PRION EXPOSURE PROTOCOL FOR THE RESEARCH LABORATORY SETTING

Organism or Agent: Prions
Exposure Risk: Human Prion Disease (Creutzfeldt-Jakob Disease/New variant CDJ, BSE, etc.)

Exposure Hotline: 415/353-7842 (353-STIC) (Available 24 hours)
Occupational Health Services: 415/885 - 7580
Office of Environment, Health & Safety: 415/476-1300 (Available during work hours)
EH&S Public Health Officer: 415/514-3531
EH&S Biosafety Officer: 415/514-2824

PROTOCOL SUMMARY

In the event of an accidental exposure or injury, the protocol is as follows:

1. Modes of Transmission:
   a. Skin puncture or injection
   b. Ingestion
   c. Contact with mucous membranes (eyes, nose, mouth)
   d. Contact with non-intact skin
   e. Exposure to aerosols

2. First Aid: There is no evidence of occupational transmission of prion disease to healthcare workers. Perform first aid as self-care according to the type of exposure/ injury.
   a. First Aid for an unbroken Skin Exposure: Wash with soap and abundant quantities of warm water (avoid scrubbing), rinse, and dry. Apply for 1 minute, 0.1N Sodium Hydroxide (NaOH) or a 1:10 dilution of bleach (sodiumhypochlorite).
      i.) When decontaminating with 0.1N NaOH or sodium hypochlorite, a face shield and eye goggles or eye goggles with mask should be worn for protection. It is important to decontaminate the wound with the appropriate agent for the appropriate length of time in order to denature the protein as soon as possible. See the special precautions for NaOH below.
      ii.) After decontamination, rinse well with soap and water to neutralize the base.
      iii.) Bring the 0.1N sodium hydroxide MSDS to the ED
   b. First Aid for lacerations or needlestick injuries: Gently encourage bleeding; wash (avoid scrubbing) with warm soapy water, rinse, dry and cover with a waterproof dressing. Further treatment (e.g. sutures) should be appropriate to the type of injury.
   c. First Aid for splashes to the Eye, Nose or Mouth: Immediately flush the area with running water or normal saline. Continue washing for 15 minutes. Do not use sodium
hydroxide or sodium hypochlorite in or around your eyes. Do not rub or keep eyes closed.

d. Following the administration of first aid, the employee will:
   
i.) Inform your supervisor of the exposure.

   ii.) Remove any garments that may have become soiled/contaminated with prions or NaOH, and place them in a double plastic bag. Close the bag securely, label it as contaminated, and wash your hands thoroughly.

   iii.) Identify any equipment involved in the exposure and the mechanism of exposure. Make sure that the area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.

   iv.) If you need immediate care for your injury, proceed to the Emergency Department(ED). When you injury is stable, Contact the Needlestick Exposure Hotline (415-353-7842) to report the exposure. The injured employee will need to follow up in the UCSF Occupational Health Clinic. Be sure to go to the clinic for medical evaluation and complete all necessary workers’ compensation paperwork.

e. Splash Affecting Garments, remove garments that may have become soiled or contaminated and place them in a double biohazard red plastic bag. Disposable gloves and gowns should dispose of by incineration, according to World Health Organization guidelines.

3. Treatment:
   
a. In the event of an acute injury resulting from a laboratory incident which requires immediate medical care, the injured employee/student should report to the emergency department for medical treatment. The injured individual must take a copy of this entire protocol document to the Emergency Department (ED).

b. In the event of a large spill, contact the emergency response team (9-911) for clean-up.

c. Contact Occupational Health Services, after first aid is complete, for follow-up care.

4. Follow up is needed in the event of any Laboratory Exposure:
   
a. After first aid has been administered, immediately inform your supervisor of the exposure.

b. In the event of a large spill, contact the emergency response team (9-911) for clean-up.

c. Contact Occupational Health Services, after first aid is complete, for follow-up care.
ROLES & RESPONSIBILITIES
AFTER ACCIDENTAL EXPOSURE TO PRIONS

1. WORKER’S RESPONSIBILITIES (Employee/Student Initial Self-Care)
   a. First Aid: Perform the recommended first aid and decontamination according to the posted instructions.
   b. Treatment: i). In the event of an acute exposure or injury resulting from a laboratory accident, the injured individual should report to the Emergency Department for acute medical treatment. The employee should bring a copy of this protocol. The employee should inform the ED physician of the exact types of toxin to which he/she was exposed. ii). In the event of an exposure, with or without an injury, call the Exposure Hotline in order to get access to medical care for the exposure and evaluation for possible post exposure prophylaxis.
   c. Access to the Exposure Hotline: Immediately, call the Exposure Hotline in the event of an exposure. Dial 415/353-7842 and provide your name and contact information to the operator. If there is no call back in 15 minutes, call again. If there is no call back the second time, proceed to the nearest Emergency Department with a copy of this protocol.
   d. Reporting: Inform your laboratory supervisor / principal investigator of the exposure.
   e. Secure the laboratory: Identify the equipment involved in the exposure and the mechanism of exposure. Make sure that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.
   f. Remove any garments that may have become soiled/contaminated and dispose in a double red biohazard bag and incinerated.
   g. Follow up: Contact Occupational Health Services (OHS) at 415/885-7580 for any needed follow-up care.

2. SUPERVISOR’S/PI’S RESPONSIBILITIES
   a. First Aid and Decontamination: Verify that the worker has washed and decontaminated himself/herself. Ensure that appropriate medical treatment has been received.
   b. Secure the laboratory: Confirm that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.
   c. Laboratory clean-up (as needed): Contact the Office of Environment, Health & Safety (OEH&S) through the UC Police Department Emergency Dispatch (from a campus telephone 9-911, from a non-campus phone 415/476-1414).
   d. Report the exposure: Call the Biosafety officer during regular hours to discuss the exposure. A report summarizing any suspected Prion exposure needs to be submitted to the Biosafety Committee by the Principal Investigator (PI). The report must include the following:
      • A brief description of the exposure event, a description of the area involved, and the extent of employee exposure
      • If applicable, specification of the amount of infectious material released, time
involved, and explanation of procedures used to determine the amount involved
- Corrective action taken to prevent the re-occurrence of the incident
- Decontamination procedures

e. **Follow Up:** Confirm that the worker has called for an appointment at the UCSF Occupational Health Clinic.


