and cosmetics are excluded from the scope of this Regulation, the principles for the determination of BSE status should be taken into account in the categorisation of the BSE status of a given country or region.

[4)] http://www.oie.int

[5)] The Scientific Steering Committee established by Commission Decision 97/404/EC shall assist the Commission to obtain the best scientific advice available on matters relating to consumer health. Starting May 2003, its functions have been taken over by the European Food Safety Agency (EFSA): http://www.efsa.eu.int

For the purposes of the present Guideline, SSC GBR classification should be used as the indicator of the status of a given country. When the countries will be categorised in accordance with Regulation (EC) No. 999/2001, this categorisation should be used.

European Commission Scientific Steering Committee Classification

The European Scientific Steering Committee has established a system for classifying geographical BSE-risk (GBR). This classification 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 found in the following table:

GBR level	Presence of one or more clinically or pre-clinically infected with BSE in a geographical region/country
I	Highly unlikely
II	Unlikely but not excluded
III	Possible, but unconfirmed or confirmed at a low level
IV	Confirmed at a high level [1)]
[1)] ≥100 cases/1 million adult cattle per year	

Reports of the GBR assessment of the countries are available on the SSC[6)] web-page. If the BSE status of a country has not been classified by the SSC, a risk assessment shall be submitted taking into account the SSC criteria for the GBR classification.

When there is freedom of choice, animals shall be sourced from countries with the lowest possible GBR risk, except the situations in which the use of materials sourced from countries with a higher GBR. Provisioning materials found in section 6, "Specific considerations", may be sourced from GBR-III countries or, in some cases, GBR-IV countries, on condition that the controls and demands specified below in the correspondent sections are carried out. Besides this exception, animals must not come from GBR-IV level, and when using animals from GBR-III, justifications should 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 could in principle be easily 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) shown to be resistant to BSE/scrapie infection shall be considered in establishing TSE free flocks. However, goats have not been sufficiently studied with regard to a genotype specific sensitivity.

Material of small ruminant origin should preferably be sourced from countries with a long history of absence of scrapie, such as New Zealand or Australia or from proven TSE-free flocks. Justification shall be required if the material is sourced from some other origin.

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

[6)] http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html

The safest sourcing is from countries where the presence of BSE is highly unlikely i.e. GBR I. However, other countries may have or have had cases of BSE at some point in time. Therefore, the practical concept of "*Negligible risk (closed) bovine herds*" has been developed by the SSC and endorsed by the CPMP 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[1].

At the time being, the quantification of the Geographical BSE-risk diminution is not possible in bovines coming from BSE negligible risk (closed) herds. However, this risk diminution is supposedly important. Therefore, the provisioning closed bovine herds will have to be taken into consideration in risk assessment, along with the GBR country status.

3.3. **Parts of animal bodies, body fluids and secretions as starting materials**

In a TSE infected animal, different organs and secretions have different levels of infectivity[8]. The tables from the annex to the present Guideline[9] summarise the present information relating to the infectivity and PrP_{Sc} repartition in BSE-affected animals and in scrapie-affected sheep or goats.

The information given in the table are exclusively based on the observations upon naturally occurring diseases primary experimental infection by the oral route (in bovines), but they do not comprise information relating to the models which use TSE strains adapted to experimental animals, because strain phenotypes which have undergone more passages may differ in a meaningful unpredictable manner from the natural occurring diseases phenotypes. Due to the immunohistochemical detection and/or western blot of the host proteins badly replicated host proteins (PrpSc) has proven to be a surrogate infectious disease marker, the PrPSc determination results were exposed in parallel to biotest data.

Tissues are classified in three major infectivity categories, regardless of the disease stage:

Category A: high infectivity tissues: central nervous system (CNS) tissues, which attain a high infectivity level in the final stages in all BSE cases, and anatomically CNS-associated tissues;

Category B: low infectivity tissues: peripheral tissues which have proven to be positive in infectivity and/or PrPSc in at least one TSE form; Category C: tissues lacking detectable infectivity: tissues that have been examined from the viewpoint of their infectivity, without any infectivity being detected, and/or from the viewpoint of their PrPSc, having negative results.

