Public health issues related to animal and human spongiform encephalopathies: Memorandum from a WHO meeting*

The transmissible spongiform encephalopathies (TSE) include bovine spongiform encephalopathy (BSE), which was first described in 1986 in the United Kingdom but has occurred subsequently in several other countries.

This Memorandum reviews the existing state of knowledge on all the known spongiform encephalopathies, and evaluates the pathways of transmission and associated hazards. The possible implications of the animal diseases, especially BSE, with regard to the use of animal tissues as animal feed, human food, and in the preparation of medicinal and other products for human use are discussed, with recommendations to national health authorities on appropriate measures to minimize the consequences of BSE to public and animal health.

Nature and occurrence

Characteristics of the spongiform encephalopathies

Bovine spongiform encephalopathy (BSE) is a member of the group of transmissible spongiform encephalopathies (TSE), whose prototype is scrapie. All these diseases are associated with a transmissible agent, the nature of which is not fully known. However, it displays many virus-like features such as strain variation and mutation, but differs from conventional viruses in being exceptionally resistant to heat, ultraviolet and ionizing radiations, and many chemical disinfectants.

The TSE agents, following long incubation periods, produce clinical diseases which are characteristically progressive and end fatally. These diseases affect the central nervous system in which characteristic spongiform changes are visible by light microscopy. Detergent-treated extracts of affected brain yield scrapie-associated fibrils (SAF) which are visible by electron microscopy. These fibrils consist mainly of an abnormally modified host-coded protein called PrP or prion protein. Because of this, the TSEs are also referred to as "prion diseases". Infection with these agents does not provoke a detectable immunological reaction in the host, so there is at present no practical means of detecting infection in healthy but possibly infected animals. Bioassay in laboratory species, such as mice, is the only way of detecting and measuring the infectivity of these agents. As with scrapie, the transmission of BSE to mice has been accomplished by intracerebral and intraperitoneal injection, as well as by feeding them with affected brain. BSE has also been transmitted to cattle by the injection of infected brain.

Occurrence of BSE and other animal TSEs

BSE was first recognized in the United Kingdom in November 1986. Initial epidemiological studies indicated an extended common source epidemic. Later studies, taking into account the disease's similarity with scrapie, identified the exposure of cattle to a

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Requests for reprints or the full report (document CDS/VPH/92.104) should be sent to Chief, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland. A French translation of this article will appear in a later issue of the *Bulletin*.

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TSE agent through feed that contained ruminant-derived protein (in the form of meat and bone meal) as the likely source of the disease. A key factor explaining the emergence of the disease was the occurrence of certain changes in rendering practices (i.e., processing of animal and abattoir wastes to produce feed) in the United Kingdom in 1981–82.

The total number of confirmed BSE cases in the United Kingdom between November 1986 and 31 August 1991 was 35 627; at the end of this period the annual incidence was 5 cases per 1000 adult cattle. BSE cases have also been reported in the following countries/territories (the cumulative number of cases as at 31 August 1991 is given in brackets): Ireland (41), France (4), Switzerland (7), Oman (2) and the Falkland Islands (1). The cases in the last two places were imported from the United Kingdom, which is the only country with a high incidence of BSE.

Other major transmissible animal spongiform encephalopathies are described below.

(1) *Scrapie* is a disease mainly affecting sheep of 2 to 5 years of age, but it can also affect goats. The disease has been diagnosed in many countries of the world and has been endemic for nearly 300 years in the United Kingdom. Scrapie was introduced into Australia and New Zealand through the importation of sheep from the United Kingdom, but stringent measures eradicated the disease in these countries. Australia and New Zealand are accepted by many countries as being scrapie-free.

Scrapie is important as the only TSE of animals which is known to exist as an endemic infection of its natural host, sheep. Epidemiological evidence shows that the transmission of infection is predominantly from infected mothers. In addition, scrapie infection can pass between unrelated sheep, either directly, or as a consequence of contamination of the environment (e.g., by infected fetal membranes). The oral route of infection is implicated in the natural spread of the disease.