### 3. Principal Investigator Responsibilities

a. The PI will ensure that all lab personnel are trained in the use of safe laboratory procedures to prevent accidental exposure before assignment to any laboratory where prions are used.

b. The PI is responsible for ensuring that all workers with potential exposure to prions have been trained regarding the clinical manifestations of spongiform encephalopathy, the hazards of prion exposure employee safety, and decontamination procedures. The supervisor is responsible for ensuring that all workers are thoroughly familiar with the post-exposure decontamination protocol. The supervisor should not allow anyone to perform work in which there is a potential for contamination with prions unless they have been trained.

1. The supervisor should ensure that all workers know that concentrated sodium hydroxide is extremely hazardous in the event of eye contact, and any eye contact necessitates immediate medical attention in an emergency department. The supervisor should ensure that all workers know the appropriate personal protective equipment that must be worn. When working with concentrated NaOH solutions, employees should wear both eye goggles and a face shield.

2. The supervisor should ensure that training is conducted on an annual basis.

c. Documentation of Training regarding initial and annual training is the responsibility of the PI or lab manager. This documentation should incorporate the following requirements:

1. Access to the laboratory must be restricted to trained personnel when work is being conducted on tissue that potentially contains prions,

2. Annual training of personnel must be documented, and

3. Personnel handling tissues must be trained in the following:
   i. Nature of CJD/BSE,
   ii. Route of transmission of CJD/BSE,
   iii. Specific hazards associated with handling of the infectious materials
   iv. Exposure follow-up procedures.

d. The PI may request assistance from UCSF OEH&S in providing information about safe laboratory procedures. For assistance, the PI should call the UCSF Public Health Officer or Biosafety Officer.

e. The PI must ensure that all researchers who will be working with prions have read the entire protocol. The PI will also ensure that the protocol will be reviewed on a yearly basis by all laboratory workers.
4. **EMERGENCY DEPARTMENT RESPONSIBILITIES**

The Emergency Department and UCSF Occupational Health Services both need to complete a Doctor’s First Report of Occupational Illness (DFR). The Emergency Department physician should leave a message for Occupational Health that an exposure has occurred. The physician administering care should forward a copy of the DFR to UCSF HR. Here is a link to the form: [http://www.dir.ca.gov/dlsr/dlsrform5021.pdf](http://www.dir.ca.gov/dlsr/dlsrform5021.pdf)
UNIVERSITY OF CALIFORNIA SAN FRANCISCO
ENVIRONMENT, HEALTH AND SAFETY/BIOSAFETY

INFECTIOUS SUBSTANCE DATA SHEET
HUMAN PRION AGENTS
FOR USE IN RESEARCH LABORATORIES

SECTION I - INFECTIOUS AGENT
Name: Creutzfeldt-Jakob agent, Kuru agent
Synonym or Cross Reference: Subacute spongiform encephalopathy, Creutzfeldt-Jakob disease (CJD), Kuru, Transmissible Spongiform Encephalopathy (TSE).
Characteristics: Filterable, self-replicating agent, slow infectious pathogen, prion protein (PrP)

SECTION II - RECOMMENDED PRECAUTIONS
Containment Requirements: Biosafety level 3 facilities, practices and containment equipment for activities involving these agents; also listed under biosafety level 2 with special precautions; level of containment will depend on the nature of the manipulations and the amount of sera, bio/necropsy materials handled
Protective Clothing: Gown and gloves when handling potentially infectious materials; eye protection may also be indicated
Other Precautions: Extreme care must be taken to avoid accidental autoinoculation or other parenteral inoculations of infectious tissues and fluids

SECTION III - HANDLING INFORMATION
Spills: Allow any potential aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1N sodium hydroxide, starting at perimeter and working towards the center; allow sufficient contact time (1 hour) before clean up
Disposal: Decontaminate before disposal; steam sterilization (132°C for 1 hour), disinfection with 1N sodium hydroxide for 1 hour, incineration
Storage: In sealed containers that are appropriately labeled

SECTION IV - HEALTH HAZARD
Pathogenicity: CJD - insidious onset of confusion, progressive dementia, myoclonic jerks with spasticity, wasting and coma; slight elevation of CSF proteins; death usually occurs in less than 1 year; 10% of cases have family history of presenile dementia; amorphous amyloid plaques in cerebellum of 15% of cases; Kuru - CNS disease with cerebellar ataxia, incoordination, tremors, rigidity, progressive wasting and death within 3-9 months
Epidemiology: CJD - Reported from 50 countries with highest incidence found among Libyan Jews in Israel; Kuru - occurred in Fore tribe of Papua New Guinea
Host Range: Mammals; humans, transmissible to chimpanzees, monkeys, guinea pigs, mice and hamsters
Infectious Dose: Unknown
Mode of Transmission: The mode of transmission of most cases is unknown; Address each mode of transmission: oral, iatrogenic, blood borne, and air borne, etc. iatrogenic cases of CJD
reported (corneal transplant, from cortical electrodes previously used on known patients, brain or eye surgery, human growth hormone therapy, exposure to infected brain tissues by pathologists), no evidence of transmission of CJD from one person to another: Kuru-handling and eating kuru infected brain during ritualistic cannibalism

**Incubation Period:** Fifteen months to 2 years or longer for CJD iatrogenic cases; 4 to over 20 years for Kuru

**Communicability:** CNS and other tissues are infectious throughout symptomatic illness; lymphoid and other organs probably infectious before signs of illness appear

