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Swiss Expert Committee for Biosafety SECB

Recommendation of the Swiss Expert Committee for

Biosafety on the classification of activities using prion genes and prion proteins

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Content

1 Introduction

Prions (***pr**oteinaceous **i**nfectious particles*) cause neurodegenerative diseases such as Creutzfeldt-Jakob Disease (CJD) and Kuru in humans, and Bovine spongiform encephalopathy (BSE) and scrapie in animals. The umbrella term for these diseases is transmissible spongiform encephalopathy (TSE), which is used in this Recommendation. A prion originates in a PrP gene. The transcription and translation of this gene generates the physiologically apathogenic PrP protein (PrP^{c}; c = ***c**ellular*). The essential, or possibly the only, component of a prion is a pathologically misfolded form of the PrP protein, PrP^{sc} (sc = **sc**rapie-associated). PrP^{sc} in turn leads to a further refolding of PrP^{c} to PrP^{sc}, producing an autocatalytic chain reaction. Thus PrP^{sc} collects in the affected tissues, primarily in the brain, sometimes leading to irreversible lethal pathological changes. Prions can be transmitted orally, iatrogenically or via wounds. Thus persons who handle prions are at risk of becoming infected, with a TSE as a consequence.

2 Legal bases

- Agents causing TSE are classified under group 3** (FOEN Classification of Organisms. Module 2: Viruses; December 2011).
- The term group 3** is used for organisms that possess a limited risk of infection to workers because air-borne transmission, according to current scientific knowledge, cannot normally occur. Transmission can only take place through contact with infected tissue, body fluids or contaminated medical instruments.
- It is now certain that humans can become infected through the oral uptake of a BSE pathogen. Thus, work must be carried out in laboratories of level 3**, although the omission of particular safety measures may be requested (see below)
- No link to human TSEs has yet been shown for other animal TSEs (scrapie, chronic wasting disease (CWD)). The safety measures of a level 2 laboratory are therefore sufficient.

3 Safety measures for work using TSE agents

- The laboratory must satisfy the safety measurements of level 3; the omission of particular safety measures may however be requested (see below).
- An airlock with washing and disinfection facilities must be available, but a shower is not necessary.
- All studies in which aerosols may be produced must be performed in a class II microbiological safety cabinet.
- An autoclave must be able to reach temperatures of 134°C (validated). It must also be available in the same laboratory if possible, or at least in the same building.
- Wastes and waste water must be decontaminated.
- A list of the laboratory personnel who have been in contact with prions must be kept for at least the period prescribed in the ContainO and the PEMO.

Measures that could be omitted when working with prions, since the danger to the environment is relatively small:

- Filtration of air exiting the laboratory with a HEPA filter
- Negative air pressure in the laboratory
- Ability to seal the area for gassing, since prions cannot be destroyed by gas (e.g. formaldehyde).

4 Risk assessment for work with prion proteins and prion genes

The following findings from scientific research must be borne in mind when the risks associated with handling prions are being assessed. Studies with native and with mutant recombinant prion proteins are also included in the considerations.

- Experimental studies on scrapie in mouse and in hamster have been carried out for several decades. There are no indications that the scrapie agent represents an increased risk either for occupationally exposed persons or for the public and the environment. To date, it has not been demonstrated that scrapie can be transmitted to humans. However, rodents infected with scrapie agents should be handled using the same safety precautions as rodents infected with natural, mouse-specific pathogens (classified into group 2).
- It has been shown that the primary structure of the prion protein plays an important role in transmission of prions from one species to another (Prusiner et al., 1990; Colby et al., 2011). The sequence of the bovine PrP gene shows greater homology to the sequence of the human gene than the sequence of the mouse or hamster gene (Gabriel et al., 1992). Although it is possible to transmit bovine TSE agents to humans, it appears unlikely that mouse- or hamster-specific TSE agents can be transmitted to humans.
- It is now possible to transform native recombinant PrP^{c} or PrP^{c} from tissue extracts into infectious prions (PrP^{sc}). Particularly when using PMCA technology (Protein Misfolding Cyclic Amplification), infectious prions reproduce efficiently in the laboratory (Castilla et al., 2005; Wang et al., 2010). It is vital that this be taken into account in the risk assessment.
- Certain activities using PrP genes should be treated with caution. Bearing in mind the current gaps in knowledge, the following activities present an increased risk to humans and the environment: (1) cloning of PrP genes in host organisms that could replicate in/on humans, and (2) the cloning and expression of modified PrP genes in prokaryotic and eukaryotic expression systems where there is a possibility of producing infectious PrP^{Sc}.
- Mutations in the DNA sequence of the PrP gene, without a host organism, poses a negligible risk according to the current state of science. This activity may thus be classified under Class 1.