(2) *Transmissible mink encephalopathy (TME)* is a rare disease of farm-reared mink associated with the feeding of animal wastes contaminated with a TSE agent. It is important in that it provides a precedent for BSE, but there are some major differences; for example, the source of infection for TME is believed to be dead stock and uncooked animal wastes, whereas the source of BSE infection is commercial feedstuffs containing heat-treated animal protein.

In contrast to scrapie, there is no evidence of transplacental or perinatal transmission of TME from infected females to their offspring. Horizontal spread of infection to unrelated mink is not known to occur, unless there is cannibalism.

(3) Other animal spongiform encephalopathies have been identified in North America in mule deer and elk (chronic wasting disease), in the United Kingdom in cats (feline spongiform encephalopathy), and in five species of exotic ungulates in zoos in the United Kingdom, all of which belong to the Bovidae family.

Human spongiform encephalopathies

These neurodegenerative diseases include kuru, sporadic and familial forms of Creutzfeldt-Jakob disease (CJD), and Gerstmann-Sträussler syndrome (GSS).

(1) *Kuru* used to occur at high incidence among the Fore people of Papua New Guinea in the 1950s. Its origin is unknown but contamination during unusual mourning rites effectively passaged neuropathogenic strain(s) of an agent in a susceptible population. Studies of several hundred kuru orphans revealed no evidence of maternal transmission of infection, unlike in scrapie. The cessation of these mourning rites in the late 1950s has led to the gradual disappearance of kuru which is now rare. Studies of recent cases indicate incubation periods of up to three decades.

(2) *Creutzfeldt-Jakob disease* (CJD) is a rapidly progressive dementia (duration, up to two years) with movement disorders (especially myoclonus) and a characteristic electroencephalogram (EEG). CJD usually occurs sporadically at a uniform worldwide incidence of about one case per million people per annum. A very small number of cases (about 35 in all) have occurred iatrogenically. Up to 10% of CJD cases are familial (see below: routes of transmission, epidemiological studies).

(3) *Gerstmann-Sträussler syndrome* (GSS) differs from typical CJD in having a longer duration (2 to 15 years), with a more slowly progressive dementia and/or cerebellar ataxia. It is a familial disease and resembles familial CJD in being associated with mutations in the human *PrP*, but not necessarily the same mutations.

Transmission

Experimental transmissibility is a key factor in the definition of spongiform encephalopathies including GSS and the familial form of CJD. The mechanisms by which pathogenesis occurs naturally vary with the disease, but an assessment of the risks of transmission to both animals and man can be made by considering certain factors, which are described below.

Routes of transmission, dose, and species barriers

The oral route has been shown experimentally to transmit spongiform encephalopathy, and it plays a

role in the natural transmission of BSE, scrapie, TME and kuru, but not CJD and GSS. However, compared to parenteral exposure, ingestion is a relatively inefficient route of transmission in all the TSEs that have been studied experimentally. For mouse scrapie, the oral dose required is about a hundred thousand times greater than the intracerebral dose. In limited studies of BSE, the amount of affected cattle brain required to produce the disease in mice was between a thousand and a million times greater by the oral route than by intracerebral injection.

The main concern is the possibility of a greater risk through accidental or intentional inoculation of contaminated material. There have already been several instances of iatrogenic transmission of CJD involving exposure within or close to the central nervous system (e.g., corneal and dura mater transplants, use of contaminated EEG electrodes and neurosurgical instruments). Particularly tragic has been the occurrence of CJD in human growth hormone recipients who received repeated intramuscular injections over many years.

It must be emphasized that the above instances of iatrogenic transmission of CJD did not involve a species barrier which is usually the limiting factor in the experimental and natural transmission of the TSE agents from one species to another. The species barrier involves two main factors. The first, the "donor/recipient species effect", depends on the species infected and the species exposed. There is now good evidence that the basis of this effect is the *PrP* gene. The second factor is the strain of the agent which can come under strong selective pressures in a new species, such that parental strains may be excluded and mutant strains favoured. It is the interaction of the host gene (*PrP*) with different agent strains that make the extent of the species barrier unpredictable.

For these reasons, it is important to consider the implications of BSE in terms of occupational exposure (e.g., in abattoir workers, butchers, farmers and veterinarians), and the use of medicinal products, including implants, which are derived from bovine tissue. This task is assisted by considering if there are any epidemiological relationships between scrapie and CJD.

