EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

OPINION ON:

THE IMPLICATIONS OF THE RECENT PAPERS ON TRANSMISSION OF BSE BY BLOOD TRANSFUSION IN SHEEP (HOUSTON *ET AL*, 2000; HUNTER *ET AL*, 2002)

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE AT ITS MEETING OF 12-13 SEPTEMBER 2002

[REVISION AND UPDATE OF THE SSC OPINION OF 26-27 OCTOBER 2000 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. (THE LANCET, VOL. 356, PP 999-1000; 955-956; 1013)]

OPINION

On 16 September 2000, Houston *et al*[1] reported in *The Lancet* on the experimental sheep-to-sheep transmission of the BSE agent by blood transfusion. (The Lancet, 2000, Vol. 356, pp 999-1000). The Scientific Steering Committee was subsequently invited to:

1. Evaluate the preliminary results presented in *The Lancet* paper and assess whether it contains important new scientific evidence and/or information that are relevant for public health.
2. Consider the relevance of these results to available scientific opinions on the safety of human and animal blood and blood products.

At its meeting of 26-27 October 2000, the SSC adopted the opinion the Implications of the Houston *et al* paper.

On September 2002, Hunter *et al*[2] reported in The Journal of General Virology on the follow up of the previous experiment and the same protocol using another TSE strain (scrapie). In the light of this new publication, the TSE/BSE *ad hoc* Group at its meetings of 25 July and 5 September 2002 re-addressed the above mandate. On the basis of the attached summary account of these meetings the SSC concluded as follows.

BSE agent is now clearly identified in sheep transfused with blood from BSE infected Cheviot sheep. This transmission via blood is also observed in natural scrapie. The data in this experiment show that the exchange by transfusion of (400 ml of) whole blood taken during the incubation period of a sheep infected with the BSE agent or scrapie agent can transmit TSE to a healthy sheep. This ovine model adds to data obtained in mouse and hamster models of scrapie and human TSE[3].

These result supports already published European Commission (SANCO) SSC, SC MPMD, and EMEA opinions and recommendations on blood safety. Therefore, although the transmission of infectivity through blood in vCJD urgently needs further study, the data presented in this paper neither justify nor add arguments for the introduction of new methods or approaches to the assessment of blood safety.

Although this information does not change the basis of risk assessment, it does reinforce the substance of previous opinions by the scientific committees regarding the safety of human and ruminant blood. It also confirms previous opinions that sheep blood from susceptible genotypes is likely to be a vehicle for scrapie transmission.

Finally, the Hunter *et al* (2002) and Houston *et al* (2000) findings should be taken into account for safe sourcing of small ruminant materials should BSE in sheep become probable or evident under field conditions[4].

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1 **Houston, F., Foster, J.D., Chong, A., Hunter, N., Bostock, C.J., 2000.** Transmission of BSE by blood transfusion in sheep. Research letter. The Lancet, **356:** 999-1000.

2 **Hunter,N., Foster, J., Chong,A., McCutcheon, S., Parnham, D., Eaton, S., MacKenzie, C., and Houston, F., 2002.** Transmission of prion diseases by blood transfusion. Journal of General Virology (2002), 83: Published ahead of print (16 July 2002).

3 Preliminary data indicate that experiments in mice (Cervenakova/Brown) and squirrel monkeys (Brown) all show transmissibility in BSE/vCJD models to be no greater than that of non BSE/vCJD models.

4 See also the SSC opinion of 12-13 September 2002, complementing the SSC opinion of 4-5 April 2002 on safe sourcing of small ruminant materials.

SUMMARY ACCOUNT OF TSE/BSE *AD HOC* GROUP MEETINGS ON THE IMPLICATIONS OF:

HOUSTON *ET AL* (2000) AND HUNTER *ET AL* (2002) ON THE TRANSMISSION OF BSE BY BLOOD TRANSFUSION IN SHEEP.

