Scrapie, chronic wasting disease (CWD), and transmissible mink encephalopathy (TME) belong to the transmissible spongiform encephalopathy (TSE) family of diseases. Scrapie is a nearly worldwide disease of domestic sheep, goats, and mouflon (*Ovis musimon*). Chronic wasting disease naturally affects mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*) in the United States and Canada. Transmissible mink encephalopathy is a rare disease of ranched mink (*Mustela vison*). The agents of scrapie, CWD, and TME are classified as Biosafety Level 2 pathogens (Centers for Disease Control and Prevention and National Institutes of Health, 1999). These best management practices address scrapie, CWD and TME.

One other TSE occurs in animals: bovine spongiform encephalopathy (BSE). BSE naturally affects domestic cattle, captive non-domestic bovids, domestic and nondomestic felids, and laboratory primates. A single case of bovine spongiform encephalopathy has been diagnosed in the United States and two cases have been diagnosed in Canada. BSE is classed as a Biosafety Level 3 pathogen in the United States. Four TSE diseases are recognized at present in humans: Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Sträussler-Shenker syndrome, and fatal familial insomnia. There are sporadic, familial, iatrogenic, and variant forms of CJD. The only zoonotic TSE of humans is variant CJD (vCJD), which is probably due to consuming BSE agent-contaminated food. Currently, there is no indication that scrapie, CWD, or TME are transmissible to people (World Health Organization, 1999; Belay et al., 2001).

Scrapie, CWD, and TME have been transmitted on primary passage following direct intracerebral inoculation to a wide variety of mammals including primates and domestic livestock. Although successful intracerebral passage indicates a degree of molecular compatibility, there is poor correlation with natural host ranges as determined through epidemiological investigation or experimental challenge via more natural routes of exposure (i.e., the oral route). With a few exceptions, notably BSE, the TSEs are typically species- or very closely related species-specific.

The TSEs of animals became a source of public concern since the mid-1990s, particularly after recognition of a relationship between BSE and vCJD, due to:

- Unknowns surrounding these agents and the diseases they cause.
- Resistance of these infectious agents to conventional denaturing and disinfection strategies.
- Impossibility of providing a categorical assurance that they will not cause disease in people.

The unusual nature of TSE agents caused public and regulatory concern in areas where the diseases are endemic in wildlife, particularly CWD in Wyoming, Colorado, Nebraska, South Dakota, and Wisconsin as well as in states where game farms populations are affected. It is worth emphasizing: no risk to animals or humans has been demonstrated to arise from carcasses or tissue wastes of animals with scrapie, CWD, or TME generated by veterinary diagnostic laboratories where TSE work is done. Veterinary laboratories are a critical element to ensure that infected carcasses are identified so that the location of the disease can be accurately mapped for management purposes by state wildlife agencies. At the same time, it is in the interests of animal health laboratories to demonstrate to regulatory agencies and the public that they take effective, responsible measures to ensure that they do not concentrate and re-circulate TSE agents into the environment. Laboratories need to work closely with the appropriate
regulatory agencies to assure they are in compliance with pertinent local, state and federal requirements when discharging wastes to sewage treatment works, leach fields, surface waters, and landfills.

The following recommendations are provided to reduce unforeseen risks to laboratory personnel and to the environment by simple, relatively inexpensive techniques. Recognizing the unique challenges that TSE agents pose for veterinary diagnostic laboratories in comparison to traditional BL2 agents, the document was prepared to assist animal health laboratories to reduce and appropriately inactivate TSE-contaminated wastes that they generate. It is a starting point. Laboratories should develop standard operating procedures specific for their circumstances.

This document will be periodically amended as knowledge of TSEs grows.

Definitions:

*TSE-suspect animal:* A TSE suspect animal is one that demonstrates clinical signs or gross lesions (e.g., neurological signs, emaciation and/or inhalation pneumonia in CWD) typical or suggestive of the syndrome for that species. The index of suspicious should be high when an animal originates from an area, herd or property where TSE was confirmed to occur.

*Highly infective tissues:* In the context of TSE agents, highly infective tissues are those in which it has been demonstrated that a high concentration of prions is typically found, particularly in brain, spinal cord, retina, and lymphoid tissues. In sheep with scrapie, other infected tissues are placenta, pituitary glands, peripheral nerves and ganglia, and lymphoid tissues.

*Biosafety level 2:* Biosafety Level 2 defines the standards that are required to work with agents of moderate potential hazard to personnel and the environment. Unlike BSL-1, (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures where infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

*Biosafety level 3:* Biosafety Level 3 is applicable to facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious materials are conducted in biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. Such laboratories have special engineering and design features. The only TSE agent in animals that requires BL-3 handling in the US at present is BSE.

**General precautions**

Use standard BL2 safety precautions for scrapie, CWD, and TME such as restricted access to laboratories, protective clothing, and facial protection, special care with sharp instruments, and avoidance of aerosols (see Centers for Disease Control and Prevention, 1999 [http://bmbl.od.nih.gov/sect3bsl2.htm](http://bmbl.od.nih.gov/sect3bsl2.htm) for detailed guidelines). In some cases, recommendations in this document exceed standard precautions for BL2 protocols in recognition of the unique properties of the TSE agents, and to demonstrate prudence in the face of uncertainty.

Appropriate care needs to be taken when handling the harsh inactivating agents required to extinguish infectivity in TSE tissues. Personnel should to be aware of the instability of such solutions over time (i.e., make up solutions fresh shortly before use), and the need to comply with state and federal environmental regulations regarding discharges of these solutions to publicly owned water treatment works. Use of commercial aqueous acid phenolic disinfectants such as Environ LpH (Steris Corporation) for inactivation of TSE agents in laboratories requires an exemption under section 18 of the Federal
Insecticide, Fungicide, and Rodenticide Act (FIFRA), until a product is registered for this use. The products may be used according to the conditions of the emergency exemption.

