

The USP Perspective to Minimize the Potential Risk of TSE Infectivity in Bovine-Derived Articles Used in the Manufacture of Medical Products

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ABSTRACT This *Stimuli* article on the potential risk of acquiring transmissible spongiform encephalopathy through the use of bovine-derived articles in the manufacture of medical products and on risk-reduction strategies to minimize this risk was prepared at the request of the USP Analytical Microbiology Expert Committee and USP Project Team 11. The article is divided into two major sections: a review of the illness and risk-reduction strategies. The review of the illness is intended to give the reader an overall view of the issues involved in transmissible spongiform encephalopathy and why it is a concern to the manufacturer, regulator, and consumer of health care products. The risk-reduction strategies are intended as a preliminary presentation of guidelines that may ultimately be included in a new general information chapter on the subject. Readers are invited to submit comments, suggested additions, changes, and revisions to the authors.

INTRODUCTION

Articles of bovine origin are used extensively in the production of pharmaceuticals, biologics, medical devices, and dietary supplements; for many of these health care products, it is difficult to identify a single product from any one of the categories that does not contain or utilize in its production at least one component that can be derived from bovine sources. Bovine articles are used as active pharmaceutical ingredients, excipients, components of medical devices, or components of cell culture media used in the synthesis of biologics and biotechnological products. Since the first description and subsequent outbreaks of a transmissible spongiform encephalopathy (TSE) in cattle (bovine spongiform encephalopathy or BSE) capable of infecting other species, including humans, by the consumption of infected bovine products, there have been some concerns about the potential risk of TSE transmission through the use of health care products. Although there have been isolated reports of TSE transmission in domesticated animals linked to the use of vaccines (Agrimi et al., 1999; Caramelli et al., 2001), it is generally accepted that the TSE transmission risk through animal-derived articles used in the manufacture of health care products is very low (Bader et al., 1997; Cohen et al., 2003). Regardless of the very low risk potential, various US and international regulatory agencies have developed guidances to help manage and further reduce the potential transmission risks. The purpose of this article is to review the issues involved in TSE transmission risks and to outline general risk reduction strategies based upon current scientific studies and regulatory guidances.

REVIEW OF THE ILLNESS

TSEs are a family of transmissible animal and human diseases characterized by spongy degeneration of the brain with severe neurological signs and symptoms. No treatments for TSEs are available, and the disease is fatal in all known cases. TSEs are also characterized by relatively long incubation periods of several years and, within the brain, are accompanied by activation of microglial cells, hypertrophy and proliferation of astrocytes, and degenerative neuronal vacuolation. Clinical features include mental changes, ataxia, and loss of fine motor control of body homeostasis (Koster et al., 2003).

The first description of a TSE was published in the mid-1700s in Europe for scrapie, a neurological disorder in sheep and goats. A human TSE, Creutzfeldt-Jakob disease (CJD) was first described in the early 1900s (Creutzfeldt, 1920). Other human spongiform encephalopathies described prior to 1995 include Kuru, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia. Additional TSEs include chronic wasting disease of elk and deer, which is endemic in certain parts of the United States and Canada (Williams and Young, 1980) and Transmissible Mink Encephalopathy (Marsh et al., 1976).

The causative agent of TSE (including BSE) is still controversial (Narang, 2002; Priola et al., 2003; Manuelidis and Lu, 2003), but most studies point to proteinaceous infectious particles devoid of any nucleic acid and termed prions (Prusiner, 1991; Uptain and Lindquist, 2002; Caughey and Lansbury, 2003; King and Diaz-Avalos, 2004). Evidence suggests that a prion is a modified form of a normal cellular protein (PrP^c). This protein is found predominantly on the surface of neurons; however, it is also found on lymphoid, lung, liver, spleen, and kidney tissues (Brown et al., 1994). Small amounts of the protein have also been reported on skeletal muscle (Thomzig et al., 2003) and blood cells (Cervenakova et al., 2003). It is a soluble glycoprotein with a

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secondary structure containing about 42 percent and about 3 percent alpha helix and beta sheet, respectively. The modified form of PrP^c, PrP^{sc} (for scrapie), is the putative disease agent. PrP^{sc} is relatively insoluble, accumulates in cytoplasmic vesicles of diseased individuals, and has the primary structure identical to PrP^c, but its secondary structure is about 30 percent and about 43 percent alpha helix and beta sheet, respectively (Pan et al., 1993). PrP^c can bind to each other, forming large aggregates. Furthermore, when it is present PrP^{sc} catalyzes the conversion of PrP^c to PrP^{sc}; expression of PrP^c is required for infection with a TSE (Brandner et al., 1996).

