

**Scientific Information Concerning the Issue of Whether Prions Are a “Pest”
under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)
(12/17/08 Draft)**

I. Introduction and Background

This paper reviews currently available scientific information pertaining to the issue of whether a prion (“**p**roteinaceous **i**nfectious particles”) should be considered to be a “pest” as defined by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The scientific information in this review has been considered by the EPA work group that developed the Notice of Proposed Rulemaking that proposes to establish a prion as a pest under FIFRA.

FIFRA provides EPA the authority to decide what entities are considered to be a “pest” under FIFRA. The sections that are potentially relevant to prions include:

- FIFRA Section 2(t): A pest is “... (2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria or other micro-organism...which the Administrator declares to be a pest under section 25(c)(1).”
- FIFRA Section 25(c)(1): “The Administrator, after notice and opportunity for hearing, is authorized—(1) to declare a pest any form of plant or animal life ... which is injurious to health or the environment.”

In September 2003, the EPA’s Office of Prevention, Pesticides, and Toxic Substances (EPA/OPPTS) decided that a prion should be considered to be a “pest” under FIFRA and that products intended to inactivate prions (i.e., “prion products”) should be regulated under FIFRA (USEPA, 2004). The Agency’s rationale may be summarized as the following key points:

- Prions are unquestionably injurious to human and animal health.
- Prions share many characteristics of other deleterious microorganisms.
- Prions share enough characteristics of “other micro-organism” or “form of life” to fall within the scope of FIFRA section 2(t)(2). They originate in living organisms, replicate within host organisms, and can be transmitted to other hosts.
- If prion products were not regulated under FIFRA, EPA could not provide assurance that such products would be effective or safe.
- It appears that the intent of Congress in the 1972 FIFRA was that EPA should regulate products used against deleterious forms of life

In order to provide sound, scientific input into the issue of whether a prion may be considered to be a “pest” under FIFRA, EPA has again examined the relevant, available scientific evidence relevant to the question. This document principally addresses the scientific evidence. Legal and policy considerations that factor into the Agency’s decision to issue a NPRM will be described in more depth in the Preamble to the NPRM.

II. The Science of Prions

A. Description

The leading theory on the causative agent for transmissible spongiform encephalopathies (TSE) is that the agent is composed of an abnormal form of a nucleic acid-free, replicating protein (Prusiner et al. 1982; Bolton et al. 1982; Brown et al. 1990a), now called a prion. The term prion (Prusiner, 1982) makes a distinction between molecular properties of the novel, proteinaceous infectious particles that cause scrapie disease and molecular properties of particles, such as viruses, plasmids, and viroids that feature a core of nucleic acid. Fueled by the catastrophic outbreak of mad cow disease, or bovine spongiform encephalitis (BSE), originating in Essex County, England (1986), research to characterize the precise nature of prions and resolve how they cause the diseases known as TSEs led to Prusiner's award of a Nobel Prize in 1997. In the absence of mechanistic evidence, the assumption prior to this body of work was that kuru in humans and scrapie in sheep and goats were caused by unconventional viruses, virus-like agents, or slow viruses, because the time interval between exposure and clinical disease was years or decades (Prusiner et al. 1982). While a number of scientists have not ruled out an unknown virus as the agent of the TSEs (Manuelidis, 2007; Manuelidis et al. 2007), no virus producing a TSE disease has been isolated after 40 years of searching. Instead, the protein-only hypothesis for the origin of the TSEs (Alper et al. 1966; 1967; Griffith, 1967; Brown et al. 1990a) is now generally accepted by overwhelming weight of evidence, especially Prusiner's hypothesis of a novel proteinaceous infectious agent – the prion (Prusiner et al. 1982; Bolton et al. 1982). In particular, experimental transmission of TSE diseases by purified prion protein synthesized or propagated in cell free systems (Legname et al. 2004; Castilla et al. 2005) is accepted by many as definitive proof of the prion hypothesis. Though prions are now generally accepted as the agent responsible for the TSEs, the precise structural properties of the prion protein responsible for infectivity and the chain of events that produce neural degeneration and fatal TSEs remain unknown (Barron et al. 2007). As a result of the novelty of the prion hypothesis and controversy surrounding it as the agent for infectious diseases, the prion protein and its isoforms have been studied with unusual intensity; yet the physiological function of the normal protein and the role of the protein in producing neurodegeneration remain obscure (review Aguzzi et al. 2008). Highlights of prion research are presented in section II of this paper.

Numerous breakthroughs have been made in the past several years. As detailed below (B. Key Characteristics), the normal isoform of the prion protein (PrP) is now known to be encoded by the PRNP gene and highly expressed in neurons on the cell membrane. The prion protein possess a unique property, so that when the normal α -helix rich conformation is converted to a misfolded, β -sheet rich conformation, the resultant protein by-passes the normal proteosomal degradation pathway, produces a TSE whose clinical features vary somewhat depending on the species (eg., cow, sheep, human, etc), and acquires the property of infectivity. Experimental intervention to restore proteosomal degradation of the misfolded protein conformation “cured” prion infectivity in cultured cells (Webb et al. 2007). The property of infectivity appears to reside in the β -sheet-rich conformation (Müller et al. 2007), which is the focal point of numerous ongoing investigations to define precisely the structural determinants of infectivity. In humans, polymorphism at PRNP gene codon 129 (129M/V) is common;

however, homozygous genotypes at 129 predispose PrP transcripts to misfolding and heterozygous transcripts resist misfolding (Mead et al. 2003). Genetic polymorphism in the PRNP promoter may also influence susceptibility to TSEs, as in the case of BSE (Haase et al. 2007). What distinguishes prion diseases from other neurodegenerative diseases featuring neurotoxic accumulations of protein plaques (such as Alzheimer's, Parkinson's, and ALS) is that misfolded PrP transcripts recruit and convert normal PrP transcripts to adopt the misfolded conformation; however, a low level of recruitment and conversion may be common to other amyloid plaque diseases. For example, experimental inflammatory amyloidosis in mice is accelerated when the mice are also administered purified amyloid fibrils (Lundmark et al. 2002). Similar seeding was observed in mice administered extracts from human Alzheimer's brain (Meyer-Luehmann et al. 2006). These misfolded β sheet proteins produce aggregate particles (known as plaques in histology), which are linked to cell death in neurons, and produce a number of fatal spongiform encephalopathies in humans, livestock and game animals (review Brown, 2005).