I. BACKGROUND AND MANDATE

On 16 September 2000, Houston *et al* reported in *The Lancet* on the experimental sheep-to-sheep transmission of the BSE agent by blood transfusion. (The Lancet, 2000, Vol. 356, pp 999-1000). The European Commission invited the Scientific Steering Committee to:

1. Evaluate the preliminary results presented in *The Lancet* paper and assess whether it contains important new scientific evidence and/or information that are relevant for public health.
2. Consider the relevance of these results to available scientific opinions on the safety of human and animal blood and blood products, including:
   - Opinion on the Risk quantification for CJD transmission via substances of human origin Adopted on 21 October 1998 by the Scientific Committee on Medicinal Products and Medical Devices
   - Update of the Opinion on The Risk Quantification For CJD Transmission Via Substances of Human Origin, adopted On 16 February 2000 by the Scientific Committee on Medicinal Products and Medical Devices.
   - Opinion on Quality and Safety of Blood, adopted on 16 February 2000 by the Scientific Committee on Medicinal Products and Medical Devices.
   - Opinion on The Safety of ruminant blood with respect to TSE risks, adopted by the Scientific Steering Committee at its meeting of 13-14 April 2000.
   - The Opinion on specified risk materials of small ruminants, adopted on 13-14 April 2000 by the Scientific Steering Committee.
   - The report of the EMEA Expert Workshop *15-16 May 2000* on human TSEs and plasma-derived medicinal products.

The SSC established a multi-disciplinary working group composed as indicated in the section IV "Acknowledgements". The Working Group (WG) met on 11 October 2000. In addition to the above mandate, it also addressed the question "W*hat risk consumers have of acquiring an infection (vCJD), resulting from the consumption of bovine meat products, taking into account the fact that sheep blood has been shown to be infectious during the incubation period of BSE."*

At its meeting of 26-27 October 2000, the SSC adopted the opinion on the Implications of the Houston *et al* paper.

On September 2002, Hunter *et al*[5] reported in The Journal of General Virology on the follow up of the previous experiment and the same protocol using another TSE

[5] **Hunter,N., Foster, J., Chong,A., McCutcheon, S., Parnham, D., Eaton, S., MacKenzie, C., and Houston, F., 2002.** Transmission of prion diseases by blood transfusion. Journal of General Virology (2002), 83: Published ahead of print (16 July 2002).

strain (scrapie). In the light of this new publication, the TSE/BSE *ad hoc* Group at its meetings of 25 July and 5 September 2002 re-addressed the above mandate. The amended report hereafter, prepared under the rapporteurship of Prof.Dr.D.Dormont, was adopted.

II. REPORT

II.1. On the results presented in Houston *et al* (2000) and Hunter *et al (*2002)

Summary of Houston *et al* (2000): 400 ml of whole blood was taken from each of 19 sheep thus far that had each been fed 5 grams of BSE-affected cattle brain. This blood was transfused into healthy BSE susceptible sheep. One recipient sheep transfused 318 days after the oral challenge of the donor sheep and 311 days before the onset of a BSE like illness in the donor sheep showed signs of a BSE-like illness 610 days later. The donor sheep itself developed disease 629 days after oral challenge. Tests on the recipient sheep that developed a BSE type illness, revealed widespread deposition of modified prion ("PrP^{sc} ") throughout the brain and the peripheral nerves. All the other sheep that received blood were still healthy at the time of the redaction of the paper, but in all but one case the observation time is shorter than 610 days. In the other case with a longer observation period, blood was transfused after one third of the incubation time in the donor animal.

