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OPINION
ON
THE SAFETY OF HUMAN-DERIVED PRODUCTS WITH REGARD TO TSE's

Adopted by
The Scientific Committee on Medicinal Products and Medical Devices
On 18 January 2002

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MANDATE

The Scientific Committee on Medicinal Products and Medical Devices, in collaboration with the SSC and its TSE/BSE *ad hoc* Group, is invited by The Health and Consumer Protection Directorate-General to identify appropriate, valuable risk assessment methods and provide examples of "good practice" in relation to evaluation, screening, detection of vCJD risk in (human) populations, human-derived products, surgical instruments, medical interventions, etc.

In the absence of common validated methodologies for diagnosis of vCJD, the Commission services would like to obtain an opinion concerning existing diagnostic tests, including newly developed blood-based tests. In the light of this, the Scientific Committee on Medicinal Products and Medical Devices, in collaboration with the SSC and its TSE/BSE ***ad hoc*** Group, is invited to address the following questions:

1. What is the value of screening vCJD in healthy subjects, potential donors, sufferers of neurodegenerative disease, using:
   - tonsil tissue tests;
   - experimental ERDF (designated erythroid differentiation-related factor) tests (Roslin Institute, UK);
   - any other available tests.

On vCJD infectivity in blood and other substances of human origin:

2. What methods and techniques are currently used for detecting infectivity in blood and other substances of human origin? Did new methods and techniques become recently available? What is the current state of knowledge on the (vCJD) infectivity of blood and other substances of human origin?

On vCJD Risk assessment:

3. What are the most important currently existing methodologies for assessing the risk of transmission of vCJD/ TSEs via blood and other substances of human origin applied by the EU Member States and countries such as Canada, New Zealand and the USA? Are they still appropriate?

4. Questions in relation to risk assessment methods of vCJD transmission via blood:

   - Are the different risk assessment methods employed by Member States to assess risk of vCJD transmission via blood compatible?
   - How should we best evaluate the different risk assessment methodologies?
   - Should a European risk area map specific to vCJD be drawn up?

5. At its meeting of 8-9 February 2001 the Scientific Steering Committee (SSC) considered that establishing a "Geographical vCJD risk " exercise (cf. GBR risk levels for BSE) is improbable because of the absence of real data.

   The Scientific Committee on Medicinal Products and Medical Devices, in collaboration with the SSC and its TSE/BSE ***ad hoc*** Group, is nevertheless invited, in relation to the "possible" geographical risk for vCJD, to list risk factors with regards to blood donors and epidemiological surveillance of vCJD in possibly exposed human populations.

On Risk reduction:

6. Questions in relation to processing procedures for blood and blood components in the risk management of vCJD transmission via blood:

   - Would leucoreduction and nanofiltration provide a significant risk reduction?
   - Is Specific filtration of plasma as a starting material a measure that should be recommended?
   - Should centrifugation be included prior to filtration and how many times (once/twice?)
   - Is there substantial evidence that leucoreduction significantly helps to reduce the risk of vCJD transmission?
   - Should leucoreduction be recommended on the basis that it is primarily beneficial in reducing risk of transmission of other diseases and secondly for a possible benefit in reducing the risk of vCJD?
   - Is there evidence to support concerns that leucoreduction may in fact be counteractive in reducing risk of vCJD transmission by liberating cellbound prions?
   - What are the best methods concerning leucoreduction?

In order to be concise and to avoid repetitions the responses are given in a slightly different order. The following table facilitates the assignments of the different chapters of this report to the number of the questions:

Question	1	2	3	4	5	6
Chapter	3	1, 2	4	5	6	7

Opinion

- During the last years the methods used for direct or indirect detection of TSE infectivity in human blood and tissues did not progress greatly. Bioassays using conventional laboratory rodents (mice, hamsters) are still the most reliable way to demonstrate infectivity but are hampered by a number of factors, most importantly by the so-called species barrier which might prevent the detection of small amounts of infectivity. This problem may be circumvented by the use of transgenic mice (bovine or human Prnp genes on a null background) but there are not yet reports using those animals for the titration of infectivity in human tissues.

- A number of reports deal with new methods to demonstrate PrPSc in body fluids. However, there is generally a lack of confirmation by other groups and a lack in validation. The same holds true for tests measuring surrogate markers (e.g. EDRF) which are assumed to correlate with an infected state. None of these tests are ready for general use. There is still no formal scientific proof for the strict correlation between detection of PrPsc and infectivity, but in a worst case scenario this correlation should be assumed.

- The presence of vCJD infectivity in human blood can still not be excluded. In some animal models (mostly rodents) a low titer of TSE infectivity in blood can be detected by bioassays. There are also reports of sporadic detection of TSE infectivity in other animals but they are met with some criticism. So far, PrPSc could never be reproducibly detected in blood. Two recent publications deal with the quantification of infectivity, respective PrPSc in vCJD patients. In tonsils and spleens lower amounts were found than in CNS. Neither infectivity nor PrPSc were detected in blood (buffy coats). Those results do neither exclude the presence of infectivity or PrPSc earlier in the incubation period of the disease nor their presence in small amounts.

- The detection of PrPSc in tonsils and appendixes of vCJD patients even in the preclinical phase opens the theoretical possibility to measure the prevalence of this disease by screening tonsils and appendixes resected for clinical reasons. However, before such studies commence, a thorough statistical evaluation should demonstrate that the outcome of such an effort leads to valid and meaningful data. It should be born in mind that even in the United Kingdom where the highest prevalence of vCJD cases is suspected the investigation of 3000 appendixes and tonsils

ended up with no positive result. In addition, such a study raises a number of difficult ethical questions which have to be resolved in advance.

- There is some progress in the clinical diagnosis and differential diagnosis of vCJD. However, despite a number of publications and announcements, in vitro tests which detect vCJD infections in the preclinical stage are not yet available. A major obstacle is the validation of such assays, mainly due to the lack of availability and standardisation of samples and reagents. Efforts should be made to overcome those problems.

- If reliable and validated in vitro diagnostics are available, their introduction into blood donor screening, despite the present lack of demonstration of blood infectivity, can be considered. Again, a number of ethical questions (information of donors, information of recipients etc.) has to be resolved.

- In the absence of reliable in vitro tests, the exclusion of donors who are assumed to be at higher risk for having acquired vCJD (mainly by food) is understood as a preventive measure to reduce the hypothetical risk of vCJD transmission by blood. This risk is believed to be associated with exposure to BSE contaminated food. It is also assumed that this risk differs between different countries. For example, the Food and Drug Administration established implicitly four categories, namely in order of decreasing risks: United Kingdom, France, rest of Europe and rest of the world. Assessments of Member States as well as other countries differ widely in their risk assignments to different geographical areas. In order to promote harmonised views, the drawing of a "European risk map" is recommended.

- In its Opinion on the Human Exposure Risk (HER) via food with respect to BSE the SSC has listed a number of factors which influence the quantitative estimation of the HER. Some of these factors (e.g. minimal infectious dose, distribution of infectivity in the tissues of an infected animal) are not needed for the estimation of relative risks between countries. Provided that the genetic susceptibility to the BSE agent does not differ between populations, the most important factors for the evaluation of relative risks seem to be the size of the endogenous BSE epizootic and the extent of importation from higher risk areas of bovine materials which entered the national food chain.

- The possible contribution of leucoreduction and nanofiltration to the reduction of the theoretical vCJD risk of blood and blood products has been discussed. However, due to the lack of knowledge of the actual state of the infectious agent in blood validation studies are difficult to

perform. The available data with both methods hint to positive effects without showing harmful effects.

