Precautions for work with human and animal transmissible spongiform encephalopathies / Advisory Committee on Dangerous Pathogens.

Advisory Committee on Dangerous Pathogens

Precautions for work with human and animal Transmissible Spongiform Encephalopathies

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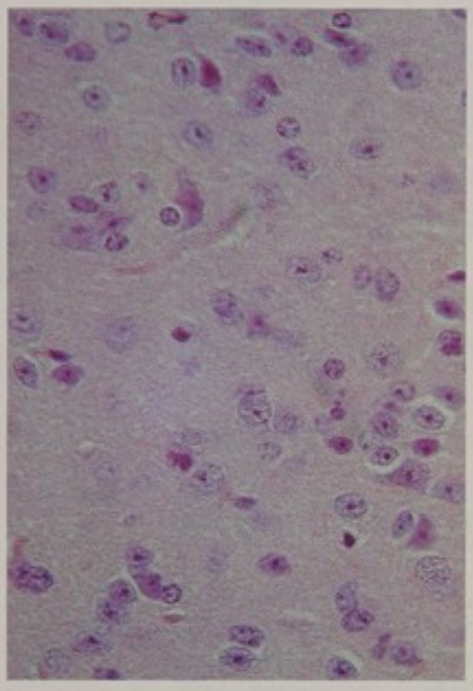
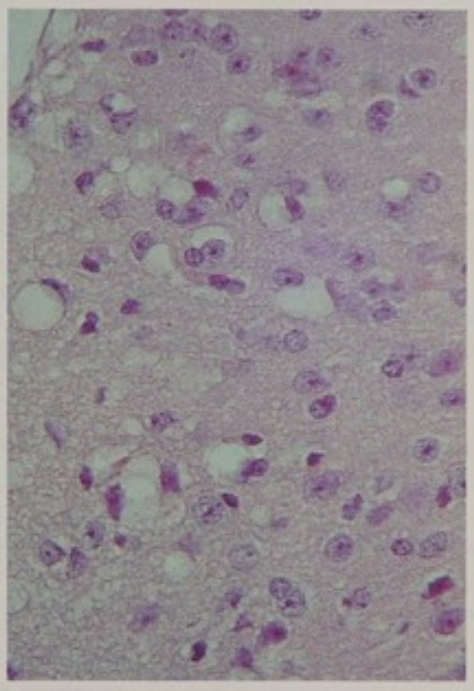


NORMAL BRAIN TISSUE



SE ASSOCIATED BRAIN VACUOLATION

Precautions for work with human and animal Transmissible Spongiform Encephalopathies

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Statement by the Health and Safety Commission about this guidance

The guidance on control measures applicable to the transmissible spongiform encephalopathies is prepared, in consultation with the HSE, by the Advisory Committee on Dangerous Pathogens, which was appointed by the Health and Safety Commission and the Department of Health, as part of a formal advisory structure. The guidance represents what is considered to be good practice by the members of the Committee. It has been agreed by the Commission and the Health Ministers. Following this guidance is not compulsory and you are free to take other action. However, if you do follow this guidance you will normally be doing enough to comply with the law. Health and Safety Inspectors seek to secure compliance with the law and may refer to this guidance as illustrating good practice.

This guidance should be read in conjunction with the COSHH regulations which are to be amended in 1994 and supplemented by an Approved List of biological agents

Further information about this and other ACDP guidance can be obtained from the Joint Secretariat of the ACDP at:

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Glossary

ACDP:	Advisory Committee on Dangerous Pathogens
ACGM:	Advisory Committee on Genetic Modification
BSE:	bovine spongiform encephalopathy
CJD:	Creutzfeldt-Jakob Disease
CNS:	central nervous system
CSF:	cerebro spinal fluid
CWD:	chronic wasting disease
GMM:	genetically modified microorganism
GSS:	Gerstmann-Straussler-Scheinker Syndrome
hGH:	human growth hormone
hPG:	human gonadotrophin
IC:	intracerebral
PrP:	naturally occurring cell membrane protein. Accumulation of a protease resistant form of this protein is characteristic of the TSEs.
TME:	transmissible mink encephalopathy
TSE:	transmissible spongiform encephalopathy
UV:	ultra-violet

Foreword

The recent emergence of bovine spongiform encephalopathy (BSE) in domestic cattle in the United Kingdom has restimulated interest in the field of human and animal transmissible spongiform encephalopathies (TSEs). The three clinical conditions in man that fall within this group, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS) and kuru, have been recognised for a number of years, but their rarity and the special technical difficulties associated with their study have always impeded extensive work on them. However, a similar condition of sheep, known as 'scrapie', has been familiar for more than two centuries and knowledge gained from studies on this disease is proving valuable.

Publication of this guidance on recommended safe methods for working with the TSEs of man and animals does not signal recognition of any known rise in the annual incidence of human disease or any newly identified risk to workers. A current national surveillance study on CJD funded by the Department of Health has shown no association between incidence of CJD and any occupational group. Nevertheless, because of the increasing activity in the field of research into the TSEs, it was considered opportune to review the existing guidance for work with these agents, and update it as necessary. The aim has been to define more closely the conditions under which various types of work should be conducted.

Aside from worker safety, there is also the consideration of possible transmission of human TSE agents to patients during medical or surgical treatments. This is known to have occurred in the past, if only rarely, from neurosurgical procedures, transplantation of certain tissues e.g. *dura mater* and *cornea*, and treatment with hormones derived from human pituitary material. The Department of Health has issued guidance on the management of patients with CJD and it is not the intention here to address the clinical issues involved in any detail.

In the light of emerging information from work with scrapie and BSE, it is timely to review and update earlier guidance on the stringent sterilisation and disinfection methods needed for this group of agents. However it should be noted that our knowledge of them and some aspects of their transmission are as yet unclear. As new evidence on the properties of the several TSE agents under study becomes available, the guidance will be kept under review.

In the meantime, it was felt to be appropriate and useful to draw together the basic facts about transmissible spongiform encephalopathies to provide information for all concerned in laboratory work, in clinical care and animal handling.

Advisory Committee on Dangerous Pathogens
June 1994

PART I Introduction

1. Transmissible Spongiform Encephalopathy – *The clinical condition.*

1. The term transmissible spongiform encephalopathy (TSE), describes a rare and fatal degenerative condition of the central nervous system occurring in man and certain animal species (Table 1) which is transmissible. It is also sometimes known as transmissible degenerative encephalopathy. A usual feature of TSE is the appearance of microscopic vacuoles in the brain from which the condition derives its name (see frontispiece).

Table 1 The Transmissible Spongiform Encephalopathies

Human:	Creutzfeldt-Jakob disease
	Gerstmann-Straussler-Scheinker syndrome
	kuru
Animal:	scrapie – sheep, goats and moufflon
	bovine spongiform encephalopathy
	transmissible mink encephalopathy
	chronic wasting disease in captive mule deer and Rocky Mountain elk
	feline spongiform encephalopathy (domestic cat, cheetah*, puma*)
	spongiform encephalopathy in captive exotic ungulates (kudu, nyala, oryx*, gemsbok*, eland*)

* assumed transmissible, but transmissibility not yet demonstrated.

The human TSEs

2. The three TSEs that are recognised in man are Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS) and kuru. All have a pre-clinical phase that may last for years, followed by symptomatic neurological illness characterised by progressive dementia with loss of memory and intellect and personality changes, or with progressive unsteadiness and clumsiness. In CJD, sudden involuntary muscular jerking is also frequently seen. In most cases, death occurs within a year of onset of symptoms and the patient is usually mute and immobile in the terminal stages.

3. **CJD** was first described in the 1920s and is very rare worldwide, affecting less than 1 per million of the UK population each year. In about 10% of cases, more than one family member is affected. The usual age of onset is 50–65 years.

4. **GSS** is an extremely rare form of TSE in which several genetically related members of a family may be affected. Affected individuals show progressive dementia, as in CJD, but the disease is usually less acute and generally starts at a slightly earlier age.

5. **Kuru** is a unique form of TSE found in the Fore tribe of New Guinea in the 1950s and associated with funeral rites involving ritual preparation of and contact with the brains of kuru victims. The established link between contact with brain tissue and the subsequent development of kuru first suggested an infective basis for all human TSEs.

6. **Scrapie** in sheep and goats has been recognised for more than 250 years and is endemic in flocks in many countries. There is no evidence that it can be passed to man. Affected animals scrape themselves against fences to alleviate itching, become unsteady on their feet and stop feeding. When they die, their brains show the characteristic microscopic changes of TSE.

7. **Bovine spongiform encephalopathy** (BSE) first occurred in 1985 and, until recently, was confined to cattle in the British Isles (small numbers of cases have now been identified on the continent). BSE is thought to have arisen from feeding cattle with protein concentrates obtained from a changed rendering process. Affected animals become unsteady on their feet, lose weight and become nervous – hence 'mad cow disease'. Their brains also show the characteristic microscopic changes of TSE.

