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Memorandum

Date: August 5, 2003
To: File
From: Dr. Kenneth H. Kortright, Assistant to the President
RE: Comment to FDA Regarding CBER Guidances 50244 & 51074

The subject of this memorandum is to repudiate the United States (U. S.) Food and Drug Administration (FDA) suggestion that CBER guidance's 63 FR 50244 and 51074 become permanent sections of the recent proposed regulations 21 CFR Part 111 (see page 12181 of 21 CFR Part 111). These guidance's cover only human cell lines and products derived from them and their control from transmission of viruses or viral related diseases. These measures have no applications to dietary products and dietary supplements nor do they relate to the transmission of Bovine Spongiform Encephalopathies (BSE) as was speculated. A thorough review of the BSE literature and food safety record will support this position and offer alternatives.

The Nobel Prize in medicine for 1997 was awarded to Stanley B. Prusiner for his pioneering work in isolating and characterizing the causative agent of BSE and its form in humans termed Transmissible Spongiform Encephalopathy (TSE) derived from cattle and transmissible to humans (Wietjens and Walvoort 1997). The name given to these 27-30 kilo-Daltons (kDa) peptides of 245 amino acids was "Prions" and their basic structure was elucidated (Boulton, McKinley, and Prusiner, 1982; Madec et al. 1997; Prusiner 1982, 1998, 1999; Scott et al., 1999). Prusiner's reports identified the normal occurring cellular non-glycosylated prion form (PrP^c) and the glycosylated and refolded disease prion form (PrP^{sc}), that also appears in brains of sheep with scrapie. The glycosylated and refolded prion (PrP^{sc}) was shown to be ultra-violet light radiation resistant, heat resistant, solvent resistant, and proteinase resistant by Gajdusek (1977) and Taylor et al. (1998). Inactivation of prion infectivity is reviewed in detail later in this review.

The identification of infectious prion peptides, PrP^{sc}, introduced an entirely new pathogenic process responsible for a group of fatal neurodegenerative diseases called TSE's. A portion of that process has recently been attributed to the presence of the PrP gene (Hope et al. 1996) which codes for prion amino acid segment 106-126. This segment was shown to be neurotoxic (Forloni et al. 1993) and when cultured with neurons from the hippocampal area of the brain caused those cells to become apoptotic. When several different prion peptides containing alpha-helix regions were cultured together they spontaneously formed amyloid complexes and Beta-sheets (Gasset et al. 1992). This Beta-sheet histologic characteristic of spongiform encephalopathy and the role of helix-regions contributing to these complex formations were reviewed by McHattie and Edington, (1999), and Hur et al. (2002). A naturally produced peptide from neuronal astrocytes called "clusterin" (Duguid et al. 1989) was found at concentrations as little as 1

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anogram (ng) per 0.1 milliliter (ml) to inhibit the aggregation of prions at amino acids 106-126 (McHattie and Edington, 1999). It was postulated that the formation of these Beta-sheets of prion amyloid complexes contributed to cell apoptosis, but the mechanism permitting PrP^{sc} prions to enter the cells was still a mystery.

A mechanism for the cellular entry of abnormal prions was discovered through the 37 kDa Laminin receptor and its precursors and was reported by Rieger, Lasmezas, and Weiss (1999). These same investigators had identified the evolution of the Laminin receptor as the cellular binding site for Venezuelan equine encephalitis virus and Sindbis virus. Abnormal prions entrance into brain neurons appeared to play the key role in the onset of spongiform encephalopathy (Rieger, Lasmezas, and Weiss, 1999; Hur et al. 2002). Another route of cellular entry for abnormal prions was shown for the B-cell receptor by Brandner, Klein, and Aguzzi (1999). These findings implicated the spread of abnormal prions throughout the lymphatic system and even to other organs and tissues of the body (Hur et al. 2002; Detwiler and Baylis 2003; Grassi, 2003; Ramasamy et al. 2003; Zanusso et al. 2003). Cellular receptor binding of heavy metals was also shown to participate in the modification of neurons in spongiform encephalopathy (Purdey 2000). A mineral imbalance was found to occur with the onset of TSE's (Purdey 2000). That imbalance was enhanced upon exposure to copper binding insecticides and manganese containing food supplements (Purdey, 2000). This mineral imbalance was shown to result from the creation of active ion transport channels for copper, iron, and manganese, (Purdey, 2000), and calcium (Courie 2001; Herms et al. 2001). These channels were formed by the complexing prions within the neuronal cells and drastically modified their cell wall charge and permeability (Hur et al., 2002). The pathogenic mechanisms of spongiform encephalopathy proposed by Prusiner (1982) identifying prion peptides responsible for onset of this neurodegenerative condition has been supported by recent molecular chemistry by Jackson and Collinge (2001) but challenged and comprehensively reviewed by Hur et al. (2002).