In summary, normal cellular protein is synthesized and eventually degraded through normal metabolic processes, whereas misfolding of the protein produces a shape change that accounts for the resistance of prion protein particles to proteosomal degradation and, consequently, for the slow accumulation of brain plaques. Furthermore, this slow progression of plaque formation and the prion diseases accounts for early speculation that the causative agent might be an unknown slow-virus. It is now clear that the transmissible encephalopathies (kuru, scrapie, mad cow, etc), in fact, feature a novel disease mechanism. In the prion hypothesis of disease, it is thought that prion protein polypeptides in the misfolded, plaque-forming isoform convert polypeptides in the normal cellular isoform to adopt the same misfolded conformation--a process that is now often described as **recruitment and conversion**. While researchers continue to describe prions as **infectious** protein particles, as defined by Prusiner (1982), it is important to note that this usage no longer refers to an unknown, slow virus, but rather to this novel process of recruitment and conversion. Furthermore, as will be detailed below, Legname et al. (2004) show evidence that normal PrP can spontaneously convert to the misfolded isoform.

While the outbreak of mad cow diseases in the UK appears to have effectively ended in cows (review Brown, 2001), the long, unresolved dormancy of prion diseases is a matter of great public concern (Prusiner et al. 1982; Carrell, 2004; Enserink, 2005), with the risk that prion disease might reappear in human populations long after the consumption of food contaminated with diseased CNS tissues. Also, surgical tools contaminated with prions are extraordinarily difficult to decontaminate (Brown et al. 1990; 2000; 2005; WHO 1999).

B. Key Characteristics

1. The prion protein and terminology

In its original description, the purified prion particles were characterized as comprised of a single protein, roughly 27,000 to 30,000 daltons in molecular weight, and resistant to proteinase K digestion. In particular, enrichment of the protein correlated with the titer of the infectious agent (Bolton et al. 1982) and failed to exhibit the spectra indicative of the presence of nucleic acids (Prusiner et al. 1984). Through the work of more than two decades, it is now

believed that the infectious isoform of the prion protein is a misfolded isoform of a normal cell membrane protein, and various terminologies have appeared as numerous researchers have joined the investigations. The normal cellular isoform of the protein is typically designated PrP^C, the infectious isoform in scrapie is PrP^{Sc}. Because a variety of isoforms produce different transmissible spongiform encephalopathies, PrP^d has been introduced recently to connote any disease isoform. Also, PrP^{res} has been used to connote a purified protein or recombinant polypeptide fragment that features the resistance to proteinase K digestion that is characteristic of TSEs. Other less common usages also appear in the literature. More recently, PrP^{TSE} has been used generically for the infectious conformation of the transcripts of any prion strain, which lends simplicity to communicating about the homologous prion proteins of different human and animal species (WHO, 2006). As used in this paper, “prion” refers to a misfolded isoform of any prion protein; “prion protein” can refer to both the normal and misfolded isoforms.

2. Current Status of Dr. Stanley Prusiner’s (1982) protein only hypothesis

Amyloid polymerization of recombinant prion protein produced in bacteria has been shown to accompany the acquisition of prion infectivity (Legname et al. 2004; Colby et al. 2007) that is characteristic of the TSEs. Between 380 and 660 days after inoculation of normal mice with these transgenic fibrils, all mice developed neuropathology, and their brain tissue, in turn, was infectious to other mice. The amino acid sequence of the human prion protein has been extensively probed and two cationic domains (PrP₁₉₋₃₀ and PrP₁₀₀₋₁₁₁) have been shown to be capable of homologous binding between PrP^C and PrP^{Sc}, which would account for the recruitment of PrP^C and conversion to infectious PrP^{Sc} (Lau et al. 2007). By using guanidine hydrochloride as a denaturant and copper binding, apparent reductions in prion infectivity have been shown to be partly reversible, which supports the concept that the property of infectivity resides with the fibrillar, protease resistant conformation (McKenzie et al. 1998).

Large quantities of infectious PrP^{res} can be produced *in vitro* by seeding the normal PrP^C isoform with small amounts of PrP^{res}, followed by protein misfolding cyclic amplification in a cell free system (Castilla et al. 2005; 2008). Hamsters inoculated with PrP^{res} produced *in vitro* developed scrapie disease and died after about 170 days.

3. Normal Prion Protein

It is now known that the infectious prion protein is any one of several isoforms of an otherwise normal cell membrane protein (Stahl et al. 1990), encoded by a highly conserved gene, variously designated as PRNP, prnp or Prnp (review Sakaguchi, 2005) located on chromosome 20 in humans and on 2 in mice (Oesch et al. 1985; Basler et al. 1986). The Prnp gene appears to be conserved across species from yeast to primates (Westaway and Prusiner, 1986) and may share an ancestral origin by divergence with a related SPRN-like gene that codes for another brain protein known as Shadoo (Premzl et al. 2003; 2004). The neuronal protein Shadoo may have neuroprotective functions similar to those of PrP^C. Shadoo is downregulated in prion disease, possibly indicating a companion role in the mechanism of prion infections (Watts et al. 2007). The prion gene family also includes Prnd which encodes Doppel, a protein with striking homology to PrP^C. Doppel does not possess the conversion and recruitment properties of the prion protein but is neurotoxic, causing cerebellar degeneration when overexpressed in the brain

(Whyte et al. 2003; Moore et al. 2001). Across the vertebrates, amino acid sequence homology between the PrP proteins of frog, turtle, chicken, and mammal is conserved at a rate of about 30%, which is sufficient to preserve the same molecular architecture of mammalian PrP^C (Harris et al. 1991; Calzolari et al. 2005).

While attention has been focused on the disease producing isoforms, it appears that the normal isoform of PrP^C is a copper binding metalloprotein (Brown DR, 1997), and it may function as an antioxidant. As noted above (McKenzie et al. 1998), copper binding status may also be an important cofactor in conversion of PrP^C and stability of the PrP^{Sc} infectious conformation. Disruption of normal PrP^C function *in vivo* by binding with monoclonal antibodies produces rapid cell death in neurons (Solforosi et al. 2004), which is consistent with indications that PrP^C functions include neuroprotection and that disruption of these normal functions produces neuronal cell death. Doppel-induced disease can be rescued by coexpression of wild-type PrP^C, attributing antioxidant properties to the cellular prion protein (Wong et al., 2001).

Similar to the aggregates of neurotoxic proteins found in other neurodegenerative diseases (review Taylor et al. 2002), the neurotoxic PrP isoforms are produced by misfolding and aggregation of β -rich isomers and accumulation of plaques in neurons. Homozygous codons for methionine (Met, M) or valine (Val, V) at codon 129 in the human PRNP gene predispose PrP to recruitment and misfolding, so that in kuru, for example, homozygotes have an earlier onset of disease following cannibalism of contaminated CNS tissues (Mead et al. 2003). Heterozygosity at codon 129 confers some resistance to prion disease, probably by inhibiting homologous protein interactions (Palmer et al., 1991).