### SECTION III – DISSEMINATION

**Reservoir:** Human cases constitute the only known reservoir

**Zoonosis:** No documented human infections acquired from animals although this has been hypothesized (consumption of scrapie-infected sheep might result in CJD; in 1996 consumption of BSE-infected beef in UK has been associated with development of CJD-like disease; variant CJD)

**Vectors:** None

### SECTION V - VIABILITY

**Drug Susceptibility:** N/A

**Susceptibility to Disinfectants:** Resistance to commonly used disinfectants is well recognized: formaldehyde, glutaraldehyde, ethanol, and iodine. Immersion in undiluted bleach (60,000 ppm available chlorine) for 1 hour is only partially effective. Disinfection should be carried out using 1N sodium hydroxide at room temperature for 1 hour (shorter treatments have occasionally not inactivated the pathogen)

**Physical Inactivation:** Resistant to ultraviolet and ionizing radiation, ultrasonication, nuclease, boiling, heat; autoclaving - 15 to 30 min at 121·C or 132·C will not effectively inactivate pathogen, 1 hour at 132·C is recommended

**Survival Outside Host:** Contaminated electrodes stored in ethanol-formalin for several years were found to cause CJD in chimpanzee

### SECTION VI - MEDICAL

**Surveillance:** Monitor for clinical signs - diagnosis based on EEG, histopathological findings, transmission to animals from biopsy specimens

**First Aid/Treatment:** Any skin contact with infectious materials should be followed by washing with sodium hydroxide or bleach followed by copious rinsing with water; no specific treatment

**Immunization:** None

**Prophylaxis:** None

### SECTION VII - LABORATORY HAZARDS

**Laboratory-Acquired Infections:** No documented laboratory-associated infections with spongiform encephalopathies. However, consequences of infection are grave and there are cases of infection from contaminated EEG electrodes and corneal transplants

**Sources/Specimens:** High titers in brain and CNS of persons with Kuru; CJD brain, spleen, liver, lymph nodes, lungs, spinal cord, kidneys, cornea and lens, blood, urine; includes formalin-fixed specimens

**Primary Hazards:** Accidental parenteral inoculation; risk of infection from aerosols, droplets, and exposure of intact skin, gastric and mucous membranes is not known
Special Hazards: Laboratory animals that have been infected and their tissues should be considered potentially hazardous

FOR THE USE OF THE EMERGENCY DEPARTMENT

SECTION VIII – EMERGENCY MEDICAL TREATMENT

Treatment Indications: Emergency department treatment will be required for immediate treatment. The treatment needs to consist of the following: 1) decontamination and debridement 2) wound repair 3) evaluation for post exposure prophylaxis and 4) follow-up with Occupational Health Services.

Exposure Indications: In the event of an exposure, with or without an injury, the Exposure Hotline must be called.

Decontamination: Ensure that the wound has been adequately decontaminated.

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: May 2012 Prepared by: Occupational Health Program

Although the information, opinions and recommendations contained in this Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

The above sections are taken directly from: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds45e-eng.php

For information about exposure risk from animal tissue, refer to WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies: http://www.who.int/biologicals/BS%202078%20TSE.pdf
REFERENCES FOR CLINICAL PROVIDERS

WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies: http://www.who.int/biologicals/BS%202078%20TSE.pdf

CDC Prion Diseases Website: http://www.cdc.gov/prions/

Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH 5th edition (see Appendix 1): http://www.cdc.gov/biosafety/publications/bmbl5/index.htm


Prion Diseases
Transmissible spongiform encephalopathies (TSE) or prion diseases are neurodegenerative diseases which affect humans and a variety of domestic and wild animal species (Tables 1 and 2). A central biochemical feature of prion diseases is the conversion of normal prion protein (PrP) to an abnormal, misfolded, pathogenic isoform designated PrPSc (named for “scrapie,” the prototypic prion disease). The infectious agents that transmit prion diseases are resistant to inactivation by heat and chemicals and thus require special biosafety precautions. Prion diseases are transmissible by inoculation or ingestion of infected tissues or homogenates, and infectivity is present at high levels in brain or other central nervous system tissues, and at slightly lower levels in lymphoid tissues including spleen, lymph nodes, gut, bone marrow, and blood. Although the biochemical nature of the infectious TSE agent, or prion, is not yet proven, the infectivity is strongly associated with the presence of PrPSc, suggesting that this material may be a major component of the infectious agent. A chromosomal gene encodes PrPC (the cellular isoform of PrP) and no PrP genes are found in purified preparations of prions. PrPSc is derived from PrPC by a posttranslational process whereby PrPSc acquires a high beta-sheet content and a resistance to inactivation by normal disinfection processes. The PrPSc is less soluble in aqueous buffers and, when incubated with protease (proteinase K), the PrPc is completely digested while PrPSc is resistant to protease. Neither PrP-specific nucleic acids nor virus-like particles have been detected in purified, infectious preparations.

OCCUPATIONAL INFECTIONS
No occupational infections have been recorded from working with prions. No increased incidence of Creutzfeldt-Jakob disease (CJD) has been found amongst pathologists who encounter cases of the disease post-mortem.

NATURAL MODES OF INFECTION
The recognized diseases caused by prions are listed under Table 1 (human diseases) and Table 2 (animal diseases). The only clear risk-factor for disease transmission is the consumption of infected tissues such as human brain in the case of kuru, and meat including nervous tissue in the case of bovine spongiform encephalopathy and related diseases such as feline spongiform encephalopathy. It is also possible to acquire certain diseases such as familial CJD by inheritance through the germline.