Creutzfeldt (1921) and Jakob (1921) were the first to report BSE in humans, hence the name given to the human infection, Creutzfeldt-Jakob Disease (CJD). A slightly modified prion was identified in the brains of teenagers during the peak of an outbreak of this disease in the United Kingdom that was called a variant of Creutzfeldt-Jacob Disease (vCJD) by Collinge (1996, 1999), Gordon (1999), Tan et al. (1999) and Wadsworth et al. (1999). Bovine Spongiform Encephalopathy, CJD, vCJD, and TSE's including other forms of neurodegenerative diseases cause loss of brain neurons and dementia as reviewed by Retzschmar (1999). Their pathogenesis is listed in Table I as reviewed by Prusiner (1998), Will (1999), and Taylor (2002). Transmissible Spongiform Encephalopathies in humans have been reported as Creutzfeldt-Jacob Disease, variant of Creutzfeldt-Jacob Disease, Kuru, Gerstmann-Straussler-Scheinker disease (GSS), and fatal familial insomnia (FFI) as reviewed by Hur et al. (2002). The forms that these neurodegenerative diseases include: sporadic familial mutations of the prion gene, infectious mode, iatrogenic exposure to infectious brain material (i.e. cadaveric pituitary hormones, dura, corneal grafts), and infection from contaminated surgical instruments (Pedersen and Smith, 2002; Bebermeyer et al. 2003; Taylor 2003). It should be noted that all transplant tissues from animals as well as humans could create recipient-recipient risk as a result of the discovery of infective prions harbored by several peripheral tissues beyond the lymphatic system as shown in Tables II and IV. (Wadsworth et al., 2001; Detwiler and Baylis 2003; Grassi, 2003; Ramasamy et al. 2003; Zanusso et al. 2003).

The Food and Agricultural Organization (1999) of the United Kingdom reported 850 breeds of sheep in

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the world, 250 reared in Europe and 24 in the U. K. (Novak et al. 2000). Transmissible Spongiform Encephalopathy in sheep, known as scrapie, was first reported in the era of Roman Times as reviewed by Gaiger (1924; Detwiler and Baylis 2003). Novak et al. (2000) phenotyped the different forms of TSE discovering the phenotype occurring in the outbreak in the United Kingdom in cattle that was traced to a shipment of Merino sheep from Spain in the 15th Century (Murphy et al. 1999; Baron et al. 1999, 2000). In 1730-32 a disease reported in England as "sui generis" presented identical symptoms as the disease in the imported Merino sheep (Novak et al. 2000). The further distribution of these disease bearing Suffolk sheep to Australia (1952), New Zealand (1952-54), now both Australia and New Zealand are free of scrapie, Brazil (1977), Norway (1958-59), Canada (1938) and from Canada to the United States (1947), South Africa 1964-72), Columbia (1968-71), and Kenya (1970), see Table III, Detwiler and Baylis (2003). In March of this year scrapie was discovered in Finland (Detwiler and Baylis 2003).

Novak's group further determined that the PrP genotype was the most critical factor determining the risk of developing TSE and that the "sip" gene determined the length of the incubation period of onset of the disease (Novak et al. (2000). Using this new genetic evidence Suffolk sheep were confirmed as the source of contaminated bone meal in feed given cattle responsible for the U. K. epidemic (Novak et al. 2000; Detwiler and Baylis 2003). A further study of the causative genotype of the U. K. epidemic was made by Ligios et al. (2002) suggesting that the strain of origin came from Sardinia, which has never been confirmed. Narang (1996) reported earlier that the strain found in U. K. infected cattle originated in sheep but was much more virulent and found evidence of vertical transmission in cattle. Cuille and Chelle (1936) successfully transmitted TSE into sheep through an intraocular injection of bacteria with an onset time of 14 to 22 months. Scrapie in sheep and goats was reported by Dickinson (1976, Detwiler and Baylis 2003), and transmissible mink encephalopathy by Marsh and Kimberline (1975), and chronic-wasting disease in mule deer and elk by Williams and Young 1982; Hamir et al. 2003). The first case of scrapie in sheep in the United States was reported in 1947 by Parry (1983) as reviewed by Narang 1996; Williams 2002; Detwiler and Baylis 2003. Intracerebral, intraperitoneal, intravenous injections and oral dosing of infected material were employed to determine if bovine spongiform encephalitis was infective in domestic pigs (Ryder et al. 2000; Wells et al. 2003). These studies demonstrated spongiform encephalopathies in all inoculated animals with incubation times of 69-150 weeks post administration. However, no infectivity was noted in orally fed pigs (Wells et al. 2003). To date there has been no naturally occurring spongiform encephalopathies in domestic pigs (Ryder et al., 2000; Wells et al., 2003) nor have there been any transmissions from goats or sheep to man (Detwiler and Baylis 2003).