Because of the apparent link between misfolded isoforms of PrP and the production of fatal, spongiform encephalopathies, the PrP has largely been studied in the brain. While most abundantly expressed in the brain, PrP^C has been detected in the liver, kidney, heart and skeletal muscle, among other tissues (Bosque et al., 2002). New evidence indicates the normal isoform of the protein has important functions in non-neural tissues. Zhang et al. (2006) show that the prion protein is a biomarker for hematopoietic stem cells where it is normally expressed on the cell surface. When bone marrow is depleted of prion protein expressing stem cells, the capacity to regenerate is also lost. This evidence indicates normal prion protein expression is required for bone marrow stem cells to regenerate.

4. Neurotoxic mechanism

Numerous breakthroughs have been made in the past several years regarding associations between the accumulation of prion protein aggregates and neuronal cell death. These aggregates are thought to result from failure of the ubiquitin-proteasome pathway to adequately degrade misfolded protein, which is a feature of prion disease share with other neurodegenerative diseases, such as ALS, Parkinson's disease, and Alzheimer's disease (Goldberg, 2007). In the case of the TSE diseases, the misfolded prion protein conformation does not appear to be intrinsically neurotoxic. Rather, it appears that the scrapie prion kills neurons by disrupting the function of the normal cell surface protein, and development of prion disease requires the presence of normal PrP expression. Uncoupling normal PrP from the cell surface also uncouples

the presence of PrP^{Sc} from the progression of disease. Key steps in resolving the mechanisms of the prion diseases include:

- Grafting transgenic tissue overexpressing PrP into embryonic forebrain of PrP deficient mice (Prnp^{0/0}) and inoculating with PrP^{Sc} shows that grafted tissues accumulate PrP^{Sc} and exhibit scrapie histology, whereas Prnp^{0/0} tissue remain unaffected (Brandner et al. 1996). In other words, the β -sheet rich prion isoform is not intrinsically neurotoxic.
- Depletion of PrP^C in neurons during established infection arrests disease progression, even though extraneuronal PrP^{Sc} continues to accumulate (Mallucci et al. 2003). Transgenic mice programmed to disable the PRNP gene in neurons at 12 weeks of age were inoculated with PrP^{Sc} several weeks before PRNP inactivation. Within days after the PRNP gene was inactivated, the mice became depleted of PrP^C and remained alive and disease free a year later. Inoculated control mice lacking the transgenic downregulation of PRNP all died.
- PrP^C protein is normally anchored to the neuronal cell membrane by a glycosylphosphatidylinositol (GTI) linkage. Disabling the GTI linkage in transgenic mice does not prevent the accumulation of PrP^{Sc} plaques in inoculated mice, but does prevent the development of scrapie disease (Chesebro et al. 2005).

Relationships between prion aggregation, plaque accumulation, infectivity, and spongiform degeneration are incompletely understood, as shown by seemingly inconsistent evidence that the formation of oligomers (or small PrP aggregates) may be an intermediate step in neuronal degeneration (Simoneau et al. 2007), although in some circumstances plaque accumulation can occur in the absence of infectivity and degeneration (Piccardo et al. 2007).

C. TSE Diseases

Prion diseases are neurodegenerative diseases also referred to as transmissible spongiform encephalopathies (TSE) due to the large vacuoles (Figure 1) that are seen upon post mortem examination of the cortex and cerebellum from affected individuals (Collinge, 2001). These diseases are seen in both humans and animals (Table 1). Scrapie is the most widely known TSE naturally occurring in sheep; it was first recognized some 200 years ago in Europe, and now present worldwide (Collinge, 2001). More recently, other types of TSE have been recognized such as chronic wasting disease in deer, moose, and elk (Williams and Young, 1980), bovine spongiform encephalopathy (mad cow disease) (Wells et al. 1987), feline encephalopathy (Wyatt et al. 1991), and transmissible mink encephalopathy (Marsh, 1992).

In humans, prion based diseases are generally thought to include Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler syndrome (GSS), fatal familial insomnia (FFI) and kuru. They are further categorized into three etiological categories: sporadic, inherited or acquired (Collinge, 2001, Wickner et al. 2004, Brown, 2005). The incidence of sporadic CJD worldwide is about one per million (Collinge, 2001). Sporadic CJD is characterized by a rapid dementia occurring in individuals between 45 and 75 years of age. Death can result as early as 2-3 months from onset (Collinge, 2001). Other symptoms include fatigue, insomnia, depression, weight loss,

headaches, ataxia, blindness and random pain sensations. No mutations have been found with sporadic or acquired prion disease in humans. In contrast, over 20 distinct mutations of the prion protein gene (Prnp) have been found in inherited cases. Fifteen percent of human prion diseases are inherited (Collinge, 2001).

Most prion disease in humans is acquired. Kuru represents the earliest known example of human acquired prion disease. Kuru is thought to have occurred in Fore natives of Papua New Guinea in the early 1900's when a case of sporadic CJD became entrenched in the population following cannibalistic rituals within the population. Because of the different roles adult men and women and children played in Fore communities, kuru was prevalent among women and children until the practice of cannibalism ended in the 1950s (Fischer and Fischer, 1961; Alpers, 1987 as cited in Collinge, 2001).