Specific Recommendations

Necropsy Laboratories (carcasses, heads, vertebral columns, and other unfixed tissues)

Disposable or dedicated equipment, instruments, and supplies should be used when possible when TSE-suspect necropsies are performed.

Wear liquid-repellent or disposable full-length gowns over necropsy coveralls.

Wear disposable gloves at all times.

Arm-length gloves are recommended to minimize contamination of non-disposable clothing.

Contaminated non-disposable clothing must institutionally washed.

Wear facial protection (face shields) to protect mouth and eyes from splashes and particles.

Mark all formalin fixed and unfixed samples from TSE suspects with “TSE Test Sample.”

Screen necropsy drain(s) to trap tissue fragments and large blood clots, to minimize the amount of potentially infectious material entering drains.

Use appropriate disinfectants (below) on potentially contaminated surfaces prior to washing.

Use minimal volumes of surface and floor wash water during clean up.

Keep instruments in disinfectant solution and maintain wet work surfaces during necropsy to minimize drying of tissues and body fluids.

Use receptacles to catch fluid wastes from necropsy tables and suspended decapitated carcasses. Absorbable materials are an alternative, but will generate undesirably large volumes of waste when many samples are processed.

Disposal of carcasses, tissues, and potentially contaminated disposable materials by one of the following methods:

- Burn in a pathological waste incinerators with one second retention times in the secondary chamber and a minimum secondary chamber temperature of 1600 – 1800°F (871° - 982°C) or
- Digest in alkali hydrolysis units for at least 6 hours at 300 F, or
- Landfill in accordance with local and state regulations.

Clean non-disposable items from necropsy with appropriate treatments as follows:

- 1:1 bleach solution to provide at least 20,000 ppm available chlorine for 1 hour (Ultra-Chlorox contains 6%, or approximately 57,000 ppm free chlorine [J.E. Bowman, Clorox Professional Products Company, Oakland, California]), or
- 1 N NaOH for 1 hour (World Health Organization, 1999, or
- Autoclave at 134 C for 4.5 hours (Centers for Disease Control, 1999), or
- 10% solution of aqueous acid phenolic solution (Environ LpH) for >30 min or 1% solution for 16 hours (Ernst and Race, 1993) for laboratories where a section 18 FIFRA exemption has been obtained or,
- Combination of autoclaving and chemical inactivating agents.

Prior to disposal according to local and state regulations, sharps should be soaked with bleach solution containing >20,000 ppm free chlorine for 1 hour, by autoclaving at 134°C for >18 minutes, soaking in 10% in aqueous acid phenolic (LpH) solution for 30 minutes or 1% solution for 16 hours, or by soaking in 1 N NaOH for 1 hour.

Following necropsy, the floor and potentially contaminated surfaces should be soaked with 10% aqueous acid phenolic (LpH) or 1:1 bleach solution kept wet for 30-60 minutes.

**Histology Laboratories**

Disposable or dedicated equipment and supplies should be used when possible.

TSE-suspect tissues should be grossed in for histology in chemical hoods using dedicated or disposable instruments and cutting boards.

Potentially contaminated formalin or other liquids should be incinerated or recycled with appropriate disposal of remaining solids (incineration, digestion, chemical inactivation), or chemically neutralized and disposed of in accordance with federal, state, and local regulations.

Sharps containers should be disposed of after treatment as described above.

Grossed in fixed tissues with potential high infectivity (brain, spinal cord, eyes, lymphoid tissues) may be treated with undiluted formic acid for 1 hour prior to washing and processing (Brown et al., 1990) in order to decrease infectivity.

All TSE-suspect tissues for histologic processing will be separately identified (i.e., unique case number or specific color of tissue cassettes for TSE-suspect tissues).

All microtomy wastes will be collected and disposed of as for fresh tissues (see above).

Formalin-fixed tissues from TSE-infected animals will be disposed of as for fresh tissues (see above).

Waste solutions will be treated by adding:

- Commercial bleach solution to make a final solution containing >20,000 ppm free chlorine (1 hour), or
- NaOH to make a final 1 N solution for 1 hour and then neutralized and disposed of down the drain.
- 10% aqueous acid phenolic (LpH) solution for 30 minutes
- 1% aqueous acid phenolic (LpH) for 16 hours

Processing, embedding, staining, and microtomy equipment will be frequently treated with appropriate TSE-inactivating solutions

**Laboratories Handling Fresh Tissues from TSE Suspect Animals**

Fresh tissues from TSE suspects will be processed in such a way that excess fluid and tissue can be captured either by receptacles or absorbent pads. This will be disposed of by incineration, alkali hydrolysis, or landfill.

Instruments used for potentially infected tissues will be placed in 1:1 bleach solution (containing >20,000 ppm free chlorine for 1 hour), or 1 N NaOH (1 hour) or 10% aqueous acid phenolic (LpH) solution (30 minutes) or 1% aqueous acid phenolic (LpH) solution (16 hours).
Waste solutions will be treated as described above prior to washing down the sink.

Sharps containers will be treated as described above.

Contaminated surfaces will be disinfected as described above.

**Literature Cited and References**


*This document was prepared by Dr. E.S. Williams and Dr. D. O'Toole. A draft was circulated, discussed and amended following discussion at the AAVLD’s Pathology Committee and the Laboratory Safety and Waste Disposal Committee, and with interested diagnosticians and TSE researchers at the annual meeting of the AAVLD in San Diego, CA in October 2003. The recommendations were approved by the AAVLD executive.*

February 18, 2004