Contents

Report of the Working Group

(1) Methods and techniques for the detection of vCJD infectivity

In 1996 the occurrence of a new variant of Creutzfeldt-Jakob disease (vCJD) was described in Great Britain (Will et al. 1996, Will et al. 1997). This variant differs from the heretofore known forms of the disease in both clinical and neuropathological characteristics (Will et al. 1996, Will et al. 1997). A whole range of observations suggests that vCJD is caused by the same agent that causes BSE in cattle: the geographical correspondence between the predominant occurrence of BSE and vCJD (i.e. in the United Kingdom), the biochemical similarity between the prion proteins associated with BSE and vCJD (Collinge et al. 1996, Hill et al. 1997), the inability to distinguish the agents in strain typing (incubation periods in different mice strains, lesion patterns in the brain (Bruce et al. 1997, Lasmézas et al. 2001), the induction of neuropathological changes in macaques after infection with BSE material, which changes are very similar to those in vCJD patients (Lasmézas et al. 1996) and finally the identical biological properties in the transmission of BSE and vCJD material to transgenic mice (Scott et al. 1999). It is therefore assumed that vCJD originates from BSE infected bovine material in food (primary transmission). It is not known to what extent secondary infections, i.e. human to human transmissions of the vCJD agent, can take place. There are no indications whatsoever for a transmission of the agent by social contact. Fundamentally, however, it cannot be excluded that vCJD could be transmitted by contaminated surgical instruments or by human tissue, especially by transplants or by blood and blood products. Should these routes of transmission be possible, vCJD could establish itself in the human population for a lengthy period, even if primary infection is prevented by appropriate measures.

Infectivity of variant Creutzfeldt-Jakob disease (vCJD) in blood or other human substances may be recognised by (a) bioassays, or (b) in vitro diagnostic assays, or (c) epidemiology.

(a) Bioassays: Transmission of TSE to indicator animals

The inoculation of blood or other substances of TSE-infected humans into indicator animals, e.g. rodents like mice or hamster, is used for investigation of TSE infectivity of human materials. An overview of studies using different bioassays is provided in Tables 1-4 of the SCMPMD opinion on the risk quantification for CJD transmission via substances of human origin (21.10.1998). Points to be considered for interpretation of respective transmission investigations are:

- the species barrier (leading to wrong interpretation or underestimation of infectivity)
- TSE strains differ in infectivity and pathogenic features between different indicator animals
- genetic background (predisposition) of the indicator animal
- transmission route (e.g. intravenous, intracerebral), with the intracerebral inoculation being the most effective
- volume of the inoculum limited by the size of indicator animals and potential detection without replication at non-affected sites

Rodents may be also genetically modified, e.g. to resemble the human situation: Transgenic mice harbouring the human (but not mouse) Prnp gene are more susceptible to human TSEs with no measurable species barrier concerning transmission of classical CJD. Transmission of vCJD to such transgenic mice still involves a species barrier which may limit the detection of low titre infectivity. However, it was reported by Scott et al. (1999) that mice transgenic for the bovine Prnp gene on a null background are highly sensitive for vCJD transmission.

The Scientific Steering Committee evaluated in a recent Scientific Opinion the use of non-human primates as potentially more appropriate models for human TSEs (SSC opinion on the use of non-human primate models for human TSEs; 6.-7. 9. 2001). These primates have special relevance as models that can be used for risk assessment in humans (infectivity studies, distribution of infectivity during incubation time), for assessment of drug and vaccine efficacy and for evaluation of diagnostic tools. At the time being, at least chimpanzees may be dispensable for research use because of the proven susceptibility of several other non-human primate species (macaques, squirrel monkeys).

(b) In vitro diagnostic assays

Successful in vitro detection of PrPsc, the pathological form of the prion protein, in certain tissues or cells is thought to be linked with TSE infectivity of these materials. Persons in the TSE incubation period might be healthy but potentially transmitting prions to others. Different candidate assays are currently in development for the screening of human blood for presence of PrPsc. So far no suitable test with sufficient sensitivity and specificity has been developed and validated. A recent publication (Saborio et al., 2001) describes the detection of PrPsc after approximately up to 16fold amplification of the protein misfolding by a number of incubation/sonication cycles. If

this principle can be further optimised, it might be applied for a sensitive assay qualified for the detection of human PrPsc already during the incubation period.
The specific binding of pathological prion forms in human brains to plasminogen is described by another group. This finding might have implications on the development of diagnostic assays for easier accessible human materials (Maissen et al., 2001).
Another report focuses on the detectability of PrPsc isoforms in urine in three TSE infected species, partially during the incubation time (Shaked et al., 2001). This report was interpreted by the SSC as a potential important contribution to preclinical diagnosis of TSEs, nevertheless the findings have to be repeated and confirmed by other groups (SSC Minutes 6-7 Sept. 2001).
Other techniques to detect PrPsc in biological fluids such as capillary electrophoresis (Schmerr et al., 1999) or scanning for intensely fluorescent targets (SIFT; Bieschke et al., 2000) have been already applied to TSE samples, but their validation remains to be done.
Identification of TSE-infected persons during the asymptomatic incubation time could also be possible by looking for TSE infection-specific surrogate markers. The recently described down-regulation of transcription of a peripheral erythroid-specific marker (EDRF) following TSE infections in different species (Miele et al., 2001) could be the basis of another approach for TSE diagnostics.

(c)Epidemiology

Epidemiological studies (surveillance, case control studies, population studies) reveal no evidence for human TSE transmissions by blood or other human substances, with the exception of transmission of CJD from one individual to another by tissues or substances which are in close contact with the central nervous system (cornea, dura mater, human growth hormone prepared from cadaveric pituitary glands). An overview is given in the SCMPMD opinion on the risk quantification for CJD transmission via substances of human origin (21.10.1998). For nvCJD no respective reports exist but the observation period is too narrow to draw conclusions from epidemiological observations.

(2) vCJD infectivity of blood and other human substances

Orally acquired TSE infections require a mechanism for the transport of infectivity from the gastrointestinal tract to the central nervous system (CNS) where peripheral tissues are involved. In a

mouse scrapie model mature B-cells, germinal centres and follicular dendritic cell (FDC) networks (lymphoinvasion) are required for the development of clinical scrapie. Replication or accumulation of prions seems to be restricted to FDCs from where infectivity is transported to the CNS through neurons (neuroinvasion). A recent publication provides evidence for a further role of dendritic cells in the process of neuroinvasion (Aucoutourier et al., 2001). Dendritic cells isolated from spleens of prion-infected mice carry infectivity and can induce disease when injected intravenously. The exact process of transmission of infectivity into the CNS and the potential role of FDCs still need to be clarified. Another recent publication again questions the pivotal role of B cells and FDCs in promoting neuroinvasion and spongiform encephalopathy in a mouse CJD model (Shlomchik et al., 2001).

Currently there is neither a disproof nor a proof for vCJD infectivity of human blood.
Epidemiological studies indicate transmission of sCJD infectivity only by tissues / substances which are in close link with the central nervous system. On the other hand, vCJD clearly differs from sCJD by detectability of the pathological agent in extracerebral tissues like the lymphoreticular system.
Investigation of biopsies of vCJD patients by an immunoblotting assay with improved sensitivity reveals the presence of pathological prion proteins in brain, retina and optic nerve, tonsils, spleen and lymph nodes of several patients, additionally in thymus, adrenal gland and rectum of one patient (Wadsworth et al., 2001). The highest extracerebral concentration of PrPsc is measured in tonsils, and the detection in tonsils of symptomatic patients is in line with the later diagnosis of vCJD at necropsy using brain biopsies. Despite the detectability in the lymphoreticular system of affected patients vCJD prions were not found in corresponding buffy coats. This may be explained by the general or temporary absence of vCJD in the blood of these patients or by the sensitivity limit of the Western Blot method. Analogous results were published for infectivity in conventional mouse strains of different materials from two vCJD patients (Bruce et al., 2001). Results of tests for infectivity in blood or blood cells using transgenic mice (human or bovine Prnp) have not yet been published.