Gajdusek (1957, 1959, 1977, 1966a,b) reported the occurrence of a neurodegenerative disease of the Fore People in Papua, New Guinea, at epidemic proportions and called it "Kuru". He described this disease as a spongiform encephalopathy characterized by a neural abundance of amyloid plaques and demonstrated that Kuru-like symptoms were transmissible to chimpanzees (Gajdusek, Gibbs, and Alpers (1966b). Vertical transmission of Kuru did not occur in the New Guinea tribesman but Gajdusek found disease transmission through the oral consumption of deceased elder's brains. Cannibalism and associated prion diseases have been suggested to date back to ancient humans (Pennisi, 2003). The origin of the New Guineas tribesman disease is linked to a mutation of codon 200 of the prion protein gene found in patients in Slovakia, Libyan and Sephardic Jews and those from other Mediterranean countries (Korczyński, 1991; Chapman and Korczyński, 1991). Hadlow (1959) was the first to report the recognition of common neural histologic features between scrapie and Kuru.

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oral transmission of TSE from both bovine and ovine origin into mice (Maignien et al. 1999), sheep and mink was demonstrated by Foster et al. (1993) and Marsh and Hadlow (1992). This transmission was shown to be effective in mice, domestic cats, Cheetahs, and cattle with tissues from animals with sheep scrapie and cattle with BSE. The first case of Feline spongiform encephalopathy (FSE) occurred in the U. K. in 1990 from consumption of contaminated feed that showed the infection had spread to several tissues including the spleen and kidneys by Ryder et al. (2001) and spleen and lymph nodes of goats and sheep (Detwiler and Baylis (2003, see Table IV). Primate centers throughout the United Kingdom reported spongiform encephalopathies from feed contamination indicating a more widespread problem than earlier suspected (Narang, 1996; Bons et al. 1999). Cases included a nyala *Tragelaphus angasi*, four eland *Tragelaphus oryx*, six greater kudu *Tragelaphus strepsiceros*, one gemsbok *Oryx gazella*, one Arabian oryx *Oryx leucoryx*, one scimitar-horned oryx *Oryx dammah*, three cheetah *Acinonyx jubatus*, one puma *Panthera pardus concolor*, and separately three ostriches *Struthio camelus* from two zoos in West Germany (Narang, 1996). Taylor (2002) estimates according to current knowledge that the oral administration of 0.5 grams of brain tissue from BSE infected cattle to goats or sheep will result in a neurodegenerative disease indistinguishable from scrapie (Foster et al. 1993). To date there have been no reports of goat or sheep scrapie successfully transmitted to humans (Detwiler and Baylis 2003). Martinsen et al. (2002) demonstrated that gastric acidity negatively affects oral transmission in mice. However, the transmission through rodent feces and other body parts remains an open threat (Cocepacion and Padlan, 2003). Therefore, other ectoparasites may also be a source of transmission of disease prions such as flies and fly larvae as reported recently by Lupi (2003).

Using animal and human brain tissue of TSE positive individuals, cerebral inoculums were administered to marmosets that were sacrificed 17-49 months post administration. The majority of those treated demonstrated histological patterns of spongiform encephalopathy (Baker et al. 1993, 1998). The part of the brain used for detection of abnormal prions using Western Blot analysis was critical to the detection in sheep and bovine species as reported by Schaller et al. 1999; Madec et al. 2000; Bruce et al. 2001; Vitale et al. 2001; Wadsworth et al. 2001; Detwiler and Baylis 2003 (see Table IV). The highest concentrations of abnormal human prions occurred in the cerebral cortex and mesencephalocortex, 5 to six times higher than other brain regions (Madec et al. 2000; Ramasamy et al. 2003). Grassi (2003) recently reported the detection of infective prions in lymph nodes in sheep prior to onset of any detectable symptoms as further reviewed by Detwiler and Baylis 2003, see Table IV). Prion proteins, both PrP^c and PrP^{sc}, were found in epididymal fluid, sperm, and genital tract tissues from French Romanov rams by Gatti et al. (2002), and throughout the lymphatic system and nasal epithelium in humans (Grassi, 2003; Zanusso et al. 2003).