Summary of Hunter *et al* (2002): 18 Cheviot sheep, imported from New Zealand, were exposed to BSE agent (17 orally: 5g of cattle brain homogenate, 1 inoculated by ic route with 0.05 g of cattle brain homogenate). 5 of the 17 orally exposed animals developed a clinical BSE at 559-761 dpi, 9 were killed for pathogenesis investigation purpose and 3 are alive 1500 dpi. The ic inoculated animal had BSE-related symptoms at day 671 pi. Blood was taken during the asymptomatic phase, and either whole blood or buffy coat were injected by iv route to 24 recipients (ARQ/ARQ Cheviot sheep). In parallel, 11 sheep of VRQ/VRQ (10) or VRQ/ARQ (1) genotype belonging to a flock with high incidence of scrapie were bled and their blood transfused into 21 recipients (VRQ/VRQ). Negative controls (transfusion of blood from healthy donors) and positive controls (infection of 10 sheep with brain homogenate from BSE affected cattle). Results were as follows: 1) Two of the 24 BSE-transfused sheep had clinical symptoms of TSE: one 610 days after transfusion (previous report from Houston et al) and one 538 days post transfusion with blood obtained from a sheep at the preclinical stage. The other 19 sheep remain healthy (68-1243 days post-transfusion). It has to be note that among the 4 animals that received blood or buffy coat from clinically affected sheep, none developed the disease. 2) Among the 21 sheep transfused with blood from scrapie infected animals (761 to 1080 days of age), 4 developed the disease between 614 and 737 days post inoculation. Buffy coat transmitted the disease when obtained from an animal at the full-blown scrapie stage. 3) Electrophoretic pattern of PrP-res was of BSE type in animals infected with blood from BSE infected animals, and of scrapie type in animals transfused with blood from scrapie affected sheep.4) PrPsc immunohistochemistry was clearly different in BSE infected animals and in scrapie affected sheep; variations could also be seen depending upon route of inoculation. 5) PrP-res immunohistochemistry signals were weaker in transfused and ic inoculated animals than in animals exposed to BSE by oral route.

The data published by Hunter *et al* are in line with the previous report; they show that a high volume blood transfusion from sheep to sheep can transmit a BSE or scrapie illness within the same species and that infectivity can be transmitted from

blood taken during the asymptomatic incubation period of the disease of the donor sheep. These data support the comment to the Houston paper, written by P.Brown (in: The Lancet, 2000, Vol. 356, pp 955-956) who considers these observations to be consistent with previous reports on blood borne infectivity in experimentally infected rodents.

The TSE/BSE *ad hoc* Group considered that the available data on infectivity in the blood of animals infected with a TSE agent show no consistently reproducible patterns and little is known about inoculum size or infectivity levels. Nevertheless, these results of successful transmission within a single species but with 2 different TSE strains should therefore be considered as additional justification for assessing the possible impact of the transmissibility of TSE agents via blood.

Although there were some doubts after analysis of the Houston paper, the Working Group members are now convinced that the agent causing a BSE like illness in the recipient sheep is the same BSE agent to which the donor sheep had been exposed orally. Although strain typing is not described in the Hunter paper, Western Blot results applied both to the original brain material, and to brain material from the donor and recipient sheep are convincing.

The TSE/BSE *ad hoc* Group considered that the findings are (i) the first published evidence for intra-species transmission of BSE agent by blood-transfusion and (ii) in line with what is currently known on TSE infectivity in blood[6] (see Annex 1; courtesy P.Brown).

The TSE/BSE *ad hoc* Group noted that studies published since October 1988 indicate that in rodents, blood is regularly infectious throughout the entire incubation period and the clinical phases of disease. It noted that the Hunter et al (2002) finding now includes blood taken in the late stage of incubation, and to the mid-incubation period. Nevertheless, the experiment does not yet tell about the percentage of BSE-infected donor sheep whose 400ml blood taken at mid-incubation transmit: i) to single recipient and ii) to the number out of 10 recipients. It also does not answer what would be the effect on successful transmission rate if transfusion volume were 200ml or 20ml versus 400ml, or if transfusion is carried out at 100 or 500 days versus 300 days post challenge.

II.2. On the possibility to use the "BSE agent in sheep" model as a proxy model for vCJD in humans or BSE in cattle.