Analysis of distribution of infectious prions in blood of asymptomatic mice infected with a human TSE agent (Gerstmann-Sträussler-Scheinker syndrome; GSS) revealed an association of infectivity mainly with the buffy coat and only weakly with the plasma fraction (Brown et al., 1999).
The successful transmission of the BSE agent by blood transfusion from an orally infected asymptomatic sheep to a recipient sheep has been reported (Houston et al., 2000). The implications

of this observation are potentially significant. However, so far this observation has not been repeated and, furthermore, the TSE strain identity in the recipient animal could not be established. The data have therefore to be regarded as preliminary and its implications unclear (Opinion of the Scientific Steering Committee on the implications of the Houston et al. paper in The Lancet of 16 September 2000 on the transmission of BSE by blood transfusion in sheep; 26.-27. 10. 2000).

(3) Application of vCJD detection tests

(a) Clinical differential diagnosis of vCJD

Advances in research into human spongiform encephalopathies are leading increasingly to the development of techniques and methods that enable the clinical diagnosis of Creutzfeldt-Jakob disease while the patient is alive. With the increasing experience in the clinical picture of vCJD, clinical criteria have now also been established. In this respect magnetic resonance imaging plays an important role. In its differentiation from vCJD, the sporadic form is still one of the most frequent clinical differential diagnoses.

Magnetic resonance imaging (MRI) is one of the methods for the diagnostic clarification of rapidly progressive dementing illnesses. In addition to exclusion diagnosis, this method can provide findings to support the clinical suspicion of CJD. In sporadic CJD, hyperintensities are observed in the caudate nucleus and putamen in approx. 2/3 of cases. In this respect, the special value of magnetic resonance imaging lies in the possible differential diagnosis of suspected vCJD: with vCJD the strongest signals are seen in the posterior thalamus (the “pulvinar sign”). Since this signal pattern has been observed in 78% of vCJD cases, magnetic resonance imaging has become one of the diagnostic criteria for vCJD.

In patients with Creutzfeldt-Jakob disease the standard cerebrospinal fluid (CSF) parameters (cell number, barrier function, inflammatory reaction) are generally inconspicuous. The rapid neural decline or astrocytic activation results in brain proteins crossing into the CSF. Proteins such as neuron-specific enolase (NSE), S100 protein, tau, brain-specific creatine kinase and G_0 protein have been measured in abnormally high concentrations in the CSF in cases of CJD. Increased concentrations of these proteins represent an indicator for a rapidly destructive process and therefore assist in the differential diagnosis of sporadic CJD and other neurodegenerative diseases. At present the most important test is the determination of 14-3-3 proteins in the CSF. In the differential diagnosis of dementias this test gives a sensitivity of 94% with a specificity of 93%. In contrast to sporadic CJD, in cases of vCJD increased 14-3-3 concentrations in the CSF were measured in only 45% of patients.
The detection of the CJD-typical proteinase K-resistant form of the prion protein in the CSF would lead to an in vivo diagnostic test; this is, however, still under development. A promising method is fluorescence correlation spectroscopy (SIFT), which yielded pathological findings in 20% of examined human CSF samples.

The tests available to date do not allow the preclinical diagnosis of CJD. They often show positive only in the advanced stages of the disease.

(b) Surveillance of vCJD

An active surveillance system was set up in the UK already in 1990 with the primary aim of identifying any changes in the characteristics of CJD that might be linked to BSE. Three years later surveillance was extended to several other European countries co-ordinated by the European Union. Results can, in part, be found in the world wide web (http://www.cjd.ed.ac.uk/figures.htm, http://www.invs.sante.fr). By the end of November 2001, there have been only few cases of confirmed or probable vCJD cases outside the UK and Ireland, namely five in France.

An active prospective surveillance of vCJD in UK children using the surveillance mechanism established by the British Paediatric Surveillance Unit, was initiated after the vCJD outbreak was established with high degree of confidence in 1996 (Verity et al., 2000). Two definite cases and one probable case were reported to this active surveillance study during a three year study period.

All affected vCJD patients have been found to have detectable amounts of PrPsc in organs of the lymphoreticular system such as tonsils and/or appendix. Furthermore, PrPsc was shown to be detectable in appendixes in the pre-clinical phase of a patient (Hilton et al., 1998). Thus a surveillance of vCJD has been further attempted by investigation of more than 3000 surgically resected appendix and tonsil specimens for detectability of PrPsc. Patients were selected in regard to their age. No positive specimens were found (Ironside et al., 2000).

It seems to be an attractive option to screen tonsils and appendices for PrPsc in order to get an impression of the prevalence of vCJD infected people. Before such a study should be commenced a thorough statistical analysis of the expected outcome and its precision should be performed. For example, a report in Germany (Gefeller et al. 2001) stated that, given a low vCJD prevalence, even the investigation of all tonsil or appendix specimens removed from patients of an age between 10 and 55 years for clinical reasons in one year would not allow a meaningful estimation of the prevalence of vCJD in Germany. It is evident that such a study would also rise a number of difficult ethical questions.

(c) Donor screening for vCJD

A significant proportion of the population receives blood transfusions at some point in their life with the majority of recipients of labile blood products being already of medium or higher age at the time point of the first transfusion. Thus, as the incubation time for clinical vCJD extends probably for several years, blood transmission as possible cause for this disease is not relevant to all blood recipients. However, the blood route could be blocked by information obtained from donor screening, i.e. by recognising asymptomatic vCJD cases who are incubating the disease.

The major challenge for an in vitro diagnostic assay is the requirement of either detecting very low levels of PrPsc in easily accessible human specimens or of sensitive testing for a surrogate marker closely linked to TSE infections. No screening test so far has yet been able to detect PrPsc in specimens like blood or plasma originating from vCJD-infected individuals (see discussions in chapter 1). A further challenge for suitable candidate tests is an adequate validation which is hampered by the current lack of availability and standardisation of reagents. The validation had to prove that a suitable blood screening assay would not result in unacceptable high numbers of false positives (who would unnecessarily be excluded from donating blood) or of false negatives (who would continue donating blood although infected).

Furthermore, the decision whether or not to perform donor screening, even if there were insufficient information of blood infectivity of incubating patients, would have to rely on a precautionary approach. As vCJD has been such a rare disease (Verity et al., 2000) very few if any blood samples from known vCJD patients are available from the incubation time. This means that the screening parameters cannot be derived from usually designed studies. In practice screening parameters would have to be indirectly assessed from living diseased patients, and even then the patient material would be limited. This would obviously mean that screening parameters including the most important information for an individual, i.e. positive predictive value of the test, would be quite unreliable. Furthermore, as the distribution of the disease is probably not uniform over Europe one should also bear in mind that, even if one had fairly reliable screening parameters from the most affected areas, positive and negative predictive values could not be generalised to other settings as prevalence varies.

Screening for an invariably fatal disease like a human TSE in yet healthy people involves also difficult ethical problems, like the extent and nature of the information to be provided to donors

who are reactive in a vCJD screening assay, or like the extent and nature of information to be given to recipients of blood transfusions which originated from a blood donor who later becomes vCJD positive. Ethical questions are not discussed in this report which is aimed to address the scientific data.