Category A tissues and derived substances must not be used in the manufacturing process of medicinal products, except when the use is justified (*see* section 5).

Although low-risk category of tissues (category B tissues) is likely to include certain types of tissues (e.g. blood) which present a lower risk than other tissues (e.g. lymphoreticular tissues), information relating to infectivity levels of these tissues are too limited to split the category into various risk levels. It is also obvious that the of a given

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

[8] If materials coming from "TSE relevant animal species" must be used, the use of materials from the lowest risk category must be taken into consideration.

[9] Tissue classification tables are based on the most recent "OMS Guidelines on transmissible Spongiform Encephalopathies concerning the pharmaceutical and biological products" (February 2003), WHO/BCT/QSD/03.01.

tissue in a category or another may be specific to the disease or species and submitted to review, as new information is gathered.

Regarding the risk evaluation (see section 4), manufacturers and/or Marketing Authorisation Holders/applicants must take into consideration the tissue classification tables included in the annex to the present Guideline[10)].

The categories presented in the table have a strictly orientative character and it is important to take into consideration the following aspects:

- In certain conjectures, a cross contamination between tissues that belong to different infectivity categories. The potential risk shall be influenced by the circumstances in which the tissues have been prelevated, especially by contact between low or undetectable infectivity tissues (category B or C tissues) and high infectivity tissues (category A tissues). Therefore, cross contamination between certain types of tissues may increase as well, if infected animals are sacrificed with the help of a bolt pistol or when the brain and/or spinal marrow are sectioned with a saw. The cross contamination risk will be minimized if the biological fluids are collected with a minimal lesion of the tissues, if the cellular elements are put away and if the foetal blood is collected while avoiding any contamination with maternal or foetal tissues, such as placenta or the amniotic and alantoic fluid. It is extremely difficult, if not impossible, to avoid a cross contamination between certain types of tissues and category A tissues (e.g. the skull). This aspect must be taken into consideration when doing the risk evaluation.

- For certain classes of substances, the methods used for the animal stunning/sacrifice may be important in view of the minimization of the potential risk[11)], due to the spreading of cerebral particles in the peripheral organs, especially in the lungs. The stunning/sacrifying methods must be described, as well as the elimination procedures of the high infectivity tissues. The employed prelevation procedures of animal tissues/organs and the measures taken in order to avoid cross contamination with a high risk material must also be accurately described.

- The contamination risk of tissues/organs of the central nervous system, potential centre of a BSE infectivity, due to the methods employed for the stunning of the animal prior to its sacrification, depends on the following factors:

- The BSE infectivity level in the sacrificed animals' brain;

- The spreading of cerebral lesions;

- The dissemination of the cerebral particles in the animal's body.

These factors must be taken into consideration along with the GBR classification of source animals, with the animals' age in bovines and *post-mortem* determination in bovines via a validated method.

The fundamental principles presented above may also be applied to sheep and goats.

The risk due to cross contamination depends on more complementary factors including:

- the measures taken in order to avoid contamination during the tissue prelevation (see above);

[10)] The split of this classification system into three categories does not block the risk evaluations based on the four-category classification previously employed in authorised medicinal products.

[11)] SSC's opinion regarding the stunning methods and BSE risk (dissemination risk of certain particles from the brain in the blood and carcass when certain stunning methods are applied), adopted in the meeting of 10-12 January 2000, http://europa.eu.int/comm/food/fs/sc/ssc/out245_en.pdf

- the contamination level (the quantity of contaminated tissue);
- the quantity and type of materials prelevated at the same time;

Marketing authorisation manufacturers or applicants/owners must take into consideration the risk associated to cross contamination.

3.4. **Age of animals**

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

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.