It is anticipated that the incidence of BSE will fall in the next few years following the ban on feeding ruminant protein to ruminants (July 1988). There is no indication that BSE is transmissible to man under normal conditions of contact or through the food chain. All animals are destroyed and disposed of as soon as the disease is suspected and all offal of specified types from all bovines other than those under 6 months is banned for human or animal consumption (Bovine Spongiform Encephalopathy Order, 1991).

8. **Other animal TSEs** A disease similar to scrapie has also occurred in captive mink, giving rise to the condition known as 'transmissible mink encephalopathy' or TME. Chronic wasting disease (CWD) in Rocky Mountain elk and captive mule deer is also considered to be similar to scrapie. TSE has also been recognised in small numbers of exotic ungulates and cats.

2. Diagnosis of TSE infection

9. The identification and diagnosis of TSEs in both man and animals is by clinical and neuropathological examination. In man, an electro-encephalogram may give a pattern characteristic of CJD but diagnosis is usually confirmed only after death, by neuropathological investigation. Brain biopsy can be unreliable and is therefore not appropriate for confirming a clinical diagnosis. The disease is characterised by the accumulation of a protease resistant form of a naturally occurring cell membrane protein (PrP). The disease related PrP accumulates in extraneuronal foci, many of which are identifiable as amyloid plaques.

10. No conventional immunological response is seen in humans or animals affected with TSEs. There are currently no diagnostic tests available that allow detection of the preclinical state. Transmission bio-assay, involving the inoculation of material into a susceptible species and looking for characteristic signs of the disease, is the only established way of identifying the pre-clinical state. Research is in progress to develop more practical diagnostic methods.

3. The nature of TSE agents

11. The epidemiology of diseases such as scrapie and kuru and experiments demonstrating transmissibility of disease both within and between species, suggest that the TSEs are caused by transmissible agents. However, to date the nature of such agents remains uncertain.

Properties of the agents

12. All agents of TSE exhibit an unusual resistance to conventional decontamination methods used in clinical and laboratory practice. They are not significantly affected by a number of standard chemical agents such as formalin and ethylene oxide, and infectivity persists after autoclaving at conventional times and temperatures (eg 121°C for 15 minutes). In addition, only extremely high doses of ionising and UV irradiation have been successful in reducing infectivity. Such treatments would be expected to destroy the most resistant conventional viruses and bacterial spores and this suggests that the agents of TSE are unconventional. The modified PrP associated with infectivity is partially protease resistant.

In addition, scrapie agent has been shown to survive in the environment for several years, indicating the persistent nature of the agent.

Scrapie agent also exhibits strain variation, expressed for example as variation in incubation period in test animals.

Theories on the nature of the agents

13. A consistent feature of the TSEs is that a normal neuronal membrane protein (PrP) becomes altered to a protease resistant form which is not degraded by the host, and accumulates in the CNS.

One hypothesis regarding the nature of the transmissible agents of TSE is that the disease specific form of PrP is, per se, the transmissible agent (**prion**), and that the transmission of prions to other hosts induces post translational modification of the normal PrP of the new host to produce further prions. This could explain the unusual resistance of the agent to conventional decontamination procedures and the absence of any immune response.

A second theory proposes that the transmissible agent is an unusual, quasi-viral hybrid (**virino**) where there is an independent genome of nucleic acid enveloped within a host derived protein coat, which could be PrP. The virino hypothesis argues that it is the association of the genomic nucleic acid with the PrP that induces the modification of PrP to its amyloidogenic form. Failure to detect nucleic acid and the resistance properties observed could be explained by only very small quantities of highly protected genetic material being present. The virino theory also could explain the absence of an immune response, since all agent protein components are host derived.

A third theory considers the agent to be a conventional, but as yet unrecognised virus. However, it is unlikely that such a candidate could have both the survival characteristics required and have defied detection by the extensive electron microscope studies that have been conducted, and fail to provoke a detectable immune response.

14. None of the present theories adequately explains all the known and unusual properties of the TSE agents. These include their unusual survival characteristics, extreme resistance to the majority of conventional chemical and physical decontamination regimes, the presence of strain variation and association with PrP. Much research effort is being directed towards identifying the nature of the causative agents of TSE.

Host genetics

15. There appears to be a host genetic component in the development of TSE, but the role of the host's prion protein (PrP) gene is unclear. Recent

work using mice transgenic for hamster PrP suggests that the prion gene influences both the synthesis of the abnormal PrP and the pattern of disease development.

16. Several specific mutations of the human PrP gene have been consistently associated with familial CJD and GSS. Similarly, it has long been recognised that certain genes in sheep (the *Sip* gene – scrapie incubation period gene) influence the incubation period of the disease. The *Sinc* gene (scrapie incubation gene) has a similar function in mice. Studies suggests that these genes are analogous with the PrP gene.

4. Transmission of TSEs

17. The natural mechanisms of transmission of human and animal TSEs are not yet fully understood. However, all experiments and epidemiological studies suggest that both human and animal TSEs are not transmissible by normal social contact. There has been no known transmission of animal TSE to man.

18. It has been shown that TSEs can be transmitted experimentally to animals by inoculation, though initially cross species transmissions can be difficult to achieve and result in very prolonged incubation periods. Animal models have been widely used to study scrapie for many years and these have demonstrated that intracerebral (IC) inoculation of scrapie infected material is usually a much more effective route of transmission than either parenteral or oral challenge. CJD, GSS and kuru brain material have produced TSE in primates after IC inoculation. Transmission experiments with BSE are still being conducted, but transmission to rodents, cattle, marmosets and pigs by IC inoculation and to rodents by oral challenge, has been demonstrated.

19. Maternal transmission i.e. from ewe to lamb has been demonstrated with scrapie, and it is possible that some spread of scrapie within a flock may be explained by consumption of the placenta by other animals. Maternal transmission has not been observed to date with BSE but experiments are still in progress. The position with human agents is unknown, but epidemiological information does not support this as a route of transmission for human agents. Familial CJD and GSS are unique and seem to occur in families with particular mutations in the PrP gene.

Iatrogenic Transmission of human agents

20. A small number of CJD cases have been associated with medical and surgical treatment.

Human Growth Hormone (hGH): A small number of individuals who had received treatment with hGH have developed CJD. The hormone used was prepared from human pituitary glands and it is believed that contamination resulted from the unwitting inclusion of some donors infected with the CJD agent. In the United Kingdom, ten of the 1,981 patients treated with hGH have developed CJD. Since 1985, hGH has been manufactured by recombinant DNA techniques and there are now no risks of acquiring CJD in this way in the UK.

Human gonadotrophin (hPG): Four cases of CJD have been identified in Australian women who were treated with this human pituitary derived hormone in the 1970s. There have been no cases of CJD attributable to treatment with this preparation in the UK.

Dura mater and corneal transplants: A few cases have been described of people contracting CJD after the use of cerebral membranes (*dura mater*) in surgical repair. The incubation period has varied from less than two years to nearly ten. There has been one case of CJD developing after a corneal transplant in 1974; none have been reported subsequently.

Neurosurgery: Three cases of CJD occurring in the UK in the 1950s in patients who had undergone brain surgery are thought to have been due to contaminated surgical instruments, despite treatment by conventional sterilisation techniques. The Department of Health issued guidance in 1981, 1984 and 1992 concerning the management of patients with or at risk of developing CJD, and recommendations for the disposal and sterilisation of surgical instruments used on known or suspect CJD patients.

Occupational Transmission of human agents

21. No confirmed cases of occupational transmission of any of the TSEs have been recorded. There have been a very few anecdotal reports of CJD in retired laboratory workers and in a pathologist, but the evidence that their disease was associated with their occupation remains at best speculative. The current national surveillance study on CJD funded by the Department of Health has shown no association between incidence of CJD and any occupational group.

PART II Hazards and risks

1. Hazard categorisation of TSE agents

22. The Advisory Committee on Dangerous Pathogens (ACDP) has categorised human pathogens according to hazard and made recommendations for appropriate categories of laboratory and animal room containment[1]. In determining the appropriate hazard grouping of a pathogen, note is taken of the pathogenicity (disease-producing capability) of the organism to man, the hazard to laboratory workers, the potential for transmission to the community and the seriousness of any illness that might result after taking into account the availability of prophylaxis or effective treatment (see Annex 1 for definitions of Hazard Groups).

The human TSE agents

23. The TSEs are invariably fatal, at least for those where the disease has been diagnosed clinically, and there is no effective prophylaxis or treatment available. This suggests that the highest of the ACDP's Hazard Groups would be appropriate for the human TSEs.

However, apart from the rare examples of iatrogenic transmission, there is no evidence that CJD or GSS has been or can spread to the community, or has occurred in workers through occupational exposure. This would suggest a Hazard Grouping of no higher than 2. The earlier guidance from the ACDP published in 1984, was reviewed for a second edition in 1990 and the Hazard Grouping of the human agents of TSE affirmed as Group 2. However, the European Community classification of Biological Agents which will be implemented as law in the UK by mid 1994 will require human TSE agents to be placed in 'Risk Group' 3, but with a derogation concerning their containment (Table 2). Thus, full Containment level 3 may not always be necessary for work with these agents.