The two forms of the prion protein differ greatly in their reaction to various biochemical, chemical, and physical agents known to either hydrolyze or denature proteins. PrP^c is completely digested by hydrolysis with proteinase K, readily hydrolyzed by relatively weak acids and bases, and denatured by high heat and chaotropic agents. Proteinase K removes only the N-terminal 67 amino acids of PrP^{sc}, leaving a residue that is still infectious. PrP^{sc} is also resistant to acid hydrolysis and denaturation by heat and chaotropic agents.

BSE was unknown until the 1980s, when it was first described in the United Kingdom (Wells et al., 1987). Before the causative agent and means of transmission were known, an outbreak of BSE occurred within the UK leading to the death of approximately 200,000 head of cattle diagnosed with the disease. In order to contain the illness, more than 4.5 million head of asymptomatic cattle were destroyed, devastating the British cattle industry (DTZ Pieda Consulting, 1998). Although the UK has had the greatest number of reported cases of BSE, the illness has also been reported throughout Europe, Israel, Japan, Canada, and, as of December 2003, the United States (OIE, 2004). In the vast majority of these diagnosed cases, the source of the BSE has been identified as originating in the UK. Because the sole incidence of BSE in the United States was in a cow traced back to Canada, the US Department of Agriculture (USDA) and the Office International des Epizooties considers this as a nonindigenous case. The only regions still reporting no cases of BSE are South America (excluding the Falkland Islands), Australia, and New Zealand.

The origin of BSE also is controversial. Two theories have been proposed. The more favored theory is that it arose from sheep and goat scrapie; this theory is, in part, supported by the fact that scrapie is widespread and remains one of the more common TSEs in domesticated animals. This theory is further supported by the discovery of a strain of sheep scrapie that has chemical properties similar to those of BSE (Hope et al., 2000). However, there are still some strain differences between scrapie prion and the BSE prion. Furthermore, scrapie and BSE differ in the signs, symptoms, and progression of the respective diseases. The alternative theory is that BSE arose spontaneously in a few cows within UK herds (Bostok, 2000). This theory is, in part, supported by the belief that the human TSE, CJD, occurs spontaneously in one in every one to five million people. It is thought that the spontaneous rate of BSE in cattle may be

similar. Although the origin of BSE is controversial, the amplification and subsequent spread of the causative agent is believed to be fairly well established (Lindenbaum, 2001; Collinge, 2001).

Prions are stabilized by fatty material, and extraction of lipids tends to make them more susceptible to heat denaturation. It is an international agricultural practice to feed cattle, especially dairy cows, high-protein supplements derived from rendered animal by-products. In the past, these animal by-products included the remains of sheep and goats, as well as cattle. Prior to the 1980s, it was common practice to extract and market tallow from rendered animals. As the demand for tallow declined around 1980, renderers switched to a less expensive rendering process, which used lower temperature without removing lipids. This produced animal feed of higher fat content. Some speculate that a few TSE-infected animals entered into the rendering process, the process failed to inactivate prions, and the subsequent infected feed was given to healthy animals. Prions were able to withstand normal digestion, passed into the animals' circulatory systems, and eventually were deposited into nervous tissues, where they became amplified. As the infected animals were recycled through the rendering process, an amplification of the infectious materials occurred, resulting in the epidemic that was experienced in the UK (Wilesmith et al., 1991; Taylor et al., 1995).

Beginning in 1988, measures to control BSE in the UK were put into place, and numerous countries began to ban the importation of cattle and bovine products from the UK. Eventually, bans on the feeding of ruminant-derived animal feed to ruminants were adopted worldwide. Statistics on the illness in the UK indicate that the regulations put into place after the BSE outbreak, following an initial lag period caused by the long incubation period of the illness, have significantly reduced the incidence within that country. Incidences of BSE are still being reported, primarily in animals that were born prior to the institution and enforcement of the feed ban (MAFF, 1998). Regardless of whether BSE represents cross-species transmission of scrapie or spontaneous eruption within cattle herds, there is a potential risk of additional BSE outbreaks in cattle herds in the future if the feed ban described above, which appears to have broken the cycle of transmission and amplification, is rescinded or if its enforcement is lax.