TABLE 1
THE HUMAN PRION DISEASES
DISEASE ABBREVIATION MECHANISM OF PATHOGENESIS
Kuru Infection through ritualistic cannibalism Creutzfeldt-Jakob disease CJD Unknown mechanism Sporadic CJD sCJD Unknown mechanism; possibly somatic mutation or spontaneous conversion of PrPc to PrPSc Variant CJD vCJD Infection presumably from consumption of BSE-contaminated cattle products and secondary blood borne transmission Familial CJD fCJD Germline mutations in PrP gene Iatrogenic CJD iCJD Infection from contaminated corneal and dural grafts, pituitary hormone, or neurosurgical equipment Gerstmann-Sträussler- Scheinker syndrome GSS Germline mutations in PrP gene Fatal familial insomnia FFI Germline mutations in PrP gene

TABLE 2
THE ANIMAL PRION DISEASES
DISEASE ABBREVIATION NATURAL HOST MECHANISM OF PATHOGENESIS
Scrapie Sheep, goats and mouflon Infection in genetically susceptible sheep Bovine spongiform encephalopathy
BSE Cattle Infection with prion-contaminated feedstuffs
Chronic wasting disease
CWD Mule deer, white-tailed deer and Rocky Mountain elk
Unknown mechanism; possibly from direct animal contact or indirectly from contaminated feed and water sources
Exotic ungulate encephalopathy EUE Nyala, greater kudu and oryx
Infection with BSE-contaminated feedstuffs
Feline spongiformencephalopathy
FSE Domestic and wild cats in captivity
Infection with BSE-contaminated feedstuffs
Transmissible mink encephalopathy
TME Mink (farm raised)
Infection with prion-contaminated feedstuffs

**Species-specificity of prions.** Most TSE agents, or prions, have a preference for infection of the homologous species, but cross-species infection with a reduced efficiency is also possible. After cross-species infection there is often a gradual adaptation of specificity for the new host; however, infectivity for the original host may also be propagated for several passages over a time-span of years. The process of cross-species adaptation can also vary among individuals in the same species and the rate of adaptation and the final species specificity is difficult to predict with accuracy. Such considerations help to form the basis for the biosafety classification of different prions.

**LABORATORY SAFETY**

**Biosafety level classification.** In the laboratory setting prions from human tissue and human prions propagated in animals should be manipulated at BSL-3. BSE prions can likewise be manipulated at BSL-3. All other animal prions are considered BSL-2 pathogens. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the guidelines applying to the source of the inoculum. Contact APHIS National Center for Import and Export at (301) 734-5960 for specific guidance. Although the exact mechanism of spread of scrapie among sheep and goats developing natural scrapie is unknown, there is considerable evidence that one of the primary sources is oral inoculation with placental membranes from infected ewes. There has been no evidence for transmission of scrapie to humans, even though the disease was recognized in sheep for over 200 years. The diseases TME, BSE, FSE, and EUE are all thought to occur after the consumption of prion-infected foods.1, 2 The exact mechanism of CWD spread among mule deer, white-tailed deer and Rocky Mountain elk is unknown. There is strong evidence that CWD is laterally transmitted and environmental contamination may play an important role in local maintenance of the disease.2

**Human prion diseases.** In the case of patients diagnosed with human prion disease, Standard Precautions are adequate. However, the human prion diseases in this setting are not communicable or contagious.3 There is no evidence of contact or aerosol transmission of prions from one human to another. However, they are infectious under some circumstances, such as ritualistic cannibalism in New Guinea causing kuru, the administration of prion-contaminated growth hormone causing iatrogenic CJD, and the transplantation of prion-contaminated dura mater and corneal grafts. It is highly suspected that variant CJD can also be transmitted by blood transfusion. However, there is no evidence for blood borne transmission of non-variant forms of CJD. Familial CJD, GSS, and FFI are all dominantly inherited prion diseases; many different mutations of the PrP gene have been shown to be genetically linked to the development of inherited prion disease. Prions from many cases of inherited prion disease have been transmitted to apes, monkeys, and transgenic mice carrying human PrP transgenes.

**SPECIAL ISSUES**

**Inactivation of prions.** Prions are characterized by resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone,
alcohols). While prion infectivity in purified samples is diminished by prolonged digestion with proteases, results from boiling in sodium dodecyl sulfate and urea are variable. Likewise, denaturing organic solvents such as phenol or chaotropic reagents such as guanidine isothiocyanate have also resulted in greatly reduced but not complete inactivation. The use of conventional autoclaves as the sole treatment has not resulted in complete inactivation of prions.\textsuperscript{5} Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. Some investigators recommend that formalin-fixed tissues from suspected cases of prion disease be immersed for 30 min in 96% formic acid or phenol before histopathologic processing (Table 3), but such treatment may severely distort the microscopic neuropathology. The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments and other materials is to discard and destroy them by incineration.\textsuperscript{6} Current recommendations for inactivation of prions on instruments and other materials are based on the use of sodium hypochlorite, NaOH, Environ LpH and the moist heat of autoclaving with combinations of heat and chemical being most effective (See Table 4).\textsuperscript{5, 6}

**Surgical procedures** Precautions for surgical procedures on patients diagnosed with prion disease are outlined in an infection control guideline for transmissible spongiform encephalopathies developed by a consultation convened by the WHO in 1999.\textsuperscript{6} Sterilization of reusable surgical instruments and decontamination of surfaces should be performed in accordance with recommendations described by the CDC (www.cdc.gov) and the WHO infection control guidelines.\textsuperscript{6} Table 4 summarizes the key recommendations for decontamination of reusable instruments and surfaces. Contaminated disposable instruments or materials should be incinerated.

**Autopsies** Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions can safely be done using BSL-2 precautions.\textsuperscript{7} The absence of any known effective treatment for prion disease demands caution. The highest concentrations of prions are in the central nervous system and its coverings. Based on animal studies, it is likely that prions are also found in spleen, thymus, lymph nodes, and intestine. The main precaution to be taken by laboratory personnel working with prion-infected or contaminated material is to avoid accidental puncture of the skin.\textsuperscript{3} Persons handling contaminated specimens should wear cut resistant gloves if possible. If accidental contamination of unbroken skin occurs, the area should be washed with detergent and abundant quantities of warm water (avoid scrubbing); brief exposure (1 minute to 1N NaOH or a 1:10 dilution of bleach) can be considered for maximum safety.\textsuperscript{6} Additional guidance related to occupational injury are provided in the WHO infection control guidelines.\textsuperscript{6} Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care at least in a BSL-2 facility.

