PROTEOPATHIC SEEDS SAFETY POLICY

Research with disease-causing prions is subject to established regulatory and safety guidance from the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). However, guidelines for work with proteopathic seeds or prion-like proteins have not been widely established. Proteopathic seeds are more frequently being used as tools to study neurological disease progression, creating an emerging need for appropriate classification and safety and containment guidance. Common research tasks involving proteopathic seeds can include in vitro seeding of cells and tissues, in vivo use to model onset of neurodegenerative disease, and purification or synthesis of proteopathic seeds in high concentrations. This policy was developed by EH&S Biosafety and the Institutional Biosafety Committee (IBC).

SCOPE

This policy encompasses research and handling of proteopathic seeds that are associated with human neurodegenerative diseases and that can induce protein misfolding when experimentally administered. The following are examples of known proteopathic seeds and the disease that they are associated with:

Proteopathic Seed	**Associated Human Diseases**
Amyloid-beta peptide	Alzheimer's disease
Tau protein	Alzheimer's disease, frontotemporal dementia
Alpha-synuclein (aSyn)	Parkison's disease, Lewy body dementia, multiple system atrophy
Transactive response DNA-binding protein 43 (TDP-43)	Amyotrophic lateral sclerosis (ALS)
Superoxide dismutase-1 (SOD-1)	ALS
Fused in Sarcoma (FUS)	Juvenile ALS
Transthyretin (TTR)	Transthyretin amyloidosis
C9orf72	ALS

Excluded from this policy are peptides and proteins that may form extracellular amyloid deposits or intracellular inclusions but are not associated with human neurological disease.

KEY VOCABULARY

- **Proteopathic seeds**, also called prion-like proteins, are misfolded proteins that are associated with human degenerative disease pathologies including tauopathy of Alzheimer's disease and synucleinopathy of Parkinson's disease. There is little to no evidence of natural horizontal transmission of proteopathic seeds.
- **Prions** are misfolded proteins capable of inducing misfolding in normal variants of the same proteins, leading to damage and fatal disease. Prion diseases are transmissible, untreatable, and fatal diseases of mammals. They are highly resistant to disinfection.

HAZARD IDENTIFICATION AND RISK ASSESSMENT

Proteopathic seeds are associated with neurodegenerative disease and may present a hazard to human health but there are no established occupational hazards. There is building evidence from animal models that proteopathic seeds administered iatrogenically, meaning introduced through experimental administration, through inoculation or ingestion can seed protein misfolding and/or aggregation. The highest risk for exposure to personnel is from percutaneous or sharps injuries during administration. Other possible routes of exposure include ingestion, inhalation, and transplantation of infected tissues. In research, precautions must be taken when there is uncertainty surrounding a hazard. Conduct an appropriate risk assessment of your work and apply the safe handling practices to understand, reduce, or eliminate potential for exposure as much as possible.

RISK ASSESSMENT

Prior to work with proteopathic seeds, consider the risks and hazards of the materials and procedures. Use the following questions to assess the risk and hazards posed by the specific seed and procedure:

- What quantity and concentration are necessary for the procedure?
- Can they form fibrils in vitro? Can cells be seeded in vitro?
- Once seeded, can cell-to-cell propagation occur in vitro?
- Can they form fibril seeds or fragments that propagate and accelerate the misfolding of protein in a chain, in vitro?
- Are ex vivo fibril seeds from tissues capable of causing cell-to cell propagation?
- Do fibril seeds administered to animals propagate within the animal?
- Is there evidence of transmission in vivo?

HIGH AND LOW RISK ACTIVITIES

Procedures involving proteopathic seeds can be classified as high or low risk based on the potential for exposure and concentration of seeds used.

High risk activities include:

- In vitro work with concentrated, purified, or amplified forms of proteopathic seeds.
- In vitro work to produce aggregates in tissue culture/cells.
- Administration to animals using sharps.
- Necropsy of exposed animals and handling of exposed tissues

Low risk activities include:

- Housing and handling of live animals after exposure to proteopathic seeds

REQUIRED BIOSAFETY LEVELS

Based on the risk level of the activity being performed, the following biosafety levels (BSLs) are required for research with proteopathic seeds:

Lab Process or Procedure	Required BSL
In vitro handling of concentrated purified or amplified forms of proteopathic seeds	BSL-2
Administering proteopathic seeds to animals	BSL-2
Necropsy of animals previously administered proteopathic seeds	BSL-2
Animal housing and handling after proteopathic seed administration	BSL-1

Research with proteopathic seeds requires Biological Use Authorization (BUA) from EH&S and the IBC. Refer to the EH&S Biological Research Approval webpage for more information.