The TSE/BSE *ad hoc* Group agreed that there were enough similarities between the pathogenesis of TSE in sheep and vCJD in humans to consider the BSE agent in sheep model as a significant addition to the CJD and scrapie rodent models as a proxy for vCJD in humans. These similarities arise mainly in terms of distribution of tissue infectivity, in particular the involvement of the LRS. (It should be noted that murine models are quite close to human vCJD in terms of dissemination of the pathological protein in the lymphoid formations; the bovine model is less pertinent as only the terminal ileum is infectious both during the pre-clinical and the clinical phase).

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[6] The SC-MPMD Opinion on *The Risk quantification for CJD transmission via substances of human origin Adopted on 21 October 1998 by the Scientific Committee on Medicinal Products and Medical Devices* contains an extensive compilation of published papers and their results.

These findings were therefore considered supportive of the existing opinions on the safety of human blood. However, caution is needed when extrapolating from an experimental model (i.e., BSE agent in sheep) to field conditions (i.e., vCJD in humans) because the conditions are likely to be different (e.g., dose; exposure; controlled environment; genetics; infectivity in blood of host-adapted BSE;…)

The model would also be relevant for assessing the risks for humans and animals, should the BSE agent(s) in sheep be confirmed under field conditions. The TSE/BSE *ad hoc* Group recommended that SSC should finalise as soon as possible its forthcoming *Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions*.

The TSE *ad hoc* Group asked whether the finding of infectivity in the blood of sheep infected with the BSE agent had relevance to the risk of consumers acquiring infection (vCJD), from the consumption of *bovine* meat products. The TSE *ad hoc* Group considered that the finding of infectivity in the blood of sheep could not be extrapolated to BSE in cattle. Indeed, the most recent research results do not support the hypothesis that bovine blood or lean meat constitutes a risk for humans. However, assay limitations have always to be born in mind, e.g., the exposure of animals vs. humans, the exposure dose, probability of infectivity in blood used for challenge, …

- In its opinion of 28-29 October 1999 *On the scientific grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom,* **the** SSC addresses the issue of infectivity being present in (bovine) spleen and muscle.
- G.Wells (pers.comm., October 2000[7], in the SSC opinion of 26-27 October 2000 on the Implications of the Houston *et al* (2000) reported on cattle (four per group) inoculated intracerebrally with a ten percent dilution of pooled spleen or lymph nodes from BSE affected cattle[8] in which the cattle did not develop a TSE. If the survival data for these animals (4/4 in the lymph node pool group surviving to 85 months post inoculation and 3/4 in the spleen pool group surviving 74-86 months) is interpreted from the titration[9] of BSE affected brain material in cattle the values suggest that if infectivity were present in these tissues it is at a concentration of less than 0.1 cattle i.c. ID50/g of tissue.

II.3. On the relevancy of the result to available scientific opinions on the safety of human and animal blood and blood products.

The TSE ad hoc Group considered that existing scientific opinions already anticipated the risks resulting from the possible presence of low levels of TSE infectivity in blood.

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[7] The study has been reported on at the 2nd Cambridge Healthtech Institute Annual TSE Conference, Alexandria, Virginia, 3-4 October 2000

[8] The donor cattle were 5 field cases of BSE, the same five from which brain was taken for the comparison of a titration of BSE affected brain material in cattle and mice

[9] There are reservations about the interpretation of titres of non CNS tissues from titrations of CNS tissues but this can be used as an approximation. A paper on these results has yet to be prepared so a more detailed interpretation is not yet available.

In fact, the opinion on the *Safety of ruminant blood with respect to TSE risks*, adopted by the Scientific Steering Committee at its meeting of 13-14 April 2000 states, amongst others:

"(…) it is concluded that there could be a risk of the occasional presence of low levels of TSE infectivity in blood collected in abattoirs. Levels of infectivity which might represent a risk to animal or human health are not known. Control measures and/or decontamination standards might need to be developed to potentially TSE-infected blood collected in abattoirs. (…)

The collective data currently available from experimental transmission studies show that there is uncertainty on the presence of infectivity in the blood of TSE-infected ruminants. If PrP^{Sc} has been detected in the blood of clinically normal sheep from scrapie-susceptible flocks using a newly-developed and highly sensitive assay system, infectivity of femtomole amounts remain to be demonstrated.