Table 2 Summary of categorisation of the agents of TSEs

	Agent	Hazard Group*
Human	CJD	3
	GSS	3
	kuru	3
Animal	scrapie	1
	BSE	1†
	Others	1†

*this categorisation is in conformity with EC Directive 93/88 on the Classification of Biological Agents (para 23)
†there is currently no evidence that these are pathogenic for man, but until more evidence is available some additional precautions are recommended. These are outlined in Part III.

[1]ACDP: Categorisation of pathogens according to hazard and categories of containment. 2nd edition 1990.HMSO ISBN 0–11–885564–6

A summary of recommended containment levels needed for experimental work with TSEs is given in Part III.

The animal TSE agents

24. Animal TSEs, like human TSEs, appear to be invariably fatal in the animals they affect. However, as there is no evidence of transmission of any animal TSE to humans, Hazard Group 1 is indicated (Table 2). In consequence, Containment Level 1 is appropriate when handling intact animals or tissue perceived to be of low risk to man.

Nevertheless, any work with lymphoid or neural tissues from or extracts derived from known or suspected cases of animal TSE should be conducted using procedures that limit exposure and, in particular, reduce the risk of accidental parenteral inoculation (Part III).

2. Hazard containment

25. The Containment Level at which a pathogen is to be handled, usually corresponds with its categorisation. Although the agents responsible for CJD, GSS and kuru are to be placed in Group 3, there is the facility to recommend a lower level of containment than is indicated by the hazard group for some agents. Therefore, given that the human TSE agents are likely to be transmitted only by parenteral routes, the recommendation in these guidelines is that Containment Level 2 may be used but with the additional precautions shown in Table 5. These reflect, in part, the incomplete state of knowledge of the TSE agents and the need to protect workers from potential risk.

The extent of use of such additional precautions should be determined by a local risk assessment that would need to take into account, amongst other factors; the agent under investigation, the tissues being manipulated, the type of experimental or other work to be performed and the concentrations of the agents likely to be encountered. In particular, experimental work involving concentration or modification of agents may require special consideration.

Containment for genetic modification work with PrP genes has been considered by the Advisory Committee on Genetic Modification (para 39 and Annex 3).

PART III Control of infection and containment

1. Introduction

26. This section applies to all work or contact with preparations, body fluids or tissues known or likely to contain the agents of both human and animal TSEs, as well as humans and animals with TSE. Preparations of purified prion proteins are included as are any hosts or vectors in which TSE associated material has been cloned by techniques of genetic modification and in which expression may be achieved. The guidance and information given in this document is provided to help employers to arrive at safe working practices but it is emphasised that this does not negate the responsibility of the employer to carry out a full risk assessment of all individual work situations.

Requirements of COSHH

27. The Control of Substances Hazardous to Health Regulations 1988 (COSHH) require employers to carry out a '*suitable and sufficient*' assessment of risk before work commences in order to be able to prevent or adequately control the exposure of workers and others likely to be affected, to substances which may be hazardous to health. In the course of the COSHH assessment it will be necessary to review all working procedures including, for example, procedural controls, arrangements for the safe disposal of waste, the potential for the dispersal of infectious material in the working environment and the contamination of equipment and apparatus. COSHH requires that the need for health surveillance also be considered if there is a reasonable likelihood that an illness may develop as a result of exposure.

Distribution of TSE infectivity in tissues

28. The nature of the TSE agents is still unknown as discussed earlier. Therefore, knowledge of which organs or systems are known to harbour the agent in natural disease is key to performing an adequate risk assessment and subsequently, in providing suitable control measures to prevent or reduce the risk of occupational exposure to the agents.

Bioassays in laboratory rodents have been used to investigate the tissue distribution of TSE infectivity in naturally occurring disease. Present knowledge of this, which is based largely on work with scrapie, is given in Table 3.

29. The data presented in Table 3 have been derived predominantly from studies with scrapie. Little work has been done on the distribution of infectivity in man; accidental human to human transmission cases suggest that human dura, cornea and pituitary contain infective agents, just as the brain does. As the diseases are similar, it is assumed that the distribution closely reflects that found in animals.

30. Occupational exposure to blood is very unlikely to present a risk of transmission of TSE. Epidemiological evidence from surveillance of CJD cases in the UK does not suggest blood transfusion is a major risk for CJD, and the agents have only rarely been demonstrated in the blood of infected animals and man, and then only by the sensitive technique of IC inoculation. The main concern in the occupational setting is accidental parenteral inoculation with lymphoid and neural tissue from cases of human or animal TSE or extracts prepared from them. Splashing of the conjunctivae or skin contamination (when there are breaks in the skin) with such material is also of concern.

Table 3 Distribution of TSE infectivity in tissues and body fluids

A. Tissues that have the highest titres of infectivity in clinical or late subclinical disease in at least some species:

brain*	pituitary gland	spinal cord*
spleen*	dura mater	thymus*
tonsil*		placenta and membranes
eye		peripheral lymph nodes
gut associated lymphoid tissue*		

B. Tissues occasionally found to have moderate or low levels of infectivity:

major peripheral nerves	pancreas
cerebro spinal fluid (CSF)	lung
adrenal gland	liver

C. Tissues regarded as unlikely to carry infectivity in any species affected by TSE:

milk	saliva	skin
semen	urine	muscle
blood†	faeces	kidney

*specified offals under the Bovine Spongiform Encephalopathy Order 1991
†See para 30

31. Accidental ingestion should be readily avoidable with standard hygiene measures and there is no evidence that the TSEs are transmitted by aerosols of contaminated material under natural conditions. Only where the agent is in high concentration and/or likely to be actively dispersed in, for example, some laboratory operations such as homogenisation of tissue, might there be a need for caution in this respect leading to active prevention of inhalation by the use of a microbiological safety cabinet or other primary enclosure. Primary containment must always be the first choice.

Basic precautions to avoid exposure

32. The following are essential points in limiting the remote possibility of transmission of the TSE agents. These are particularly important when there is exposure to the lymphoid and neural tissue of TSE infected individuals or animals or extracts prepared from them. The basic precautions that should be adopted whenever materials or tissues known to contain the agents of TSEs are handled, are given in Table 4. More stringent measures may be required in certain occupational settings.

2. Experimental laboratory work with TSE agents, materials and infected animals

Containment of laboratory work with the TSE agents and associated materials

33. The principles underlying the need for containment have been discussed earlier in this document under 'Hazard and Risks'. In most situations the

Table 4 Basic protective measures

1. protect skin wounds, eczematous lesions etc
   – use waterproof dressings;

2. wear appropriate protective clothing routinely
   – consider use of disposable gowns and aprons;

3. wear disposable gloves routinely;

4. protect eyes and mucous membranes if splashing is possible
   – use eye protection or full-face visor where appropriate;

5. avoid active uncontrolled dispersal of material (eg in mixing, homogenisation, centrifugation, or splashing)
   – use enclosed systems (eg sealed centrifuge buckets or, where appropriate, a microbiological safety cabinet);

6. avoid or minimise the use of sharps wherever possible (needles, knives, scissors and laboratory glassware)
   – use plastic disposable items (eg containers, pipettes, inoculating loops and other minor instruments); consider use of suitable hand protection eg armoured glove(s) where use of sharp instruments are essential in postmortem examinations or collection of human or animal brain/spinal cord;

7. dispose of contaminated waste safely
   – use secure leakproof containers (eg double-bagging, where appropriate) avoiding external contamination; use only incineration or recommended autoclaving procedures (Annex 2);

8. recycle durable items for re-use only after appropriate decontamination
   – use only recommended autoclaving procedures or recommended chemical disinfection methods (Annex 2);

9. decontaminate surfaces thoroughly
   – use only recommended decontamination procedures (Annex 2);

10. record all accidents involving parenteral exposure to TSE material or contaminated wastes
    – a local record should be kept of all the above exposures (paras 70–73)

containment required for the work would correspond with the hazard group of the agents. Therefore, the majority of work with animal TSE agents and tissues containing them can be performed at Containment Level 1 (with attention being given to the basic precautions outlined in Table 4). As there is only limited experience of BSE at present, additional precautions are advised when handling tissues identified in part A and B in Table 3 from BSE infected animals (Table 5).

Work with human agents can generally be performed at Containment Level 2 as a minimum.

34. However in certain experimental situations where the amount of agent likely to be present is significantly enhanced above levels normally encountered in naturally occurring disease, modified containment, additional precautions

Table 5 Summary of the laboratory containment levels and additional precautions for experimental work with the TSE agents

Type of work	Containment level	Additional precautions
Work with human TSEs	2	Dedicated room Eye protection Wear gloves, gown and apron Certain dedicated equipment* Special decontamination Use microbiological safety cabinet if potential for aerosol production
Work with scrapie	1	Use microbiological safety cabinet if potential for aerosol dispersion of high titre material Use Containment level 2 for concentration of and high titres of agents, plus eye protection and gloves Special decontamination
Work with BSE and other animal TSEs (except scrapie)		
Tissue identified in Part A and B of Table 3	2	As for work with human TSEs apart from need for dedicated room
Other material (including tissue identified in Part C of Table 3)	1	—

Ensure good hygiene and avoid inoculation injuries for all types of work.
*For example with electron microscopes and ultracentrifuges only the grid holders and rotors need be dedicated.

and dedicated facilities may be required on the basis of a local assessment of risk (Table 5).