Quality assurance systems, such as ISO 9000 certification, HACCP[12)] or GMP, must be put in place for monitoring the production process and 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, such as 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 probably more appropriate than chemical treatments.

A description of the manufacturing process, including in-process controls applied, shall be presented, and the stages which could contribute to the contamination diminution or elimination via TSE agents will have to 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.

Cleaning process **-** Cleaning of process equipment may be difficult to validate as far as the elimination of TSE agents is concerned. It has been signaled the fact that after being exposed to preparations containing a high concentration of TSE agents, there can remain a detectable infectivity connected to the surface of the inoxidable steel. The removal of all adsorbed protein via the use of sodium hydroxide or chlorine releasing disinfectants (e.g. 20,000 ppm. chlorine for 1 hour) is considered an acceptable approach where equipment which cannot be replaced, having been exposed to potentially contaminated material. In the case of using category I materials in the manufacture of a product, dedicated equipment shall be used, unless otherwise justified.

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.

[12)] *Hazard Analysis Critical Control Point* (risk analysis and critical points control)

Elimination/Inactivation Validation. - Validation studies of removal/inactivation procedures are difficult to interpret, since 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 however needed to develop an understanding of the most appropriate "spike preparation" for validation studies.

Therefore, validation studies are currently not generally required at the time being. 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 validation studies.

In addition to appropriate sourcing, manufacturers are encouraged to continue their investigations into removal and inactivation methods to identify steps/processes, which would have benefit in assuring the removal or inactivation of TSE infective 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 infective agents.

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 illustrated in section 3.1 (Scientific Principles for Minimising Risk).

As indicated in the introduction to the present Guideline, regulatory compliance is based on a favourable outcome from a risk/benefit analysis. This risk assessment, carried out by manufacturers and/or the Marketing Authorisation Holders or applicants, for various materials or substances derived 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, that risk has been minimised by application of the principles described in this Guideline.

TSE conformity certificates, issued by EDQM, can be used by Marketing Authorisation owners or solicitors as a base for the risk evaluation.

A global risk evaluation for a medicinal product, carried out by Marketing Authorisation owners or solicitors, must take into consideration the risk evaluation for each of the various materials derived from the "TSE relevant animal species" and, if needed, the TSE agents minimising or elimination via the manufacturing process stages of the active substance and/or the finished medicinal product.

The final evaluation of the regulatory compliance is conducted by the Competent Authority.

It is incumbent upon the manufacturers and/or the Marketing Authorisation Holders or applicants for medicinal products for human use to select and justify the control measures for a given "TSE relevant animal species" derivative, taking into account the state of the art of science and technology.

5. Risk/benefit evaluation

In addition to the parameters as mentioned in the sections 3 and 4, the acceptability of a particular medicinal product containing materials derived from a "TSE relevant animal species", or which as a result of the manufacturing process could contain these materials shall 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 dosage and duration of treatment);

- intended use of the medicinal product and its clinical benefit.

Unless otherwise justified, high infectivity tissues (Category I) and substances derived from them must not be used for the manufacture of medicinal products, starting materials or intermediary products (including active substances, excipients and reagents). A justification will have to be uttered, according to which no other material can be used. In these exceptional and justified circumstances, the use of high infectivity tissues will be able to be taken into consideration in the manufacture of active substances, if, following a risk assessment, as described in section 4 and considering the clinical use in force, a positive analysis of the risk/benefit ratio effectuated by the Marketing Authorisation solicitors. Substances derived from category I materials, if their use is justified, must be produced from animals of GBR I countries.

6. Specific considerations

The following materials prepared from the "TSE relevant animal species" are considered in compliance with the present Guideline, 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 the present Guideline 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 gelatine are applicable (see below).

- Collagen produced from tissues such as hides and skins 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 strictly avoided during their procurement.

6.2. **Gelatine**

Gelatine is a soluble natural protein, gellifying or not, obtained via partial hydrolysis of collagen derived from animal bones, skin, tendons or muscles.