The relationship between PrP^{Sc} and infectivity is not understood. The two do not always correlate; the presence of PrP^{Sc} does not necessarily imply presence of infectivity. Moreover, the methods for detecting PrP^{Sc} need to be validated for the pre-clinical stage. As far as ruminant blood is concerned, it is considered that the best approach to protect public health at present is to assume that it could contain low levels of infectivity. However, even if this is true, it becomes almost irrelevant compared with the level of contamination that could occur as a result of the methods of stunning used in abattoirs. (…)

Citation from the October 1998 SC-MPMD opinion:

"(…) An evaluation of all animal experiments [on all TSEs, not only vCJD - rapporteur] has to take into account, that the results depend on several factors: strain and sometimes breed of the host animal, type of TSE agent, its dose and its route of administration (Asher 1976). Therefore, an extrapolation from animal experiments onto the situation in men may be difficult. A conservative conclusion may be, that in animal models, if at all, a low infectivity in blood may be measurable in late stages of the incubation period and during clinical stages.

The Houston *et al* and Hunter *et al* finding does not bring a new scientific concept to the consideration of BSE in terms of risk assessment of vCJD and does not provide reason to change the scientific bases of the risk assessments carried out so far. However, the Hunter *et al* (2002) finding, does provide additional support to the opinions listed in Section I and strengthens the recommendations made in these opinions. A selection of these recommendations is as follows:

- From the SSC opinion on the safety of ruminant blood:

  "The SSC also recommends that intraspecies recycling of ruminant blood and blood products should be avoided in situations when a TSE risk exists."

- From the SC-MPMD 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:

  "(…) the SCMPMD cannot make a clear-cut recommendation with respect to the general introduction of leukodepletion, unless a number of studies has been performed to answer the open questions. The SCMPMD recommends supporting of such research. (…) In the meantime, it might be advisable to introduce leukofiltration as a precautionary step, as it is assumed that it will contribute to diminishing infectivity in blood. (…)

  (…) The SCMPMD repeats its recommendation to support efforts in the development of easily applicable screening tests for CJD/vCJD."

  (…) the SCMPMD recommends a careful consideration whether the exclusion of donors who stayed for a defined period of time in areas with increased risk of exposure to the BSE agent would provide an increase in safety balanced to its negative impact on

supply and donor population. In order to be able to make the optimal decision three sets of data have to be collected and evaluated:

1. The travel pattern of European donors which may differ between Member States.
2. The exposure to UK bovine derived material in food between 1980 and 1996 in different Member States.
3. The prevalence of HIV, HBV and HCV in first time donors in different Member States."

From the February 1998 CPMP recommendations:

"Given the lack of specific information on nvCJD, as a precautionary measure it would be prudent to withdraw batches of plasma-derived medicinal products from the market if a donor to a plasma pool is subsequently strongly suspected, by de reference centre, of having nvCJD.

Since a recall involving albumin used as excipient has the potential to cause major and widespread supply difficulties for essential products, manufacturers should avoid using, as an excipient , albumin derived from countries where a number of nvCJD cases have occurred."

- From the report of the EMEA Expert Workshop *15-16 May 2000* on human TSEs and plasma-derived medicinal products: See Annex 2.

