UNIVERSITY OF CAMBRIDGE
Occupational Health and Safety Service

Guidance on laboratory research using proteopathic seeds (taken from Advisory Committee for Dangerous Pathogens Transmissible Spongiform Encephalopathy Subgroup guidance).

Introduction

Over recent years there has been increasing evidence of the ability of proteins (other than the prion protein) to adopt abnormal conformations, self-propagate and cause transmissible pathologies and diseases in humans and laboratory animals.

Such proteins and peptides share a range of pathological properties with PrP prions and may be referred to as “prions,” “prion-like,” “prionoid,” or “proteopathic seeds” in the scientific literature but are also distinct from PrP prions in several ways, including importantly, that there are no known animal or human epidemics or established occupational risks.

In this document we use the term “proteopathic seeds” and include only those proteins known to associate with human neurodegenerative diseases (amyloid-beta peptide, microtubule associated protein tau, alpha-synuclein, TDP-43 and FUS).

Human-human transmission of proteopathic seeds has only been observed for amyloid beta in some specific circumstances that historically transmitted prion infection (e.g. use of cadaver derived human pituitary hormones or dura mater in neurosurgery), after long latencies.

This document is based directly on guidance issued by ACDP and gives advice on safe working practices to help prevent the unwanted transmission of proteopathic seeds during laboratory work with such agents or material that contains or may contain them.

It covers experimental work with synthetic, amplified, or concentrated laboratory preparation forms.

Definitions and hazard classification

Proteopathic seeds are defined as mis-folded protein assemblies usually found in aggregated states in neurodegenerative disorders like Alzheimer’s disease, Parkinson’s disease, frontotemporal dementia, motor neuron disease, and that share some pathological properties with prions. It excludes monomeric proteins in their native state.

Synthetic - Proteopathic seeds made from purely recombinant material.

Amplified - Proteopathic seeds made using methodologies like protein mis-folding cyclic amplification (PMCA) or real-time quaking induced conversion (RT-QuIC) that may involve use of substrates that are either synthetic or derived from mammalian tissues.

Concentrated - Proteopathic seed preparations concentrated from mammalian tissues or bio fluid, e.g. brain tissue from patients with Alzheimer's diseases. Preparations might be made for any of a number of purposes: structural characterisation, biochemistry, Western blot, or transmission experiments. Techniques for concentration might include filtration, affinity purification or centrifugation (pelleting and resuspension), with the end result of an increased concentration of proteopathic seed relative to the starting material.

Classification as Hazard Group 2 applies to synthesized, amplified or concentrated materials containing misfolded protein assemblies in forms known to share pathological properties with prions (including the ability to self-propagate) and are associated with human neurodegenerative diseases. Includes proteopathic seed forms of amyloid-beta peptide, tau protein, alpha-synuclein, TDP-43 and FUS.

At least CL2 working with such materials is recommended.

Risk assessment and containment measures

Risk assessments should be done for laboratory work with preparations of non-PrP proteopathic seeds. The hazards are greatest when handling in vitro materials containing concentrated, amplified, purified or synthesised material which has or could have seeding (self-propagating) functions in humans.

Risk assessment should also be done for the handling of materials derived from cells or animals that are inoculated with proteopathic seeds and engineered to express human forms of these proteins (or proteins sequences that might propagate the human protein).

Containment Levels recommended for work with proteopathic seed agents -

Laboratory work CL2.

Animal work CL1. The guidance issued gives no further detail on what may or may not constitute animal work. CL implies this refers to 'infected' animal work and while such animals may be considered safe at the lowest containment, the administration steps could be regarded as higher risk.

General, basic protective measures should be used wherever there is a risk of exposure to potentially infectious proteopathic seed preparations as defined. These measures are summarised below.

• Laboratory staff must read and understand the Codes of Practice that are specific for their laboratory. These MUST be followed at all times.

• Never work alone in a laboratory without informing others of your presence there.

• Apply general good hygiene measures, never eat, drink, smoke, apply make-up in a laboratory or pipette by mouth.

• Disposable gloves should be worn at all times when handling proteopathic seeds. Consider the use of suitable special hand protection such as armoured glove(s) (https://www.hse.gov.uk/biosafety/blood-borne-viruses/use-of-gloves.htm#what-gloves-should-be-worn) where the use of sharp instruments is essential.

• All workers in the laboratory must cover cuts and abrasions with a waterproof dressing before entering the laboratory

• Wear the appropriate protective clothing routinely for all work with proteopathic seeds. This includes wearing eye protection or full-face visor to protect eyes and mucous membranes from splashes with potentially infectious material. If a liquid-repellent laboratory coat is not available a disposable plastic apron should be worn over existing laboratory coat, or disposable gown.

• Manual handling of material should preferably be undertaken on non-absorbent surfaces in a Microbiological Safety Cabinet (MSC) if one is available.