Human to human transmission was shown by Gajdusek (1957) and reviewed by Pennisi (2003) by cannibalistic practices. Transmission of TSE from cattle to man was dramatically demonstrated by the outbreak of vCJD in the United Kingdom as reviewed by Collinge (1999), Dormont (2002a,b), and Prusiner (1998, 1999). Current evidence suggests that the cause of this epidemic was the consumption of contaminated meat and brain material resulting from recycled carcasses from scrapie infected sheep and BSE infected cattle meat used in producing bone-meal (MBM) protein nutritional supplements (Wilesmith et al. 1991, 1992, 1996; Wilesmith 1994; Kimberlin and Wilesmith 1994; Collee and Bradley 1997;) as reviewed by Fishbine (1998) and reported by Ratzan 1998; Baron et al. 1999, 2000; Novak et al. 2000; Conway 2001; Taylor 2002; and Dormont 2000b. Tan et al. (1999) reviewed the current risk of transmission of BSE to humans to be minimal as reported by Hueston (1997) and Brown (1998) and in North America by Williams (2002). BSE in the form of vCJD has not been shown to exist in the United

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States as reviewed by Holman et al. (1996), Schwetz (2001), and Horby (2002). However, three recent and separate reports of vCJD in U. S. patients aged from the teens through 31 years of age has occurred in Michigan (Peltier et al, 2002, in Colorado (Belay et al. 2002; Bosque 2000) and Wisconsin (2002, 2003). All eight cases were known to have consumed venison as reported by Peltier et al. (2002) and Belay et al. (2001) and reviewed by Bosque (2002). One case of vCJD in a 22-year-old Florida resident was reported in April 2002, owing to extensive travel in the U. K. and consumption of contaminated food (Morb. Mort. Weekly, October 18, 2002, CDC).

The epidemiology for the Michigan deaths has not been verified by scientific proof but was based on patient life-styles and history. Histopathology of the deceased brain material from the Colorado patients were confirmatory for spongiform encephalopathy (Belay et al. 2001). Brain tissue phenotyping by Western Blot and PCR uncovered a genetic variant in the Colorado patients that was different from any reported to date. No additional reports have appeared. Three more U. S. men who participated in wild game feasts in northern Wisconsin died of neurodegenerative diseases, one a confirmed case of vCJD, as reported by the Center for Disease Control (CDC) in the Journal of the American Medical Association (2003) and the Morbidity Mortality Weekly Report (2003). The tabulated global incidence of BSE and vCJD is shown in Table V developed from data by Will (1999) and Table VII by Smith (2003). To date there have been only 129 cases of vCJD globally (Smith, 2003; see Table VII).

Current methods for the control of BSE, TSE, CJD, and vCJD in the U. S. involve cattle, sheep, pig, farmed elk, deer, mule deer, and mink populations (United States Department of Agriculture (USDA, 1998), Tan et al. (1999), Schwetz (2001), Williams (2002) and Detwiler and Baylis (2003). Control of entry into the U. S. of herds of beef, chicken, sheep, or pigs or raw materials from them are regulated by the USDA (1991), FDA (1997a,b, 1999), as reviewed by Porter and Drazek (1999). Control of feed composition and shipment from foreign sources are also controlled by the FDA (1997a). Slaughtering guidelines, inspections, and testing of slaughter animal meats are controlled in the U. S. (USDA 1998). Hett et al. (2002) has recommended a 99.6% specific set of criteria for identifying BSE suspects in slaughtered cattle. Tissue grafts and related products are also controlled as reviewed by Hellman (1997). Brown (1998) summarized these regulations as adequate to prevent entry of foreign sources of BSE into the United States, and adequate regulations exist to prevent undetected cases of BSE from uncontrolled amplification within the U. S. cattle population. Brown (1998) emphasizes that adequate regulations already exist for controlling the entry of high-risk bovine materials from contaminating products intended for human consumption. Bosque (2002) supports these observations and emphasizes that measures to minimize human exposure to infective animal prions, particularly deer, elk, or sheep scrape materials are an essential part of preventing CJD in the U. S. Bosque (2002) found it encouraging that over the last 10-13 years, following the period of greatest exposure of humans to BSE prions, there have only been 129 confirmed cases of human vCJD which was further supported by Smith in January of 2003. To date there have been eight U. S. recorded cases of neurodegenerative diseases, over half that are confirmed cases of vCJD resulting from consuming venison as previously reviewed in this report. Additionally, the incidence of one case per million population per year of spontaneous CJD has been reported in the U. S. (CDC Morbidity and Mortality Weekly Report, February 21, 2003; Smith 2003). However, there have not been any vCJD from cattle within the U. S. (Tan et al. 1999). This U. S. record was threatened when on May 20th, 2003 the Canadian Agriculture Minister, Lyle Vanclief (Associated Press), announced the detection of a BSE infected cow in the province of Alberta. The infected animal reportedly was