**Bovine spongiform encephalopathy** Although the eventual total number of variant CJD cases resulting from BSE transmission to humans is unknown, a review of the epidemiological data from the United Kingdom indicates that BSE transmission to humans is not efficient.\textsuperscript{8} The most prudent approach is to study BSE prions at a minimum in a BSL-2 facility. When performing necropsies on large animals where there is an opportunity that the worker may be accidentally splashed or have contact with high-risk materials (e.g., spinal column, brain, etc.) personnel should wear full body coverage personal protective equipment (e.g., gloves, rear closing gown and face shield). Disposable plastic ware, which can be discarded as a dry regulated medical waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 1N NaOH and rinsed with water. HEPA filters should be bagged out and incinerated. Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that impervious gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids. Animal carcasses and other tissue waste can be disposed by incineration with a minimum secondary temperature of 1000°C (1832°F).\textsuperscript{6} Pathological incinerators should maintain a primary chamber temperature in compliance with design and applicable state regulations, and employ good combustion practices.
Medical waste incinerators should be in compliance with applicable state and federal regulations. The alkaline hydrolysis process, using a pressurized vessel that exposes the carcass or tissues to 1 N NaOH or KOH heated to 150°C, can be used as an alternative to incineration for the disposal of carcasses and tissue. The process has been shown to completely inactive TSEs (301v agent used) when used for the recommended period of time.

**TABLE 3**
**TISSUE PREPARATION FOR HUMAN CJD AND RELATED DISEASES**
1. Histology technicians wear gloves, apron, laboratory coat, and face protection.
2. Adequate fixation of small tissue samples (e.g., biopsies) from a patient with suspected prion disease can be followed by post-fixation in 96% absolute formic acid for 30 minutes, followed by 48 hours in fresh 10% formalin.
3. Liquid waste is collected in a 4L waste bottle initially containing 600 ml 6N NaOH.
4. Gloves, embedding molds, and all handling materials are disposed as regulated medical waste.
5. Tissue cassettes are processed manually to prevent contamination of tissue processors.
6. Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated as in Table 4.
7. In preparing sections, gloves are worn; section waste is collected and disposed in a regulated medical waste receptacle. The knife stage is wiped with 2N NaOH, and the knife used is discarded immediately in a "regulated medical waste sharps" receptacle. Slides are labeled with "CJD Precautions." The sectioned block is sealed with paraffin.
8. Routine staining:
   a. slides are processed by hand;
   b. reagents are prepared in 100 ml disposable specimen cups;
   c. after placing the coverslip on, slides are decontaminated by soaking them for 1 hour in 2N NaOH;
   d. slides are labeled as "Infectious-CJD."
9. Other suggestions:
   a. disposable specimen cups or slide mailers may be used for reagents;
   b. slides for immunocytochemistry may be processed in disposable petri dishes;
   c. equipment is decontaminated as described above or disposed as regulated medical waste.

**Handling and processing of tissues from patients with suspected prion disease**
The special characteristics of work with prions require particular attention to the facilities, equipment, policies, and procedures involved. The related considerations outlined in Table 3 should be incorporated into the laboratory's risk management for this work.

**TABLE 4**
**PRION INACTIVATION METHODS FOR REUSABLE INSTRUMENTS AND SURFACES**
2. Immerse in 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. Transfer into water rinsing well and autoclave (gravity displacement) at 121°C for 1 hour. Clean and sterilize by conventional means. Treat with disinfectant and rinse with water. Treat waste as contaminated liquid waste.
3. Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. Rinse instruments with water, transfer to open pan and autoclave at 121°C (gravity displacement) or 134°C (porous load) for 1 hour. Clean and sterilize by conventional means.
4. Surfaces or heat-sensitive instruments can be treated with 2N NaOH or sodium hypochlorite (20,000 ppm) for
1 hour. Ensure surfaces remain wet for entire time period, and then rinse well with water. Before chemical treatment, it is strongly recommended that gross contamination of surfaces be reduced because the presence of excess organic material will reduce the strength of either NaOH or sodium hypochlorite solutions.

5. Environ LpH (EPA Reg. No. 1043-118) may be used on washable, hard, non-porous surfaces (such as floors, tables, equipment, and counters), items (such as non-disposable instruments, sharps, and sharp containers), and/or laboratory waste solutions (such as formalin or other liquids). This product is currently being used under FIFRA Section 18 exemptions in a number of States. Users should consult with the State environmental protection office prior to use. (Adapted from www.cdc.gov, 10, 11)

**Working Solutions** 1 N NaOH equals 40 grams of NaOH per liter of water. Solution should be prepared daily. A stock solution of 10 N NaOH can be prepared and fresh 1:10 dilutions (1 part 10 N NaOH plus 9 parts water) used daily. 20,000 ppm sodium hypochlorite equals a 2% solution. Most commercial household bleach contains 5.25% sodium hypochlorite; therefore make a 1:2.5 dilution (1 part 5.25% bleach plus 1.5 parts water) to produce a 20,000 ppm solution. This ratio can also be stated as two parts 5.25% bleach to three parts water. Working solutions should be prepared daily. CAUTION: Above solutions are corrosive and require suitable personal protective equipment and proper secondary containment. These strong corrosive solutions require careful disposal in accordance with local regulations.

**Precautions in using NaOH or sodium hypochlorite solutions in autoclaves** NaOH spills or gas may damage the autoclave if proper containers are not used. The use of containers with a rim and lid designed for condensation to collect and drip back into the pan is recommended. Persons who use this procedure should be cautious in handling hot NaOH solution (post-autoclave) and in avoiding potential exposure to gaseous NaOH, exercise caution during all sterilization steps, and allow the autoclave, instruments, and solutions to cool down before removal. Immersion in sodium hypochlorite bleach can cause severe damage to some instruments. This procedure should only be used on instruments that need be re-used. It should be avoided if at all possible in order to prevent instrument and personnel injury.