• Use closed systems such as sealed centrifuge buckets and fully enclosed autoanalysers, or when manually handling samples, a Microbiological Safety Cabinet (MSC) to protect against splashing of material when mixing, centrifuging or homogenising samples (see next bullet for what to do if an MSC is not available).

• If an MSC is not available, risk assessments should consider the potential for dispersal of the agent, for contamination of workers, equipment or surfaces at all stages of the activity, for example using disposable plastic trays when aliquoting CSF on an open bench, and have protocols in place for dealing with spillage.

• Keep your working area clean and free from obstructions. Do not leave coats, bags or unnecessary books on the benches or on floors to trip over.

• Remove waste material from your area as soon as possible and dispose of it in the appropriate waste container.

• When you have finished, leave your area tidy and follow any instructions for clearing and cleaning equipment.

• Minimise the use of sharps (needles, knives, scissors and laboratory glassware) wherever possible. Any use of sharps must be risk assessed.

• Remove protective equipment (e.g. your laboratory coat) and wash your hands thoroughly before leaving the laboratory.

• Use plastic single-use disposable items (containers, pipettes, inoculating loops and other such instruments); in the case of large items this could be interpreted as specified parts of the item e.g. dedicated ultracentrifuge rotors or electron microscope grids.

• Use recommended decontamination procedures.

Cleaning and decontamination

As many of the standard methods of decontamination cannot ensure complete inactivation of prions, and so potentially proteopathic seeds, the emphasis must be on prevention of spillages, single use equipment and the removal of the agent by specific cleaning protocols. An annual check of competency at handling spillages ("spill drill") is recommended.

Examples of procedures for decontamination of prions; these procedures may be relevant to routine work with or after a spillage of proteopathic seeds.

Spillages or contamination with low risk human bio fluids can follow standard local procedures.

Any work involving unsealed biological material should be performed in a defined area such as over a plastic spill tray. In the event of a spillage outside of a spill tray, the area should be decontaminated before cleaning.

Laboratory equipment that has been exposed and is not in a dedicated location within a laboratory should be decontaminated after the exposure.

WHO recommended methods for prion destruction are exposure to a hypochlorite solution containing a final concentration of >20,000 ppm free chlorine or 1M NaOH for 1 hour at room temperature. Where such treatments are not possible (for example because of reactions with chemicals or surfaces) consideration should be given to alternatives that, although not formally validated, may have inactivating action against prions and/or proteopathic seeds.

These could include:

Autoclave at ≥134°C for ≥20 minutes.

Exposure to high concentrations of ionic detergents in aqueous solution at elevated temperature, e.g. >2% w/v SDS at >45°C.

Exposure to strong chaotropes such as guanidine hydrochloride (>5M) or guanidine isothiocyanate (>3M).

Treatment with high concentrations of broad specificity alkaline proteases, e.g. proteinase K at >1mg/ml. Sequential treatments are likely to increase the probability of inactivation.

Other considerations

There should be plans in place (including competency testing) in the laboratory to deal with accidents involving proteopathic seeds, for example dealing with spillages or first aid arrangements for inoculation injuries.

The training of employees working with proteopathic seeds should include highlighting the readily foreseeable incidents that could occur and the procedures for dealing with accidents, incidents and emergencies, and the name of the person or people to whom accidents should be reported.

Competency should be tested at intervals.

Any inoculation injury with proteopathic seeds (or material that contains these agents) if not bleeding should be gently encouraged to bleed, washed (not scrubbed) with warm soapy water and covered with a waterproof dressing. Similarly, any contamination of skin should also be removed with soapy water. Disinfectants should not be put onto cuts or broken skin, as this could worsen the injury and impair the body's localised defence reaction.

Employees may be distressed about a potential exposure and should be offered local psychological support if needed. National services (e.g. National Prion Clinic, www.nationalprionclinic.org, and National CJD Research and Surveillance Unit, https://www.cjd.ed.ac.uk/, can provide additional advice if needed).

An official local record should be kept of any inoculation or mucosal membrane (e.g. face splash) incident involving proteopathic seeds. Except in rare circumstances, incidents will need to be reported to HSE under RIDDOR. Due to the length of incubation period of human TSEs these records should be retained for 40 years.

References

www.thelancet.com/article/S1474-4422(21)00379-3/fulltext

www.gov.uk/government/publications/guidance-from-the-acdp-tse-risk-management-subgroup-formerly-tse-working-group (see Part 3 on laboratory guidance)

www.ncbi.nlm.nih.gov/pmc/articles/PMC7687189/

Addendum

Genetic modification and notification.

Any protein (and its use) produced by whatever system falls outside of the scope of the GM regulations as there is no associated genetic material.

The risks from the production systems (bacteria or cell cultures) will be in scope and may require notification. Where systems produce significant amounts of protein with seed potential, the risks from exposure leading to propagation or aggregation events must be assessed and if requiring adoption of CL 2 control measures, will be notifiable.

Modified animals would only be notifiable if they represented a greater risk of harm, such as from transmission by biting.