III. PROPOSALS FOR FURTHER RESEARCH

The TSE *ad hoc* Group, in the course of its discussions, identified the following areas for further research (not exhaustive list):

- identification of the components of blood in sheep - or other species - that carry the source of infectivity. It should be researched whether prion infectivity is present in plasma, in the cellular part of blood, or in both. (This research has direct bearing on leucodepletion.. Also, cellular blood components [platelets, erythrocytes] have completely different therapeutic usage than plasma derived components, most of which (with the exception of fresh frozen plasma) are used for the manufacturing of stable blood products (immunoglobulin preparations and so on) and are actually easier to decontaminate from many agents.
- diagnostic testing;
- if feasible, challenge of sheep with blood from a vCJD victim and verification of infectivity in the recipient sheep blood;
- challenge of sheep with brain material from a vCJD victim and verification of infectivity in the blood of these sheep;
- broadening the Hunter *et al* (2002) protocol to:
  i) a larger range of volumes of transfused blood, for example from 20 to 400 ml.
  ii) infectivity titration of the donor sheep brain.
- species-barrier determination between cow and sheep.

As a first step, a comprehensive paper should be prepared, critically setting out all the experiments on transmissibility of blood a) in cattle, b) in sheep.

IV. SUMMARY CONCLUSIONS

The agent at the origin of TSE in this transmission is clearly now BSE in BSE-blood transfused sheep and scrapie in scrapie-blood transfused sheep. The data in this experiment are new to the extent that they show that the exchange by transfusion of (400 ml of) whole blood taken during the incubation period of a sheep infected with the BSE agent or with the scrapie agent can transmit TSE to a healthy sheep. This ovine model adds to data obtained in mouse and hamster models of scrapie and human TSE.

These results support already published European Commission (SANCO) SSC, SC MPMD, and EMEA opinions and recommendations on blood safety. Therefore, although the transmission of infectivity through blood in vCJD urgently needs further study, the data presented in this paper neither justify nor add arguments for the introduction of new methods or approaches to the assessment of blood safety.

Although this information does not change the basis of risk assessment, it does reinforce the substance of previous opinions by the scientific committees.

Finally, the TSE *ad hoc* Group members recommend that the possible implications of the Hunter et al results for mother-to-child transmission of vCJD are assessed and the ethical aspects of the question of informing recipients of vCJD blood should be addressed.

V. ACKNOWLEDGEMENTS

The following scientists contributed to the preparation of the present report:

The Working Group established in 2000: Prof.Dr.K.Jones (chairperson), Dr.E.Vanopdenbosch (rapporteur), Prof.Dr.S.Bird, Dr.P.Brown, Prof.Dr.H.Budka, Prof.Dr.D.Dormont, Dr R.Geertsma, Dr N.Hunter, Prof.Dr.H.Kretzchmar, Dr.F.Lantier, Prof.Dr.J.Löwer, Dr.J.Schlatter, Dr.G.Silvester, Dr D.Taylor, Prof.Dr.J.H.Trouvin, Prof.Van Aken, Prof.Dr.M.Vanbelle, Mrs.E.Voets, Prof.Dr.G.Wells, Prof.Dr.M.Wierup.

The TSE/BSE *ad hoc* Group, meeting on 25 July and 5 September 2002 under the rapporteurship of Prof.Dr.D.Dormont.

V. REFERENCES

Brown, P., 2000. BSE and transmission through blood. Commentary. The Lancet, **356:** 955-956.

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Hunter,N., Foster, J., Chong,A., McCutcheon, S., Parnham, D., Eaton, S., MacKenzie, C., and Houston, F., 2002. Transmission of prion diseases by blood transfusion. Journal of General Virology (2002), 83: Published ahead of print (16 July 2002).

Taylor, D.M., Fernie, K., Reichl, H.E., Somerville, R.A., 2000. Infectivity in blood of mice with a BSE-derived agent. Letter to the Editor. Journal of Hospital Infection, 46: 78-79.

Annex 1: Summary of research findings on TSE infectivity in blood.