**REFERENCES**

SOURCE
Appendix 2

SUSPECTED HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES:
MODIFICATIONS OF STANDARD PRECAUTIONS AND PROCEDURES

I. POLICY: It is the policy at UCSF to confine and contain materials contaminated by patient fluids or tissues in order to prevent the transmission of prions.

II. OBJECTIVES:

1. To confine and contain contaminated materials generated during the course of performing autopsies and during brain cutting conferences.

2. To confine and contain contaminated materials generated upon receipt and processing of surgical biopsies from the operating room.

3. To prevent transmission of prions to pathology personnel or others.

III. BACKGROUND INFORMATION:

Human Transmissible Spongiform Encephalopathies (TSE)

Creutzfeldt-Jakob Disease (CJD)
- sporadic CJD (90% of human TSEs)
- familial CJD
- iatrogenic CJD
- new variant CJD (vCJD) caused by Bovine Spongiform Encephalopathy (BSE) (Mad Cow Disease in the UK & Europe)

Fatal Insomnia
- sporadic fatal insomnia (SFI)
- familial fatal insomnia (FFI)

Gerstmann-Sträussler-Scheinker disease (GSS)

CJD is a rare, fatal disease of the central nervous system characterized by rapid, progressive dementia, sensory disturbances, myoclonus and a characteristic electroencephalogram. CJD is present worldwide with an incidence of 1 case per million people.

Most cases of CJD are sporadic and the length of the incubation period for these cases is unknown; although, once clinical signs present, death follows in an average of 6 months. In contrast, cases with defined exposure events to high-risk tissue appear to develop symptoms within months to years. Death in CJD patients occurs within 6-12 months post onset of symptoms and is often due to pneumonia or other complicating conditions of dementia. In recent years there has been described a “new variant” form of CJD (vCJD) occurring primarily in the United Kingdom and less frequently in Europe. This form of CJD differs from the sporadic disease. All of the evidence indicates that vCJD is acquired by ingestion of food contaminated with a highly virulent form of bovine spongiform encephalopathy (“mad cow disease”).

The causative agents or prions are small, proteinaceous particles that are resistant to the conventional methods of sterilization and decontamination. However, because prions are proteins, they are inactivated by procedures that denature or hydrolyze proteins such as exposure
to Sodium Hydroxide. The patient’s brain tissue, cerebrospinal fluid, and cornea should be considered infectious. Due to the hardness of the causative agent, special precautions must be taken when handling items that have come into contact with the patient’s brain tissue, CSF, or cornea.

**RISK OF CJD TRANSMISSION AS A FUNCTION OF TYPE OF TISSUE**

<table>
<thead>
<tr>
<th>RISK</th>
<th>TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIGH</td>
<td>Brain, dura mater, cornea</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>CSF, kidney, liver, lymph node, spleen, lung</td>
</tr>
<tr>
<td>LOW TO NONE</td>
<td>Blood, urine, adrenal gland, feces, heart, bone marrow, muscle, nasal mucus, peripheral nerves, saliva, gingiva, sputum, tears</td>
</tr>
</tbody>
</table>

CJD prions have been transmitted among humans by medical procedures (iatrogenic CJD) and to animals in the laboratory. In laboratory animals, the most effective method of infection is by intracerebral inoculation of prions. Intraperitoneal and percutaneous inoculation is significantly less effective. Ingestion of prions is least effective.

**Iatrogenic CJD:** Iatrogenic spread of CJD from person to person is exceedingly rare (240 cases total as of June 2001): It has occurred after transplantation of CJD-infected corneas (3 cases), dura mater grafts (>105 cases), or stereotactic depth electrodes previously used on infected individuals (3 cases). An increasing number of episodes of iatrogenic CJD have been described in young patients who received injections of human cadaveric growth hormone for treatment of endocrine disorder (>105 cases) or cadaveric pituitary gonadotropin for infertility (5 cases).

**Occupationally acquired CJD:** There have been no documented reports of transmission of disease from patients to hospital or mortuary staff. Epidemiological studies have documented occupationally-acquired CJD in three laboratory workers. All three had percutaneous exposures to high-risk tissues.

**IV. GENERAL PRION SAFETY ISSUES AND GUIDELINES:**

**NOTE:** *TRANSMISSION OF CJD HAS NOT BEEN ASSOCIATED WITH ENVIRONMENTAL CONTAMINATION OR FOMITES.*

Prion infectivity is strongly stabilized by drying. Therefore, contaminated reusable instruments should be kept wet between time of use and disinfection in chemical disinfectants (WHO Infection Control Guidelines).

The WHO also recommends that, “Policy makers should be guided by …. expectations of how the instrument will be re-used. In this way, the most stringent recommendations are applied to instruments contacting high infectivity tissues of a person with a known TSE, which will also subsequently be re-used in the CNS or spinal column (of a living patient).” The goal in the surgical pathology and autopsy suites is to eliminate the risks to personnel.

**Disposable instruments:** WHO emphasizes that “…the safest and most unambiguous method for ensuring that there is no risk of residual infectivity on … instruments is to discard and destroy them by incineration.”
Instruments which must be reused: WHO recommends … “that, where possible, two or more different methods of inactivation be combined in any sterilization procedure for these agents.” Procedures that employ heat and NaOH (either consecutively or simultaneously) appear to be sterilizing under worst-case conditions (e.g., infected brain tissue partly dried on to surfaces).”

The CDC notes that sodium hypochlorite may be corrosive to some instruments. This procedure should be only be used on instruments that need to be re-used. Persons who use this procedure should be cautious in handling hot NaOH solution (post-autoclave) and in avoiding potential exposure to gaseous NaOH; exercise caution during all sterilization steps; and allow the autoclave, instruments, and solutions to cool down before removal.

Waste: Waste management of suspect Human Spongiform Encephalopathy (CJD, etc.) contaminated equipment, supplies and liquids requires confinement and containment of waste followed by high temperature autoclaving or chemical treatment of contaminated equipment and solutions generated during autopsy. Waste containment and decontamination is required in subsequent processing steps in the department. Therefore, hazard communication must be maintained throughout.

