ENVIRONMENT, HEALTH & SAFETY
UNIVERSITY OF MICHIGAN

Prion and Prion-like Protein Guidance

What are Prions?

A protein is made as a linear chain of amino acids, and interactions between these amino acids allows the chain to fold into a specific three-dimensional (3D) shape. In some instances, proteins can misfold and clump together to form aggregates, which can be toxic to normal, healthy cells[1]. Prions are misfolded versions of a protein, that can be cytotoxic. Prions are considered infectious because the toxic fold of the protein that can spread by self-propagating and binding to normal copies of the protein causing the misfolded shape. Prions are the infectious agents responsible for prion diseases, also known as Transmissible Spongiform Encephalopathies (TSE).

Prion Diseases

TSE's cause neurodegenerative disease and can be fatal in humans and other animals. Examples of prion diseases:

Human Prion Diseases	
Kuru	Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease (CJD)	Sporadic, variant, familial/genetic, Iatrogenic.
• Sporadic	Unknown mechanism, or mutations
• Variant	Possibly from consumption of contaminated cattle products, or bloodborne transmission
• Familial	germline mutations
• Iatrogenic	From contaminated corneal or dura mater grafts, pituitary hormone or neurosurgical equipment
Gerstmann-Straussler-Scheinker syndrome	Germline mutations
Animal Prion Diseases	
Scrapie	Infections in genetically susceptible sheep, goats
Bovine Spongiform Encephalopathy (BSE)	Cattle infected from contaminated feedstuffs
Chronic wasting disease (CWD)	Deer, elk, moose infected from contaminated feces, urine, feed, water, environment

Characteristics

- Highly resistant to inactivation by heat and chemicals.
- Transmissible by inoculation, ingestion (e.g. consumption of infected tissues), or transplantation of infected tissues or homogenates (e.g. medical procedures using contaminated materials).
- Under experimental conditions, prion diseases have been transmitted through aerosols.

- Historically, a primary route of infection was through medical procedures with prion-contaminated materials. In this procedure, people were treated with human growth hormone derived from the pituitary glands from cadavers contaminated with prions and incubation periods exceeded five decades[2].
- Prion infectivity is highest in the brain and other central nervous system tissues.
- Prions are most effective in infecting homologous species but cross-species infection is possible.
- There are no vaccines or effective treatments/therapeutics for prion diseases.

Prion-like, Prionoid, Proteopathic Seeds[3]

Proteins (other than the PrP protein) with the ability to have abnormal conformations and share pathological properties with PrP prions. Currently, the key difference between these proteins and prions is that although the prion-like proteins are considered to be infectious, they are not considered to be transmissible, by standard definition. Additionally, there are no known animal or human epidemics or established occupational risks. However, there is increasing evidence that these proteins can self-propagate in a prion-like manner and cause transmissible diseases in humans and laboratory animals. See examples below[4]:

- Alpha-synuclein protein (Parkinson's disease)
- Tau protein (tauopathies)
- Beta-amyloid protein (Alzheimer's disease)
- Polyglutamine-containing proteins (polyQ) (Huntington's disease)
- Ability to "seed" a pathology or cause a disease

UM-EHS conducted a literature review of previous and current research to provide biosafety guidance for conducting research with these materials.

The misfolded form of proteins associated with neurodegenerative diseases have been shown to be transmissible in animal models and cell culture[5]. Findings in experimental models have raised concern about the human-to-human transmissibility of such proteins. In recent years, increasing experimental data has shown emerging similarities and overlapping properties between many neurodegenerative disease related proteins and prions. Although there is limited data to support infectivity of these prion-like proteins, evidence has shown that their replication and propagation have similar pathogenic mechanisms to prions.