In the years intervening between the detection of BSE in the UK and the institution of measures to control its spread, infected cattle entered into the human food chain. Although CJD had been first described in the 1920s, starting in 1994 a new form of CJD, variant CJD (vCJD), began to appear in persons residing or having resided in the UK. The signs and symptoms of CJD (Will et al., 1996) include a mean age of onset of about 65 years; mean onset of clinical signs before death of about 4 months; displays of degrees of confusion and ataxia without abnormal behavior; and, upon autopsy, "florid" amyloid plaques usually absent from brain tissue. In contrast, the signs and symptoms of vCJD include a mean age of onset of about 29 years, mean onset of clinical signs before death of about 12 months, displays of abnormal be-

havior, and, upon autopsy, “florid” amyloid plaques always present (Ironsides et al., 1997). Another difference between vCJD and CJD is that, to date, 100% of all vCJD patients are homozygous at codon 129 of the prion protein gene for methionine, whereas no such distinction is present in cases of CJD. In addition, when the brain material from the vCJD patients was strain typed using the mouse system, vCJD and BSE were found to be the same strain (Bruce et al., 1997). Furthermore, the size and glycosylation pattern of the prion molecule from CJD and vCJD are different; prions from patients with vCJD are identical to those found in cattle with BSE (Collinge et al., 1996). It has also been shown that for transgenic mice containing the human prion protein and infected with BSE, the incubation times are very similar to those of mice containing the bovine prion protein (Korth et al., 2003). These last three characteristics, along with geography of both the BSE and vCJD outbreaks, indicate that the source of vCJD was from the consumption of human food products of bovine origin. This indication suggests there may be a potential risk of TSE transmission to humans from the use of bovine-derived medicinal products. Currently, fewer than 150 patients with vCJD have been diagnosed in the UK; however, recent examination of lymphoid tissues surgically removed from appendicitis and tonsillitis UK patients leads to estimates that UK residents may harbor the infectious TSE agent at a rate of 49–692 per million (Hilton et al., 2004).

Prions at high titer are found primarily within tissue of the central nervous system of infected animals. This includes the brain, spinal cord, and dorsal root ganglia; these tissues are referred to as high-infectivity tissues (WHO, 1999). In infected cattle, lower titers have been found in cerebrospinal fluid, lung, lymph tissue, spleen, kidney, liver, and ileum (Brown et al., 2001; Terry et al., 2003); these tissues are referred to as low-infectivity tissues. In cattle, no detectable infectivity was found in skeletal muscle, cardiac muscle, prostate, adrenal glands, clotted blood, fat, gingiva, intestine (except for the ileum), testis, thyroid, milk, mucus, feces, saliva, semen, serous exudate, sweat, tears, and urine; these tissues are regarded as noninfectious. However, other studies of TSE-infected hamsters have found prions in skeletal muscle (Thomzig et al., 2003). Studies using rodents have demonstrated the presence of TSE infectivity associated with blood (Cervenakova et al., 2003). This infectivity is associated mostly with the leukocyte and plasma fractions, and residual infectivity is associated with red blood cells. The transfusion of blood from preclinical sheep infected with either BSE or scrapie can infect naive sheep (Hunter et al., 2002). Moreover, the December 2003 report of a case of a human vCJD fatality in the United Kingdom, purportedly from a blood transfusion, indicates a potential TSE-infection risk associated with blood and blood products (Matthews et al., 2003). In addition, studies have found prions in the urine of TSE-infected rodents, cattle, and humans as detected by immunological methods; however, in-

tracerebral inoculation of hamsters with urine-derived prions did not cause clinical signs of prion disease even after a prolonged incubation period, suggesting urine-derived prions may differ in pathogenic properties from brain-derived prions (Shaked et al., 2001).

Animals and humans are currently diagnosed with TSE post-mortem using several types of tests. The classic diagnostic test is histological examination of brain tissue, looking for the characteristic vacuolar degeneration. There are also immunohistochemical tests that can confirm the presence of PrP^{sc} in the vacuolated regions of the brain (Haritani et al., 1994). In addition, there are other, immunologically based tests, such as Western blots (Oesch et al., 2000) and ELISA (Grassi et al., 2001), that can quantitate the PrP^{sc} present in tissues having high or moderately high titers. These tests typically take less time to perform than histological examination (6–8 hours versus weeks, respectively) and can be partially or fully automated. Although most of these are post-mortem tests, some of them can be used as ante-mortem tests of lymphoid tissue samples from the tonsils (Schreuder, et al., 1998) or from the third eyelid (O'Rourke et al., 2000) of infected animals. However, the immunochemical tests still require extensive sample collection and preparation and are expensive, limiting their feasibility for routine testing and monitoring the disease state of large herds. Current tests lack the sensitivity to detect prions in certain tissues, such as blood, where infectivity has been demonstrated. Moreover, current tests may not be able to detect infectivity in infected animals not yet showing clinical signs; negative results do not ensure the absence of infectivity. In these cases, the detection of infectivity is possible if suspect tissue is inoculated intracranially into experimental animals where the prions can become amplified. The disadvantage of the intracranial inoculation approach for detection of low infectivity is that it can take months to years to obtain a positive result. Although other analytical methods are under development for the detection and quantitation of prions from low-infectivity tissues such as blood (Schmerr et al., 1999; Schmitt et al., 2002), no currently available and fully available method is sensitive enough for routine ante-mortem screening of asymptomatic animals. Other factors hampering method development include an unclear understanding of what constitutes an infectious dose, the lack of Biosafety level 3 laboratories necessary for carrying out method development and validation, sample matrix interferences, and the lack of readily available, standardized reference materials for validation. In this situation, the best current approach to reduce the risk of transmitting TSE either via foods or health care products is through the appropriate sourcing of the animal-derived articles and the use of practices and procedures that have been shown to eliminate or destroy the infectivity. The following section will address these issues.