Personal protective equipment (PPE): Extreme caution must be taken to avoid puncture of the skin. Wear cut resistant gloves and 2 pair of latex gloves during autopsy and tissue slicing procedures. If accidental contamination of skin occurs, swab area with either undiluted bleach or 1N sodium hydroxide, allowed to remain for 5 minutes, then wash with copious amounts of water. Call the Exposure Hotline and report injury to your supervisor.

Preparation for an autopsy or neurosurgical biopsy specimen: Prepare all materials needed ahead of time, lay down disposable plastic sheeting on work surfaces and apply non-contaminating technique so that the exterior of containers and other work surfaces do not become contaminated. Use disposable supplies and instruments to the extent possible to reduce sources of transmission and minimize the number of items requiring decontamination.

Hazard communication and labeling: Hazard communication must continue through every step in the processing cycle to include autopsy storage containers, specimen cassette containers, specimen cassettes, paraffin blocks, light slides, EM vials (blocks, slides & grids), log books, and notation with the computer request, i.e. CoPath Histo Data Entry block detail field. Use the following warning statement, “CJD Precautions”, since different disinfection protocols apply.

V. DECONTAMINATION OF INSTRUMENTS:

1. A Two-Step Procedure for Autoclavable Materials (as Recommended by WHO)
   a. 1N Sodium Hydroxide (Fisher #LC24380-4)
      Use: Apply to equipment 2-3 times over the course of one hour. Wipe down and clean as usual. Soak instruments in a plastic basin with a minimal amount of solution. Rinse well with water. Collect all contaminated solutions in the NaOH Waste Collection Container for pick up by EH&S, 6-1300. Follow with autoclaving of equipment where possible.
   b. Autoclave NaOH treated instruments at 134°C in water (gravity displacement steam autoclaving for 90 minutes). Or porous load steam autoclaving for one 18 minute cycle at 30 lbs psi or six three-minute cycles at 30 lbs psi.

2. Disinfection, non-autoclavable equipment:
   1N sodium hydroxide stock 2 Hr. Wipe equipment.
3. Disinfection of Liquid Waste:

Waste Collection Container: 10N Sodium Hydroxide (Fisher #LC24460-4)

**Instructions:** Place 400 ml 10N in a 4L waste container. Add liquid waste and dilute to 4L. Dispose through EH&S Hazardous waste pick-up, 6-1300. Hazardous-waste pick-up tag labeling: “CJD contamination in 0.1N NaOH, may contain up to 10% formalin”.

NOTE: Obtain a 1N final concentration of NaOH in the waste collection containers.

VI. AUTOPSIES OF PATIENTS WITH SUSPECTED PRION DISEASE:

1. Modifications of Standard Necropsy Procedure

**Staff:** Attendance is limited to two staff including the Neuropathologist.

**PPE:** Standard autopsy attire is mandatory. However, a disposable, waterproof gown is worn in place of a cloth gown. Wear cut-resistant gloves underneath two pairs of surgical gloves or chain mail gloves between two pairs of surgical gloves. UCSF has approved use of an Air-Mate® HEPA 12 Powered Air-Purifying Respiratory System (PAPR) (RACL Health and Safety, Inc., Frederick, MD) during the brain removal when aerosols are mainly created as a result of opening the skull with a Stryker saw. The respirator is composed of a polycoated Tyvek head cover, HEPA filter unit containing battery pack, belt and HEPA filter, AC adapter/charger, airflow indicator, breathing tube assembly.

**Dedicated equipment and instruments:** The Morgue contains a complete set of duplicate instruments for CJD autopsies including Stryker saw & blade & wrench, skull breaker & hammer, 5” forceps & 5” scissors, rib cutter.

**Containment:** Confinement procedure to the infectious procedure room, and keep the door closed during the procedure. Collect all liquid waste and mix 1:1 with 2N NaOH in the waste bottle. Collect all disposable solid wastes in double-lined biohazard container.

**Non-disposable equipment:** Stryker saw & blade & wrench, skull breaker & hammer, 5” forceps & 5” scissors, rib cutter. HEPA bone dust collector.

**Disposable equipment:** Head rest, Cutting Board, Scalpels, forceps, scissors plastic pre-filled formalin containers, HEPA filters, Brain knife.

**Pre-necropsy preparation:**

a. Drape the autopsy table completely with waterproof sheeting.

b. Drape instrument trays, work surfaces and weighing pans with plastic or disposable plastic underpads. Use clear 2-inch plastic tape to connect seams and to secure edges against the table.

c. Fill one 165 oz. plastic bucket half full with 10% formalin for fixing the brain and prepare a second formalin container if required for in situ sampling of other tissues.

d. Line waste cans with red biohazard plastic bags.

e. Place the electronic balance in a clear plastic waste bag, and secure the cord out of the work area.

2. Body Dissection Procedure:

a. All procedures are performed in the body bag in which the body was received.
b. Place body on the plastic sheet-protected autopsy table.
c. Place CJD instruments and pre-filled formalin containers on the draped instrument table. Confine contaminated instruments on plastic underpads.
d. Perform thoracic/abdominal procedures first, using one scalpel blade at a time and to prevent aerosols, use a manual rib cutter to remove the rib plate. Line the rib cage with disposable blue underpads to prevent cuts or abrasions to prosector. Assist the Neuropathologist as required to perform in situ sampling of organs.
e. Aerosols are mainly created during opening of the skull with the Stryker saw. To reduce bone dust aerosols in the room, place a plastic bag over the head and securely tie it around the neck. Open the sealed end of the plastic bag. The scalp is incised and the skull cap is removed with the Stryker saw within the bag. An alternate procedure is use of a bone dust aspirator attached to the Stryker saw which removes all aerosols and deposits them in HEPA filter. During bone sawing, the UCSF-approved Air-Mate® HEPA 12 Powered Air Purifying Respiratory System (“space suit”) are worn by the prosectors. The respiratory “space suit” can be removed for all other procedures since they do not generate aerosols.
f. Remove the brain as usual. Place the intact brain in a lined weighing pan, weigh it, and place the brain on a disposable cutting board. Assist the Neuropathologist as needed while they dissect appropriate samples for snap freezing. After sampling is completed, place the brain immediately in the 165 oz container of 10% formalin. DO NOT place a suspected prion disease brain in with other specimens in the brain tank.
g. After the procedure, suture the decedent and wipe down suture areas with dilute bleach.
h. Discard all disposables in red biohazard waste bags and secure the bags.
i. Do not flush contaminated liquid down drains. Mix 1:1 with 2N NaOH in a waste collection bottle.