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slaughtered on January 31, 2003, and only recently tested for BSE. Unfortunately the animal gave no indication of infection at the time of slaughter and supposedly went to a rendering plant and did not enter the food chain. An exhaustive quarantine and testing program has resulted with the elimination of Canadian cattle and sheep and related meat products from export into many countries including the United States, Japan, South Korea, Australia, Taiwan, and Mexico (J. Jones, May 21, 2003, Reuters News Service). That blockade continues as of July 21st, 2003.

Inactivation of the infectivity of infectious prions (PrP^{Sc}) using heat and solvent extraction had little effect as shown by Gajdusek (1977) and Taylor et al. (1998) with the exception of one of the most effective agents to date, guanidine thiocyanate (Meyer et al. 1999). Guanidine hydrochloride was also shown to block growth of yeast prions (*Saccharomyces cerevisiae*) that no longer would self-propagate and complex similar to the same behavior of transmissible spongiform encephalopathy prions (Ferreira et al. 2001). The focus of this action of guanidine was shown to occur at residue 184 of the yeast prion, the Hsp 70 gene chaperone, and affected its propagation and thermal tolerance (Ferreira et al. 2001; Jung, Jones, and Masison, 2002). Moreover, a naturally occurring mutation found in yeast demonstrated the same blockage of propagation and reduced thermal tolerance occurring at the identical loci (Jung, Jones, and Masison, 2002). Other agents such as peracetic acid (Antloga et al. 2000) and epoxides like glycidol (Yamamoto et al. 2001) were shown to inactivate the infectivity of abnormal prions from spongiform encephalopathies. An improved approach for inactivation in plasma was shown for methylene blue (Hornsey et al. 2001), low temperature sterilization with gas plasmas (Moisan et al. 2001) and filtration with leukocyte depletion (Karger and Kretschmer (2002). The most significant and practical method of sterilization of neurosurgical instruments was found to be exposure to 1 M sodium hydroxide followed by autoclaving at 121 °C (Taylor 2003). For dental instruments 1 N sodium hydroxide solution for 1 hour followed by autoclaving in a vacuum at 134 °C was also found to be totally effective (Bebermeyer et al. 2003). Miekka et al. (2003) employed a 25% human albumin solution containing 1:100-10% scrapie infected hamster brain in phosphate buffered saline and exposed it to gamma-irradiation of 50 kilogray (kGy) at a dose rate of 49kGy per hour at room temperature. The Syrian hamster cerebral inoculation of varying doses followed by observation of onset of scrapie characteristics demonstrated inactivation of infective prions by 1.6 log₁₀ ID₅₀ (50% of infective dose) with 70% of the albumin remaining intact (Miekka et al. (2003).

Therapy for TSE, BSE, CJD, or vCJD has not been available. However, recent reports of the effectiveness of mouse prion PrP^{Sc} specific monoclonal antibodies in mice with experimentally induced spongiform encephalopathy have indicated immunotherapeutic approaches are worthy of attention (White et al. 2003). Forloni et al. (2002) recently reviewed all of the compounds that antagonize prion propagation in cellular and animal models showing that most of these agents either did not cross the blood brain barrier and/or had severe toxicity. Syrian hamsters were intracerebrally inoculated with 1 mM tetracycline hydrochloride co-incubated cerebral extracts from scrapie infected Syrian hamsters creating a significant delay in the onset of Magnetic Resonance Imaging (MRI) detectable brain abnormalities, neuropathological changes, and PrP^{Sc} accumulation (Forloni et al. 2002). When these same investigators reincubated the scrapie inoculum with tetracyclines and then injected the animals, one-third of the hamsters injected did not develop neurodegenerative disorders. Therefore, tetracyclines may play a future role in the treatment of cattle with BSE and humans with vCJD (Forloni et al. 2002). In May of 2003 chondroitin polysulfate (PP), or Elmiron from Alza Pharmaceuticals was used in the experimental therapy of terminal teenager in the U.K. with vCJD (Bhattacharya, 2003; Dyer, 2003). The early