(4) Transmission of vCJD/ TSEs via blood: Risk assessment technologies applied by different countries

(a) Europe

In Europe, there were assessments both on community level (scientific committees SSC and SCMPMD of the European Commission; CPMP and scientific working groups of the EMEA), and by several member states (UK, France, Germany).

- The **SCMPMD** convened a working group of experts who addressed questions, which had been asked by the Commission, concerning the risk of CJD/vCJD transmission via materials of human origin. Answers were discussed and formulated in the light of a careful and comprehensive review of the available literature (AE 1 – AE 3; see Annex of this Opinion). A special opinion was published by the SSC after assessment of a Lancet paper with a preliminary report of a sheep experiment, after discussion with one of the authors (AE 3).
- The **EMEA** published a position statement (AE 4) on vCJD and plasma derived medicinal products in 1998 (CPMP/201/98), taking into account both scientific papers and previous regulatory documents, and stating that no recall of batches was required upon diagnosis of sporadic CJD in a donor who had contributed to the starting material. However, it was recommended to recall in case of vCJD diagnosis of a donor, and it was recommended to avoid the use of albumin as excipient from a country with a number of vCJD cases. Several workshops have been organised by CPMP/BWP, convening experts from academia, regulatory bodies and industry, presenting recent experimental findings and new epidemiological data, and discussing possible technical and regulatory consequences. Reports summarising the present status and conclusions were published (AE 5). A further workshop is planned for spring or early summer 2002.
- **UK** has been the first member state, where the new health problems BSE and vCJD emerged, and thus performed very early and intense risk assessments. These were based on both epidemiological and experimental observations. The only risk factors for contracting vCJD identified to date are young age and residence in the UK. UK decided not to use domestic plasma for fractionation from 1998 on. The surveillance programs so far did not allow for firm conclusions on the extent of the vCJD epidemic, and did not reveal any epidemiological information on the possibility of vCJD transmission by blood or medicinal products derived from blood. There are very detailed and comprehensive assessments of the vCJD risk of human

cells and organs (A 9), surgical instruments (A10), and a special paper on the question how to deal with cases of incidents of potential vCJD transmission (A11).

- In **France**, experts convened by the AFSSAPS elaborated two reports published in February 2000 and on 11 December 2000. The first report (AE 6) dealt with the question, whether an exclusion (as proposed by the FDA, see below) of donors who spent a cumulative time of more than 6 months in UK between 1980 and 1996 was appropriate also for France. The group attempted to compare the "endogenous" risk, i.e. the exposure to BSE risk materials of the population within France, with the (additional) risk or exposure of those individuals who had stayed in UK. The group assessed, as far as possible, the food chain, and in particular the importation of cattle and bovine material from UK to France, and came to the conclusion that an adaptation of the FDA exclusion criteria would bring about only a marginal reduction of the risk. On the other hand, the estimate showed that a really substantial reduction of the hypothetical risk would require an unaffordable loss of donations. Since there had been cases of vCJD in France, the second report (AE 6) assessed the available experimental data on transmissibility of TSE, and focused on the question, whether blood components and plasma for fractionation sourced in France could still be used. The group made assumptions derived from available data concerning the maximum load of infectivity in whole blood, and its distribution between cells and plasma, and performed model calculations of the infectivity of blood components and plasma derivatives under worst case scenarios. The group came to the conclusion that a low (hypothetical) risk could not be ruled out for blood components, which, however should be further used given the lack of alternatives. Further, the group concluded that domestic plasma for fractionation could still be used, since an elimination of spiked material, pointing to an effective removal of infectivity during manufacture, could be shown. As general principles, this group recommended to strictly respect therapeutic indications, and to consider the use of alternatives (e.g. recombinant coagulation factors), as appropriate.
- In **Germany**, the question of exclusion of donors having spent more than 6 months in the UK was discussed shortly after the FDA proposal at the end of 1998 in the Blood Advisory Group ("Arbeitskreis Blut") appointedby the federal Ministry of Health, and the group could not adopt that measure at that time. The matter was revisited in November 2000, after the first BSE case was detected, and a survey by blood donation organisations had revealed that the loss of donations would not compromise supply. It was recommended to adopt the FDA policy at that time, to exclude donors who spent a cumulative time of more than 6 months in UK between 1980 and 1996. An argument for this recommendation was also that a difference of standards for source plasma between Germany and U.S.A. should be avoided.

By the end of the year 2000, the Ministry of Health mandated an expert group to develop a strategy for blood supply in face of variant CJD. This group performed a comprehensive review of available scientific information on the epidemiology of BSE in Europe, the movement of cattle and risk materials, the epidemiology of vCJD, the particular endogenous and exogenous risk of primary vCJD infection from the food chain, the available experimental data on vCJD pathogenesis, and the potential occurrence of infectivity in blood. On the basis of this review and assumptions derived thereof, an assessment of the potential risk of blood components and plasma derivatives under worst case assumptions was performed. Conclusions were drawn, and recommendations on a strategy involving precautionary measures (e.g. exclusion of former recipients of a blood transfusion from blood donation), enhanced donor motivation in order to enable further measures such as the exclusion of transfusion recipients, and the optimal use of blood products were derived. The paper (AE 8) was introduced to the public on 16 October 2001, in the meantime the English translation is also available.

(b) Countries outside Europe (USA, Canada, New Zealand)

- In the **USA,** the situation is profoundly different from Europe, since no BSE cases have been observed yet in the country, and no significant importation of cattle, bovine materials or feed had taken place. Thus, the FDA adopted early a very cautious approach to avoid the risk of spreading human TSEs by blood products. In 1996, the FDA recommended (AO 8) to withdraw any batch of product, when a donor contributing to the source material was identified later on as having CJD (there was no differentiation from vCJD at that time), or to be at risk for developing CJD. This policy was revised later (AO 9), since it lead to severe supply problems, particularly with immunoglobulins for intravenous (iv) use , and epidemiological evidence showed that even if there was a risk of transmission of CJD by blood it would be of such a low level that it would be not detected.

  In August 1999, the FDA recommended (AO 10) to defer donors who spent a cumulative time of more than 6 months in UK between 1980 and 1996. The basis for this recommendation was the assessment of the risk factors for vCJD, where so far besides young age only residence in the UK had been identified. Therefore, the hypothesis was coined that the risk of contracting vCJD is proportional to the time spent in UK. A survey of travel to UK among donors was performed by the American Red Cross. Based on these data, and defining a stay of one day in UK as “one risk unit”, model calculations were performed, how several scenarios of donor

exclusion would have impact both on the total hypothetical risk and the blood supply. From these calculations, the above recommendation was derived balancing a reasonable reduction of the hypothetical risk against a sustainable loss of donations. This method of assessment, and particularly the underlying hypothesis of a stay of one day in UK being a "risk unit" was challenged by some, but the approach was adopted more or less also by assessments in other countries. For instance, the French and the Canadian assessments (AE 6, AO 3) used similar model calculations.

Under the impression of the ongoing increase of vCJD incidence in UK, the occurrence of vCJD in other European countries (France, Ireland), the background of new BSE cases over Europe, and the preliminary results of a sheep experiment, the FDA revised the recommendations on donor exclusions in August 2001 (AO 12); measures announced by the American Red Cross (AO 13) are even stricter. The background of the revised FDA recommendations is the extent of the BSE epidemy in different countries, the time points for introduction of effective measures in different countries to prevent BSE transmission to humans, the higher association of potential vCJD infectivity with cellular components compared to plasma and the number of vCJD cases. The recommendations include, among others,

* the deferral of donors who have spent three months or more cumulatively in the U.K. (from 1980 to 1996),

* the deferral of donors who have spent five years or more cumulatively in France (from 1980 to the present) and

* the deferral of whole blood, but not source plasma, donors who have spent five years or more cumulatively in Europe (from 1980 to the present).