- Injection of proteopathic seeds, especially amyloid β, into the brain of transgenic mice who overexpress amyloid-beta precursor protein were shown to develop amyloid β aggregates[5].
- Experimental data in animals has shown that the inoculation of misfolded tau derived from human brain can be taken up by neurons and cause misfolded mouse tau[6].
- The inoculation of brain-derived or synthetic alpha-synuclein seeds within mice resulted in progressive neurodegenerative disorders that were similar to that of human Parkinson's disease or multiple system atrophy[7].
- Protein "seeding" (a misfolded protein acts as a seed and initiates aggregates of the same unfolded protein species) was observed experimentally in Alzheimer's studies. Findings in mice showed that β-amyloid extracts derived from brains of Alzheimer's disease caused the deposition of β-amyloid[7]. Further experiments looked at the ability of tau to enter cells in culture and concluded the possibility that tau can move from one brain cell to another[8].

- Mutant human tau injected into mouse brains was shown to induce aggregation of wild-type mouse protein and spread from the injection site to neighboring brain regions[9].
- Three mechanisms of transmission of protein aggregates from cell to cell have been seen. Tunneling nanotubes, secretion as naked aggregates and packaging into extracellular vesicles[10].
- Prion-like proteins can be transmitted to a phylogenetically unrelated cell as long as the recipient cell expresses a soluble protein and template its amyloid conformation[11].
- Aerosol transmission of prions has been observed experimentally, therefore, it should not be ruled out that prion like proteins may be transmitted in a similar manner[12] and the standard microbiological practice of minimizing the generation of aerosols should always be followed.

Laboratory Acquired Illnesses

2019 – French laboratory worker died at the age of 33 from variant Creutzfeldt-Jakob disease (vCJD), 10 years after puncturing her thumb during an experiment with prion-infected mice. The lab worker stabbed her thumb piercing two layers of gloves with curved forceps while cleaning a cryostat, used to slice brain sections from transgenic mice infected with a sheep-adapted form of BSE[13].

2021- A French retired lab worker who handled prions in the past was diagnosed with (CJD). This incident is under investigation as to whether or not the person contracted the disease on the job[13].

Biosafety

Due to the infectious nature of prions and prion-like proteins, consequence of disease potential, long latencies, and the resistance to traditional biohazardous inactivation methods, work with these materials requires added biosafety considerations and practices to minimize exposure risk.

Human Prions[14]

Prions are classified as risk group 3 by the NIH Guidelines. Biosafety level may be BSL2 or BSL3 based on facility specific risk assessment.

Examples of situations requiring BSL3;

- Direct work with prions
    - Extraction, purification
- Work with BSE proteins which pose a high human and agricultural risk

Examples of situations requiring BSL2;

- Use of human tissues with suspected or confirmed prion positivity for clinical or diagnostic purposes
- Immunostaining

Additional practices include;

- Use of prion-dedicated equipment
- Prion specific decontamination procedure
- Avoid use of needles or sharps whenever possible
- Perform all manipulations in Biosafety Cabinet (BSC) or containment device

Higher risk materials include brain, dura mater, CNS tissues, samples taken from known prion disease, and samples taken from a donor with unknown medical history. Low risk materials such as blood, urine, saliva, cerebral spinal fluid and most non-CNS tissues are believed to contain very low concentrations of prions.

Sample Type Containment Guide		
Risk Level	**Tissue Type/Source**	**Biosafety Level**
High	Sample from known prion disease	BSL2/3
High	Sample from donor with unknown history	BSL2/3
High	Brain, dura mater, CNS tissues	BSL2/3
Medium	Sample from donor with known neurodegenerative disease	BSL2
Low	Blood, saliva, non-CNS tissues	BSL2
Low	Sample from donor with known history excluding prion or other neurodegenerative diseases	BSL2

Animal Prions[14]

Bovine spongiform encephalopathy (BSE) is the only animal prion disease considered zoonotic and capable of causing variant Creutzfeldt-Jakob disease (vCJD) in humans[15]. There is no evidence that other animal prion diseases are associated with human disease but data is limited. Experiments in non-human primates and mice suggest that other diseases such as Scrapie, Chronic Wasting Disease (CWD), and L-type atypical BSE might have zoonotic potential[15]. Biosafety level will be based on facility specific risk assessment.