For gelatine, documentation to demonstrate compliance with the present Guideline 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) <u>The source material used</u>

Gelatine used in medicinal products can be manufactured from bones and hides.

- *Hides as the starting material* - On the basis of current knowledge, hides used for gelatine production represent a much 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 gelatine, more stringent production conditions shall be applied (see below). In any case, the removal of skulls and spinal cords from the starting material is considered as a first precautionary measure which largely affects the safety of the product. As far as practicable, bones should be sourced from countries classified as GBR I and II. Bones from category GBR III countries can be used if the gelatine is manufactured under defined conditions as indicated

below and if the vertebrae of bovines aged over 12 months are removed from the raw/starting materials is assured[13].

(ii) Manufacturing methods

No specific measures with regard to the processing conditions are required for gelatine 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.

However, the mode of manufacture must be taken into account where bones are used as the starting material.

- Bones (including vertebrae) for the production of gelatine using acid treatment shall be sourced only from GBR category I or II countries. An additional alkaline treatment of bones/ossein (13.1-hour pH) may further increase the relative TSE safety of acid derived bone gelatine.

For bones sourced from a GBR category III country, the alkaline process shall be applied. However, this manufacturing method is optional for bones coming from GBR category I and II countries.

- For a typical alkaline manufacturing process, bones are finely crushed, degreased with hot water and demineralised with dilute hydrochloric acid (at a maximum 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 (a minimum pH of 12.5) for a period of 20 to 50 days. The gelatine is extracted, washed, filtered and concentrated. A "flash" heat treatment step using 138-140°C for 4 seconds is applied. Bovine hide gelatine can also be produced by the alkaline process. Bovine bones may also be treated by an acid process. In this case, the liming step in a Calcium hydroxide treatment is then replaced by an acid pre-treatment where the ossein is soaked overnight at pH < 4.

6.3. **Bovine 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 foetal blood sampled by cardiac punction, under sterile conditions on a separate place.

New born calf serum is obtained from calves under 20 days old, and calf serum, from animals under 12 months old. In the case of donor bovine serum, on condition that these animals are aged under 36 months old, the TSE 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 derivatives, documentation to demonstrate compliance with this Guideline 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

[13] Regulation (EC) No. 1774/2002 of the European Parliament and of the Council which establishes sanitary norms concerning animal by-products not destined for human consumption will be applied, unless otherwise justified. As regards the gelatine and collagen manufacturing process or the raw material importation needed for such a manufacturing process in order to be used in pharmaceutical products, only materials from animals destined for human consumption shall be used. The use of these vertebrae coming from GBR II animals, which is sure, according to the risk assessment, shall continue to be allowed.

Traceability to the slaughterhouse of origin 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, traceability records must be available for each serum batch which assures the traceability to the farms of origin.

(ii) Geographical origin

Whilst tissue infectivity of BSE in cattle is more restricted than scrapie in sheep and goats, as a precautionary measure bovine blood must be sourced from countries classified GBR I and II, unless otherwise justified.

(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 bold stunner with or without pitching as well as by pneumatic stunner, especially if it injects air, can destroy the brain and disseminate brain material into the blood stream. Negligible risk can be expected from a non-penetrative stunner and from electro-narcosis[14]. The stunning methods must therefore be described for the bovine blood collection process.

If sourcing is allowed from countries where cases of BSE have been detected (GBR III) a nonpenetrative stunner shall be used for 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[15] 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, have been the subject of specific consideration by CPMP and CVMP and are thought unlikely to be infectious. For this reason, such materials manufactured under the conditions at least as rigorous as those given below shall be considered in compliance for the present Guideline, 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 ore;
    - Continuous process: at not less than 140 °C, under pressure for not less than 8 minutes, or equivalent.
- Distillation at 200°C.

It's barely probable that tallow derivatives obtained in these given conditions present a TSE risk and shall, therefore, be considered in compliance with the present Guideline.

For tallow derivatives manufactured in other conditions, regulatory compliance with the present Guideline must be proven.