SAFE HANDLING PRACTICES

PREPARATION AND TRAINING

- Handle only the minimum concentration and mass of proteopathic seeds necessary.
- Maintain the pH of working solutions at a suitable range to reduce the amount of spontaneous assembly when working with monomers, if allowed by the characteristics of the monomer.

- Develop lab-specific SOPs for work with proteopathic seeds including agent-specific hazard assessment, required personal protective equipment (PPE), decontamination protocol, and established routes of disposal before starting work.
- Document training for anyone who will handle proteopathic seeds.

SHARPS SAFETY

- Eliminate sharps whenever possible. Utilize cannulas or fixed sharps, such as stereotaxic injections.

ENGINEERING CONTROLS

Conduct aerosol-generating tasks such as fibrilizing, agitating, sonicating, centrifugation, and homogenizing with appropriate engineering controls:

- Work inside a certified biosafety cabinet
- Centrifuge with aerosol containment safety cups
- Pipet using filtered tips
- Use flasks with vented caps

PERSONAL PROTECTIVE EQUIPMENT (PPE)

For procedures requiring BSL-2, utilize the following at a minimum:

- Disposable lab coat or gown
- Gloves
- Eye and face protection if potential for splash/spatter, especially for animal necropsy

Additional PPE may be required based on risk assessment. Procedures requiring BSL-1 (i.e., animal housing) require a lab coat and gloves at a minimum.

DECONTAMINATION AND DISPOSAL

Because proteopathic seeds may be resistant to inactivation by heat and chemicals, it is important to plan ahead for decontamination and eliminate the need to disinfect reusable items as much as possible.

- Conduct work on absorbent pads for easy clean up and disposal after work.
- Use disposable items whenever possible.

Incineration is the preferred method of disposal for solid waste containing proteopathic seeds.

- Collect contaminated disposable items including used PPE as biohazardous waste and ship as regulated medical waste for off-site incineration. For questions about shipping regulated medical waste, contact EH&S Biosafety at ehsbio@uw.edu or 206-221-7770.

If incineration is not possible or for liquid waste: contact EH&S Biosafety for guidance to ensure proposed route of decontamination or disposal is viable to inactivate proteopathic seeds. Some proposed mechanisms of inactivation include:

- Flood with 2 N NaOH or sodium hypochlorite solution (20,000 ppm available chlorine) and ensure surfaces remain wet for one hour. Wipe up with paper towels and follow with a water rinse.
- Immerse in 1 N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour. Transfer into water and autoclave (gravity displacement) at 121ºC for 1 hour.
- Autoclave at 134°C for 18 minutes or at 132°C for 1 hour.

Fixing specimens with formalin, ethanol, or glutaraldehyde may not inactivate proteopathic seeds and may make them more difficult to inactivate. Therefore, fixation is not considered a method of decontamination for proteopathic seeds.

EXPOSURE RESPONSE

For any exposures to proteopathic seeds, follow the Exposure Response Poster. Call 9-1-1 for any life-threatening emergencies. Wash the site for 15 minutes with sudsing soap or use an emergency eye wash for 15 minutes. Then call for medical help. Finally, report the incident to EH&S.

CONTACT

For questions, contact EH&S Biosafety at ehsbio@uw.edu or 206-221-7770.

REFERENCES

- Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition, Section VIII-H: Prion Diseases.
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Appendix B-III-D: Risk Group 3 (RG3) Viruses and Prions.
- Prion and Prion-like Protein Guidance. University of Michigan. Accessed 03/2025.
- Prions and Prion-like Proteins. University of Minnesota. Accessed 03/2025.
- Prions and proteopathic seeds: Safe Working and the Prevention of Infection. UK Advisory Committee for Dangerous Pathogens. 2021.