5 Classification of work with TSE agents

Based on the risk assessment above, the following recommendations can be made for work with prions and prion proteins (see also Table on p. 5):

5.1 Animals

- Autopsy of sick cattle should be done in accordance with the Epizootic Diseases Ordinance and according to special clinical regulations, in principle using the safety measures of level 2 (see Diagram on p. 6). Infected cattle used for research purposes can be kept in safety level 1, since transmission via aerosol can be virtually ruled out.
- Activities with animals infected with rodent-adapted scrapie prions, i.e. animal husbandry and care, invasive procedures (e.g. inoculation with prions), and post-mortem examinations and tissue sampling from animals, are classified as class 2.
- Activities with live mice and hamsters infected with BSE prions, BSE-related prions or human TSE agents (e.g. animal husbandry and care), are classified as class 2. The risk

of bite and scratch wounds is reduced by handling the animals with long, blunt forceps. Invasive procedures (e.g. inoculation with TSE agents) as well as post-mortem examinations of infected animals and tissue sampling are classified under class 3.

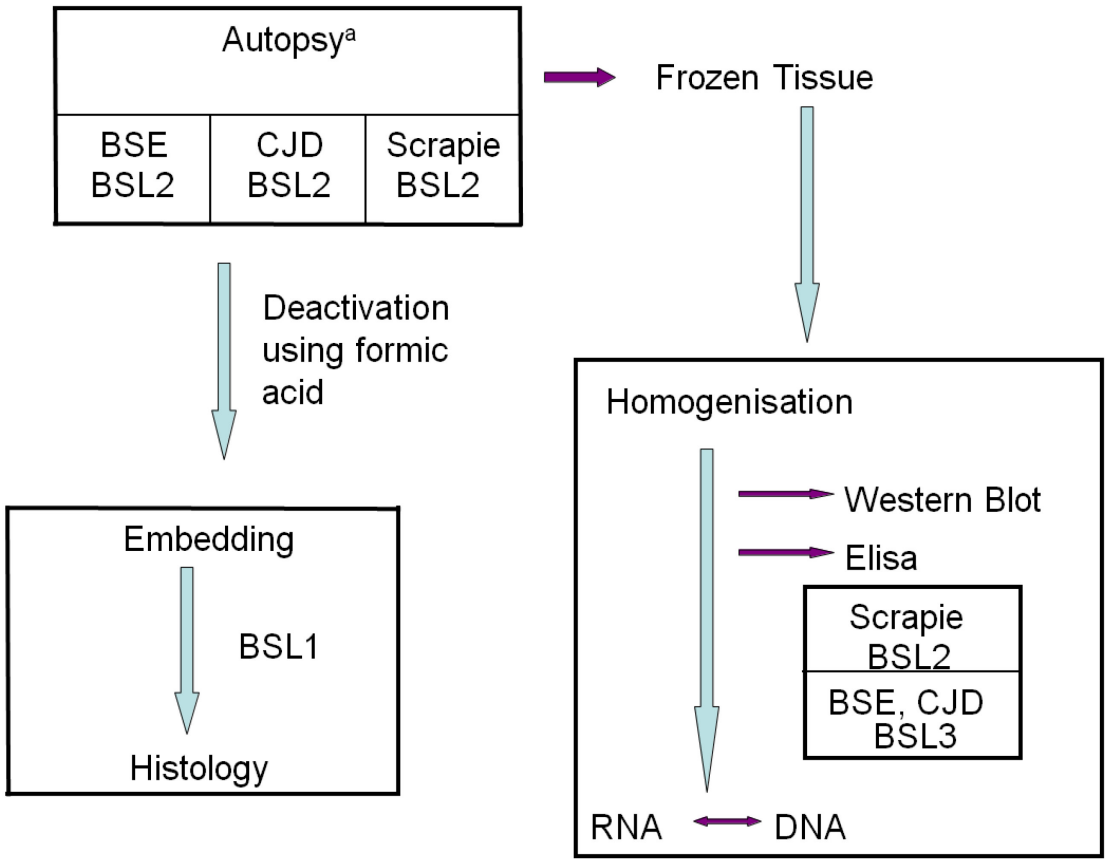
5.2 Tissue

All experimental studies involving lymphoid and neural animal tissue that contains rodent-adapted scrapie prions are classified as class 2. Activities using tissue from humans and animals that contains human or bovine TSE agents are classified as class 3.

5.3 Classification of work using genetically modified PrP genes and proteins

	Origin of the PrP genes			
	sheep, rodent		cattle, human	
Cloning of DNA in plasmid with inactive promoter	1		1	
	State of the protein			
	PrP^{c}	PrP^{Sc}	PrP^{c}	PrP^{Sc}
Expression in prokaryotic and eukaryotic expression systems that don't replicate in/on humans	1	2	1	2
Expression in hosts that replicate in/on humans	1	2	2	3

5.4 Overview of the classification of pathological work using prions



a) Special clinical measures must be applied for autopsies of humans and cattle

6 References

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