---

[14] 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 in the meeting of 10-11 January 2002, http://europa.eu.int/comm/food/fs/sc/ssc/out245_en.pdf

[15] OJ L 273, 10.10.2002, p.1.

6.5**. Animal charcoal**

Animal charcoal is prepared by carbonisation of animal tissues, such as bones, using high temperature at >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 their geographical origin and the nature of the tissue, for the purpose of regulatory compliance, animal charcoal shall be considered in compliance with the present Guideline.

It's barely probable that, being obtained in these given conditions, animal charcoal presents a TSE risk and will, therefore, be considered in conformity with the present Guideline. For the animal charcoal manufactured in other conditions, conformity with the present Guideline must be proven.

6.6**. Milk and milk derivatives**

In the light of the current scientific knowledge and irrespective of its geographical origin, milk is unlikely to present any risk of TSE contamination.

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 the abomasums or rennet derived from other ruminants. The CPMP/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[16]. The conclusion was endorsed by the SSC[17] which has also performed an assessment of the TSE risk of rennet in general[18].

It's barely probable that milk derivatives obtained in these given conditions present a TSE risk and shall, therefore, be considered in compliance with the present Guideline:

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

- no other ruminant material, 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 be proven in compliance with the present Guideline.

6.7. **Wool derivatives**

Derivatives of wool and hair of ruminants, such as lanolin and wool alcohols derived from hairs shall be considered in compliance with the present Guideline, provided the wool and hair are sourced from live animals.

It's barely probable that wool derivatives produced from wool which are sourced from slaughtered animals declared "fit for human consumption" and whose manufacturing

---

[16] Committee for Proprietary Medicinal Products and its Biotechnology 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 replacers used as feed for the animals from which abomasums are obtained is particularly important. The report can be found on http://www.emea.eu.int.

[17] 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://europa.eu.int/comm/food/fs/sc/ssc/out255_en.pdf)

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

process in relation to pH, temperature and duration of treatment meets at least one of the stipulated processing conditions listed below present any TSE risk and shall therefore be considered in compliance with the present Guideline:

- Treatment at pH ≥13 (initial; corresponding to a NaOH concentration of at least 0.1 mol NaOH) at ≥ 60°C for at least 1 hour, which normally occurs during the reflux stage of the organic-alkaline treatment;

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

In the case of wool derivatives manufactured in other conditions, regulatory compliance with the present Guideline must be proven.

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.

It's barely probable that amino acids prepared using the following processing conditions in accordance with Commission Decision 98/256/EC[19] and Commission Decision 2001/376/EC[20] present any TSE risk and shall therefore be considered in compliance with the present Guideline:

- 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 >11, followed by heat treatment at 140°C for 30 minutes at 3 bar;

- the resulting amino acids or peptides must be filtered after production; and

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

For amino acids manufactured in other conditions, regulatory compliance with the present Guideline must be proven.

[19] OJ L 113, 15.4.1998, p. 32
[20] OJ L 132, 15.5.2001, p. 17

ANNEX
to Guideline

MAJOR INFECTIVITY CATEGORIES

The tables below are adapted from "OMS Guideline for Transmissible Spongiform Encephalopathies relating to pharmaceutical and biological products" (February 2003).

Legend:
+ = infectivity or $PrP^{TSE(1)}$ presence,
- = infectivity or PrP^{TSE} absence,
NT = non-tested,
? = questionable or uncertain results.

Category A : High infectivity tissues

Tissues	Bovines		Sheep and goats	
	BSE		Scrapie	
	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}
Brain	+	+	+	+
Spinal marrow	+	+	+	+
Retina, optic nerve	+	NT	NT	+
Rachidian ganglions	+	NT	NT	+
Trigeminal ganglions	+	NT	NT	+
Hypophyse[1]	-	NT	+	NT
Dura mater[2]	NT	NT	NT	NT

1. Biologic infective dosages of bovine tissues were tested either on bovines, either on mice (or on both); and the majority of infectivity biologic dosages of sheep and/or goat tissues were only tested on mice. As regards sheep and goats, not all results are similar in for species.