3. Modifications of Autopsy Suite Decontamination Procedures

a. Place instruments (open box locks and jaws) and saw blades into a large stainless steel dish.
b. Soak instruments for 1 HR in Kleenzyme.
c. Immerse for 1Hr in 1N Sodium Hydroxide.
d. Rinse instruments for 2-3 min in water. (Collect all waste).
e. Transfer instruments into red autoclavable biohazard waste bags and hand deliver or place instruments on a steel cart for delivery to Material Services Decontamination Room M-01, along with a completed Material Services Interdepartmental Request form to request sterilization of the instruments by Material Services. Requisition copies: place the original copy with the instruments to be sterilized. Retain duplicate copies and provide the yellow copy back to Material Services when picking up the sterilized instruments.
f. Clean the Stryker saw by wiping with 1N sodium hydroxide solution and the exterior of the bone collector to the extent possible.
g. Place absorbent table cover and instrument pads, disposable clothing, etc. in double bagged infectious waste bags for incineration.
h. Any suspected areas of contamination of the autopsy table or room are decontaminated by wetting surfaces over one hour with 1N sodium hydroxide, followed by thorough rinsing and washing.

4. Unfixed, Frozen Samples of Brain Obtained by Biopsy During Life or At Autopsy.

The most effective and precise techniques for establishing or ruling out the diagnosis of prion disease is by the histoblot technique and Western analysis for PrPSc, both of which require samples of unfixed frozen brain. Ideally at autopsy, 1-2 cm³ samples should be taken from
the frontal cortex, parietal cortex and the cerebellar cortex. In each case, the tissue block must include the full thickness of the cortex with underlying white matter. In the case of a surgical biopsy, a 1 cm$^3$ biopsy of cerebral cortex should be bisected from the cortical surface through to the underlying white matter with one-half formalin-fixed and the other frozen. Alternatively, the brain can be bisected sagitally. One half is sectioned coronally into slabs 1-2 cm thick. The other half is immersion fixed in formalin.

In each case, the sample to be frozen is placed in a labeled plastic bag and sealed. The outside of this bag is assumed to be contaminated with prions and other pathogens. With fresh gloves or with the help of an assistant with uncontaminated gloves, the bag containing the specimen is placed into another plastic bag which does not have a contaminated outer surface. The samples can then be frozen on dry-ice or in a -70°C freezer.

**VII. MODIFICATIONS OF BRAIN CUTTING PROCEDURES:**

1. After adequate formaldehyde fixation (at least 10-14 days).

2. Brain cutting is performed on the morgue table in the Infectious Disease Containment room. This table is fitted with its own ventilation system which reduces formalin-fume hazard.

3. The brain is not rinsed with running water as is done with non-prion disease brains.

4. The brain is examined and cut on a disposable cutting board which is placed on the morgue table which has been covered with an absorbent pad with an impermeable backing. Dedicated disposable brain cutting knives are available and must be used. Discard disposables in the appropriate biohazard waste containers.

5. Samples for histology are placed in cassettes labeled with "CJD precautions." In the Neuropathology Research Laboratory where all of the CJD cases are currently processed for neurohistopathological analysis, tissue blocks are only subjected to formalin fixation. If tissue is to be processed in the general pathology laboratory, the formalin-fixed tissue blocks must be placed in 95-100% formic acid for one hour, followed by fresh 10% formalin solution for at least 48 hours. The latter eliminates all prion infectivity in the embedded specimen.

6. All instruments and surfaces coming in contact with the tissue are decontaminated as described previously.

7. Tissue remnants and cutting debris must be discarded in the biohazard waste containers for incineration. Contaminated formaldehyde solution must be discarded in a prepared container with NaOH at final dilution of 1N, for disposal through EH&S hazardous waste pick-up.

**VIII. SURGICAL BIOPSIES:**

Contact Neuropathology upon receipt of brain biopsy specimens labeled with “CJD Precautions” so that proper processing may be initiated. This may typically require contacting the clinician and/or neurosurgeon to confirm required processing methodology. Retain the specimen container in the biohazard bag it is received in until the Neuropathologist has given direction on how to proceed.

**IX. TISSUE PREPARATION:**
At UCSF, processing and embedding of tissue blocks, cutting sections, and neurohistological and immunohistochemical staining of tissue sections are all performed in the Neuropathology Research Laboratory.

X. ELECTRON MICROSCOPY:

Processing precautions would include preparation of a 500 ml waste bottle containing 80 ml of 6N NaOH for collection of liquid waste. Standard PPE precautions apply with the addition of cut-resistant gloves when slicing wet tissue. Dispose of contaminated beakers, underpads, etc. in a biohazard bag.

1. Obtain disposable forceps from autopsy laboratory if possible –or- disinfect forceps using 2N NaOH for 1 hour, both grid forceps and wet tissue forceps. Wipe, then use regular disinfection.


3. Collect thick section trimmings using scotch tape, dispose in biohazard container.

4. Collect staining waste solutions and water used in washing in the NaOH waste bottle.

5. Treat the tip of the specimen holder with 2N NaOH for 1 HR, followed by regular polishing and cleaning.


References:
Schulster, L.: Draft CDC Guidelines; 2-8-99