With these recommendations FDA undertook the effort to balance the estimated differences of the vCJD risk in different countries against precautionary measures. So far, there is no FDA policy to introduce leucoreduction as precaution against vCJD; however, leucoreduction was recommended for other reasons (AO 11). The current perspectives and policies regarding vCJD in the U.S.A. and elsewhere were recently reviewed by P. Brown et al. (2001), who made a strong case for rigorous attempts towards global elimination of animal TSEs.

- There was an intense discussion about the blood system in **Canada** after the Krever inquiry had been completed in 1997. The first group to propose an exclusion of residents of areas with significant incidence of vCJD as blood donors (AO 2) was the Bayer Advisory Council on Bioethics convened in Canada. The regulatory assessments (AO 3) followed more or less the same line as those of the FDA, after discussing the background and recent findings with stakeholders. The so far latest, very strict directive (AO 4) on donor exclusions according to

their stay in European countries was released by Health Canada in August 2001. In that assessment, also the historic relations to France, where cases of vCJD had been observed, was an important aspect. The policy in Canada was recently reviewed (Wilson et al., 2001), and the authors identified some progress in decision making in the period from 1995 to 1999, e.g. in terms of a transition from reactive to proactive approach, more thorough assessments, and a more consultative and transparent process involving stakeholders and consumer groups.

- **New Zealand** is a country with a particularly strong impact of the cattle industry. Recently, a comprehensive assessment of potential human health risks of imported food products with respect to BSE was published (AO 7). The decisions regarding the vCJD risks and product recall (AO 5) and blood supply (AO 6), more or less followed the international trends.

Taking together the methodologies used for assessment, there is generally the problem of decision making in the absence of solid, reliable evidence. In this situation, the only possible way is to convene experienced experts who are asked to review the available epidemiological and experimental data, and to derive assumptions thereof. This basis can be used for exploring risk scenarios and model calculations, for deriving recommendations on precautionary measures, and for identification of priority issues for further research. Such an approach was particularly used by the European expert groups.

There are, however, some important observations, concerning the scope of, or the attitude behind the available documents and recommendations. The assessments of the SCMPMD are focused on a basic and differentiated scientific estimation of the risk, while the EMEA, according to its direct responsibility for medicinal products derived from human materials, has a more practical and regulatory approach. The assessments of France and Germany dealt with a comprehensive analysis of the particular situation in their countries in the context of the BSE and vCJD evolution in Europe, and particularly in relation to the situation in the UK. The U.S.A. and Canada, as well as New Zealand have a much more defensive approach, since they do not consider that their countries have a substantial “endogenous” risk; thus their focus is more the prevention of the “importation” of the vCJD risk from Europe.

Table 1

National measures concerning blood donations in European countries

	Exclusion criterion defining minimal period spent in UK (1980-1996)	Performance of leucoreduction
Greece	6 months (introduced 1.3.01)	Universally recommended for blood components.* (Stepwise implementation in 2001.) Proposed for therapeutic plasmapheresis and other apheresis procedures.
Portugal	6 months	universal*
United Kingdom	UK plasma is not used; no restrictions for cellular blood products of UK donors	Yes for blood components, ? plasma for fractionation
Austria	6 months	Yes for labile blood components
Finland	6 months	Yes (for thrombocytes)
Belgium	6 months proposed	All platelets and part of red blood cells (packed cells), proposal for universal use
Netherlands	No exclusion	Yes
Germany	6 months for blood components, intended for plasma derived medicinal products	Yes
Sweden	No exclusion	partially introduced
Spain	No exclusion	being introduced
Denmark	No exclusion	Proposed for labile products
Luxembourg	(probably >1 year)	Yes (specific plasma filtration)
France	1 year	Universal
Ireland	5 years (introduced 31.3.01)	Yes (for labile products). Ireland does not produce plasma for fractionation at present.
Norway	No exclusion	Yes for blood components, no for plasma for fractionation
Italy	6 months	No

Provisional information courtesy G. Silvester, EMEA
Table 1 reflects the status in 2001, to be confirmed and updated in 2002
* universal = for all blood components; not only for selected at risk patients

(5) Compatibility of different risk assessments

The risk assessments employed by Member States as well as by other countries consist of two separate parts:

1. Assessment of the risk whether or not TSE infectivity can be transmitted by blood (via blood components and plasma derivatives) of individuals infected with the vCJD agent.
2. Assessment of the risk that a blood donation was donated from a vCJD infected individual in populations of different geographical areas (prevalence of vCJD infected blood donors).

ad 1.

The assessment is generally based on an evaluation of

- the distribution of PrPsc and of infectivity in peripheral tissues of vCJD patients;
- the results of blood transmission studies as well as studies on TSE infectivity in blood in a number of animal model systems;
- the distribution of infectivity between plasma and the cellular components in blood of animal models.

Only a limited number of data addressing these issues are published. These data are taken into account in the assessments of different Member States as well as of other countries leading to compatible conclusions. It is generally assumed in all assessments that the possibility of the presence of vCJD infectivity in blood of infected individuals should be acknowledged and that most of this infectivity, if it is present, will be associated with the cellular components rather than with the fluid part of blood although the latter will not be free of infectivity. A more extensive discussion of this issue is found in chapter 2 of this report.

ad 2.

The assessment of the prevalence of vCJD infected donors which might be directly correlated with the prevalence of vCJD infected individuals in a given population is less uniform. The basis for the assumptions is usually less well elaborated, but in general, the following parameters are considered:

- the number of probable and confirmed vCJD cases in a given country,
- the number of expected vCJD cases in a given country,
- the extent of the BSE epizootic in the cattle herd of a given country.

While the number of probable and confirmed vCJD cases are published irrefutable figures, the methods to estimate the upcoming number of vCJD cases range from sophisticated statistical methods (Ghani et al., 2000, Valleron et al., 2001) to simple extrapolations of UK data (AE6, AE8). All results are associated with huge uncertainties.

So far, 115 vCJD cases have been recognised in UK (as of Dec. 3, 2001, including the two cases, one in Ireland and one in Hong Kong, where the patients spent a considerable part of their life in UK) and 5 in France (as of Dec. 1, 2001). Those data lead the FDA implicitly to assign European countries to three risk categories: UK to the highest risk category, France to an intermediate risk category and the rest of Europe to a lower risk category.

The rest of Europe is taken as being at risk for the appearance of vCJD cases because of the BSE cases found in most European countries (including GBR II countries like Finland and Austria who announced their first BSE cases on 7 December 2001). Therefore, the extent of the BSE epizootic in the different national herds could also be taken as a parameter for the categorisation of countries. However, due to differences in counting (only clinical cases, clinical cases and cases with positive rapid tests, differences in test populations (over 30 months vs. over 24 months)) the figures as they are published by OIE are hardly comparable between points in time and countries.

In its Opinion on the Human Exposure Risk (HER) via Food with Respect to BSE (10 December 1999) the SSC clearly stated that the human exposure risk (which would be paralleled by the prevalence of vCJD infected cases) does not only depend on the likelihood that an animal infected with BSE enters the human food chain (extent of the BSE epizootic) but also on other factors (see next chapter).

As a basis for risk management it is not sufficient to evaluate the risk in foreign countries. It is also necessary to evaluate the risk in the country itself in order to be in a position to estimate the risk reduction by possible measures (e.g. the exclusion from blood donation of individuals who stayed for a specified period of time in a country with a presumed higher risk). Of course, the criteria used to evaluate the risk in a foreign country should be the same as those used for the evaluation of the country itself.