Epidemiological studies

Since the demonstration of the transmissibility of CJD in 1968, many epidemiological surveys have been carried out to identify the natural reservoir of CJD infection and the mode of infection. Although these studies have not revealed the cause of CJD in the great majority of patients, the following conclusions can be drawn from some consistent findings.

(*a*) Sporadic CJD has a worldwide distribution and uniform incidence of 0.5 to 1 case per million par annum. This incidence has been confirmed in systematic surveys, and there is no convincing evidence of spatio-temporal aggregation of cases. The geographical distribution of cases largely parallels population density, although in two countries a slightly higher incidence is found in urban populations.

(*b*) Some 5–10% of cases are familial with an apparently dominant pattern of inheritance, but it is not known whether this represents contact transmission or an inherited influence on disease expression. In the past two years the application of molecular biological techniques has shown that mutations of the *PrP* gene on chromosome 20 correlate in some pedigrees of Gerstmann-Sträussler syndrome and CJD with affected individuals, while unaffected family members do not have the mutation. This provides strong evidence for a genetic influence in CJD, although it is known that mutation of the *PrP* gene is neither necessary nor sufficient by itself for the development of disease.

(*c*) Iatrogenic transmission (see above: routes of transmission, paragraph 2) is fortunately extremely rare, and cannot be invoked as an explanation for the development of CJD in the great majority of patients.

(*d*) There is no convincing evidence of case-to-case transmission by conventional contagious routes. Personal contact between individual sporadic cases is exceedingly rare, and the great majority of cases appear to be geographically isolated. The protracted incubation period (18 months to 25 years in iatrogenic cases) compromises the assessment of the geographical distribution of cases, but the study of cases at various epochs in the past, by examining lifetime residential history, has also failed to reveal any clustering of cases in space and time.

(*e*) There is no convincing evidence to support zoonotic transmission, and in particular no evidence to suggest a causal link between scrapie and CJD. There is an extraordinarily high incidence of CJD in Libyan-born Israelis, and recently an aggregation of cases in Czechoslovakia has been reported. However, these exceptional occurrences of CJD are related to a high incidence of familial cases, associated with mutations in the *PrP* gene, rather than to an increased exposure (e.g., dietary) to scrapie. Furthermore, there is no evidence of an increased risk of CJD due to occupational exposure to the scrapie agent, and case-control studies have not shown an increased exposure to sheep meat in affected patients compared with controls. CJD has been described in a lifelong vegetarian. In addition, a relatively low incidence of CJD has been reported in one country despite endemic scrapie and the histori-

cal practice of consuming scrapie-affected animals. Most significantly, CJD occurs with the expected incidence in countries such as New Zealand and Australia where no scrapie cases have been reported during the past 38 and 40 years, respectively, and also in Japan where scrapie has only recently been reported in imported stock.

Man has potentially been exposed to high levels of scrapie agent via diet and occupation for more than two centuries, but no epidemiological link between scrapie and CJD has been established. The same could well apply to BSE, but the uncertainties of the species barrier between cattle and man means that precautionary measures are necessary to reduce the risks to the lowest possible level.

TSE agents

Distribution in tissues

Based on present knowledge, there is little or no risk of infection as a result of oral exposure to tissues that contain no detectable TSE infectivity as determined experimentally using the most efficient routes of inoculation (e.g., intracerebral). There is a large literature on the amounts of infectivity in different tissues of animals infected experimentally or naturally with one or other of the TSE agents. Although the general patterns are similar, the amounts of infectivity in tissues vary with the different diseases. For the purposes of risk avoidance, natural scrapie in Suffolk sheep and goats is the most appropriate "worst case scenario", for three reasons:

— First, only a very small species barrier effect was encountered when performing the bioassays of infectivity in mice.
— Secondly, it is important to extrapolate from examples of TSE which reproduce the timescale of BSE (i.e., the incubation periods of natural scrapie and BSE are comparable).
— Thirdly, the infectivity titres of TSE agent outside the central nervous system are usually higher with scrapie than with the other diseases.