RISK-REDUCTION STRATEGIES

This section considers measures for minimizing the potential risk of TSE transmission during the use of animal-derived materials, especially if the animal is a ruminant. Such materials may be used for the preparation of:

- active substances
- excipients
- raw or source materials and reagents used in production (e.g., bovine serum albumin, enzymes, culture media—including those used to prepare working cell banks or new master cell banks)
- fermentation culture media (media used in establishment of stock microbial cultures should be evaluated only for contribution to final fermentation volume)
- medical devices
- reagents used to clean manufacturing equipment
- media used for media fills on equipment used to manufacture sterile products.

It is recommended that alternative, nonanimal source ingredients be substituted for animal-source ingredients whenever possible. However, in some cases, nonanimal source ingredients may not be suitable for the intended purpose. In these situations, animal-source ingredients are a necessity, and the implementation and maintenance of risk-reduction strategies are a requirement.

These risk-reduction strategies are also applicable to materials that come into direct contact with the equipment used in the manufacture (and therefore have the potential to allow contamination) of, for example, test media used in plant and equipment validation. The proposed measures are especially applicable for bovine and other ruminant-derived material and may be adapted to include other animals if they are later shown to have the potential risk of transmitting the TSE agent.

In light of the current scientific knowledge and regardless of the geographic origin, milk is unlikely to present any risk of TSE contamination (Bader et al., 1997; Cohen et al., 2003). Therefore, milk and materials derived only from milk may be excluded from the scope of these strategies, provided the milk is sourced from healthy animals under the same conditions as milk collected for human food consumption. Derivatives of milk from ruminants prepared with the use of other ruminant materials (such as pancreatic-enzyme digest of casein), are not excluded from the scope of this article because of the use of these other ruminant materials.

Derivatives of wool and hair of ruminants, such as lanolin, wool alcohol, and amino acids are also excluded from the scope of this article, provided the wool and hair are sourced from live animals, because hair by itself is considered to have no potential of infectivity (Bader et al., 1997). Derivatives of wool and hair from ruminants prepared with the use of other ruminant materials (such as pancreatic enzymes) are not excluded from the scope of this article because of the use of these other ruminant materials.

Manufacturing (Including Collection of Source Materials)

Ideally, the sourcing of materials of ruminant origin should be established closed herds. A closed herd is maintained in such a way as to minimize contact with other domestic ruminants to reduce the risk of contracting diseases, such as TSE, that are transferable to humans. The animals in a closed herd have not been suspected of scrapie, BSE, or other herd-threatening diseases. Members of the closed herd have documented female lineage, and each animal is uniquely identified. Adequate steps are taken to ensure that the animals of a closed herd do not come in contact with other animals not of the closed herd, although exposure to animals not thought to be of high risk of being TSE infected, such as horses, cats, and dogs, may be acceptable. Food provided to the animals of the closed herd contains no ruminant-derived protein or offal, and, preferably, all animal feed is produced within the confines of the closed herd facility to help guard against accidental exposure to TSE-infected feed. Once established, all cattle in a closed herd should be born to that herd. Furthermore, because research indicates that semen does not transmit TSE (WHO, 1999), new genetic variation should be introduced into a closed herd only as semen. A herd should be maintained under the conditions stated above for at least 6 years to ensure no latent TSE is present before it can be declared a closed herd (ES, 2000). The lowest possible potential risks of TSE transmission are through the use of bovine-derived articles originating from animals from closed herds, especially for bovine-derived articles that do not undergo processing to reduce infectivity.

Currently, due to the relatively small number of closed herds, bovine and other ruminant-derived articles from closed herds are in limited supply and tend to be more expensive than articles derived from open herds. When using closed herd-derived material is not feasible, additional details should be sought about the source of the bovine-derived materials and other measures taken to minimize the risk of transmission of TSE agents (as well as other animal diseases); the remaining part of this discussion will focus on the use of open-herd, animal-derived material. The manufacturer of the medicinal product should audit the supplier of these materials to ensure that the latter are sourced and handled in conformity with the principles outlined in this article and appropriate quality control systems.