In summary, the different risk assessment methods employed by Member States to assess the prevalence of vCJD infected blood donors are not compatible. The major problem is the assignment, between Member States, of the relative risk for a blood donor to be infected with vCJD.

In order to allow consistent risk management decisions it is advisable to draw a European risk area map specific to vCJD.

(6) Transmission routes of vCJD: Risk factors in regard to blood donors

According to our current understanding, there are two in principle different possible ways to contract vCJD, exposure to bovine material carrying BSE infectivity and exposure to human derived material carrying vCJD infectivity. The former route is called primary transmission, the latter secondary transmission.

A number of measures have been implemented to interrupt primary transmissions, notably the removal of specified risk material at slaughtering and the exclusion of animals scoring positive in a BSE rapid test. Since October 2000 specified risk material is removed in all Member States. Animals over 30 months of age are either excluded from slaughtering at all or are tested by a rapid BSE test with animals entering the human food chain if they score negative in the test. Provided all these measures are fully implemented the risk of acquiring vCJD through exposure to BSE infected bovine material is approaching zero. Unless BSE is found in sheep in non-experimental conditions new infections via food are unlikely. However, before the implementation of adequate measures an unknown number of individuals have certainly been infected in UK, in France and, most probably, in some other Member States.

The number of individuals infected by food will hopefully not increase, but they form the “source” of secondary transmissions via substances of human origin, most probably by blood donations and organs for transplantations. Also surgical instruments having had contact to infected tissues may cause secondary transmissions. Individuals infected by these routes will themselves become a source for secondary transmissions. Therefore, if such secondary transmissions occur at all (and, at the time being, this possibility cannot be excluded) these routes will sustain the occurrence of vCJD cases even if all cases infected by food have been identified.

Early in the second round of vCJD transmissions, the risk for secondary transmissions depends merely on the prevalence of individuals infected by primary transmissions. If there are significant differences between different areas in the prevalence of those individuals it might be sensible to prevent, in lower risk areas, the use of human material originating from higher risk areas. This consideration may justify attempts to evaluate or even quantify the extent of the primary vCJD epidemic in different Member States.

In its Opinion on the Human Exposure Risk (HER) via Food with Respect to BSE (10 December 1999) the SSC lists the factors on which the HER will depend in any country and at any time point:

1. the likelihood that an animal infected with BSE enters the human food chain;
2. the amount and distribution of infectivity in that animal;

3. the ways in which the various tissues that could contain infectivity are used in the food chain; and
4. the marketing of infected food produced in other countries.

The SSC refused to estimate the size of the human exposure, predominantly because one of the most important parameters, namely the minimal infectious dose for humans, was, and still is, not known. However, in the context of this opinion, there is no need to estimate the absolute risk rather the relative risk between different countries. As it can be assumed that the minimal infectious dose is similar in all areas knowledge of this parameter is not necessary for the evaluation of relative risks. For the same reason, it is also not necessary to know the amount and distribution of infectivity in a given animal (factor 2 in the list above) during the incubation period assuming it is the same for all cattle breeds.

Another factor which seems to be difficult to specify is the use of various infectivity containing tissues in the food chain. It seems to be important to know whether there are profound differences in the use of highly infectious tissues like brain and spinal cord. However, according to the ancient attitude to use as much as possible from a slaughtered animal as well as to the economic advantages in using cheap animal materials one may assume that those tissues have been taken for the preparation of food in all countries (e.g. pâtés, sausages) to a similar extent. In a first approach, the regional differences, therefore, may be neglected.

The remaining factors are the extent of the BSE epizootic in a given country as well as the use of bovine derived food imported from countries with a higher BSE risk. In the French reports the assumption was made that the French vCJD cases originated from exposure to bovine food imported from UK. During 1980 to 1996 the percentage of bovine material from UK used in France was estimated from import data to be 5 percent. At the time of assessment, the number of French vCJD cases was also 5 percent of the number of vCJD cases in UK (with the same population size in both countries). Until now, the ratio between French and UK vCJD cases remains approximately constant (5/115 = 4,35%).

The extent of the BSE epizootic in different countries may be best determined by the well controlled use of the rapid BSE test. The collected figures may not only allow to estimate the BSE epizootic in a given country at the time the test is used, but also, with the accumulation of data over time, to calculate back the time course of the epizootic.

Data on the trade of bovine material are available. Their interpretation is hampered by the fact that the collected data do not directly show the shipments of risk material, instead those shipments have to be extrapolated from the categories of goods which have been used in the collection of import and export data and which may not be identical in different countries. The task might be simplified

by the fact that only the trade between countries with significantly different BSE epizootics has to be analysed.

To determine the relative extent (relative to the UK) of the primary vCJD epidemic in the different Member States based on an analysis of the BSE epizootic in that Member State and its trade of bovine material with countries with a significantly greater extent of the BSE epizootic is a valid possibility which allows to estimate the relative numbers of individuals who are infected with the vCJD agent.

Another possibility which should also be explored is to estimate the relative number of infected people from the number of clinically ill people. Such an approach did not work for the estimation of the geographical BSE risk most probably due to the inadequate surveillance of BSE cases. In contrast, the surveillance of CJD as well as vCJD cases in the Member States seems to be well established (van Duijn et al., 1998). Therefore, it might be possible to categorise Member States according to their endogenous vCJD cases.

For both approaches, it has to be taken into consideration that the susceptibility to the vCJD agent is genetically determined. So far, all vCJD patients are homozygous for the codon that codes for methionine in position 129 of the prion protein gene, while the frequency of this homozygocity in the European population is about 40%. Therefore, the relative risk of two populations may also depend on the distribution of the different genotypes for codon 129. Although some data have been collected in control groups for CJD and vCJD patients (Alperovitch 1999) a systematic study of the healthy population in all Member States has not been published.

(7) Processing procedures for blood and blood components and vCJD transmission risks

(a) Leucoreduction of blood components

With the most sensitive tests available to date, no infectivity was found in the blood of vCJD patients (Wadsworth et al., 2001). The detection of such an infectivity in the blood of vCJD patients would be a prerequisite for experiments to find out, whether, and if yes how tightly, it would be bound to or associated with cells.
However, there has been some evidence in rodent systems that most of the infectivity in whole blood is associated with the buffy coat containing the bulk of white blood cells (leucocytes) and platelets. An estimate by P. Brown, used in the French and German assessments, is about 90 %. In normal blood, the physiological cellular PrP^c is associated mainly with platelets. However, most of the buffy coat infectivity in experimental rodent systems was found in the white cells (Brown and Cervenáková, 2000). Therefore, leucoreduction, i.e. a reduction of leucocytes by 3 to 4 logs, was proposed as a precaution against vCJD transmission by blood transfusion.

Leucoreduction is a technically demanding and expensive process, and is usually carried out by means of special filters, which do not work by their pore size, but by specific adherence of white cells to the surface of the filter material. In case of collection of blood components or plasma by apheresis, leucoreduction may be achieved also by special programming of the software of the apheresis machines, without need for filters. Leucoreduction has already been introduced in many member states (table 1), and it had been established in transfusion practice for certain indications for some time before. Several commercial systems are available for blood components, and their use has been validated in numerous blood establishments. It could be shown (Prowse et al., 1999) that the different types of commercial leucoreduction filters are effective in reducing the number of residual white cells below 10^6 per unit, and no evidence of overt cell fragmentation was found.
So far, there is no evidence, to indicate which conditions of leucoreduction would be most effective for the purpose of preventing vCJD transmission.