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results in this effort show a complete diminution of detectable infective prions and disappearance of vCJD clinical manifestations. Although never before used for vCJD therapy, Elmiron is approved for successful therapeutic use in the United States and U. K. for interstitial cystitis (Nickel, 2000; Moldwin and Sant, 2002; Parsons, 2002, 2003) and in France for inflammation and osteoarthritis (Ghosh and Cheras, 2001; Ghosh and Smith, 2002). Experimental therapy with Elmiron in the rescue of later radiation proctitis was demonstrated by Denton et al. (2002). Further potential for the use of Elmiron in elimination of potential blood transmission of TSE's is reviewed later. Although no sustained benefit has been shown, open-label clinical trials are in progress with quinacrine and chlorpromazine (Ware et al., 2003; Macleod, 2003). However, Barret et al. (2003) just reported that from *in vitro* studies on cell-lines and the mouse spongiform encephalopathy model it is most unlikely either quinacrine or chlorpromazine will have any affect on infective prions *in vivo*. A significant breakthrough may have resulted in the development of a novel vaccine for both animals and humans using a repeating motif of tyrosine-tyrosine-arginine reported in one of the prion folding points on the molecule by Paramithiotis et al. (2003).

Analytical techniques for the detection of BSE and vCJD and its variants have been based upon histologic examination of brain tissue, immuno-histochemical staining of histologic sections of the brain, feed microscopy, Western Blotting, Polyacrylamide gel electrophoresis (PAGE) with sodium dodecyl sulfate (SDS), Capillary Electrophoresis (CE), Chemiluminescence Enzyme Linked Immunosorbant Assay (ELISA), Sandwich Immunoassay (SIA, Dot-Blot DNA analysis, and Polymerase Chain Reaction (PCR) as reviewed by Prusiner (1998), Harmeyer, Pfaff, and Groschup (1998), Hill et al. (1998), Meyer et al. (1999), Schmerr et al. (1999), Momcilovic and Rasooly (2000), Commission of the European Communities (1999, 2000), Biffiger et al. (2002), Germain and Goldman (2002), Gatti et al. (2002) and Williams (2002).

Current sensitivity, specificity, and reliability requirements for a diagnostic test for PrP^{Sc} have reduced these types of analytical tests to several possible choices. These are Western Blot Analysis (Schaller et al. 1999; Wadsworth et al. 2001; Bradley 2002; Grassi 2003), Capillary Electrophoresis (Schmerr et al., 1996, 1997, 1999) and Jackman and Schmerr, 2003) and luminescence immunoassay (Biffiger et al. 2002). These techniques have been validated by current standards and involve pretreatment of the sample with at least protease K digestion, while the CE technique also requires two additional pretreatment steps. Those steps are incubation of the protease K digest supernant with first a PrP^{Sc} specific antibody followed by fluorescence labeling of the immune complexes formed (Schmerr et al. 1999). The CE technique demonstrates sensitivity of picogram (pg, 1×10^{-12}) levels of detection in sheep blood using less than a nanoliter sample size (1×10^{-9} liters). An immunocompetitive capillary electrophoresis assay (ICCE) was reported to have a sensitivity of between 2-0.6 nanomoles (2000-600 picograms) for synthetic prion peptides but was incapable of distinguishing normal from abnormal prions in chimpanzee and human blood (Cervenakova et al. 2003). This technique obviously did not employ the method of Schmerr and others of first exposing their samples to proteinase K digestion to remove normal prions from the sample before conducting the analysis. Biffiger et al. (2002) established and validated a Luminescence Immunoassay (LIA) for the automated detection of infective prions in post protease K digested brain samples reporting 100% detection and no false positives out of 336 infected samples and 1064 normal samples from cattle and sheep. This analytical method has been commercialized by Prionics of Switzerland and has been endorsed by the European Community for screening bovine and ovine diseased animals and slaughter animals prior to distribution of their meat. Just recently Kang et al. (2003)

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took advantage of an earlier report of infective prion inactivation with guanidine thiocyanate (Meyer et al. 1999) and used this same agent to inactivate samples prior to analyzing them in an sandwich ELISA for successful detection of mouse and hamster abnormal prions without prior proteinase K digestion.