The patterns of distribution seen with natural scrapie and BSE, based on extensive studies, are described below.

In natural scrapie. No scrapie agent was detected in any tissue from lambs of up to 8 months of age. At 10–14 months of age, low infectivity was present in the large masses of lymphoreticular tissue in the intestines (Peyer's patches), lymph nodes associated with the gastrointestinal tract and elsewhere, spleen and tonsil. The titres in these tissues increased subsequently and, before clinical signs appeared, infectivity was detected in the spinal cord, medulla and some other areas of the brain. By the time the animals showed clinical disease, levels of infectivity in the central nervous system, including the spinal cord, had risen above those in the lymphoreticular system.

Attempts have been made to detect scrapie infectivity in a wide range of other tissues (see Box). Those that have *not* been shown to harbour detectable infection include skeletal muscle (i.e., carcass meat), heart, kidney, milk and colostrum, mammary gland, uterus, blood, saliva, and skin.

In BSE. Transmission experiments in mice have been undertaken with a wide range of tissues from confir-

Categories of infectivity

Relative scrapie infectivity of tissues and body fluids from naturally infected Suffolk sheep and goats with clinical scrapie are given below.

- *Category I* . High infectivity
  Brain, spinal cord
- *Category II.* Medium infectivity
  Spleen, tonsil, lymph nodes, ileum, proximal colon
- *Category IIIa.* Some infectivity
  Sciatic nerve, pituitary, adrenal, distal colon, nasal mucosa
- *Category IIIb.* Minimal infectivity
  Cerebrospinal fluid, thymus, bone marrow, liver, lung, pancreas
- *Category IV.* No detectable infectivity
  Skeletal muscle, heart, mammary gland, colostrum, milk, blood clot, serum, faeces, kidney, thyroid, salivary gland, saliva, ovary, uterus, testis, seminal vesicle

The above is based on data obtained by bioassays of infectivity using mice injected intracerebrally (*1, 2*).

Note 1: Under experimental conditions with strains of agent adapted to laboratory animals, higher titres may occur resulting in slightly different classifications.

Note 2: For some other tissues not listed in this box, see text (section on "type of bovine material", page 189). Conditions under which certain tissues are collected may alter this classification (see sections on "type of bovine material" and "conditions under which materials are collected", page 189).

med cases of BSE. So far, the brain was found to be the only tissue from which BSE has been transmitted, either by feeding or by injection. Experiments with mice that were fed milk and mammary gland, placenta, lymph nodes or spleen have failed to transmit the disease within the natural lifespan of the animals, or even to establish subclinical BSE infection of the lymphoreticular system. Furthermore, mice exposed parenterally to spleen, placenta, skeletal muscle, buffy coat (leukocytes from centrifuged blood) and semen did not succumb to a spongiform disease within their natural life span.

Prevention and risk reduction

Prevention and control of BSE in cattle

Epidemiological studies, including computer simulation of the epidemic in the United Kingdom, suggest that:

— feed contaminated by a TSE agent is the cause of the disease; two possible hypotheses as to the original source of this agent are consistent with the epidemiological findings (either that it was the agent of scrapie itself, or that it was a cattle-adapted strain of a scrapie-like agent); whichever of these hypotheses in correct, there is evidence that the epidemic was amplified by the recycling of infection, via feed, from cattle to cattle;
— even when the source of infection is cut off, the long incubation period (4 to 5 years) can lead to the appearance of new cases for several years, after which the disease incidence is likely to fall markedly;
— sources of exposure other than feed (e.g., maternal transmission) are not likely to be important in the spread of BSE or to be able to sustain the epidemic.

Activities of the veterinary services should be designed primarily to control the disease in cattle; to evaluate those control activities in countries where it is present; and, in countries where BSE is absent, to avoid the occurrence of the disease and to establish appropriate surveillance systems for early detection.

Detailed guidelines covering these aspects were proposed by the specialists advising the Office international des Epizooties (OIE) during a meeting held in Paris on 30–31 October 1991. Recommendations to national veterinary administrations importing live animals, fresh meat, and meat products from areas with either a high or low incidence of BSE have been specified.[a] The participants agreed that the basic requirement for the control of the disease, where it is present, is to eliminate the exposure of cattle to TSE agents through feed. This has been achieved in countries where BSE occurs by means of a ban on the use of ruminant proteins (other than milk) to feed cattle.