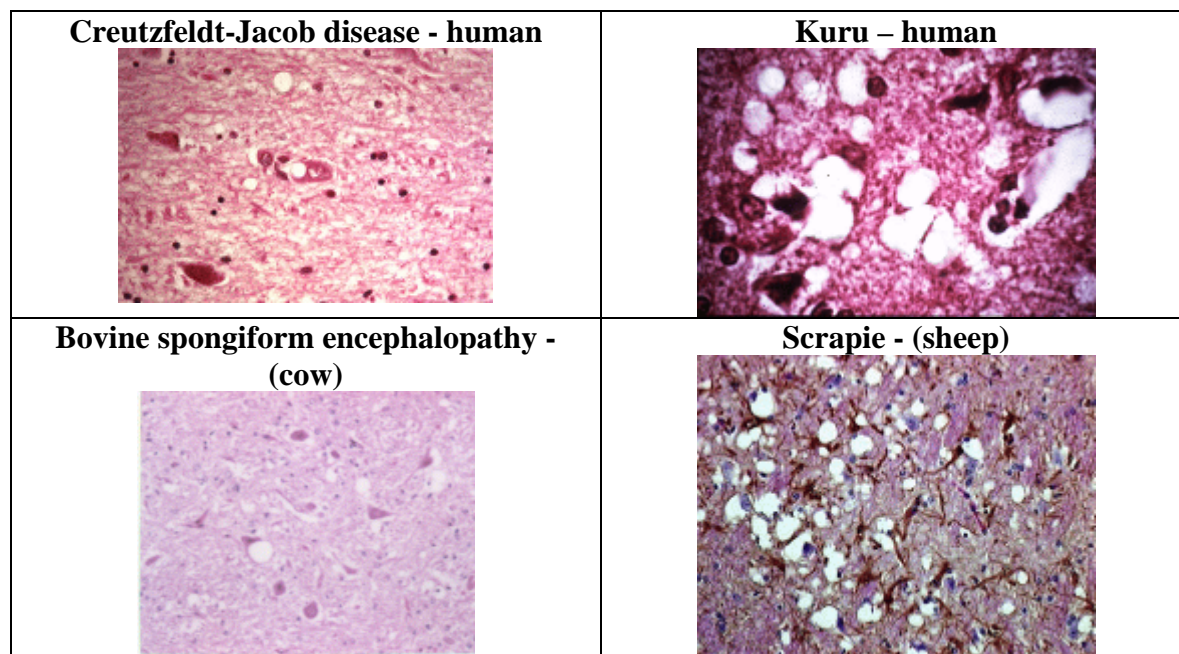


Figure 1. Pathology of Prion Disease in Humans and Animals

Table 1. Prion Diseases in Humans and Animals

Human	Animal
<p>Spongiform</p> <ul style="list-style-type: none"> ▪ Creutzfeldt-Jacob disease (CJD): sporadic, iatrogenic, familial ▪ Kuru ▪ Sporadic and familial insomnia ▪ Acquired variant CJD 	<p>Spongiform</p> <ul style="list-style-type: none"> ▪ Scrapie – sheep ▪ Bovine spongiform encephalopathy ▪ Transmissible mink encephalopathy ▪ Chronic wasting disease of mule deer & elk

(vCJD)	<ul style="list-style-type: none"> ▪ Feline spongiform encephalopathy ▪ Encephalopathies in zoo animals*
Dominantly Inherited <ul style="list-style-type: none"> ▪ Gerstmann-Straussler-Scheinker Syndrome (GSS) 	
Acquired by ingestion <ul style="list-style-type: none"> ▪ Variant CJD 	

* ankole, Arabian oryx, bison, cheetah, eland, gemsbok, greater kudu, nyala, ocelot, puma, Scimitar horned oryx, and tiger (Jeffrey and Wells, 1988).

D. History of TSE Diseases

Though sporadic CJD occurs spontaneously in the human population at the rate of one per million people per year, prion-related disease epidemics tend to result from acquired TSEs. The first prion-related epidemic disease was documented in 1957 in Papua New Guinea among native tribes known to practice ritualistic cannibalism (Zigas and Gadjusek, 1957). The disease was called "kuru" and was closely related to Cruetzfeldt Jacob Disease (CJD). However, the first major outbreak of transmissible encephalopathies was not diagnosed until 1985 when mad cow disease (i.e., bovine spongiform encephalopathy; BSE) first appeared in Great Britain. By 1990, 14,324 cases of BSE were confirmed in British cattle, out of an estimated population of 10 million cattle. In 1992 and 1993, more than 1,000 cases of BSE were reported each week, and in 1995 at the height of the epidemic, there were 146,000 cases of BSE. Other European countries also reported cases of BSE during this time including Switzerland (200 cases), Ireland (120 cases), and Portugal (30 cases). As the practice of including animal parts in cattle feed was stopped, the epidemic waned (Brown et al., 2001; Univ. of Wisconsin, 2004). However, since the introduction of monitoring programs to detect BSE in dead and slaughtered cattle, 12 countries have found their first cases of BSE: Austria, Czech Republic, Finland, Germany, Greece, Israel, Italy, Japan, Poland, Slovakia, Slovenia, and Spain. Small numbers of cases have also been reported in Canada, the Falkland Islands (Islas Malvinas) and Oman, but solely in animals imported from Britain (WHO, 2006).

In 1995 and 1996, a cluster of cases of variant CJD was diagnosed in humans in Britain. The link between BSE and vCJD was announced publicly on March 20, 1996. By the end of 2003, 145 cases of vCJD had appeared in Great Britain. Since then, the annual number of new vCJD cases has declined, from a high of 28 in 2000 to 1 in 2007 (<http://www.cjd.ed.ac.uk/cjdq56.pdf>). The first case of mad cow disease reported in the United States was on December 23, 2003 on a dairy farm in the state of Washington in a Holstein purchased from Canada. As a result, 4,000 cattle on the ranch were destroyed. A second case of BSE was detected in November 2004 in a cow of unknown origin and born before the U.S. ban on the use of mammalian protein in ruminant feed (diagnosis final in June 2005) (<http://www.cfsan.fda.gov/~comm/bsefaq.html>). The third and most recent case of BSE was discovered in Alabama

(http://www.aphis.usda.gov/newsroom/hot_issues/bse/downloads/EPI_Final5-2-06.pdf; Heaton et al., 2008).

To date, three cases of vCJD have been reported in the United States. The first and second patients (dates of onset 2001 and 2005, respectively) were both born in the United Kingdom and resided there during the defined period of risk for transmitted TSEs from cattle (1980-1996). A third US case of vCJD (onset and diagnosis, 2006) is likely to have originated in Saudi Arabia where the patient was born, raised and resided until 2005. None of the patients had medical histories to which transmission could be attributed (http://www.cdc.gov/ncidod/dvrd/vcjd/factsheet_nvcjd.htm).

Chronic Wasting Disease (CWD) was first recognized as a clinical "wasting" syndrome in 1967 in captive mule deer in a wildlife research facility in Colorado. It was identified as a Transmissible Spongiform Encephalopathy (TSE) in 1978. In 1981, CWD was detected in a wild elk and in 1985 it was detected in a wild mule deer in Colorado; shortly thereafter it was shown to be present as an endemic focus in wild deer and elk in northeastern Colorado and southeastern Wyoming. Since nationwide wildlife surveillance was instituted in 1997, CWD has been identified in wild deer and elk in several additional states: Illinois, Kansas, Nebraska, New Mexico, New York, South Dakota, Utah, West Virginia, and Wisconsin (www.aphis.usda.gov). However, numbers of positive animals remain low. For example, in 2002, only 302 samples were positive out of 91,636 samples tested (a prevalence of 0.3%) (www.aphis.usda.gov). Within endemic areas, the prevalence of CWD has been estimated at <1% in elk and <1% to 15% in mule deer (Williams et al. 2002). Despite these low endemic levels, models suggest that epidemics of CWD could lead to local extinctions of infected deer populations although it is not known how the disease might become epidemic (Miller et al. 2000). There is no evidence of human cases of prion disease linked with CWD.