The risk of transmission of infectious agents can be greatly reduced by controlling a number of parameters. These parameters include:

- geographic source of animals
- nature of animal tissue used in manufacture
- production process(es).

It is unlikely that any single approach will establish the safety of a product, and therefore the three approaches cited above need to complement each other to minimize the risk of contamination.

Animals as Sources of Materials

Careful selection of source materials is the most important criterion for the safety of medicinal products. The most satisfactory sources of materials are closed herds, followed by open herds from countries that have not reported cases of endogenous BSE and have (OIE, 2003):

- compulsory notification
- compulsory clinical and laboratory verification of suspected cases.

Official certification of the origin should be available from the supplier, obtained and kept on file. In addition, firms should ensure that a risk of BSE infection is not introduced from the following factors:

- the importation of cattle from countries where a high incidence of BSE has occurred
- the importation of progeny of affected females
- the use in ruminant feed of meat and bone meal containing any ruminant protein.

Current US FDA recommendations prohibit the use of any bovine-derived materials that originate from countries reporting indigenous cases of BSE in any FDA-regulated product (CBER, 2000), with the exception of gelatin. For veterinary biologics, current Center for Veterinary Biologics (USDA) regulations indicate that ingredients of animal origin should be sourced from countries whose BSE status is either no or low risk as defined by the US National Center for Import and Export and 9 *CFR* 94.18 (CVB, 2001). However, suppliers and manufacturers should not rely on country of origin alone to ensure the safety of the animal-derived materials when additional and reasonable practices and procedures are available to further reduce the potential risk. The absence of reports of indigenous cases of BSE should not be interpreted as evidence of complete absence of BSE within a country or region.

Source animals should be born after the feeding ban was imposed. If the date of birth of the animals is not known, both the implementation date of the ban and the incubation period of TSE should be considered to determine the safety of the sourcing.

Along with these measures, manufacturers of a medicinal product should justify their strategy for sourcing, in relation to the category of materials, the quantity of source material, and the intended use of the finished medicinal product. In supplying countries, source materials from well-monitored herds may provide an extra safety margin.

Parts of Animal Bodies, Body Fluids, and Secretions as Starting Materials

Table 1 is a partial list of articles of bovine origin commonly used to manufacture medical products; the table also includes the common source tissue from which the articles are derived. In a TSE-infected animal, different organs and secretions have different levels of infectivity. On the basis of data on natural scrapie, organs, tissues and fluids have been classified into four main groups bearing different potential risks, as shown in *Table 2* (EMEA, 2001). Although the dis-

tribution of infectivity in BSE-affected cattle may be different, the classification of tissues and body fluids in *Table 2* can be a consideration for the selection of bovine-source materials; cross-referencing the information contained in *Table 1* with *Table 2* can yield some indications about the relative potential risk of a particular bovine-derived article to carry TSE infectivity. It is important to note the following points:

- the classification of tissues shown in *Table 2* is based on titration of infectivity in mice inoculated by the intracerebral route. In experimental models using strains adapted to laboratory animals, higher titers and a slightly different classification of tissues may occur
- in certain situations there could be cross-contamination of tissues of different categories of infectivity. The potential risk will be influenced by the circumstances in which tissues were removed, especially contact of material of a low-risk group with that of a high-risk group. Thus, the cross-contamination of some tissues may be increased if infected animals are slaughtered by penetrative brain stunning or if the brain and/or spinal cord are sawed. The risk of cross-contamination will be decreased if body fluids are collected with minimal damage to tissue and cellular components are removed and if fetal blood is collected without contamination from other maternal or fetal tissues, including placenta or amniotic and allantoic fluids
- the risk posed by cross-contamination will be dependent on several complementary factors, including:
 - precautions adopted to avoid contamination during collection of tissues (see above)
 - level of contamination (amount of the contaminating tissue)
 - amount of material to be used
 - processes to which the material will be subjected during the manufacturing process
- no detectable infectivity does not necessarily mean no infectivity. Suppliers and manufacturers should not rely upon the tissue origin of the ruminant-derived article alone to ensure the lowest potential TSE risk when additional and reasonable practices and procedures also are available to reduce the risk even further
- the potential risk of a bovine-derived article carrying TSE infectivity is a function of both the potential risk associated with a particular tissue and the processes involved in the extraction and isolation of the article from the tissue. The potential risk of articles derived from high TSE infectivity tissue can be reduced by processes that can clear the TSE agent.