In BSE-free countries where the relevant risk factors are present (e.g., use of ruminant protein in ruminant feed, the occurrence of scrapie, size of sheep population relative to that of cattle, etc.), consideration might be given to the exclusion from ruminant feed of selected tissues which might contain high titres of the agent. These tissues are the "specified offals" described below (section on prevention of hypothetical foodborne transmission).

Minimizing the risks to humans

The results of studies carried out mostly on scrapie suggest that the risk, if any, of infection of humans with the BSE agent would arise only from exposure to certain tissues of infected animals, or products prepared from those tissues. Significant infectivity is likely to be present only in the central nervous system, and in certain organs containing lymphoreticular tissues. Experiments, albeit incomplete, suggest that the distribution of infectivity in BSE cases is no more widespread than in scrapie and is probably more restricted. In addition, there is a range of tissues (e.g., skeletal muscle, milk, etc. (see Box, p. 186)) in which, on the basis of what is known for scrapie, no detectable infectivity is expected to occur at any time, even in clinically affected animals.

Furthermore, BSE epidemiological and tissue infectivity studies that have been completed so far support the hypothesis that maternal transmission is not likely to be a significant factor in the transmission of BSE. By analogy with scrapie, infection would not be detected in an animal incubating the disease until it is six months old, even if it had been infected before or at birth.

Prevention of hypothetical foodborne transmission from cattle. As none of the food-processing technologies, such as heat treatment (cooking, pasteurization, sterilization), freezing, drying, chemical treatment including acidification, fermentation including pickling, and irradiation, are fully effective for inactivating the infectious agent, prevention of foodborne transmission must be based on identifying healthy animal sources and, in countries with a high incidence of disease, excluding those tissues which in an incubating animal may be infective. Infection is unlikely to be detectable in calves which are incu-

[a] Draft chapter 3.2.13 in OIE Code on Bovine Spongiform Encephalopathy, to be submitted to the OIE Committee during the 60th General Session in May 1992.

bating BSE before they are six months old. Any detectable infectivity in older animals is likely to be confined to the central nervous system and/or lymphoreticular system. Therefore, in countries where there is a high incidence of BSE, the "specified offals" (brain, spinal cord, tonsils, thymus, spleen, and intestines—from duodenum to rectum inclusive) taken from cattle over six months old, and any protein material derived from them should not be used in human food or animal feed, including that of pet animals and birds. "Intestines" exclude oesophagus and stomachs, because these organs, when prepared for food, do not contain significant quantities of lymphoid tissues; casings derived from intestines are included because lymphatic tissue from Peyer's patches may be partially retained after processing. The above list of specified offals should be reviewed in the light of new scientific knowledge, particularly on the infectivity titres in the tissues of BSE-affected cattle.

Where BSE occurs with a high incidence, the above ban is considered to be sufficient for protecting the health of humans including that of infants, children, the elderly, immunocompromised persons, etc. and of animals.

Reassurance can be provided by the following measures:

- the removal from meat (skeletal muscle) of visible nervous and lymphatic tissue during the cutting process (United Kingdom);
- compulsory slaughter of suspect cases, with total destruction of carcasses (United Kingdom, Switzerland, France, and Ireland);
- non-marketing of milk from suspect cases (United Kingdom);
- voluntary slaughter of herds in which BSE cases occur (France, Ireland).

Tallow is one of the two end-products of rendering (the other being meat meal, or meat and bone meal). The higher grades of tallow are used in the manufacture of human food, animal feed (including pet food) and the preparation of toilet soaps and detergents. The lower grades have various industrial uses. Because of their proteinaceous nature the TSE agents would during the extraction process tend to remain with the cellular residues of meat and bone meal, rather than be extracted with the lipids of tallow. This is consistent with epidemiological observations which show that the geographical distribution of BSE in the United Kingdom is related to the use of meat and bone meal, not tallow. Tallows are filtered at the rendering plant to remove protein and other impurities and then subjected before use to further rigorous processing. For these reasons, tallow does not appear to be a risk for human and animal health.