Distribution of CWD (www.aphis.usda.gov)

Scrapie, a neurological disease of sheep, has been present in Europe since the early 18th century. Affected sheep were observed rubbing, or "scraping," their coat against a tree or a fence post as if it itched. The disease is generally fatal after a long incubation period. It is estimated that scrapie costs the U.S. sheep industry \$20 million per year in direct losses (USDA, 2000). There are no known human cases associated with scrapie infected sheep. Scrapie infected offal in cattle feed may have been the source of BSE.

Approximately 100 cases of feline spongiform encephalopathy (FSE) have been reported in Great Britain and Europe during the BSE epidemic in the early to mid 1990's (Cornell Feline Health Center, 2004). British cats were believed to have gotten the disease by eating BSE-contaminated commercial cat food and butcher scraps. The number of new cases in domestic cats dropped off once measures were put into place to prevent materials potentially contaminated with BSE prions from entering the food chain. No new cases have been reported since 1999. No cases in U.S. cats have ever been reported (Cornell Feline Health Center, 2006). Britain also reported cases in captive exotic cats including nine cases in cheetahs (three were diagnosed

abroad but originated in Britain), three in pumas, three in ocelots, two in tigers and two in lions (Cornell Feline Health Center, 2004).

E. Transmission and Potential Routes of Exposure

TSEs can be transmitted naturally and experimentally. The most efficient route of experimental transmission is intracerebral injection of infectious brain tissue (Weissman et al. 2002a). Intraocular, intraspinal, intravenous, intramuscular, intraperitoneal, or subcutaneous injections, scarification, or oral administration have also been shown to be effective methods of transmission in the laboratory setting. Experimental transmission via the dental route has also been reported (Ingrosso et al. 1999). Consumption of contaminated food is generally thought to explain the natural pathogenesis of scrapie in sheep, the spread of kuru, and the human acquisition of vCJD from BSE-contaminated beef; however, the precise details about the fate of infectious prions in association with other food constituents as they pass through the alimentary canal and cross the gut mucosa to produce CNS disease remains elusive (Jeffrey et al. 2006; Rose et al. 2006; Austbø et al. 2007; Scherbet et al. 2007; Solomon et al. 2007).

Natural transmission routes of prions vary among TSEs. Transmission of scrapie among sheep and goats is not completely understood. The scrapie agent is believed to originate from the placentas of infected sheep and goats (Pattison et al. 1972) although scrapie-contaminated feces have also been proposed (Weissmann et al. 2002b). Both scenarios represent direct horizontal transmission. Some studies suggest that indirect, horizontal transmission occurs via the oral route, such as from contaminated environments (Palson, 1979) or by prionuria (Ligios et al. 2007). Vertical transmission (mother to offspring) of scrapie may be possible, but probably not significant. It is thought that a more likely route of transmission involves an early horizontal, post-natal event (Andreoletti et al. 2002).

Transmission of CWD among deer and elk is not completely understood. Both direct and indirect horizontal transmissions are known to occur via contact with infected animals and environments contaminated with excreta or decomposed carcasses of infected animals (Miller et al. 2004). In this regard, prion contaminated material in soil remains infectious for years (Brown and Gajdusek 1991; Seidel et al. 2007) and may serve as a reservoir of disease. Purified PrP^{Sc} protein has been shown to adhere to soil minerals and remain infectious (Johnson et al. 2006; Ma et al. 2007), and this soil binding may actually enhance infectivity (Johnson et al. 2007), possibly by a mechanism comparable to the recognized durability and seemingly enhanced infectivity of prions bound to surgical steel instrument surfaces (Zobeley et al. 1999; Flechig et al. 2001; Peretz et al. 2006). It is also recognized that, as with BSE, maternal transmission of CWD likely occurs, but at low levels (Miller and Williams, 2003; Wells and Wilesmith, 2004). A recent study confirms transmission of CWD in deer via blood and saliva, raising caution regarding contact with body fluids from infected animals (Mathiason et al. 2006). Currently, there is no evidence of a link between CWD and unusual cases of CJD in humans, though data are limited and more studies are necessary (Belay et al. 2004).

Several animal TSEs are associated with consumption of contaminated feed. BSE represents a large-scale common source epidemic transmitted by the feeding of BSE prion-contaminated meat and bone meal to cattle (Wilesmith et al. 1991). Meat and bone meal are

prepared from the offal of cattle, sheep, pigs, and chicken and fed to cattle as a high protein nutritional supplement. For the U.S., a recent study by Heaton et al. (2008) surveyed over 6,000 cattle, including five commercial beef processing plants, and determined that BSE cases with the K211 allele that occurred in Alabama would be unlikely. Similarly, in cats, feline spongiform encephalopathy (FSE) has been linked with consumption of commercial cat food and butcher scraps contaminated with BSE, and transmissible mink encephalopathy (TME) is associated with the feeding of tissue from scrapie-infected sheep or BSE-infected cattle (Chesebro, 2003).

Human TSEs are associated with a variety of transmission routes. Most cases of CJD are classified as sporadic and are likely to result from a somatic mutation, the spontaneous conversion of PrP^C to PrP^{Sc}, or reduced clearance of low levels of PrP^{Sc} that may normally be present (Peretz et al. 2001). However, CJD can be transmitted by exposure to contaminated surgical instruments or electroencephalogram electrodes, or exposure to infectious brain, pituitary, or ocular tissue or associated products via organ transplantation or other medical procedures (Blattler, 2002).

Both kuru and vCJD are caused by ingestion of contaminated material. Kuru, the TSE of New Guinea natives, is associated with consumption of infected human tissue during cannibalistic rituals. Exposure is also believed to have occurred through abrasions of oropharyngeal or conjunctival mucous membranes, as well as via open wounds on the hands (Gajdusek, 1977). Variant CJD (vCJD) is associated with consumption of BSE-contaminated beef products (Will et al. 1996, Collinge et al. 1996, Bruce et al. 1997, Will et al. 1999). Additionally, two cases of transmission of vCJD via blood transfusion have also been reported (one case was manifested clinically and the second was identified at autopsy following death from an unrelated cause) (Llewelyn et al. 2004, Peden et al. 2004).