Leucoreduction has several documented areas of benefit. It reduces the frequency of non-haemolytic febrile transfusion reactions (NHFTR), the alloimmunisation of transfusion recipients, and thus the incidence of platelet refractoriness of multi-transfused patients, and the transmission of intracellular pathogens (micro-organisms which may be harboured by white cells), such as

cytomegalovirus (CMV). These effects were an important argument for mandatory introduction of leucoreduction for cellular blood components, e.g. in Germany, and such benefits were the main reason to recommend universal leucoreduction (Nightingale, 2001) in the USA.
Leucoreduction has been in place for quite some time in order to provide leucocyte-poor blood components for special indications. Certain patients are particularly susceptible to side effects such as NHFTR, and an alloimmunisation should be avoided in patients needing multiple transfusions. Some patients, particularly those who are immunocompromised, are at risk for a severe course of CMV infections. In general, leucocytes are considered as an "impurity" of blood components for transfusion (with exception of granulocyte or stem cell concentrates), and their reduction brings about a benefit at least for a considerable subset of patients.

The mandatory introduction of universal leucoreduction is currently considered or has been adopted by most member states (table 1), but in a variable way; by some member states only for cellular blood components, or even only for platelets. In several member states arguments other than a precaution against vCJD, i.e. benefits as outlined above, were relevant. Advantages and disadvantages of the introduction of leucoreduction are summarised in table 2.
The existing scientific data are so far not conclusive, as summarised e.g. in the assessments of France and Germany (AE 6, AE 8). But it appears likely that the (still hypothetical and not quantifiable) risk of vCJD transmission by blood components for transfusion would be reduced, albeit probably not eliminated. Since in animal models, the infectious dose was inversely correlated with the incubation time (i.e. the lower the infectious dose, the longer the incubation time), a considerable reduction of vCJD infectivity in a blood component would potentially prolong the incubation time, eventually beyond the natural life span of the recipient.
In summary, the following judgement expressed in the SCMPMD Opinion on Update of the Opinion Given by the Scientific Committee on Medicinal Products and Medical Devices on the Risk Quantification for CJD Transmission Via Substances of Human Origin (16 February 2000) is still valid:
"There are still many unknowns, but assuming that, in contrast to the classical forms of CJD, infectivity is present in peripheral blood of vCJD cases (as extrapolated from models of small laboratory animals with a peripheral distribution of the pathological form of the prion protein similar to that in vCJD patients) and assuming that this infectivity is predominantly associated with white blood cells (again inferred from models of small laboratory animals) it might be a precautionary step to remove white blood cells as completely as possible in order to interrupt the potential, but not proven transmission of vCJD by transfusion of blood components. A

recommendation for the general use of leucofiltration would be in line with the belief that many if not all transfusion recipients would benefit from the removal of white blood cells for other reasons. However, with respect to vCJD there are a number of caveats: lack of experimental proof (in animal model system) of the effect of leucoreduction on TSE infectivity, lack of knowledge on which cell types carry the infectivity and to which degree they can be removed, unknown effects of different filter types, and lack of validation."

Table 2. Arguments in favour and against universal leucoreduction of blood components.

Pros	**Cons**
Efficacy to reduce white cells to $<10^6$ per blood component validated	Technically demanding, complex sets of bags, filters and tubing, risk of leakage (bacterial contamination); extensive training of staff required
Technology developed, several commercial systems available	High cost, depending on domestic markets ca. 20 to 30 Euro
Accepted benefits: reduction of febrile reactions (NHFTR), alloimmunisation, cytomegalovirus (CMV) infection	Clinical benefits proven only for a subset of patients
Precaution against vCJD transmission	Presence of vCJD infectivity in blood hypothetical; efficacy in vCJD not proven

(b) Leucoreduction or filtration of plasma for fractionation

The capacity of leucoreduction of plasma for fractionation to reduce the risk of vCJD transmission appears to be more uncertain than in the case of blood components, as e.g. pointed out in the report on the EMEA expert workshop on human TSEs and plasma-derived medicinal products (compare AE 5). In rodent experimental systems, infectivity was found after leucoreduction of platelet poor plasma, and this infectivity did not appear to be associated with cells or cell debris (Brown et al., 1999): Infectivity in platelet poor plasma of intracerebrally inoculated mice after leucoreduction was found only marginally reduced (symptomatic mice), or even significantly increased (asymptomatic mice) after leucoreduction. This raised concerns that infectivity might even be "liberated" by this process. The relevance of these experiments to the prevention of vCJD transmission in humans is not known. So far, there is at least no specific evidence that

leucoreduction of human blood would lead to significant fragmentation of cells (Prowse et al., 1999) or liberation of prions (Brown et al., 1999).

Also the use of membrane filtration was proposed, in order to remove cells and cell-derived particles from plasma for fractionation. The efficacy of this procedure in reducing vCJD infectivity in plasma, and how it compares to the use of leucoreduction filters, remains to be demonstrated. Moreover, there is some uncertainty on the impact of membrane filtration on the integrity of labile plasma proteins. These questions should be further investigated, before specific filtration of plasma as a starting material should be recommended.

The question, whether centrifugation should be included prior to filtration and how many times (once/twice?), is again connected to the general question, whether vCJD infectivity, if present in plasma for fractionation, would be associated with (cell-derived) particles. So far, only data from rodent experiments are available, which suggest that there is cell free infectivity. In one experiment, such cell-free infectivity could not be precipitated by high speed centrifugation (Brown et al., 1999). Centrifugation would at least not be detrimental, since it should not impact on plasma protein integrity. However, the particular conditions to be used and the value of centrifugation alone or in combination with filtration havenot been established.

Taken together, there is no compelling scientific evidence to date for the introduction of leucoreduction of plasma for fractionation, or other methods aiming at removal of cells and debris, as a precaution against vCJD transmission. This question should be further explored by suitable experiments.

(c) Nanofiltration of plasma derivatives

The technology of nanofiltration has been developed for the elimination of very small infectious particles, such as parvovirus B19. There have been claims that nanofiltration would remove TSE infectivity from plasma derived products. This claim is due to experiments using brain homogenates or prion fibrils as spiking material for filters with a pore size below 35 nm. However, the effect was in some experiments almost abolished by the addition of detergent (sarcosyl), which would dis-aggregate prion fibrils (Tateishi et al., 1998). This suggests that, if the infectivity would be present in smaller aggregates or even single molecules, it might pass nanofiltration membranes (for further discussion, compare appendix D of the German assessment, AE 8). However, as recently summarised in a SSC assessment (Updated report and scientific opinion on the safety of hydrolysed

proteins produced from bovine hides. 25-26 May 2000), there are some data obtained with several methods suggesting the size of infective prion particles to lie between 15 and 40 nm.

Thus, nanofiltration seems to be a promising approach, but its general feasibility needs to be further explored. So far, this measure has been adapted by a French manufacturer. Its new factor VIII product is on the market since January 2001. Since then, 300-350 patients either switching to this product or treated with this product de novo have received it on a daily basis. There has been neither breakthrough of inhibitors, or allergic/anaphylactic reactions, nor lack of effect or need to increase the dosage. No report were made to either the French National Pharmacovigilance system, the Company's pharmacovigilance nor the French haemophiliac patients follow-up (Réseau FranceCoag). Therefore, based on this early experience (11 months follow-up) for a 15 nm nanofiltrated factor VIII product, there seems to be no signal towards a detrimental effect of the nanofiltration on the molecular integrity for Factor VIII. It is noteworthy that the same conclusion can be drawn for Factor IX, for which the nanofiltration step was introduced in 1999.