Prevention of hypothetical occupational transmission. Although Creutzfeldt-Jakob disease has been reported in a small number of individuals who might have been occupationally exposed to the agent, descriptive and analytic epidemiological studies have not provided evidence of a link between specific occupations and an increased risk of developing CJD. In particular, there is no evidence of an increase in the incidence of CJD in those individuals occupationally exposed to the scrapie agent. However, the possibility that the BSE agent may exhibit different biological properties from scrapie has resulted in concern that specific occupational groups in the United Kingdom, such as abattoir workers and veterinarians, may now be at greater risk due to the accidental inoculation of the BSE agent in cuts or lacerations. Guidelines, equivalent to those applicable in other potential zoonoses, have been issued to all relevant occupational groups in the United Kingdom to create awareness, to minimize the possibility of occupational exposure, and to advise on procedures to be followed if an accident occurs. Specific recommendations include:

- taking care to avoid injuries especially with instruments that have been in contact with specified bovine offals;
- covering open cuts and grazes with waterproof dressings;
- wearing suitable protective gear;
- minimizing direct contact with specified bovine offals.

CJD has not been reported in any laboratory worker involved extensively in research on the TSEs, despite the significant number of individuals potentially exposed. But appropriate precautions should be taken when handling or processing potentially infected material such as brain. The possibility should be considered that new laboratory techniques may lead to a greater theoretical risk than previous techniques.

Prevention of hypothetical risks to humans from medicinal products and medical devices derived from bovine material

Considering the current scientific knowledge about the agents causing BSE and other TSEs, the careful selection of source materials is the best way to secure maximum safety of ingredients or reagents of bovine origin used in the manufacture of medicinal products. Therefore the epidemiological situation of BSE in countries and herds as well as the age of animals from which tissues originate (see below) should be taken into account by manufacturers of medicinal products wishing to procure raw material of bovine origin. Depending upon the information available on

the source and type of material used, additional measures may need to be taken to further reduce the hypothetical risk of contamination. These include the control exercised over collection and the steps taken to inactivate or remove possible BSE contamination (if applicable). Other theoretical risk factors include the amount of material administered and the route of administration. Consideration should also be given by the licensing authorities to the risk-to-benefit ratio of the medicinal product.

Type of bovine material. Studies of the maximum infectivity titres of tissues from Suffolk sheep and goats (measured intracerebrally in mice) at the clinical stage of natural scrapie (*1, 2*) can be used as a guide to the possible BSE infectivity in bovine materials (see Box, p. 186). As can be seen, ovine tissues have been classified on the basis of relative infectivity titres, and following a decreasing order, into four categories—from category I (high infectivity) to category IV (no detectable infectivity within the limits of the bioassay).

For practical purposes, other considerations should influence the classification of bovine tissues according to their potential risk. As an example, all of the bovine intestines, from duodenum to rectum, should be included in category II, even though corresponding ovine tissues (ileum, proximal colon, distal colon) were found to have different scrapie titres. Similarly, unless contamination with adjacent brain tissue can be avoided during collection, pituitary and cerebrospinal fluid (in category IIIa and IIIb, respectively) should be moved to a higher risk category. Since scrapie infectivity in the adrenal was found to be higher in goats than sheep, adrenal might also be moved to a higher risk category, i.e., from IIIa to II.

There are several tissues which are not listed in the Box (p. 186) because their scrapie infectivity titres were not determined. These tissues can, however, be classified on the basis of the overall pattern of tissue titres, supplemented by data from some of the other TSEs (experimental or natural). For example, it is logical to place bovine dura mater and pineal gland in either category II or I, depending on the extent of the contamination with brain material when the tissues were collected. Because the ovine placenta is known to be a source of infection in natural scrapie, it is recommended that bovine placenta be included in category II. It is also recommended that several other bovine tissues, such as bile, bone, cartilage, connective tissue, hair, skin and urine be placed in category IV. In addition, all bovine tissues from animals aged six months or less should be regarded as belonging to category IV.