Prions have presented a major public health problem in the UK with the emergence of mad cow disease. Through a major effort in the UK and other countries, this disease appears to be under control. However, because so little is understood about prions, they will remain an important public health issue for the foreseeable future. Certain prion diseases are infectious to humans, including BSE or mad cow disease, but it is not clear if the measures taken so far will adequately prevent future occurrences. In addition, scientists do not completely understand several aspects of prion diseases, or whether effective treatments might be found through understanding of the misfolding and infectivity of the fibrillar structure (Karpuz et al. 2007; Supattapone et al. 1999, 2001). Finally, the epidemic of chronic wasting disease in deer and elk populations in the Western USA continues to spread, but the implications of this disease for humans remains unknown.

Rapid advances in recombinant and cell free technologies are attacking the most puzzling question about the prion diseases (Dong et al. 2007; Müller et al. 2007; Tessier and Lindquist 2007). Why are they transmissible whereas other neural protein misfolding diseases such as Alzheimer's and similar amyloidoses are not? In addition to known hereditary prion diseases, it is an especially worrisome health problem that normal prion protein can also misfold under conditions that have not been defined to produce spontaneous TSE disease. For example, the UK commission report concluded that the catastrophic epidemic of mad cow (1986 – 2000) began with the spontaneous emergence of an abnormal prion, followed by using contaminated

bone meal to feed other cattle. The resultant intense surveillance now shows low level BSE in other countries – including three recent cases in the USA.

In summary, prions are a persistent problem but the extent to which these diseases will pose a public health threat in the future is unclear.

F. Existing Occupational Safety Procedures

Because TSEs are transmissible to humans by various routes (as described above), standard protective measures have been put in place to prevent or minimize occupational exposure to human or animal tissues or waste materials contaminated or potentially contaminated with prions. These measures are pertinent to health-care and veterinary care providers, mortuary workers, and laboratory workers, all of whom may handle or be exposed to contaminated blood, tissues, or waste materials. Standard protective procedures include administrative and engineering controls, special work practices, personal hygiene measures, personal protective clothing, and decontamination methods.

The Centers for Disease Control and Prevention (CDC) has published two documents that provide guidance for health-care workers pertaining to environmental infection control generally and to “special pathogens” (including prions) specifically. *Guidelines for Handwashing and Hospital Environmental Control* (Bolyard et al. 1998; CDC/HICPAC 2003) encompasses general infection control, with emphasis on the handling of air, water, and environmental surfaces; environmental sampling; and disposal of regulated and unregulated medical waste. *Guidelines for Environmental Infection Control in Health-Care Facilities* (CDC 2003) provides extensive, detailed guidance on all aspects of infection control in health-care facilities and specifically addresses Creutzfeldt-Jakob Disease (CJD) in patient-care areas. This guidance notes that a limited number of cases result from direct exposure to prion-containing material (usually central nervous system tissue or pituitary hormones) acquired as a result of health care (i.e., iatrogenic cases). Six documented iatrogenic cases were associated with neurosurgical instruments and devices that introduced residual contamination directly to the recipient’s brain. Because there is no evidence to suggest that either CJD or vCJD has been transmitted from environmental surfaces (e.g., during housekeeping activities), CDC states that routine procedures are adequate for cleaning and disinfection of a CJD patient’s room. However, the CDC recommends that hospitals identify patients with known or suspected CJD and implement prion-specific infection-control measures for the operating room and for instrument reprocessing. Such measures should include:

- Use of disposable, impermeable coverings during autopsies and neurosurgeries to minimize surface contamination
- Cleaning and decontamination of surfaces that have been contaminated with central nervous system tissue or cerebral spinal fluid (see decontamination methods described in next section).

In 2000, the World Health Organization issued its guidance document, *WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies* (WHO, 2000), which focuses specifically on prion diseases. In this document, the WHO designated central nervous

system tissues and fluids as “high-infectivity,” which should be managed more conservatively than others. Key recommendations include the following:

- Covering patient treatment surfaces with disposable non-permeable covers and uncovered surfaces are to be decontaminated.
- Clinicians involved in patient handling are to wear single-use disposal coverings (mask or visor, goggles, cut-resistant gloves, and liquid-repellent aprons over plastic gowns).
- Treatments are to be conducted with equipment and tools that either are dedicated to a single patient, or are disposed after a single use.
- Instruments that have contacted high-infectivity tissues are to be disposed by incineration, as are all single-use coverings and tools.
- Reusable instruments are to be kept moist until cleaning and decontamination; this measure is needed to prevent drying of tissues or fluids thereon.
- Low- and high-infectivity instruments are to be stored separately between and during uses.
- Bulk fluids are to be decontaminated or absorbed into sawdust, then incinerated.
- Contaminated medical wastes are to be incinerated, as are the disposable materials.
- All incineration is to be conducted in hazardous-waste facilities.

The WHO document provides similar specific guidelines for mortuary and laboratory workers who may contact contaminated materials, and for decontamination of clinical or laboratory workers after occupational exposures. Safety recommendations are provided also for the transport of contaminated specimens by air.

With respect to veterinary laboratories, the American Association of Veterinary Laboratory Diagnosticians (AAVLD) has issued recommendations aimed at reducing potential risks to laboratory personnel posed by TSE agents in animal tissues and waste materials (AAVLD, 2004). The AAVLD recommends:

- Using standard BSL2 safety precautions such as restricted access to laboratories, protective clothing, and facial protection, special care with sharp instruments, and avoidance of aerosols.
- Numerous specific protective measures for necropsy laboratories, histology laboratories, and laboratories handling fresh tissues from TSE suspect animals, such as extensive protective clothing, screened drains to trap tissue fragments, use of appropriate disinfectants to inactivate TSE agents on surfaces and non-disposable items, and disposal of contaminated tissues by certain methods.

G. Decontamination Methods

A number of decontamination methods have been developed and tested for their ability to inactivate prions on inanimate surfaces and on objects that may have contacted infected animal or human tissues. This development and testing has been especially spurred by the successful migration of BSE into the human population during the mad cow epidemic (UK 1986-2000) and by the compelling need for methods to decontaminate environmental surfaces and instruments in

veterinary facilities, research laboratories, health care settings, and other settings in which prion-contamination is a possibility .