Factors that will need to be considered when performing a risk assessment to decide whether the extra precautions detailed in Table 5 will need to include:

- nature of the TSE
- assessment of type of task (eg concentration/purification)
- if work is likely to result in a high titre of infectivity
- frequency of contact with the agents or materials likely to contain them
- type of tissue handled – likely level of agents present (Table 3)
- genetic modification
- possible routes of exposure
- knowledge of expression of agent in experimental model
- the potential for inoculation injury in the work

Work with disrupted tissues and concentrated TSE agents

35. When tissue known to carry TSE infectivity is to be disrupted (eg by homogenisation and centrifugation in biochemical or other analytical work), the assumption should be that infectious material could be readily dispersed. Such operations should be appropriately contained (Table 5). For work with human TSE material and animal TSEs, apart from scrapie, Containment Level 2 with extra precautions as outlined in Table 5 are required. For work with scrapie, Containment Level 1 is indicated, with a further requirement to use Level 2 and extra precautions, if concentration of agents or material containing high titres of agent are being handled.

Genetic modification work with TSE

36. Experimental work involving the genetic modification of the PrP gene and the production of animals transgenic for the PrP gene, is subject to regulatory control under the Genetically Modified Organisms (Contained Use) Regulations 1992. It is acknowledged that there may be particular difficulties involved with working with the TSE agents in this context and the Virus Working Group of the Advisory Committee on Genetic Modification (ACGM) has drawn up guidelines specific for this type of work. These appear at Annex 3.

Clinical diagnostic work

37. Handling of clinical specimens is covered in paras 48–57.

Containment of animals experimentally infected with TSE

38. There is no rationale for handling animals infected experimentally with animal TSEs any differently from the same animals with natural infection. Consistent with the hazard group ratings given in Part II, and provided that there are no other special circumstances, it is suggested that no extra precautions are needed with live animals.

Animal Containment Level 1 is adequate for animals inoculated with scrapie and other animal TSEs. Animal Containment Level 2 should be used for animals inoculated with human TSEs. However, to prevent cross-contamination of the sort that might invalidate experiments, local guidelines may insist on a higher level of isolation and containment.

39. Additional containment precautions would need to be considered in experiments where the special circumstances given in Table 6 apply.

Table 6 Criteria for selection of additional precautions for animal containment

A.	concentrations of infectivity above those found naturally could be expected;
B.	routes of inoculation are used in which leakage of infectious material externally could occur or during the oral dosing phase when the feed material remains exposed;
C.	there are other experimental circumstances that might enable external release of infectivity;
D.	experiments are subject to the Genetically Modified Organisms (Contained Use) Regulations 1992.

40. If the agent is of human origin, or is an animal agent that is primate-adapted, and direct exposure to fluids or tissues could arise, Containment Level 2 is recommended with additional precautions to protect against sharps injuries and splashing of the eye. If abnormally high levels of scrapie agent, for instance, are anticipated, but would be secure within the nervous system of the experimental animals in question, then Containment Level 1 would suffice. Following this broad guidance, local rules should be drawn up to ensure safe practice.

3. Exposure to human TSE agents in the health care setting

General

41. It is not the intention of this guidance to address the prevention of iatrogenic transmission of the human TSE agents. Advice on this has already been issued by the Department of Health and is referenced in full at Annex 4. That advice covers those known or suspected to have CJD or GSS, as well as those groups considered to be at higher risk of developing CJD or GSS (Table 7), and detail procedures to be adopted during neuro, ophthalmic and general surgery to minimise the risk of transmission. They include protocols for the disposal and disinfection of instruments used in health care practices.

Table 7 Patient risk groups

- known or suspect cases of CJD and GSS;
- recipients of hormone derived from human pituitary glands such as growth hormone and gonadotrophin;
- recipients of human *dura mater* grafts;
- members of recognised familial CJD or GSS families.

42. However, several types of clinical and other procedures in the health care setting may result in the exposure of healthcare workers to the agents of human TSE with the potential for transmission, and these aspects are addressed below. In the majority of cases, the precautions recommended for the prevention of iatrogenic infection coupled with the basic precautions outlined in Table 4 will serve to minimise the exposure of individuals involved in the clinical care of patients who have or may develop TSE, and protect them from the very remote possibility of infection.

43. For the purpose of the guidance that follows, '*known, suspect or at risk TSE patients*' shall be taken to include the groups of patients identified in Table 7.

Nursing in the ward and community

44. Lack of any epidemiological evidence for spread within the population suggests that normal social or routine clinical contact with a CJD patient does not present a risk. Patients with TSE can be nursed in the open ward or the community with no particular precautions beyond those employed for all other patients.

45. Most TSE cases are unlikely to be of child-bearing age, but in the event that a *known, suspect or at risk TSE patient*, for example a recipient of hormone

derived from human pituitary glands (hGH or hPG), becomes pregnant, childbirth should be managed using the precautions recommended for any woman with an infection such as hepatitis B. The placenta and membranes should be treated as if infected.

46. Drug administration by injection and the collection of blood specimens should involve the precautions used for all work of this type with any patient ie avoidance of sharps injuries and other forms of parenteral exposure, and the safe disposal of sharps and contaminated waste. Particular care should be taken with the collection of cerebro-spinal fluid (CSF) or tissue biopsy specimens (see below).

Surgical Procedures

47. As discussed above, the guidance on the prevention of iatrogenic transmission of TSE should also serve to protect health care staff who are involved in surgical procedures. The PL(92)CO/4 identifies patients who may be at risk of developing CJD or GSS (Table 7) and who may transmit the disease via neural tissue and for whom additional precautions are required when undergoing neuro or ophthalmic surgery.

The additional precautions for neuro, ophthalmic and general surgery for these groups of patients are detailed in DA(81)22 and DA(84)16 and PL(92) CO/4 (Annex 4).

Collection, labelling, transport and handling of clinical specimens

48. The distribution of TSE infectivity in natural disease has been discussed earlier and is summarised in Table 3. In the main, most infectivity is likely to be concentrated in the CNS and particular care should be taken with such specimens from patients with *known, suspect or at risk TSE*. Blood is very unlikely to present a risk of transmission of TSE.

49. Basic precautions for work with TSEs are given in Table 4. Clinical specimens from *known, suspect or at risk TSE patients* will be handled at Containment Level 2 as a minimum. General guidance on the handling of clinical specimens has been issued by the Health Services Advisory Committee[1].

50. **CSF and biopsy of tissue other than neural tissue:** Only staff aware of the hazard should collect CSF and tissue biopsy specimens from *known, suspect or at risk TSE patients*. They should wear gloves and eye protection where splashing could occur. Such specimens should be marked simply with a 'biohazard' label.

51. Because of the unusual resistance of the TSE agents, disposable equipment should be used wherever practicable and all small items contaminated by such specimens should be destroyed by incineration, or else autoclaved or disinfected to the required standard (Annex 2).

52. In the clinical laboratory, when handling CSF or biopsies, use of the usual control of infection procedures should provide adequate protection for staff. Particular care should be taken to avoid accidental inoculation or other injury when preparing samples for microscopy or culture. Special arrangements

[1]HSAC: Safe Working and the Prevention of Infection in the Clinical Laboratory, 1991 ISBN O-11–8854446–1

may be needed to minimise any residual contamination of equipment. Disposable equipment should be used wherever feasible (cell counting chambers etc). Where manual analysis with disposables is not feasible and automated equipment is to be used, the potential for residual contamination must be considered and be dealt with appropriately before equipment is serviced. Where stringent decontamination procedures are inappropriate, as in the case of microscopes, for example, the stage should be suitably protected.

53. **Neuropathology specimens:** General precautions for the collection and handling of CSF and tissue biopsies given above, apply for similar work with brain and neural biopsy specimens from *known, suspect or at risk TSE patients*. Specimens should only be collected and handled by trained personnel taking appropriate precautions and wearing appropriate protective clothing.

54. However, if large numbers of specimens are handled, additional precautions are necessary in the laboratory because of an increased problem of residual contamination. It may be appropriate for such specimens to be handled exclusively in a specialist neuropathology laboratory or centre.

55. Where there are appropriate facilities locally, limited histological processing can be undertaken with care wearing the protective clothing detailed in Table 5. All work with known or suspected CJD brain and neural material must be undertaken in a defined area of the laboratory where the working surface has been covered in advance with disposable non-permeable material. This and all washings, other waste material and protective clothing, should be disposed of by incineration. Absorption by sawdust is a convenient way of making small quantities of formalin and other liquid waste suitable for incineration.