2. Experimental data on human hypophyse or dura mater haven't been reported, but corpse dura mater remains and growing hormones derived from corpse

(1) In the main text of the present Guideline, the abnormal isomorphs of prion proteins are named PrP^{Sc}. Yet, these tables being directly transcripted from the OMS Guideline mentioned above, the OMS nomenclature has been preserved for the abnormal prion proteins PrP^{TSE}.

hypophyses have transmitted the disease to tens of persons, which is why hypophyse and dura mater must be included in the high risk tissue category.

Category B: Low infectivity tissues

Tissues	Bovines		Sheep and goats	
	BSE		Scrapie	
	Infectivity[1]	**PrP^{TSE}**	**Infectivity[1]**	**PrP^{TSE}**
Peripheral nervous system				
Peripheral nerves	-	NT	+	NT
Enteric plexus	NT	+	NT	+
Limphoreticular tissues				
Spleen	-	-	+	+
Lymphatic ganglions	-	-	+	+
Tonsils	+	NT	+	+
Nictating membrane	NT	-	NT	+
Thymus	-	NT	+	NT
Digestive tract				
Esophagus	-	NT	NT	+
Pre-stomach[2] (only for ruminants)	-	NT	NT	+
Stomach/ abomasum[2]	-	NT	NT	+
Duodenum	-	NT	NT	+
Jejunum	-	NT	NT	+
Ileum[3]	+	+	+	+
Large intestine	-	NT	+	+
Reproductive tissues				
Placenta	-	NT	+	+
Other tissues				

Lung*	-	NT	-	NT
Liver	-	NT	+	NT
Kidneys*	-	-	-	-
Adrenal gland	NT	NT	+	NT
Pancreas	-	NT	+	NT
Bone marrow	+	NT	+	NT
Blood vessels	-	NT	NT	+
Olfactive mucous membrane	-	NT	+	NT
Gingival tissue*	NT	NT	NT	NT
Salivary gland	-	NT	+	NT
Cornea[4*]	NT	NT	NT	NT
Organic liquids				
Cephalo-rachidian liquid	-	NT	+	NT
Blood[5]	-	NT	+	-

1. Limited in bovine distal ileum.

2. Just like the real stomach (abomasum), ruminant stomach (reticulum, rumen and omasum) is for current use. Bovine abomasum (and sometimes sheep abomasum) is also a clot source.

3. In bovines and sheep, only the distal ileum is subject to biological infective dosage.

4. Since out of hundreds of thousands of receivers, only one or two CJD cases have been plausibly attributed to the corneal transplant, cornea belongs to the low-infectivity tissues. The tests effectuated on other tissues of the anterior chamber (crystalline lens, aqueous humor, iris, conjunctiva) have given vCJD and TSE negative results, no epidemiological proof has allowed the association of these with the iatrogenic transmission of the disease.

5. First reports on the transmission of the disease at rodents via infected blood of sCJD patients haven't been confirmed, and the experimental and epidemiological data evaluation relating to TSE transmission via blood, blood compounds or therapeutic plasma compounds do not indicate the transmission via the blood of patients affected by a "classical" TSE form. Gathered information is not sufficient in order to utter the same affirmation relating to the blood of vCJD-affected patients. Fetal calf blood does not contain any detected infectivity, but in sheep genotipically susceptibile to natural scrapie or experimentally induced BSE, high blood quantity transfusions have allowed the transmission of the disease to healthy sheep. Moreover, infectivity has been demonstrated in studies of rodent-adapted TSE strains.
* These tissues belong to category II: low infectivity tissues, because infectivity and/or PrP^{TSE} have been detected in human CJD (vCJD or other).