- Work with BSE proteins pose a high human and agricultural risk and will predominantly be classified as BSL3.
- All other animal prions may be manipulated at BSL2 with standard BSL2 practices.

Prion shedding occurs extensively in blood, saliva, urine, feces, milk by preclinical and clinically infected animals demonstrated in CWD and Scrapie[16,17].

Cross-species infection (with reduced efficiency) is possible. When a prion from one species is inoculated into another the infected animal should be treated according to the biosafety guidelines for either the source or recipient, whichever is more stringent.

Prion-like Proteins

Biosafety level of Prion-like, Prionoid, Proteopathic seeds may be BSL1 or BSL2 based on risk assessment. It is not unreasonable based on current research to consider additional biosafety practices for the handling of these materials.

Examples of situations that may require BSL2;

- Use of misfolded forms of prion-like proteins associated with neurodegenerative disease.
    - Purification or concentration of misfolded form
    - Manipulations to change native form to misfolded form
- Large volumes
- Aerosol-generating procedures
- Use of sharps

- Use of animals

Additional biosafety practices for BSL2;

- Laboratory specific standard operating procedure (SOP)
- Prion/Prion-like protein specific training
- Inactivation of sample(s), reusable materials, and surfaces: use treatment effective for prions
- Use of centrifuge safety cups
- Recommend performing all manipulations in BSC or other containment device whenever possible
- Recommend disposable instruments/materials instead of reusable

Prion-like, Prionoid, Proteopathic seeds Containment Guide		
Risk Level	**Sample Type**	**Biosafety Level**
High	Concentrated or amplified materials containing misfolded proteins	BSL2
High	Forms shown to share properties with prions such as self-propagating	BSL2
Medium	Genetically modified forms	BSL1/2
Low	Native normal protein	BSL1
Low	Proteins that are not associated with human neurodegenerative diseases	BSL1

Inactivation of prions

Most effective treatments include; incineration, enzymatic treatments with SDS, vaporized hydrogen peroxide, 4% SDS in 1% acetic acid at 65-134 degrees C, or mildly acidic hypochlorous acid[14].

Disposable instruments, materials & waste	Incineration
Biological Safety Cabinet	1 N NaOH or 50% v/v of 5.25% sodium hypochlorite bleach, and rinsed with water
Reusable instruments and surfaces	1 N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour followed by water rinse. 1 N NaOH equals 40 grams of NaOH per liter of water. Solution should be prepared daily
Routine staining	Slides are decontaminated by soaking them for 10–60 min in 2 N NaOH or sodium hypochlorite (20,000 ppm) followed by distilled water
Fixation of small tissue samples	96% absolute formic acid for 30 minutes, followed by 45 hours in fresh 10% formalin
Formalin-fixed and paraffin-embedded tissues remain infectious	Immerse for 30 minutes in 96% absolute formic acid or phenol before histopathologic processing

The following table from the World Health Organization lists ineffective/sub-optimal disinfectants[18]

Table 8 Ineffective or sub-optimal disinfectants

Chemical disinfectants	Gaseous disinfectants	Physical processes
Ineffective[17] alcohol ammonia ß-propiolactone formalin hydrochloric acid hydrogen peroxide peracetic acid phenolics sodium dodecyl sulfate (SDS) (5%)	Ineffective ethylene oxide formaldehyde	Ineffective boiling dry heat (<300°C) ionising, UV or microwave radiation
Variably or partially effective chlorine dioxide glutaraldehyde guanidinium thiocyanate (4 M) iodophores sodium dichloro-isocyanurate sodium metaperiodate urea (6 M)		Variably or partially effective autoclaving at 121°C for 15 minutes boiling in 3% sodium dodecyl sulfate (SDS)

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