56. For optimal fixation of whole brain for general histopathological purposes, standard formalin should be used. However, formalin fixed TSE tissue retains infectivity for long periods if not indefinitely and should be handled with the same precautions as fresh material. Similarly, tissue for electron microscopy fixed in glutaraldehyde retains its infectivity. This is of equal importance when handling archive material stored in fixative, blocks or as mounted slides. As evidenced by work with both CJD and scrapie, formalin fixed TSE tissues can be decontaminated largely if not completely by formic acid treatment (method at Annex 5). However, as the full extent of the efficacy of the formic acid treatment is still uncertain, even mounted histological preparations of known TSE brain and neural tissue should be regarded as potentially infected and special care taken to avoid breaking the slides or similar accidents during which penetrating injuries could occur. Once tissue blocks are fixed and acid treated, sections can be cut on a routine microtome (but using a disposable knife) and processed as usual. Debris from section cutting should be contained and disposed of preferably by incineration.

57. **Other clinical specimens:** Blood, urine, faecal specimens and swabs can be collected, handled and processed in the normal fashion.

Pathology: Post-mortem examination of known, suspect or at risk TSE patients

58. Only fully trained staff should undertake any necessary post-mortem examination on these cases. Post-mortem technicians and others attending out of necessity should be fully trained in or informed of procedures for such post-mortems and made aware of the relevant history. Post-mortem examination procedures appropriate for such cases are given in Annex 6. Unless instruments are subjected to the stringent autoclaving practice recommended in Annex 2, they may retain residual infectivity thus posing

a risk for staff whilst being cleaned and when in use again. A set of dedicated instruments for known, suspect or at risk TSE cases is therefore recommended in order to minimise the frequency of their use and the risk in handling. Instruments should be appropriately decontaminated *before* cleaning and storage.

Anatomy and pathology teaching

59. The bodies of patients who have died from CJD or GSS or had neurological disease suggestive of CJD or GSS or had familial association with either should not be used for teaching anatomy or pathology. The same should apply to recipients of hormones derived from pituitary glands, and *dura mater*.

Body handling and embalming

60. Concern about possible unsuspected CJD does not warrant a level of precaution for handling intact bodies other than those used generally for all work of this nature. No extra precautions are necessary. In cases of traumatic injury, it is a sensible general practice to minimise contact, particularly in circumstances under which penetrating injuries could arise. Embalmers, who are exposed principally to blood, need have no undue anxiety over CJD, as there is no evidence that the disorder is associated with blood transfer. However as a precaution, embalming of patients with *known, suspect or at risk TSE* should be discouraged.

4. Exposure to animals with TSE

Naturally infected animals

61. Scrapie is widespread around the world in sheep and goats but not apparently present in all countries. Although the primary focus of BSE has been within the UK, small numbers of cases have now been recognised in cattle in continental Europe. There is no evidence to suggest that close contact with TSE infected animals presents an infection risk and no special precautions are required when handling an intact diseased animal.

62. However, with certain invasive veterinary procedures, such as intervention at parturition and inoculation, basic precautions to minimise human exposure are appropriate. The specific precautions to be taken are detailed in Annex 7. In most cases, these will constitute the normal good hygiene measures taken by veterinary staff to protect themselves from any other infection carried by the animals they deal with. MAFF has already issued guidance on the handling of animals infected with BSE (Guidance for Veterinary Surgeons handling known or suspect cases of BSE, January 1990, Annex 7). Attention to the safe disposal of placenta and membranes from parturient animals should be standard practice.

Laboratory animals with TSE

63. Containment of laboratory animals with TSE is covered in paragraphs 38–40. The preferred method of disposal of carcases of all animals *experimentally* infected with TSE is incineration.

Carcases of animals with TSE

64. **BSE:** Those involved in the slaughter of known or suspected cases of BSE and in subsequent disposal or transport of the head to a laboratory for

examination, should take precautions to minimise exposure, particularly to the neural, lymphoid and placental tissue. Other than the necessary decapitation, the carcase should not be incised. Table 4 indicates that all workers exposed to TSE agents should observe certain basic precautions, and should cover all existing cuts and abrasions with waterproof dressings, wear protective clothing including gloves, and take particular care to avoid puncture wounds or cuts. Where a risk of splashing exists, eye protection is needed. Simple washing and decontamination measures should suffice for dirty protective clothing except where contamination with brain tissue has taken place in which case disposal by incineration is recommended. Carcases of known or suspect cases of BSE must be disposed of in accordance with MAFF advice. Advice for workers involved in the handling and transportation of BSE carcases for disposal was issued by HSE (BSE and Carcase Disposal, 1990).

65. **Scrapie:** No particular precautions are needed for the slaughter and disposal of known or suspected cases of scrapie.

66. **Other animal TSEs:** Similar precautions to those taken with BSE carcases should apply.

Post-mortem examination of animals with TSE

67. The precautions and procedures for post-mortem examination of large and small animals infected with TSE are detailed in Annex 8.

Animal neuropathology

68. In view of the uncertainty surrounding BSE, the neuropathological examination of animal TSE material (except scrapie) should be conducted at the standard more usually applied to Hazard Group 2 pathogens. Thus Containment Level 2 should be used and precautions taken to prevent dispersal of infected material. Extra care will be needed to avoid penetrating injuries, and eye protection used to avoid splashing onto the conjunctivae. Autoclaving and disinfection procedures should be as recommended in Annex 2. Further guidance on the decontamination of formalin-fixed tissue for neuropathology is given in Annex 5.

Abattoirs

69. The practice of exclusion of known or suspect BSE cases is the primary preventative measure for abattoir workers. In addition, the recommended precautions to minimise transfer of infections from humans onto the edible parts of animal carcasses should provide more than adequate protection for most abattoir workers against the possibility of contamination with TSE agent. Guidance for the abattoir industry has been issued and endorsed by the HSE (British Meat Manufacturers' Association, Guidance Note 05, March 1990).

5. Accident reporting and health surveillance

Accident reporting and recording

70. All accidents and occurrences with infectious or potentially infectious material involving the exposure of individuals should be the subject of local records. In addition, in some cases the HSE must be notified in conformity with the Reporting of Incidents, Diseases and Dangerous Occurrences Regulations 1985.

71. Accidental injuries or inoculation wounds involving known or suspected material should be washed immediately and thoroughly in running water with further treatment being given as appropriate to the type of injury. Splashes into the eye or mouth should be dealt with by thorough irrigation. An official local record should be made of all such exposures.

Health surveillance

72. Regulation 11–(2)–(b) of the Control of Substances Hazardous to Health Regulations 1988 (COSHH) requires that health surveillance of employees is appropriate when:

> *'the exposure of the employee to a substance hazardous to health is such that an identifiable disease or adverse health effect may be related to the exposure, there is a reasonable likelihood that the disease or effect may occur under the particular conditions of his work and there are valid techniques for detecting indications of the disease or the effect.'*

73. The human TSEs have not been associated with occupational exposure but proof of their transmissibility does exist. While there are no laboratory techniques that can indicate that transmission has occurred, clinical examination when early signs and symptoms of TSE appear may allow a diagnosis. Given that if TSE were to develop as a result of occupational exposure, it would only appear after some years, keeping a health record (Appendix to COSHH general Approved Code of Practice) is appropriate for those working with the human TSEs. For routine clinical care of patients with CJD or GSS however this should not be necessary.

PART IV: Decontamination and waste disposal procedures for TSE agents

74. The agents of TSE exhibit quite unusual resistance to conventional decontamination methods used in clinical and laboratory practice. As the longest recognised and most intensively studied of the TSEs, scrapie is the acknowledged model for the group. Much of what is recommended here about decontamination in general is derived from studies involving scrapie.

Ineffective methods of decontamination

75. The agents of TSE are well recognised as being particularly resistant to all standard physical and chemical methods of laboratory decontamination. The standard laboratory autoclaving regime of 121° C for 15 minutes, which is known to inactivate the hardiest pathogenic bacterial spores, is ineffective with TSE agents. Similarly, the cycle normally used for the sterilisation of surgical equipment (134°C +4/-0 for 3 mins) cannot be relied upon. The TSE agents are not significantly affected by a number of common chemical disinfecting agents (see Annex 2). The doses of ionising or UV irradiation required to produce significant reductions in TSE infectivity are too great to be of practical value.

Effective methods of decontamination

76. Details of effective decontamination regimes and procedures are included at Annex 2. For the majority of situations porous load autoclaving is the decontamination method of choice.

77. Where autoclaving is not practicable, or the use of chemicals is the preferred method of decontamination, the disinfectant of choice is sodium hypochlorite. A treatment of at least one hour with sodium hypochlorite containing 20,000 ppm available chlorine is appropriate for TSE agents.

Disposal of waste

78. Advice on the disposal of clinical and laboratory waste is given in the Health Services Advisory Committee document: Safe Disposal of Clinical Waste (1992)[1]. There are also statutory requirements under the Department of the Environment's Controlled Waste Regulations 1992. Carcases of animals that have been experimentally infected with TSE should be disposed of by incineration. Advice on the disposal of other waste is given in Annex 2.