The infectious conformation of prion proteins is extraordinarily durable, and the measures necessary to destroy such prion proteins are extreme compared to decontamination methods for other biological pathogens, including bacterial spores. In particular, prions bind tightly to stainless steel and are most difficult to inactivate on this surface (Zobeley et al. 1999)

Some of the currently recommended decontamination methods include treatment with 1-2 N NaOH or NaOCl (2%, or 20,000 ppm chlorine) for one hour followed by heat in a gravity displacement autoclave at 121° C for one hour, autoclaving at 134° C for 1 hr (or up to 5 hrs as in Peretz et al. 2006), or complete incineration (850° C or higher as in Brown et al. 2004). General descriptions of these procedures are found in several advisory reports, such as those of the CDC (1999), WHO (2000), AAVLD (2004), Canadian Food Inspection Agency (2005) and MSU (2005). However, these recommended decontamination methods are based on limited academic research, and a number of issues bring into question whether these methods can demonstrate a disinfectant's ability to totally inactivate prions on inanimate surfaces and objects.

1. The recommended prion-inactivation methods have not been tested using a validated efficacy test method, and such a method does not yet exist. Currently, EPA requires that disinfectants and sterilants be tested using specific, validated efficacy test methods before EPA will register such products, and such test methods are available (e.g., AOAC Use Dilution Test). However, no one has formally validated any of the prion-inactivation efficacy test methods used to date, so it is difficult to know whether such efficacy test methods provide consistent, reliable data.
2. The risk of transmissible infectivity persists at prion titers far below the limits of detection using analytical methods, such as gel electrophoresis and Western blot assay (Yamamoto et al. 2001; Solassol et al. 2006). For example, Gregori et al. (2003) found that assaying for surviving prion infectivity in hamsters was 1000 fold more sensitive than visual detection by Western blot assay. Consequently, adequate confirmation in an animal model is required to confirm effective elimination of all prion infectivity.
3. What constitutes an adequate animal test model for determining the prion-inactivating efficacy of chemicals is uncertain. Although the Syrian hamster (Marsh and Kimberlin, 1975) and mouse (Chandler, 1961) have a long history of utility in prion research, the life span of these laboratory animals is relatively short, whereas the time interval from prion exposure to the development of clinical disease can span decades of time, as in the human prion diseases, for example. In experimental models, it is known that as the titer of infective material decreases, the incubation time for the development of clinical disease in test animals increases (Jackson et al. 2005; McDonnell et al. 2005; Solassol et al. 2006). Hence, the life span of the test animal may be too short to detect residual prion infectivity at very low titers, as might be expected with cleaning procedures that have been largely, but not entirely, effective. Ideally, efficacy studies should include positive controls to determine the relationships between prion titer, infectivity, and the life span of the test animal. Otherwise, the absence of clinical signs during the short lifespan of the

animal model might be mistaken as evidence of successful elimination of infectivity. Attempts to accelerate the progression of infectivity by using synthetic mammalian prions (Legname et al. 2004), as well as PrP over-expressing cell lines (Solassol et al. 2004) and animals (for example, Peretz et al. 2006) potentially point to ways to solve the lifespan problem, but these approaches create new uncertainties by using animals already predisposed to overexpression of host or foreign prion strains.

4. In addition to the common use of different species of test animals, hamster and mouse, many different strains of prions are employed in decontamination research. These experimental variables might account for the discrepant results for Envion LpH obtained by Ernst and Race (1993) and Race and Raymond (2004), using Syrian hamsters as the test model, with those of Jackson et al. (2005), using mice.
5. There are important barriers to the transmission of prion diseases between animal species and humans, and the degree of transmission of prion diseases from animals to humans varies greatly. For example, experimental studies using human and elk prion genes expressed in a transgenic mouse indicate that elk chronic wasting disease may not transmit readily to humans (Kong et al. 2005). On the other hand, the mad cow epidemic in the UK (1986-2000) demonstrates that BSE readily crosses the species barrier and is infectious to humans as vCJD (Brown, 2001). Since there are no systematic studies of comparative infectivity of prion strains across different animal species and humans, it is not clear how precisely one can extrapolate human risk from efficacy studies performed in various animal species. Specifically, some of the strains used in efficacy studies may pose little or no risk to humans. Conversely, it seems possible that some prion strains dangerous to humans may not be testable in lab animals commonly used in efficacy studies. Finally, imperfect decontamination procedures may have consequences for risks to humans that vary from inconsequential to very significant, depending on the strain of prion being inactivated. For example, sheep scrapie is clinically unknown in humans and so the risks to humans resulting from imperfect decontamination of scrapie would be expected to be negligible compared to the risks from imperfect inactivation of BSE prions on surfaces to which humans are exposed.
6. Similarly, comparisons of the prion proteins of different species in a common animal model indicate there may be profound differences in the prions of different species with regard to their stability and resistance to inactivation during decontamination procedures. For example, Peretz et al. (2006) compared the resistance of hamster scrapie and human CJD prions in transgenic mice expressing either hamster PrP or a chimeric mouse-human PrP transgene and found that human sCJD prion is 100,000 fold more difficult to inactivate than hamster Sc237 prion. Preliminary additional studies indicate that the cow BSE prion may be even more resistant to inactivation than the human CJD prion (Giles et al. 2006; 2008 in press). This approach and the results obtained by the direct comparison of different prion strains in common test models indicate that decontamination procedures tested with rodent prions may not be effective when tested with more durable human and cow prions.

Accordingly, although a number of academic studies have demonstrated that certain chemical and/or physical decontamination methods provide some assurance of reduction of prion infectivity, current test methods cannot demonstrate that a disinfectant can totally inactivate prions or TSE agents on inanimate surfaces and objects.

III. The Science of Prions As It Relates to the FIFRA Definition of “Pest”

The Introduction to this paper described two sections of FIFRA that define or give the EPA Administrator the authority to define the term “pest.” This section addresses the extent to which the available scientific information on prions summarized in Section II relates to the issue of whether prions fit within the definition of “pest” specified in FIFRA Sections 2(t) or 25(c)(1).

A. How Do Prions Relate to Common Definitions of “Microorganism”?

The definition of “pest” in FIFRA Section 2(t) has two parts. The first part is whether the pest occurs in “circumstances that make it deleterious to man or the environment.” Based on the available information described in Section II., prions clearly are deleterious to humans and animals. Exposure may lead to infectious acquisition of irreversible and fatal TSE diseases.

The second part of the definition of “pest” relevant to prions revolves around whether prions are “any other form of ... virus, bacteria or other microorganism....” Prions are neither viruses nor bacteria, as discussed in the preceding sections, so if prions are to be considered pests under FIFRA they would have to be “other micro-organisms”.