Suppliers and manufacturers of medicinal products should assess the potential risk and adjust their practices and procedures accordingly to minimize the potential risk of TSE infective to the lowest practical level.

STIMULI TO THE REVISION PROCESS

Stimuli articles do not necessarily reflect the policies of the USPC or the USP Council of Experts

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Table 1. Partial list of articles of bovine origin used in the production of pharmaceuticals, biologics, medical devices, and dietary supplements

Bovine Article	Tissue of Origin	Use	Alternatives Currently Available
Amino acids	Hair/skin	API and cell culture media	Yes
Aprotinin bovine	Lung	API	No
Bone and bone derivatives	Bone	Medical devices	Limited
Bovine colostrum	Colostrum	Colostrum	No
Bovine immunoglobulins	Blood, colostrum	Cell culture media	Limited
Bovine serum	Blood	Cell culture media	Limited
Bovine serum albumin	Blood	Cell culture media	Limited
Calf serum	Blood	Cell culture media	Limited
Casein derivatives	Milk	Excipients and cell culture media	Limited
Cholesterol	Brain	Excipient	Yes
Chondroitin sulfate sodium	Bones	API and dietary supplement	Yes
Creatine	Muscle	Dietary supplement	Yes
Chymotrypsin	Pancreas	API and cell culture media	Limited
Collagen	Bone/hide	Medical device and cell culture media	Yes
Corticotropin	Pituitary	API	Yes
Diglycerides	Tallow	Excipient	Yes
Fatty acids	Tallow	Excipient	Yes
Fetal bovine serum	Bone	Cell culture media	Limited
Fibroblast growth factors	Multiple (including brain)	Cell culture media	Limited
Fibronectin	Blood	Cell culture media	Yes
Gelatin	Bone and hide	Excipient and cell culture media	Limited
Glucagon	Pancreas	API	Yes
Glycerin	Tallow	Excipient and cell culture media	Yes
Hard fat	Tallow	Excipient	Yes
Hemoglobin	Blood	Blood replacement	Limited
Heparin	Intestine	API	Limited
Hyaluronidase	Testis	API	Yes
Insulin	Pancreas	API	Yes
Lactitol	Milk	Excipient	No
Lactoferrin	Milk	Excipient	Limited
Lactose	Milk	Excipient	Limited
Lactulose	Milk	API	Limited
Levothyroxine sodium	Thyroid	API	Limited
Monoglycerides	Tallow	Excipient	Yes
Oxytocin	Pituitary	API	Yes
Pancreatin	Pancreas	API	Yes
Pancrelipase	Pancreas	API	Yes
Pegademase bovine	Intestine	API	No
Pepsin	Stomach	API and cell culture media	Yes
Peptone	Muscle	Cell culture media	Limited
Phospholipids	Brain	Excipient	Yes
Pituitary gland extract	Pituitary gland	Cell culture media	Limited
Polysorbate 80	Tallow	Excipient and cell culture media	Yes
Smallpox vaccine	Living skin	Live vaccine	No
Stearates	Tallow	Excipients and cell culture media	Yes
Thrombin	Blood	API	No
Thyroid	Thyroid	API	Yes
Trypsin	Pancreas	API and cell culture media	Limited
Vasopressin	Pituitary	API	Yes

Table 2. Relative scrapie infectivity titers in tissues and body fluids from naturally infected sheep and goats with clinical scrapie¹

Category I	High infectivity Brain, spinal cord, (eye)
Category II	Medium infectivity Ileum, lymph nodes, proximal colon, spleen, tonsil (dura mater, pineal gland, placenta), cerebrospinal fluid, pituitary, adrenal gland
Category III	Low infectivity Distal colon, nasal mucosa, peripheral nerves, bone marrow, liver, lung, pancreas, thymus, blood
Category IV	No detectable infectivity² Clotted blood, feces, heart, kidney, mammary gland, milk, ovary, saliva, salivary gland, seminal vesicle, serum, skeletal muscle, testis, thyroid, uterus, fetal tissue, (bile, bone), cartilaginous tissue, connective tissue, hair skin, urine)

¹ Tissues in parentheses were not titrated in the original studies, but their relative infectivity is indicated by other data on spongiform encephalopathies. On the basis of their composition, materials not listed may be classified by analogy to those mentioned.

² No infectivity was transmitted in bioassays involving inoculation of up to 5 mg of tissue into rodent brains.

Process Validation

Due to the documented resistance of TSE agents to most inactivation procedures, controlled sourcing is the most important criterion in achieving acceptable product safety. However, whenever possible, validation studies of processes developed to reduce or eliminate prions from potentially infected materials should be performed.