[1] HSAC: Safe Disposal of Clinical Waste, 1992. HMSO, ISBN 0–11–886355–X

ANNEX 1 Categorisation of pathogens by hazard group

Advisory Committee on Dangerous Pathogens: Definitions of Hazard Groups

Hazard Group 1: An organism that is most unlikely to cause human disease.

Hazard Group 2: An organism that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylaxis or effective treatment is usually available.

Hazard Group 3: An organism that may cause severe human disease and present a serious hazard to laboratory workers. It may present a risk of spread to the community but there is usually effective prophylaxis or treatment available.

Hazard Group 4: An organism that may cause severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread to the community and there is usually no effective prophylaxis or treatment.

ANNEX 2 Decontamination procedures for TSE agents

1. The recommended treatments given in this annex are largely based on work with the scrapie agent. Where findings have been paralleled by work on the agent of CJD, this is mentioned specifically. For most situations, porous load autoclaving is the method of choice for decontamination and rendering waste material safe to handle.

1. Physical and chemical methods of decontamination

Heat

2. **Boiling:** The agents are not greatly affected by boiling, and boiling water treatment for decontamination is not adequate.

3. **Autoclaving – Gravity displacement autoclaves:** Gravity displacement autoclaving at 132°C for one hour inactivates both CJD and the scrapie agent. The lower commonly used temperature of 126°C is unreliable despite extension of exposure to two hours.

4. **Autoclaving – Porous load autoclaves:** The use of a porous load autoclave is preferred. A cycle of 18 minutes at 134°C to 138°C is recommended or six cycles of 3 minutes at the same temperature.

Table I Autoclaving regimes currently recommended (*porous load*)

- a single cycle 134°C (+4/-0) (30lbs psi), 18 mins (holding time at temperature)
- six separate cycles 134°C (+4/-0) (30lbs psi), 3 mins (holding time at temperature)

Although no practical problems appear to have arisen with this time and temperature combination, recent preliminary studies of a scrapie agent under rigorous experimental conditions have shown some residual infectivity. This may be due to the use of relatively high-titred material and more thermostable strains. Further work is planned to confirm the appropriate lower temperature limit.

5. **Dry heat:** Dry heat treatment of macerated infected tissue at 160°C for 24 hours, leaves some residual infectivity. Lyophilised (freeze-dried) tissue homogenates exposed to 360°C for one hour also remain infectious. As the water content of material to be heat-treated has an influence, desiccation confers a particular resistance to inactivation. The infectivity of moist tissue is destroyed in 60 minutes at 200°C. Substantial, but not complete, inactivation of both CJD and scrapie is attained after one minute at 240°C.

Chemical decontamination.

6. The TSE agents are not significantly affected by the majority of chemical disinfecting agents, such as those listed in Table II. Autoclaving is the method of choice for decontamination, but where this is not practicable, the disinfectant of choice is sodium hypochlorite. A one hour treatment with sodium hypochlorite at 20,000 ppm available chlorine is appropriate for TSE agents.

Table II Chemical disinfectants shown to be ineffective against the TSE agents

alcohols	ethylene oxide
formaldehyde	formalin
glutaraldehyde	hydrogen peroxide
iodophors	phenolics
B-propiolactone	

Ionising and UV radiation

7. Doses of ionising and UV radiation which inactivate conventional micro-organisms have little effect on TSE agents. The doses required to produce a significant reduction in infectivity are too great to be of practical value.

Desiccation and environmental exposure

8. CJD agent can survive at room temperature for at least 28 months and residual scrapie infectivity has been found after burial for 3 years. Therefore, unless appropriate chemical or physical decontamination methods are used there is the potential for the accumulation of infected material on work surfaces and equipment.

2. Specific decontamination procedures

Treatment of heat stable equipment and non disposable protective clothing

9. Autoclaving (to the regimes detailed in Table I) is the decontamination method of choice for heat stable items such as non disposable protective clothing, surgical and post mortem instruments and laboratory equipment. Exceptionally, some instruments may be immersed in sodium hypochlorite containing 20,000 ppm available chlorine for at least one hour.

Treatment of work surfaces and non-heat stable equipment

10. **Hypochlorite:** A one hour exposure to sodium hypochlorite containing 20,000 ppm available chlorine is effective in destroying scrapie infectivity on open surfaces. Repeated wetting with the disinfectant is necessary over the treatment period. As this concentration of hypochlorite can be corrosive for metals and some commonly used surface finishes, work that involves the handling of infected materials should be conducted only on resistant surfaces or work benches shielded by disposable absorbent plastic-backed temporary coverings. Users should note that the concentration of available chlorine in hypochlorite solutions may be significantly affected by the presence of organic matter, especially blood. The use of enamel, heat-stable plastic or disposable trays is recommended to confine contamination. These should be autoclaved after use (see above). Temporary bench coverings and disposable trays should be bagged for incineration.

11. **Sodium hydroxide:** Sodium hydroxide solution 2M is also active against scrapie but may not completely inactivate high concentrations of agents especially if protected by dried organic material. Constant re-wetting during the treatment of surfaces is necessary.

12. **Detergent washing:** This may result in a dilution of the agent or contaminating material, but is not an effective way of decontaminating surfaces.

Decontamination and disposal of liquids (fixatives/solvents/scintillation fluids etc)

13. TSE contaminated fluids should be disposed of regularly to limit the volumes to be dealt with at one time. Large volumes of organic solvents should be disposed of by the commercial controlled incineration techniques generally used for these materials. Small volumes of fluid may be conveniently absorbed in containers carrying sawdust ready for incineration. Water-based fluids may be autoclaved (see above) or treated with hypochlorite to achieve a final concentration of at least 20,000 ppm of available chlorine allowing for the effects of any organic matter present (see above).

Formaldehyde solutions must not be autoclaved or mixed with other chemical disinfectants. Contaminated formalin solutions should be disposed of by incineration. Workers must ensure that alternative methods of formalin disposal such as discard to the sewerage system are in accordance with local Water Authority rules.

Decontamination of microbiological safety cabinets

14. As indicated above, formalin or rather in this context gaseous formaldehyde, which is the conventional medium for the fumigation of safety cabinets, is not effective against the TSE agents; it may in fact stabilise them. Nonetheless, fumigation will need to be carried out as a precaution against other infectious agents that may be impacted on the surface of the cabinet's HEPA filter. Service engineers will require the unit to be decontaminated before changing filters.

15. Due to the difficulties associated with their decontamination, it is recommended that safety cabinets used for work with TSEs should be of the type with the facility for removing HEPA filter units by bagging. Whether or not bagging of the filter as it is withdrawn is possible, spraying the filter face after fumigation and before removal with eg hair spray will help to limit the shedding of particulate matter. Where a Class II cabinet (BS:5726:1992) is to be used, a model that has the main HEPA filter immediately below the work surface is preferred as this will prevent contamination of the plenum of the cabinet. With the filter in this position, use may be made of liquid latex to seal the filter surface before removal. Pre-filters (dust filters) are generally easily removed and after immersion treatment with 2M sodium hydroxide solution (see above) to limit dust dispersal they should be contained securely for incineration or safe transport to the autoclave. If made of durable but not heat stable material, they may alternatively be treated with hypochlorite solution containing 20,000 ppm available chlorine.

16. Working in a shallow tray in the cabinet will limit dispersal onto work surfaces by splashing but it is *essential* to ascertain by testing the cabinet with the tray **in situ** that containment for operator protection is not affected. (See BS 5726:1992 for detail of containment testing.) Another option is to tape disposable plastic backed absorbent paper to the working surface in order to minimise contamination. The covering must be renewed regularly (preferably after each period of work) and incinerated.

Fixation for histology

17. Conventional methods of tissue fixation employing formalin or glutaraldehyde are known to be ineffective in destroying CJD and scrapie infectivity. It is reasonable to assume that the other TSE agents are similarly

resistant. Exposure to 96% formic acid for one hour after formalin fixation has been shown to be effective in reducing scrapie and CJD infectivity substantially (Annex 5).

Disposal of tissues

18. Final disposal of processed tissue should be by incineration using appropriate precautions during handling and packaging for carriage. Stored fixed material (bulk tissue, blocks and stained or unstained slides) from known or suspected cases of TSE must be handled as though it were infectious, and attention paid to the possibility of sharps exposure.

ANNEX 3 Genetic modification

1. This section of the guidance document has been drawn up by the Virus Working Group of the Advisory Committee on Genetic Modification (ACGM). It draws attention to the particular difficulties of work with the agents of transmissible spongiform encephalopathy (TSE). This guidance must be brought to the attention of local genetic modification safety committees, biological safety officers and those involved in this area of genetic modification work.