A more comprehensive classification of bovine tissues based on the scrapie agent content of organs from infected sheep is contained in the "Note for guidance for minimizing the risk of transmitting agents causing bovine spongiform encephalopathy via medicinal products", which was adopted by the European Community's Commitee for Proprietary Medicinal Products on 11 December 1991 (document No. III 3298/91 Final).

Cell lines (e.g., neuroblastoma cells and PC12 cells), known to be capable of concentrating or amplifying agents causing spongiform encephalopathies, should not be used in the manufacture of medicinal products, apart from reasoned exceptional cases.

The information currently available suggests that, given assurances of adequate collection and/or processing, certain materials and their derivatives are unlikely to present any risk of contamination. These include milk and its derivatives (e.g., lactose and casein), skin and its derivatives, and hair and wool and their derivatives (e.g., wool alcohols and lanolin).

Conditions under which materials are collected. It is recognized that the potential risks will be influenced by the circumstances in which tissues were removed. For example, contamination of some tissues might be increased if infected animals are slaughtered by penetrative brain stunning, or if the brain or spinal cord is sawed.

Body fluids should be collected with minimal damage to tissue. Fetal blood should be collected without contamination from the placenta and amniotic fluids.

Procedures capable of reducing or removing infectivity. Inasmuch as the properties of medicinal products derived from bovine tissues are not adversely affected by the procedures indicated below, manufacturers should consider including such procedures in their manufacturing processes. None of the following procedures may guarantee complete inactivation of the infectious agents, but the efficiency of the first three methods listed below is considered greatly superior to that of the remaining ones:

(i) autoclaving under appropriate conditions (recommended parameters are 134–138 °C for 18 minutes for porous-load autoclaving, and 132 °C for 1 hour for gravity-displacement autoclaving);
(ii) treatment with sodium hydroxide (preferably 1 mol, for 1 hour at 20 °C);
(iii) treatment with sodium hypochlorite (preferably a solution containing at least 2% available chlorine, for 1 hour at 20 °C);

(iv) autoclaving for shorter times and/or at lower temperatures than those given above;
(v) extraction by organic solvents (use the organic phase);
(vi) removal of protein by precipitation, ultracentrifugation or absorption;
(vii) preparation of filtrates by passage through 10-nm filter;
(viii) passage through appropriate chromotographic columns; before being reused, columns should be treated for 4 hours with at least 0.1 mol sodium hydroxide.

If the potential risks of BSE from source materials are such that reliance has to be placed on the removal of infectivity during processing, validation studies should be carried out using appropriate model systems. Since the BSE status of many countries is uncertain at the moment, validation studies are recommended whenever the amount of medicinal product administered is equivalent to a substantial quantity of a material derived from a potentially high-risk tissue (categories I and II).

Materials derived from rendered carcasses and subjected to highly rigorous processes of extraction and purification (e.g., triglycerides, glycerol, sorbitan esters, etc. manufactured from tallow) may be considered unlikely to be contaminated. Similarly, gelatine extracted from skin and bones is unlikely to be contaminated.

Amount of bovine material. In evaluating the potential risk of infecting humans with the BSE agent, it is logical to consider the amount of bovine material of whatever type in the dose to be administered to humans.

The number of doses should also be taken into account because multiple exposures over long periods of time will increase the total amount of potentially infective material administered. Particular attention should be paid to implants and medicinal devices where the "exposure time" may be very long.

Route of administration. The hypothetical risk of transmission of BSE to humans by medicinal products will be influenced considerably by the route of administration. Data obtained from studies of experimental scrapie in mice show that direct injection into the CNS is the most efficient route of infection. Among the non-neural routes, intravenous is the most efficient (but less so than intracerebral), followed by intraperitoneal and then intramuscular/subcutaneous. The oral route is less efficient than the parenteral routes.

Comments

The potential risks associated with a given medicinal product administered to humans should be considered on a case-by-case basis, taking into account all the above-mentioned factors and the benefits to patients.

Although the recommendations given above relate particularly to materials of bovine origin, they might also be considered as applicable to other materials used in the manufacture of medicinal products, when these are obtained from sheep, goats, deer and other animals that are infected with TSE agents.

Manufacturers of cosmetics might consider the possibility that use of their products may present a risk to public health if they are manufactured from raw materials infected with agents that cause TSE.

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