Although there is no single, definitive definition of “microorganism”, the definition in the Oxford Dictionary of Biology (Fourth Edition, 2000) is representative.¹ The Oxford Dictionary of Biology defines “microorganism” as “Any organism that can be observed only with the aid of a microscope. Microorganisms include bacteria, viruses, protists (including certain algae), and fungi.” “Organism” is further defined by the Oxford Dictionary as “An individual living system, such as an animal, plant, or microorganism, that is capable of reproduction, growth, and maintenance.” These latter terms are defined by Dorland’s Medical Dictionary as follows:

- **Reproduction**—the sexual or asexual process by which organisms generate others of the same kind
- **Growth**—the normal process of increase in size of an organism by accretion of tissue similar to that originally present
- **Maintenance**—the process of holding a stable, steady state over a long period

Therefore, whether prions fall within this definition of “microorganism” as it is generally used by biologists and the medical community would depend on whether they exhibit reproduction, growth and maintenance. The research studies summarized in Chapter II indicate

¹ This definition and those that follow were chosen from among several available to EPA. Those discussed herein were selected based on their inclusion in authoritative sources, their completeness, and their utility in addressing the scientific arguments considered.

that a prion protein (whether normal or misfolded) does not have these characteristics. The normal prion protein is a cellular protein that is transcribed by a gene ubiquitous in vertebrate species. It cannot reproduce, grow or perform self-maintenance as microorganisms do. When a prion protein becomes misfolded, it converts normal prion proteins to adopt the same misfolded conformation through recruitment and conversion. Thus, a misfolded prion protein cannot reproduce itself; it can only induce other prion proteins to take on a misfolded shape. All other infectious agents—algae, fungi, bacteria, viruses and viroids—produce infectious diseases by infecting and propagating their numbers in the host species, which is accomplished through genetic reproduction and the propagation of new generations from genes comprised of DNA or RNA. Unlike these other infectious agents, the aggregated particles of a prion protein are devoid of any genetic element and have no mechanism for genetic reproduction. The property of infectivity is limited to that of misfolded prion protein converting other, existing, homologous protein to adopt the same misfolded conformation. There is neither synthesis of new prion protein nor of genetic material. Finally, recognition that prions are distinct from the entities that biologists and the medical community generally recognize as microorganisms lies at the heart of Nobel prize for the discovery that prion particles are (a) comprised solely of protein and (b) are the causative agent of a novel infectious process (Prusiner, Nobel Prize, 1997).

Although prions do not fit the criteria biologists generally use to define microorganisms, the term “microorganism” is sometimes used more broadly. For example, the Oxford Dictionary of Biology’s definition for “microorganism” cited above includes viruses as microorganisms, even though they do not have all of the characteristics of an “organism” as listed above. Viruses cannot reproduce by themselves, but do replicate themselves by injecting their DNA into host cells and taking over the host cell’s reproductive machinery. Nevertheless, they are often grouped with bacteria and other entities that meet the entire microorganism criteria described above.² In the absence of a more general term that expressly includes viruses and other infectious agents that do not meet all the criteria of microorganisms, this broader use of the term “microorganism” is common among professionals in the biological, medical and public health sciences, at least in casual speech.

B. Are Prions Alive?

As mentioned above, FIFRA Section 25(c)(1) authorizes the EPA to declare “a pest any form of plant or animal life...which is injurious to health or the environment.” Because prions are already known to be injurious to human and animal health, the remaining issue is whether prions are a “form of life.”

Dorland’s Medical Dictionary (2004), defines “life” as:

² Microbiology textbooks also routinely devote significant space to viruses; see, for example: Textbook of Diagnostic Microbiology, Connie Mahon, George Manuselis, Donald Lehman (3d ed., Elsevier 2006); Microbiology: Principles and Explorations, Jacquelyn G. Black (6th ed. Wiley 2005); Bailey & Scott’s Diagnostic Microbiology, Betty A. Forbes, Alice S. Weissfeld, Daniel F. Sahm (Elsevier Health Sciences 2007); Medical Microbiology, Patrick R. Murray, Ken S. Rosenthal, Michael A. Pfaller, Ken S. Rosenthal (Elsevier Health Sciences 2005); Koneman’s Color Atlas and Textbook of Diagnostic Microbiology, Elmer W. Koneman, Stephen D. Allen, Gail L. Woods, William M. Janda, Paul C. Schreckenberger (Lippincott Williams & Wilkins 2005); Sherris Medical Microbiology: An Introduction to Infectious Diseases, Kenneth J. Ryan, C. George Ray, John C. Sherris (McGraw-Hill 2003).

“The aggregate of vital phenomena; a certain peculiar stimulated condition of organized matter; that obscure principle whereby organized beings are peculiarly endowed with certain powers and functions not associated with inorganic matter. Generally, living things share, in varying degrees, the following characteristics: organization, irritability, movement, growth, reproduction, and adaptation.”

This definition adds four more characteristics to the characteristics beyond those included in the definition of “microorganism” and “organism” and addressed in the preceding section (i.e., reproduction, growth, metabolism). These additional characteristics of living things are:

- **Organization**—the act or process of being organized.
- **Irritability**—the property of living organisms that allows them to respond to stimuli.
- **Movement**—the act or process of changing of place or position.
- **Adaptation**—adjustment to environmental conditions by modification of an organism to make it more fit for existence.

Of the six properties of living things identified in the Dorland definition, the only property of living things that prions share is organization. Prions are made of protein, which has four levels of organization (Leninger, 1970):

- Primary structure is the sequence of amino acids that comprise one polypeptide chain.
- Secondary structure describes the polypeptide chain's repetitive domains of alpha (helical) and beta (pleated sheets) structures.
- Tertiary structure describes the full, 3-dimensional folding conformation of the polypeptide, which is determined by the content of alpha and beta structures, as well as by disulfide cross-linkages.
- Quaternary describes the organization of individual polypeptide chains to form a larger, more complex molecule comprised of individual subunits, such as first described in hemoglobin. [Note: prions are single polypeptide chains, so they do not have this level of organization.]

Although prions feature the above kinds of “organization,” they do not differ in this respect from any other biological proteins. Inasmuch as other biological proteins are not generally considered to be forms of life, these kinds of “organization” do not appear indicative of forms of life. With respect to “irritability” and “movement,” prions are not capable of reacting to stimuli or initiating movement on their own. Finally, with respect to “adaptation,” prions have no known mechanism for either adapting to their environment or passing along their characteristics via DNA because they contain no nucleic acids.

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