2. Since 1st February 1993, all work involving the contained use of genetically modified organisms has been subject to regulatory control under the Genetically Modified Organisms (Contained Use) Regulations 1992[1]. 'Contained use' includes any operation in which organisms are genetically modified or in which genetically modified organisms are cultured, stored, used, transported, destroyed or disposed of and for which physical barriers (plus biological and/or chemical barriers) are used to limit contact with the general population and the environment. Such operations must comply with the requirements of the Contained Use Regulations; among other things this means carrying out a risk assessment for both human health and safety and environmental protection, submitting a notification to the Health and Safety Executive, and in certain circumstances receiving the Executive's formal consent.

3. ACGM has advised that given the current uncertainty surrounding the relationship between TSE agent and prion protein (PrP) genes in man and animals, certain genetic modification work of PrP genes should be approached with caution. In particular, ACGM has highlighted two areas of work that are considered to pose an increased risk to human health and safety:

i. cloning PrP genes into hosts capable of colonising humans;

ii. expression of 'modified' PrP in prokaryotic and eukaryotic systems.

4. Recognising the difficulties that genetic modification centres have in making a satisfactory risk assessment for work with PrP genes, ACGM has made the following recommendations (taken from Annex II of ACGM/HSE/DoE Note 7):

A. As part of a risk assessment under the Contained Use Regulations 1992, all genetically modified organisms must be classified into either Group I or Group II according to the criteria in Schedule 2 to the Regulations. The ACGM consider that in most cases a genetically modified micro-organism (GMM) containing cloned PrP genes **cannot** be considered to be a Group I GMM because the insert is not "known to be free from harmful sequences" and it may result in a "harmful or pathogenic phenotype. . ." to humans and animals (Schedule 2, Contained Use Regulations). Therefore, all work involving the cloning of such genes must be notified to HSE before work commences and, depending on the type of operation, may require a specific consent.

B. However, as an exception to this general principle, a GMM containing a cloned sub-fragment which lacks harmful biological properties (for example, a small probe sequence for a PrP gene) may, after detailed review, be judged to fulfil the criteria for Group I status.

[1] For further details see "A guide to the Genetically Modified Organisms (Contained Use) Regulations 1992", 1993. HMSO ISBN 0–11–882049–4

C. Work involving the cloning of genes associated with prion proteins should be assessed with respect to the risks posed to human health and safety and the environment using (where relevant) the parameters contained in Schedule 3 to the Contained Use Regulations.

D. Work involving the genetic modification of higher animals should continue to follow the guidance contained in ACGM/HSE Note 9 (Guidelines on work with transgenic animals). Advice on additional risk assessment considerations for animal work should be discussed with ACGM/HSE. Users should also consider whether the resulting GMO fulfils the criteria of Part III of Schedule 2 of the Contained Use Regulations. The containment appropriate for such animals should be assigned on the basis of Note 9 and should take into account the current ACDP guidance for animals infected with TSE if appropriate.

E. The risk assessment scheme for GMMs detailed in ACGM/HSE/DoE Note 7 using Access, Expression and Damage is **not** considered to be applicable to work with human or animal PrP genes. ACGM therefore made the following recommendations:

i. the cloning of whole or truncated PrP genes should use a host incapable of colonizing humans. Hosts with an Access factor of 10^{-6} or less in ACGM/HSE/DoE Note 7, or those assigned to the "disabled" category (Note 7, Annex I) by the local GMSC are considered to be incapable of colonizing humans. Details of eukaryotic viral vectors which are considered incapable of colonising humans can be found in ACGM/HSE/DoE Note 5.

ii. All work with whole or truncated PrP genes cloned into prokaryotic or eukaryotic micro-organisms should continue to be assigned to the minimum containment level set out in ACGM Newsletter 13 as updated below. Particular attention is drawn to the provision for a case-by-case review of the assigned containment level in the light of a detailed risk assessment containing data which may support a down-grading of containment. For Type A work, the submitted risk assessment should be more detailed than that normally required for other Group II, Type A notifications.

Recommended minimum containment levels for work involving the cloning of human or animal prion protein genes.

Expression[1] of any human PrP gene OR Expression[1] of **modified**[2] animal PrP gene.	ACGM Level 3*
Expression[1] of naturally-occurring animal PrP gene.	ACGM Level 2
Non-expression[3] work in disabled hosts	ACGM Level 1

Notes.

1. **'Expression'** is taken to mean high level expression (over-expression), typically associated with the high protein concentrations produced by some bacterial host-vector systems or with baculovirus-insect cell culture systems. Host-vector systems that are associated with lower levels of protein expression, such as the *lac*ZY and SV40 promoters used in many vectors or certain *in vitro* promoter systems (e.g. T7), may be suitable for a lower level of containment and such requests for down-grading should be included along with the prior notification as indicated above.

2. **'Modified'** refers to prion proteins which have an altered amino acid sequence, particularly those analogous to, or identical with, polymorphisms associated with human disease. The term may also apply to prion proteins which are not post-translationally processed (e.g. those lacking glycosylation, glycolipids or retaining the C-terminal portion) or those which are expressed as a fusion or truncated peptide. Again, the latter two categories of 'modified' may be suitable for down-grading to a lower level of containment on a case-by-case basis as indicated above.

3. **'Non-expression'** is taken to mean either insertion into a site of limited promoter activity (eg. *Bla* in pBR322, Expression = 10^{-6}) or insertion at a site specifically engineered to prevent expression (Expression = 10^{-9} in ACGM/HSE/DoE Note 7) and may include promoters which are not recognized by normal host RNA polymerase, such as the T7 or SP6 promoters.

*The use of inwards air flow via a room ventilation system or via the use of a safety cabinet is not considered essential.

ANNEX 4 Department of Health guidance on the management of patients with TSE (CJD, GSS and kuru)

DA(81)22

Report of the Advisory Group on the Management of Patients with Spongiform Encephalopathy (Creutzfeldt-Jakob Disease)(CJD).

DA(84)16

Management of Patients with Spongiform Encephalopathy (Creutzfeldt-Jakob Disease)(CJD).

PL(92)CO/4

Neuro and Ophthalmic Surgery Procedures on Patients with or suspected to have, or at risk of developing Creutzfeldt-Jakob Disease (CJD), or Gerstmann-Straussler-Scheinker Syndrome (GSS).

Anyone involved in the management of patients with TSE should be familiar with the contents of these documents which can be obtained from the Department of Health at the address given below.

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ANNEX 5

Formic acid treatment of formalin-fixed tissue for human and animal neuropathology

i. Fix tissues* in 4% formaldehyde solution (10% formol-saline) for minimum time necessary for optimal tissue preservation;

ii. Immerse in 96% formic acid for one hour;

iii. If tissues are to be processed by hand, they may be taken directly from formic acid into ascending alcohol solutions;

iv. If tissues are to be processed by machine, they should be washed again with formalin (formic acid may damage plastic containers).

(* blocks should be of a size to ensure adequate penetration)

Note 1: Formic acid should not be used on brain tissue that has been exposed to phenol as well as formalin as this causes a deleterious tissue reaction.

Note 2: Phenol has been shown to be ineffective in decontaminating formalin treated tissue and should not now be used. Some archive material is likely to have been exposed to phenol in the past, and care should be exercised when handling these tissues.

Note 3: To control noxious fumes, it may be appropriate to conduct this work in a fume cupboard.

ANNEX 6 Post-mortem examination of known, suspect or at risk TSE patients

The guidance given here also applies to the groups of patients given in Table 7.

1. A post-mortem examination is very important in CJD for validating the clinical diagnosis. Detailed guidance has been prepared by the Department of Neuropathology, Western General Hospital, Edinburgh on the collection of specimens for the CJD surveillance programme. Further guidance on techniques useful in CJD post-mortem examination is available from this source and has been published[1]. It is advised that the brief notes given here are read in conjunction with the more detailed published guidance.

Key points

2. Only fully trained pathologists and post-mortem technicians should undertake these examinations. Three people should be present during the examination: the pathologist assisted by one technician, and a further 'clean' technician to open or label specimen containers. Access, including telephone calls during the examination should be restricted to avoid distraction.

3. Disposable protective clothing should be worn including theatre suit, gown, apron, hat and double gloves, and a face visor, preferably of the ventilated type which completely encloses the operator's head to protect the eyes. If a face mask is worn (as an alternative to a visor) to protect against bone dust it should be of an approved type (BS 6016 type 2 or 3 or manufactured to European Standard BS EN 149, type FFP2 or FFP3). Consideration should be given to the use of suitable hand protection e.g. armoured gloves/liners during post-mortem.

4. The extent of the post-mortem and the processing of tissues will depend on the facilities available. Restricted post-mortem examinations on CJD cases can be undertaken in any mortuary. If only an examination of the brain is to be undertaken, the scalp is reflected in the normal way with absorbent wadding underneath the head to soak up CSF and other material when the cranium is opened. The head and neck of the cadaver should then be enclosed in a large polythene bag. The bag serves to contain bone dust while opening the cranium with either an electrical oscillating saw or hand saw. The bag and skull cap can be detached together before sampling the CSF and removing the brain and pituitary.