Category C : Tissues with no detectable infectivity

Tissues	**Bovines**		**Sheep and goats**	
	BSE		**Scrapie**	
	Infectivity[1]	**PrP^{TSE}**	**Infectivity[1]**	**PrP^{TSE}**
Reproductive tissues				
Testicles	-	NT	-	NT
Prostate/ Epididim/ Seminal bladder	-	NT	-	NT
Sperm	-	NT	NT	NT
Ovar	-	NT	-	NT
Uterus (non-pregnant)	-	NT	-	NT
Placental liquids	-	NT	NT	NT
Foetus[1]	-	NT	-	NT
Embrion[1]	-	NT	?	NT
Musculoskeletal tissues				
Bone	-	NT	NT	NT
Skeletal muscle[2]	-	NT	-	NT
Tongue	-	NT	NT	NT
Heart/ pericardium	-	NT	-	NT

Tendon	-	NT	NT	NT
Other tissues				
Trachea	-	NT	NT	NT

Skin	-	NT	-	NT
Adipose tissue	-	NT	NT	NT
Thyroid gland	NT	NT	-	NT
Mammary gland/ udder	-	NT	-	NT
Organic liquids, secretions and excretions				
Milk[3]	-	NT	-	NT
Colostrum[4]	NT	NT	-	-
Cordonal bood	-	NT	NT	NT
Saliva	NT	NT	-	NT
Perspiration	NT	NT	NT	NT
Tears	NT	NT	NT	NT
Nasal mucus	NT	NT	NT	NT
Urine[4,5]	-	NT	NT	NT
Fecals	-	NT	-	NT

1. Embryos coming from BSE affected bovines haven't transmitted the disease to mice, but no other infectivity determination on calf foetal tissues has been effectuated, excepting the ones in blood (negative biological assay in mice). Calves born from cows which have received embryos coming from BSE affected bovines have survived on observation periods up to seven years, and brain examination of unaffected cows and their calves has revealed neither spongiform encephalopathy nor PrP^{TSE}. 2. Intracerebral inoculation of muscle homogenates has not transmitted the disease to: 1) primates, from sCJD-affected people; 2) mice or bovines, from BSE-affected bovines; 3) mice, from sheep and goats affected by natural or experimentally induced scrapie. However, older reports describe isolated cases of transmission from goat and muscular tissue, and a more recent study describes a transmission from wild and transgenic mice muscles, but since each of these studies have been carried out with TSE strains which were subject to more passages, their relevance relating to natural disease remains undetermined.

2. Intracerebral inoculation of muscle homogenates has not transmitted the disease to: 1) primates, from sCJD-affected people; 2) mice or bovines, from BSE-affected bovines; 3) mice, from sheep and goats affected by natural or experimentally induced scrapie. However, older reports describe isolated cases of transmission from goats and muscular tissue, and a more

recent study describes a transmission from wild and transgenic mice muscles, but because each of these studies has been carried out with TSE strains which were subject to more passages, their relevance relating to natural disease remains undetermined. A recent report relating to a human case describes a CJD-affected patient, subject to an inclusion body myositis characterized by an abundant quantity of PrP^{TSE} in the affected muscle. However, following numerous deliberations, the committee has decided to preserve the muscle in the "no detectable infectivity" category, until more information on uncomplicated natural infections will be available.
3. The proof that infectivity is not present in milk resides in: tempo-spatial epidemiological observations, which did not allow the detection of maternal transmission; the clinical observations on more than one hundred calves nursed by infected cows, which have not developed BSE; and experimental observations showing that oral or intracerebral administration in mice of milk coming from infected cows hasn't led to the transmission of the disease. Presently, experiments are being tested, consisting in the concentration of a large volume of milk coming from experimentally infected cows and in its testing in order to determine the presence of PrP^{TSE}.
4. Isolated cases of CJD through cordonal blood, colostrum and human urine have never been confirmed and are considered improbable.
5. A PrP type which has never been previously signaled, named PrP^{U}, has been identified in the urine of sporadic or familial CJD-affected patients, yet its significance relating to the transmission risk remains to be determined.