Gramithiotis et al (2003) in June reported the identification of tyrosine-tyrosine-arginine repeat motifs in infective prions responsible for generating monoclonal antibodies that specifically discriminate between infective and non-infective prions. These new approaches offer promise but await confirmation. To date there are no currently available analytical techniques that are U.S. government approved for screening for normal prions in any kind of sample (Cervenakova et al. 2003).

Application of sensitive and specific testing of raw materials for infective prions has been suggested by Momcilovic and Rasooly, 2000; Horby, 2002; Grassi, 2003. Most nutritional products of animal origin are severely heated, subjected to organic solvent fat extraction, dried at high temperature and milled to fine powders. This process denatures and degrades most proteins and DNA (Momcilovic and Rasooly, 2000). However, none of these treatments have been effective in the inactivation of infective prions (Ajduksek 1977; Conway, 2001; Taylor et al. 1998, 2003). Commercial testing procedures have been developed and utilized in European communities but not in the U. S. Domestic analyses utilize sample pre-digestion with protease K followed by DAS-ELISA (Bio-Rad Laboratories) or Agri-Screen immune complex test strips (Neogen). Neither method has been used exclusively, but in combination with histological and histochemical examination of brain tissue for reliable diagnoses. Moreover, the part of the brain from which the sample is taken is critical to the likelihood of detection of abnormal prions as shown by Schaller et al. 1999, Madec et al. 2000, Bruce et al. 2001, Vitale et al. 2001, Wadsworth et al. 2001, Detwiler and Baylis 2003, and Ramasamy et al. 2003 (see Tables II & IV). Grassi (2003) has recently reported using Western Blot analysis to pre-clinically detect abnormal prions in sheep lymph nodes. Horby (2002) points out that sensitive diagnostic tests will create yet further dilemmas when one considers the implications in human testing and current incubation times for the onset of vCJD of 10-14 years. Germain and Goldman (2002) address this subject in the screening of blood supplies.

Studies in the United States by Vamvakas (1999) suggested that vCJD infection resulting from transfusion of blood, plasma, and plasma derivatives could not be excluded from investigations he had conducted. However, blood supply safety has been reviewed and summarized as being quite safe in the U. S. (Brown, 2003; Busch, Kleinman and Nemo, 2003) including enhanced screening of donors with questionnaires and serologic testing (Germain and Goldman, 2002). Blood infectivity and the prospects of a diagnostic screening test for CJD were reported by Brown, Cervenakova, and Diringer (2001) at NIH that agreed with recent findings on the safety of the current U. S. blood supply. The United States blood supply and its components are carefully monitored and regulated by the FDA (1997c, 1999). Moreover the restriction of travelers to BSE, TSE, CJD and/or vCJD positive countries as donor candidates in the U. S. and Canada has further reduced the risk of degenerative encephalopathy related to prion contamination of the blood supply (Germain et al. 2000; Germain and Goldman, 2002; Wilson et al. 2003). Caughey and Raymond (1993) demonstrated the inhibition of scrapie-associated prion accumulation in cultured cells using pentosan polysulfate. Farquhar, Dickinson, and Bruce (1999) demonstrated the potential use of pentosan polysulfate in the elimination of the transmission of transmissible spongiform encephalopathies. Ringue et al. (2000) demonstrated inhibition of infection in scrapie-infected mice using pentosan polysulfate at therapeutic levels. Fricker (2001) reported the clearance of vCJD infective prions from by

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heparin-like complexing of the prions followed by precipitation. This same Elmiron stored blood using pentosan polysulfate ("Elmiron" by Alza Pharmaceuticals, Mountain View, California) mechanism of action was demonstrated in the inhibition of HIV-Tat protein and cellular HIV-1 infection in vitro (Rusnati et al., 2001). Recently 1 M sodium hydroxide and lower concentrations were used to inactivate the agent of bovine spongiform encephalopathy by gelatin manufacturers in Europe (Taylor 2003). Karger and Kretschmner (2002) further reported inline filtration and leukocyte-depletion as treatments of the blood supply as potentially effective measures for preventing the transmission of vCJD. However, this has implications of reducing the current availability of blood by 5-10%. A recent review quotes not only a very low risk of transmission in the current blood supply but also in orthopedic surgery (Doerr et al. 2003). Similar safety levels were reported for use of contact lenses by Hogan (2003).