Caveats to performing such studies are that validation studies of removal/inactivation procedures may be difficult to interpret because 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), after spiking and after the treatment. Further research is needed to develop an understanding of the most appropriate methodology for validation studies. An additional problem in performing prion clearance validation studies is the lack of readily available, suitably well-characterized and standardized reference materials for spiking. Regardless, if claims are made for the ability of a specific manufacturing process to remove or inactivate TSE agents, these claims will have to be substantiated by appropriate validation studies with suitably characterized reference material. Because there are currently no validated analytical methods for the detection of small amounts of the TSE agent, TSE clearance validation studies typically employ the intracranial injection of in-processed material into rodents for amplification and detection of potential residual infectivity (Blum et al., 1998). Analysts should consult *USP* general chapter *Design and Analysis of Biological Assays* (111) when developing their validation studies.

Beyond the particular limitations that apply to TSE validation studies and their interpretation, the major hurdle is identifying steps that will effectively remove or inactivate TSE agents during the manufacture of biological medicinal

products. Manufacturers are encouraged to continue their investigations into removal and inactivation methods to identify steps/processes that will have benefit in ensuring the removal or inactivation of TSE agents. In any event, a production process whenever possible should be designed taking note of available information about methods that are thought to inactivate or remove TSE agents. For example, certain production procedures such as those used in the manufacture of tallow, tallow derivatives, and gelatin may contribute considerably to the reduction of the risk of TSE contamination.

TSE Reference Materials

As indicated previously, there are no readily available, well-characterized, and standardized TSE reference materials with which to perform TSE clearance validation studies. However, the acquisition of some forms of TSE reference materials, most frequently TSE-infected brain homogenate, is sometimes possible via academic sources. Within the United States, the possession, use, and transfer of TSE agents are regulated by USDA (9 *CFR* Part 121, 2002). Potential users of TSE reference materials should consult and comply with the regulations. When selecting materials to be used in validation studies, researchers should consider the relative resistance of the TSE reference materials to be cleared by a given procedural step. For example, certain TSE strains, such as mouse-adapted BSE strain 301V, appear to be the most resistant to heat inactivation (Bostock, 2000). It is recommended that this strain, or a strain showing similar resistance to heat, be used to validate a heat treatment step. Although TSE-infected brain homogenate appears to be the most available type of TSE reference material, such material may not be the best for all situations. The brain matrix in which the TSE agent is found may promote or prevent the inactivation of a given procedural step. In such cases, the use of isolated prion protein may be more appropriate.

Age of Animals

Because the accumulation of TSE infectivity occurs during an incubation period of several years, sourcing from young animals may be prudent. Because this consideration will be lot specific, the audit of the raw material supplier should include a review of records establishing this fact. In addition, the purchase contract should stipulate the maximum age of the source animals.

Specific Products

Tallow. Tallow used as the starting material for the manufacture of tallow derivatives should be produced by a method at least as robust and rigorous as those referred to in international regulations (EMEA, 2001 and APAG, 2003). Tallow derivatives such as glycerol and fatty acids that are manufactured from tallow by rigorous processes have been the subject of specific consideration and are thought unlikely to be infectious. Examples of rigorous processes are:

- transesterification or hydrolysis at not less than 200 °C for not less than 20 minutes under pressure (glycerol, fatty acids, and fatty acid ester production)
- saponification with 12 M NaOH (glycerol and soap production)
- batch processes: at not less than 95 °C for not less than 3 hours
- continuous processes: at not less than 140 °C, at a pressure of 2 bars (2000 hPa) for not less than 8 minutes, or equivalent.

Gelatin. Current US FDA guidance for industry on the use and sourcing of gelatin for health care products (FDA, 1997) allows for the use of bovine-derived gelatin from cattle from countries reporting BSE or from countries not meeting the latest international BSE-related standards (OIE, 2003) provided:

- importers, manufacturers, and suppliers of bovine materials determine the tissue, species, and country source of all such materials to be used in processing gelatin for human use. This requires thorough record keeping with a clear indication of chain of custody
- bones and hides from cattle, from any country, that show signs of neurological disease are not to be used as raw material for the manufacture of gelatin
- the slaughterhouse removes the heads, spines, and spinal cords immediately after slaughter and that the heads, spines, and spinal cords are not used in gelatin production
- the gelatin produced from the above countries is not to be used either in injectable, ophthalmic, or implanted FDA-regulated products, or in their manufacture. The gelatin may be used for oral dosage forms and cosmetics.

