UNIVERSITY OF MINNESOTA
Environmental Health & Safety
IMPROVING THE QUALITY OF OUR WORK AND STUDY ENVIRONMENT

Bio Basics Fact Sheet:
Prion Research Procedures

Introduction

All protocols for working with prions must be approved by the Institutional Biosafety Committee (IBC) before work is started. Submit SOPs and an application form to the committee, http://cflegacy.research.umn.edu/ibc/download/index.cfm. Approved protocols are effective for three years with annual review.
Note: *Laboratory space assignment and animal housing biosafety levels must be included as part of the IBC approval process.*

There are no known effective treatments or vaccines for prion (also known as Transmissible Spongiform Encephalopathies or TSEs). It is necessary to handle prions with extreme caution, both for worker protection and for environmental protection. The highest concentrations of prions in infected animals are in the central nervous system and its coverings. Studies indicate that it is likely that high concentrations of prions may also be found in spleen, thymus, lymph node, and lung tissue.

Scrapie is a prion disease that affects sheep and goats. Chronic Wasting Disease (CWD) is a prion disease that affects deer, moose, and elk. There has been no documented evidence that either scrapie or CWD can be transmitted to humans. Bovine Spongiform Encephalopathy (BSE) is a prion disease that normally affects cattle.

Cruetzfeldt-Jakob Disease (CJD) is a rare human prion disease. A form of CJD called variant Creutzfeldt-Jakob disease (vCJD) has been diagnosed since 1996 and is thought to be linked to the consumption of meat products derived from BSE-infected cattle.

A USDA permit is required to receive and work with animal prions.
Note: *Import includes crossing state lines.*

Exposure Control Methods

- Personnel working with prions cannot have contact with any other animal colonies or with susceptible animal species. Prion animal colonies must have separate air supply and exhaust systems (per USDA).
- Follow Biosafety Level 2 requirements as outlined in the Biosafety Level 2 Procedures Fact Sheet.
- The principle investigator or lab supervisor must train all workers in specific handling procedures for prions. Written Standard Operating Procedures (SOPs) must be available for worker reference.
- Lab access must be limited to trained individuals. If non-trained individuals need to enter the lab they must be accompanied by a trained individual.
- One of the main precautions to be taken when working with prions is to avoid puncture of the skin. Therefore, sharps and glass should not be used unless it has been determined that there is no other alternative.
- All work surfaces should be protected with disposable bench covers that are handled as low level prion waste.

- All manipulations with prions should be done in a biological safety cabinet. Cabinet needs to be certified annually.
- Open centrifuge rotors in a biological safety cabinet.
- Transport of all prion materials outside a biological safety cabinet requires secondary containment.
- All workers should wear appropriate personal protective equipment:

1. Double gloves
2. Lab coat (disposable back closing preferred)
3. Goggles to prevent touching of face
4. Full face protection if it is necessary to handle prion material outside the biological safety cabinet

Spill Clean-up and Disinfection

Prions are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and many chemicals (formalin, betapropiolactone, alcohols). Fixation with alcohol, formalin, or glutaraldehyde strongly stabilizes the infectivity of prions and makes them more difficult to inactivate. Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. As a consequence, contaminated materials should not be exposed to fixation reagents, and should be kept wet between the time of use and disinfection by immersion in chemical disinfectants. Fixed material that contain or may contain prions must be disposed of as "prion waste".
Note: *Be sure to decontaminate all equipment prior to maintenance or service work.*

Effective Disinfectants

- Environ LpH for a minimum of 30 minutes. Environ LpH from Steris *(other LpH formulations are not as effective)* is effective and not as corrosive to surfaces as bleach or NaOH. This was formerly known as Canadian LpH.
  Note: *Minimize breathing LpH fumes. Use squirt bottles and saturated lab wipes rather than spray bottles that create a mist. Containers with LpH should have tight covers. Wear eye protection if using outside a biological safety cabinet or chemical fume hood including pouring down drain for disposal. User must observe the precautions and safety requirements as listed on the registered product label or MSDS.*
- 40% household bleach, per USDA requirements (2% free chlorine by making a final concentration of 2.5% sodium hypochlorite solution) for one hour
- Freshly made 2N NaOH for one hour

In addition to the above disinfectants, prions can be inactivated by:

- Steam autoclaving at 134°C for 1 hour or
- Incineration

Spill Clean-up

- Notify other lab workers that a spill has occurred.
- It is important to keep contaminated surfaces moist until decontamination is complete as the infectious agents become even more resistant to chemical inactivation when dry.
- Cover spill with paper towels or other absorbent material.
- Saturate with Environ LpH or other disinfectant listed above and let sit for at least 30 minutes (60 minutes if using bleach or NaOH). Use sufficient Environ LpH to have a final concentration of 10%. If possible, leave the lab to avoid prolonged breathing of fumes. Direct other workers not to enter the lab.
- Dispose of absorbent material and any other clean-up material in yellow waste bag.
- Report all spills to lab supervisor.

Decontaminating Non-Disposable Items

- **For small items** (e.g., glassware, instruments, and small animal cages):
  - Submerge in 5% LpH for 30 minutes followed by autoclaving for 90 minutes at 134°C or
  - Submerge in 5% LpH overnight or
  - Submerge in 10% LpH for 30 minutes
    Note: *Container should be placed in a chemical fume hood or have a tight top to reduce exposure to fumes.*
- **For larger items:**
  - Wipe down equipment, lab surfaces, and other non-submersible low level prion contaminated items with 10% LpH. Re-treat as necessary to keep wet throughout the 30 minute time period.
- Following the contact time, surfaces may be rinsed with water and allowed to air dry.

Waste Handling

The following procedures are to be followed for disposing of prion waste. Other methods of waste disposal must first receive IBC approval.

Non-Tissue Low Level Solid Prion Waste (Includes Animal Bedding)

- Place waste in yellow bag.
- Place in yellow barrel for incineration.
- Call Facilities Management (4-2900) to arrange to have a yellow barrel delivered to the lab and for pick-up / replacement of yellow barrels.

Liquid Prion Waste

- **Low titer waste**: treat with sufficient Environ LpH to have a final concentration of 5% overnight or 10% for a minimum of 30 minutes.
  Note: *Container should be placed in a chemical fume hood or have a tight top to reduce exposure to fumes.*
- **High titer waste**: follow above LpH treatment by autoclaving at 134°C for 1 hour.
- Dispose treated liquid waste down the drain. Wear gloves, disposable lab coat, and full face protection. If possible, dispose in a vented sink area. Rinse sink with copious amounts of water (at least 2.5L per ml of LpH).

Note: *LpH waste disposed down the drain must not contain other chemicals and must have a pH between 5.0 and 9.0. Do not put concentrated LpH down the drain.*

Sharps & Histology Slides

Place sharps in a puncture proof red sharps container. When container is no more than 3/4 full, close and seal the container. Place container in a yellow waste bag for incineration.
Note: *Small quantities of sharps may be placed in other small, sealed, puncture-proof containers and put in yellow bag.*

Infected Animal Carcasses and Tissue

Dispose of all infected carcasses and tissue in the tissue digester adjacent to Vet Diagnostics on the St. Paul campus. Call 625-0255 to make arrangements.

Accident Response

- Needle sticks or laceration: gently encourage bleeding, wash with warm soapy water, rinse, dry and cover with a waterproof dressing. Seek medical attention immediately and report incident to the Biosafety Officer, 612-626-6002.
- Accidental contamination of skin: wash with warm soap and water for 5 minutes. (May clean briefly, 1 minute, with 0.1N NaOH, 10% bleach, or 5% LpH followed by washing with warm soap and water for 5 minutes.)
- Splashes to the eye or mouth: irrigate with copious amounts of water for 15 minutes. Seek medical attention immediately.
- Report all incidents to supervisor immediately. Report any incident requiring medical treatment to Biosafety Officer, 612-626-6002.

References

WHO infection control guidelines for transmissible spongiform encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23-26 March 1999, http://www.who.int/csr/resources/publications/bse/whocdscsraph2003.pdf

Section VIII-H. Prion Diseases. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_VIII_h.pdf

BL2 TSE Laboratory Standard Operating Procedures, U.S. National Institutes of Health (NIH), Rocky Mountain Laboratories (RML). Hamilton, Montana

Inactivation of Transmissible Spongiform Encephalopathy (Prion) Agents by Environ LpH, Richard E. Race and Gregory J. Raymond, *Journal of Virology*, Feb. 2004, p. 2164-2165