(8) Acknowledgement

This opinion of the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) of the European Commission is based on the report of a Working Group consisting of members of the SCMPMD, members of the BSE/TSE ad hoc working group and external experts. The membership of this Working Group was as follows:

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(9) Appendix: Selected official documents (assessments, guidance)

: identification for citation of the documents in the text; AE means assessment by European country/institution, AO means assessment by overseas country/institution. Within the two groups, the countries/organisations are listed in alphabetic order.

#	Country/ Institution	Document (Identification)	Date	Remarks
		Europe:		
AE 1	European Commission – SCMPMD	Opinion on the Risk Quantification for CJD Transmission Via Substances of Human Origin (XXIV/SCMPMD/98.048 Final)	adopted by SCMPMD on 21 October 1998	Scientific assessment to answer questions asked by the Commission
AE 2	European Commission – SCMPMD	Opinion on Update of the Opinion Given by the Scientific Committee on Medicinal Products and Medical Devices on the Risk Quantification for CJD Transmission Via Substances of Human Origin (SANCO/SCMPMD/2000/0006 Final)	adopted by SCMPMD on 16 February 2000	Scientific assessment to answer questions asked by the Commission
AE 3	European Commission – SSC	Opinion on the Implications of the Houston et al. Paper in The Lancet of 16 September 2000 on the Transmission of BSE by Blood Transfusion in Sheep.	adopted by SSC at its meeting of 26-27 October 2000	Discussion with one of the authors
AE 4	EMEA	CPMP position statement on new variant CJD and plasma derived medicinal products	25 February 1998	Statement on recall policy and use of albumin as excipient
AE 5	EMEA	Expert Workshop on Human TSEs and Plasma-Derived Medicinal Products – Report Meeting on 15-16 May 2000 (CPMP/BWP/1244/00)	27 July 2000	Presentations with original work and reviews
AE 6	**France** (Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps**))**	Report – Revision of Measures to Minimising the Risk of TSE Transmission Via Blood Products	February 2000	assessment of the impact of risk of stay in UK versus intrinsic risk in France
AE 7	**France** (Agence Française de Sécurité Sanitaire	Risk Analysis of New Variant Creutzfeldt-Jakob Disease Transmission by Blood and Blood Products – Recommendations	11 December 2000	Comprehensive risk analysis, assessment of various products, conclusion that further use of domestic

	des Produits de Santé (Afssaps))			plasma is justified
AE 8	**Germany** (Paul-Ehrlich-Institut, Robert Koch-Institut)	Bericht der Arbeitsgruppe "Gesamtstrategie Blutversorgung angesichts vCJK"	August 2001	comprehensive risk assessment and strategy for safe blood supply
AE 9	**United Kingdom** (Department of Health)	Guidance on the Microbiological Safety of Human Organs, Tissues and Cells Used in Transplantation (Advisory Committee on the Microbiological Safety of Blood and Tissues for Transplantation MSBT)	August 2000	
AE 10	**United Kingdom** (Department of Health)	Risk Assessment for Transmission of vCJD Via Surgical Instruments: A Modelling Approach and Numerical Scenarios – Economics and Operational Research Division (EOR4)	February 2001	very detailed analysis of risks and possible interventions; assessment of scenarios
AE 11	**United Kingdom** (Department of Health)	CJD Incidents Panel – Management of Possible Exposure to CJD through Medical Procedures – A Consultation Paper	October 2001	risk assessment of iatrogenic transmission and recommendations for dealing with incidents

		Overseas Countries:		
AO 1	**Australia**	Australian Health Ministers Agree to Take Precautionary Action over Possible Link between vCJD and Blood	21 September 2000	teleconference after Lancet paper; follow measures of USA, NZ and Canada
AO 2	**Canada** (Bayer Advisory Council on Bioethics)	Creutzfeldt-Jakob Disease, Blood and Blood Products: A Bioethics Framework (Working Paper)	15 October 1998	first recommendation to exclude residents with sign. incidence of vCJD
AO 3	**Canada** (Health Canada)	BSE and vCJD Risk to Canadians; slides available on internet: http://www.hc-sc.gc.ca/sab-ccs/sep2000_BSE_vCJD_slide11_e.html	24 July 2001	details of risk assessment
AO 4	**Canada** (Health Canada)	Donor Exclusion to Address Theoretical Risk of Transmission of Variant Creutzfeldt-Jakob Disease (vCJD) through the Blood Supply from United Kingdom, France & Western Europe (Directive)	30 August 2001	additional reduction of theoretical risk by 16 to 18%, on top of 72% reduction by 1999 and 2000 directives

AO 5	**New Zealand** (Ministry of Health)	CJD Blood Recall Policy in line Internationally	26 May 1999	Follow others, to recall because vCJD, but not CJD
AO 6	**New Zealand** (Ministry of Health and the New Zealand Blood Service)	Measures in Place to Protect Blood Supplies	18 November 1999	follow USA and Canada Exclusion of stay in UK > 6 months; estimate of donor loss 10%, 1 Mio. $ recruitment campaign
AO 7	**New Zealand** (Ministry of Health)	Measure to Provide Ongoing Management of the Human Health Risks Associated with Imported Food Products Potentially Containing the Bovine Spongiform Encephalopathy Agent	October 2001	Detailed analysis of BSE risk of imported food
AO 8	**USA** (FDA/CBER)	Revised Precautionary Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products	11 December 1996	Quarantine products from donors developing classical CJD
AO 9	**USA** (FDA/CBER)	Change to the Guidance Entitled "Revised Precautionary Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"	09/08/98	Restricting previous guidance to products of vCJD donors
AO 10	**USA** (FDA/CBER)	Guidance for Industry – Revised Precautionary Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products	November 1999	“original” recommen-dation to exclude stay in UK > 6 months; following TSEAC advice on the basis of donor survey and modelling calculations
AO 11	**USA** (FDA/CBER)	Pre-Storage Leucocyte Reduction of Whole Blood and Blood Components Intended for Transfusion (Draft – not for Implementation)	January 2001	Considering as benefits reduced immunisation and CMV infection; does not consider vCJD precaution
AO 12	**USA** (FDA/CBER)	Guidance for Industry – Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products – Draft Guidance	August 2001	Tightening of previous recommendations: to defer stay in UK > 3 months, include France and other European countries
AO 13	**USA** (American Red Cross)	American Red Cross Implements New Deferral Policy	12 October 2001	Stricter recommendation than FDA

(10) References

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Abbreviations

AFSSAPS	Agence Française de Sécurité Sanitaire des Produits de Santé
BSE	Bovine spongiform encephalopathy
BWP	Biotech working partry
CJD	Creutzfeldt-Jakob-Disease
CNS	Central nervous system
CPMP	Committee for Proprietary Medicinal Products
CSF	Cerebrospinal fluid
EDRF	Erythroid differentiation-related factor
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Agency
FDC	Follicular dendritic cell
FFP	Fresh frozen plasma
GBR	Geographical BSE risk
GSS	Gerstmann-Sträussler-Scheinker-Syndrome
HER	Human exposure risk
IU	Infektious Unit
iv	intravenous
ic	intracerebral
MRI	Magnetic resonance imaging
OIE	Office International des Epizooties
PrP	Prion protein
PrPc	Cellular form of the prion protein
PrPsc	Pathogenic form of the prion protein (sc=Scrapie)
sCJD	Sporadic Creutzfeldt-Jakob-Disease
SCMPMD	Scientific Committee on Medicinal Products and Medical Devices
SIFT	Scanning for intensely fluorescent targets
SSC	Scientific Steering Committee
TSE	Transmissible spongiform encephalopathy
vCJK	Variant Creutzfeldt-Jakob-Disease