In order to reduce the potential risk of transmitting the TSE agent in gelatin produced from cattle originating and residing in BSE-free countries, it is recommended that the practice described in the third item above — the removal

of heads, spines, and spinal cord immediately after slaughter and the nonuse of heads, spines, and spinal cords in gelatin production — also be followed. Regardless of the country of origin of the bovine material, it is recommended that the alkaline process of manufacturing gelatin be performed, because this method has been shown to clear the TSE agent (Inveresk Research International, 1998a, 1998b, and 1999). Systems should be in place for monitoring of the production process and for batch delineation (i.e., definition of batch, separation of batches, cleaning between batches, etc.). The potential for cross-contamination with possible infectious material is to be avoided.

Other bovine-derived articles. The extraction and production of both tallow and gelatin from bovine tissues involve the prolonged exposure of the tissues to high moisture and/or high pH, agents that have been shown to inactivate the TSE agent. However, such treatments are inappropriate for the extraction of many other types of bovine-derived articles because those treatments will lead to the destruction of the article of interest. In these cases, other means to remove or destroy the TSE agents must be employed. In some situations, conventional chemical and biochemical extraction and isolation procedures may be sufficient to remove the infectious agent. For example, it has been shown that an extraction and isolation procedure involving multiple ammonium sulfate fractionation steps and ion-exchange chromatography, plus other conventional techniques, were sufficient to significantly reduce the TSE infectivity from an aprotinin preparation derived from bovine lungs that had been spiked with TSE-infected brain homogenate (Blum et al., 1998). Similar techniques may be effective for other bovine-derived articles.

CONCLUDING REMARKS

The assessment of the risks associated with TSE requires careful consideration of all of the parameters cited, and the preferred option should be to avoid the use of material derived from animals known to be susceptible (other than by experimental challenge) to TSEs in the products produced by the pharmaceutical industry. The acceptability of a particular medicinal product containing the materials, or which as a result of manufacture could contain these materials, will be influenced by a number of factors, including:

- documented and recorded source of animals
- nature of animal tissue used in manufacture
- production process(es)
- route of administration
- quantity of tissue used in the medicinal products
- maximum therapeutic dosage (daily dosage and duration of treatment)
- intended use of the product.

Manufacturers of medicinal articles containing ingredients of animal origin are responsible for the selection and justification of adequate measures to reduce the potential risk of TSE infectivity. The state of science and technology must be taken into consideration. Notwithstanding the points and considerations raised above, it should be empha-

sized that the potential risks associated with a given medicinal product should be considered individually in the light of specific circumstances and any new development in the current understanding of TSEs. The various steps to be taken by USP should provide an additional and complementary prospective to the strategies developed in this *Stimuli* article, with the ultimate goal of benefiting the patient.

USP's BSE–TSE Initiative

In 2002, USP initiated the formation of a Project Team to advise the USP Council of Experts on steps that USP could initiate to help ensure the safety of pharmaceutical products in terms of BSE–TSE contamination. The Project Team includes the following national and international experts:

Brian Nunnally – Eli Lilly and Co.
Byron Rippke – US Department of Agriculture
Chuck Filburn – Nutramax Laboratories, Inc
David Schoneker – Colorcon
Everett Flanigan – Advanced ChemTech
Hannelore Wilkommen – Clearant GmbH
James Akers – Akers, Kennedy and Associates
Jordi Ruiz–Combalia – BIOIBERICA, S.A.
Joseph Knapp – University of Pittsburgh, School of Pharmacy
Judd Aiken – University of Wisconsin, School of Veterinary Medicine
Kristen Blancard – Nutramax Laboratories, Inc.
Louis Blecher – International Specialty Products
Mary Jo Schmerr – Iowa State University, College of Veterinary Medicine
Peter Ganz – Health Canada
Ralph Gomez – Hoffmann–La Roche Inc.
Richard Moreton – Penwest Pharmaceuticals Co.
Scott Sutton – Vectech Consulting
Susan Schniepp – Abbott Laboratories
Taryn Rogalski–Salter – Merck & Company
Thomas Kreil – Baxter BioScience
Ana Padilla – WHO
Debbie Cooper – Wyeth

The Project Team discussed the role of USP and highlighted the need for standardized methods and reference materials. USP is investigating the elaboration of reference materials, including brain homogenate, reagents, and analytical procedures, that could be useful in the manufacture of bovine-derived articles and medical products. USP recognizes that the next step could be the development of an information chapter based on this *Stimuli* article and the need to monitor the new analytical procedures in order to be able to standardize those procedures in the future. Furthermore, USP is developing two new general information chapters, *Glycoprotein and Glycan Analysis* (1084) and *Biological Assay Validation* (1033), which will provide additional framework for the standardization and validation of biological assays and glycoprotein analysis. These general information chapters should assist suppliers and medical

product manufacturers in the development and validation of procedures to minimize the potential risk of TSE contamination of bovine-derived articles.

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