5. More extensive examination, including sampling the abdominal organs and peripheral nerves, requires the entire body to be placed into an open body bag with absorbent wadding. The head and neck can be enclosed, as indicated above, for opening the cranium. The chest and abdomen should be approached anteriorly and tissues sampled rather than organs removed. On completion, the body should be sewn up leaving the wadding **in situ** in the body bag. This has the advantage of absorbing fluids. Any excess wadding should be incinerated. Care should be taken in sewing up the body that 'burning' through gloves does not occur by pulling too hard on the twine. The body bag is then sealed. With this technique, contamination of the working environment should be minimised.

[1]JE Bell, JW Ironside, How to tackle a possible Creutzfeldt-Jakob disease necropsy.J Clin Pathol 1993; **46**, 193–197.

6. If a full-scale post-mortem examination of a case of CJD is indicated, including removal of the viscera and spinal cord, it is recommended that the body is removed for special handling in a high risk autopsy suite. Funds have been provided by the Department of Health to cover costs related to such a referral.

7. On completion of the work, all disposable clothing and non sharp disposable equipment should be double bagged and removed for incineration. Instruments that are not disposable should be autoclaved or dealt with as recommended in Annex 2. If, as with electrical saws, full decontamination is not feasible, the equipment should ideally be dedicated to CJD work and retained in a suitably labelled sealed polythene bag for future use. Surfaces should be decontaminated with sodium hypochlorite containing 20,000 ppm chlorine as detailed in Annex 2.

ANNEX 7 Veterinary care of animals naturally infected with TSE

1. The brief notes given here are intended to give broad guidance for the handling of animals naturally infected with animal TSEs. Guidance on the handling of BSE animals has been issued by MAFF[1], and should be read in conjunction with these notes.

Parturition

2. Epidemiological and experimental evidence suggests that the placenta and membranes can be infectious to other animals in natural sheep scrapie. The infectivity of the placenta in BSE is unknown, but appropriate oral and parenteral inoculation experiments are in progress.

3. There are good animal health reasons for collecting and disposing of the placenta (cleansings) safely even from animals not known to be infected. Advice to that effect has already been issued by MAFF in regard to cattle and zoo species. Other infectious diseases can be contracted from animal placentae so direct skin contact should be avoided. Cuts and grazes should be covered and gloves worn if direct handling of the placenta cannot be avoided, and normal hygiene measures adopted. The measures necessary to reduce the possibility of spread of disease between animals, such as disinfection after parturition, should serve to minimise human contact with potentially infected tissue. For cows with suspect BSE, some of these measures are a legal requirement on grounds of animal health.

Injections, artificial insemination, surgery, carcase disposal

4. Parenteral inoculation is more efficient than topical or oral exposure in the transmission of TSEs. Iatrogenic transfer of scrapie to sheep has been described following the use of contaminated louping ill vaccine, and is a direct parallel to the instance of CJD infection from human growth hormone derived from pituitary glands. All animal (and human) medicines have been investigated by the appropriate regulatory authorities, and the possibility of transmission of disease by this route is now eliminated in the UK. However, needles that have been used on known scrapie infected animals and which are likely to be contaminated with scrapie could still cause cross-infection. The situation with other animal TSEs is less clear. However, it would be prudent to ensure that all needles and other equipment, such as that used in surgery, are disposed of safely after use in any animal with a disease. If they are to be reused, sterilisation should match the exacting standards recommended in Annex 2. The risk of transfer of animal TSEs to humans by needlestick injury is unknown but is likely to be extremely remote.

5. There have to be compelling grounds for embarking on elective surgery, including caesarian section, in any animal with suspected TSE. If considered essential, then exceptional precautions must be taken to minimise the possibility of parenteral inoculation of staff and to ensure all material is sterilised or disposed of safely. The procedures employed in high risk human surgery will be useful in the drawing up of local guidelines as to safe practice in such circumstances. Extra precautions are also essential in post-mortem work on any animal with suspected TSE and any 'high risk' tissue.

[1]Guidance for Veterinary Surgeons handling known or suspect cases of BSE. January 1990.

ANNEX 8 Post-mortem procedures for animals with TSE

1. General

1. Before a post-mortem examination is performed on animals with natural or experimental TSE a risk assessment must be carried out to assess the appropriate level of containment and controls necessary for the procedure. In general, animals that are transgenic for human PrP or that have been experimentally infected with agents of human TSEs should be treated at post-mortem with the same degree of caution as humans with the same conditions.

2. The procedures for large and small animal post-mortem given in this annex should serve as a guide to draw up appropriate local codes of practice. Detailed advice on decontamination is given in Annex 2.

2. Post-mortem procedures for large animals

Practice

3. Only essential persons should be present in the post-mortem room when diagnosed or suspect animals are being dealt with. For other submissions, examination should take place at other times. If separation cannot be maintained, then an area of the room should be set aside for examination to enable high standards of disinfection to be adequately maintained.

Protective clothing

4. Full protective clothing must be worn at all times when handling material. This should include a waterproof gown or disposable rubber suit, with waterproof apron, thick rubber or nitrile gloves, wellington boots and full face visor. As an alternative to a visor, a positive pressure respirator helmet complete with visor can be used (this obviates problems of the visor misting up and also affords operator comfort).

5. When knives and saws are in use, an armoured or cut-proof liner should be worn under the glove to protect against cuts. If a disposable dust respirator is worn to protect against hazards other than, eg bone dust, it should meet the requirements of BS 6016, type 2 or 3 or be manufactured to European Standard BS EN 149, type FFP2 or FFP3. Protective clothing used for work, other than disposable items, should be dedicated for that purpose and kept separate from other items of protective clothing.

Instruments

6. A separate set of instruments should be kept for examinations. These should include a hand saw with blades that are disposable or dedicated. When removing whole brains, a double jawed pillar vice that can easily be cleansed should be used.

Cleansing and disinfection

7. **Clothing and equipment:** Before removal, all items of reusable waterproof clothing should be washed with water and detergent and rinsed well. Items may then be removed and immersed in sodium hypochlorite solution containing 20,000 ppm available chlorine for one hour and then rinsed well in running

water. All disposable clothing and equipment should be double bagged to await removal to the incinerator. Non disposable equipment should be either immersed in sodium hypochlorite as above or autoclaved to the required standard.

8. **Carcases**: If the carcase cannot be removed intact to the incinerator by mechanical means it should be dismembered taking care to avoid injury, and placed with other unwanted material into bins to await incineration. Particular care should be taken to include the placenta and associated fluids of pregnant animals with this material.

9. **Benches and Floors:** These must be cleaned immediately after the examination with water and detergent, rinsed and then swamped with sodium hypochlorite as above. After one hour surfaces should be washed with copious amounts of running water. A similar procedure should be used for the head vice and the incinerator bins. No other disinfectants should be used simultaneously for safety reasons.

Accidental injury

10. Inoculation injuries should be immediately washed thoroughly in running water and further treatment given as appropriate to the type of injury. An official record must be made of this, as with any other type of work accident.

3. Post-mortem procedures on small animals and laboratory rodents

Practice

11. Ensure that the containment level of the post-mortem area is appropriate for the agent/s involved (Section II, Table 5). Where it is not possible to dedicate a room for examinations, an area of the post-mortem room should be set aside. For work on open benches it may be considered advantageous to fabricate these in stainless steel, and in such a manner that they can be dismantled into sufficiently small sections to permit autoclaving. Alternatively, work may be conducted in a stainless steel or enamelled tray that will fit into the autoclave. Working surfaces should be protected by disposable coverings. Routine, non-disposable items, eg specimen racks should be autoclavable. Work should be organised so that there are no interruptions (eg answering the telephone), and that everything required is within reach while seated. A 'clean' assistant should be available to take care of record-keeping, and to procure instruments, sample containers etc which have been overlooked or are required unexpectedly.

Protective clothing

12. Surgical gloves, gowns, hats and masks should be worn, together with visors or safety spectacles. Alternatively, positive-pressure respirators (with integral visors) may be worn instead of separate hats, masks and visors/spectacles.

Disinfection

13. **Clothing and equipment:** Disposable items should be bagged for incineration. Routine, non-disposable items should be decontaminated by autoclaving prior to washing; instruments should then be packed and autoclaved again to inactivate putative cross-contamination which would compromise experimental studies. An autoclavable bin should be available for discarded gowns which should be decontaminated only by porous-load autoclaving. If this option is not available, disposable gowns should be used.

14. **Carcases:** Carcases should be bagged and disposed of by incineration.

15. **Surfaces:** Contamination of unprotected surfaces can be dealt with by flooding the affected area with sodium hypochlorite solution (containing 20,000 ppm available chlorine) for one hour, and then washing off with water. Even when there has been no obvious contamination of bare working surfaces, it is recommended that these should be treated with sodium hypochlorite or (where appropriate) dismantled and autoclaved from time to time. It is inappropriate to attempt to decontaminate formol-fixed tissue by autoclaving; formalin should not be permitted to come into contact with sodium hypochlorite.

Accidents

16. Inoculation injuries should be immediately washed thoroughly in running water and further treatment given as appropriate to the type of injury. An official record must be made of this, as with any other type of work accident.

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