U. S. food safety has been recently reviewed and shown to have a minimal risk for BSE related disease contamination by Conway (2001), Dormont (2002a,b) and Williams (2002). The highest risk to the worlds food supply is food poisoning according to Conway (2001). A sample population survey was conducted and out of 42 potential causes of food poisoning between 1996-1999 in Ireland, 90% were due to sources of poorly cooked or handled food in institutions outside the home (Anderson, 2000). Sources of food contamination are listed in Table VIII. All represent pathogenic bacteria with no mention of transmissible spongiform encephalopathy (Anderson, 2000; Conway 2001). As a matter of comparison the risk of cancer from the carcinogens in one cup of coffee is equivalent to one year's pesticide residue in the normal U. S. diet (Ames and Gold, 2000). Food additives create an even greater risk of food contamination through allergic reactions as reported by Conway (2001). A recent survey of United Kingdom residents showed 0.01-0.23 percent (between one in 23 people in 10,000) proved to be intolerant of food additives as reviewed by Conway (2001). In summary, the risk of BSE in the food supply is minimal and in contrast to other current issues is 0.02 percent in the U. S. or minimal as reported by the CDC (2003). Moreover, this risk is related to the diligent inspections of the producers and steadfast promulgation of the laws governing the production of food and dietary supplements by federal inspectors (Tan et al. 1999; Bosque, 2002; Taylor 2002). Elimination of remaining risks would be achieved by the extermination of infected herds of deer, elk, goats, and sheep (Bosque 2002). This has recently been started in Wisconsin as reported by Yam (2003). However, Paramithiotis et al. (2003) has just reported the discovery of a potentially promising peptide vaccine for both animals and humans that may modify this drastic approach of slaughtering U. S. wild life.

Conclusion: The origins of transmissible spongiform encephalopathy, it's development, pathogenesis, routes of infection, history of the United Kingdom epidemic, risk and incidence of infection, prion inactivation agents, experimental therapeutic approaches, current analytical tools available for PrP^{Sc} detection, and blood supply and food safety in relationship to BSE, TSE, CJD, and vCJD related disease incidence has been thoroughly reviewed. There can be found no relevance of CBER guidance's 50244 and 51074 in promoting or securing the safety of dietary components, dietary ingredients or dietary supplements. Significant strides in finding inactivation and disinfection agents (Gajdusek 1977; Prusiner 1998; Meyer et al. 1999; Taylor 1998, 2003) strongly indicate guanidine thiocyanate (Meyer et al. 1999), guanidine hydrochloride (Ferreira et al.2001) and 1 M sodium hydroxide and heat sterilization (Bebermeyer et al. 2003; Taylor 2003) eliminate nosocomial routes (Weber and Rutala, 2002) of

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transmitting infection. The recent findings on the potential for treating stored blood to eliminate infective prions (Fricker, 2001) and the human treatment of vCJD patients with tetracyclines (Forloni et al. 2002), pentosan polysulfate (Bhattacharya 2003; Dyer 2003) quinacrine or chlorpromazine (Macleod 2003; Ward et al. 2003) have raised promising potential. Developments by Schaller et al. (1999), Wadsworth et al. (2001), Bradley (2002), Grassi (2003), Biffiger et al. (2002), Jackman and Schmerr et al. (2003), and Kang et al. (2003) yield strong possibilities of sensitive and specific analytical tools employing Western Blot, Luminescence Immunoassay, Capillary Electrophoresis with antibody tagging and fluorescence staining of the complexes, and guanidine hydrochloride sample pre-treatment followed by ELISA. New in-vitro diagnostics developing from the Paramithiotis et al. (2003) report of a potential peptide vaccine that generated infective prion specific monoclonal antibodies is further proof of the progress in the field.

The lack of availability of U.S. federally approved diagnostic tests for BSE related prions have not contributed to near elimination of occurrence of cases of CJD or vCJD in the U. K. (Smith 2003). In fact, the incidence of occurrence of BSE and vCJD has dwindled in the UK to a rate existing ten years before this pathogen was discovered, namely 1 case per million per year (Morbidity Mortality Weekly Report, February 21, 2003; Smith, 2003) which is the rate for sporadic CJD globally (see Table VII). In short, the diligent hire and education of inspectors, active promulgation of the current laws, aggressive application of severe fines for offenders, and the approval of a rapid and sensitive commercial test for PrP^{sc} are the current priorities (Taylor 2002; Detwiler and Baylis 2003; Grassi 2003). The use of an approved and reliable analytical test to identify and rid the U. S. of scrapie infections in deer, elk, and sheep would further reduce the current minimal risks and necessity of large wild life purges as reported recently in Wisconsin (Yam, 2003). **The FDA and USDA need to apply their resources to establishing that test before creating more regulations.**