Table 1. Attempts to detect infectivity in the blood of animals with TSE

Species Donor	Assay	Inoculum[1]	Route of Inoculation	Pos./total donors	Reference
Scrapie (natural)					
Goat	Mouse	Blood clot/serum	ic	0/3	Hadlow 1980
Sheep	Mouse	Blood clot/serum	ic	0/18	Hadlow 1982
BSE (natural)					
Cow	Mouse	Blood clot/serum/	ic + ip	0/2	Fraser, 1994, cited
		Buffy coat	ic + ip	0/2	in Bradley 1999
Scrapie (experimental)					
Goat	Goat	Whole blood	ic	0/14	Pattison 1962
Mouse	Mouse	Whole blood	ic	0/39	Eklund 1967
Goat	Mouse	Blood clot	ic or sc	0/20	Hadlow 1974
Sheep	Mouse	Serum	ic	1/1	Gibbs 1965
Rat	Rat	Serum	ic	1/1 (pool)	Clarke 1967
Mouse	Mouse	Serum	ic	1/1 (pool)	Clarke 1967
Mouse	Mouse	Whole blood	ic	3/13	Dickinson 1969
Hamster	Hamster	Whole blood	ic	0/9	Diringer 1984
Hamster	Hamster	Blood extract	ic	5/5 (pools)	Diringer 1984
Hamster	Hamster	Blood extract	ic	10/11 (pools)	Casaccia 1989
Hamster	Hamster[2]	All blood components	ic	1/1 (large pool)	Rohwer 1999
		Whole blood	ic	25-50%	Rohwer 1999
		Whole blood	iv	<1%	Rohwer 1999
Sheep	Sheep	Whole blood	iv		Hunter 2002
Mink encephalopathy (experimental)					
Mink	Mink	Serum	ic	0/1	Marsh 1969
Mink	Mink	Whole blood, plasma, red cells, white cells, platelets	ic	0/8 (pools)	Marsh 1973
BSE (experimental)					
Cow	Mouse	Buffy coat	ic + ip	0/11 (pools)	Wells 2000
	Cow[2]	Buffy coat	ic	0/4 (pools)	Wells 2000
Mouse	Mouse	Plasma	ic	4/48	Taylor 2000
Sheep	Sheep[2]	Whole blood	iv	1/19	Houston 2000
Sheep	Sheep	Whole blood	iv		Hunter 2002
CJD (experimental)					
Guinea pig	Guinea pig	Buffy coat	ic,sc,im,ip	10/28	Manuelidis 1978
GSS (experimental)					
Mouse	Mouse	Buffy coat	ip	4/7 (pools)	Kuroda 1983
Mouse	Mouse	Buffy coat/plasma	ic	5/5 (pools)	Brown 1999
		Buffy coat/plasma	iv	2/2 (pools)	Brown 1999

[1]In several of the studies, assays were conducted on serial specimens obtained during both the incubation and clinical phases of disease. [2]Ongoing experiments.

Table 2. Attempts to detect infectivity in the blood of humans with CJD

Diagnosis	Pos./total subjects	Assay Animal	Inoculum	Route of inoculation	Pos./total Animals	Reference[1]
Sporadic CJD	1/1	Guinea pig	Buffy coat	ic	2/2	Manuelidis 1985
Sporadic CJD	1/1	Guinea pig	Buffy coat	ic	0/5	
		Hamster	Buffy coat	ic	2/2	
Sporadic CJD	1/3	Mouse	Whole blood	ic	2/13	Tateishi 1985
Sporadic CJD	1/1	Mouse	Leukocytes	ic	0/10	Tamai 1992
		Mouse	Plasma conc. X3	ic	3/8	
Sporadic CJD	0/3	Chimpanzee	Whole blood units	iv	0/3	Gajdusek/Gibbs/
Sporadic CJD	0/1	Guinea pig	Whole blood	ic,ip	0/2	Brown 1994
Sporadic CJD	0/1	Spider monkey	Whole blood	ic,iv,ip	0/3	
Sporadic CJD	0/1	Squirrel monkey	Whole blood	ic,ip,im	0/1	
Sporadic CJD	0/4	Squirrel monkey	Buffy coat	ic,iv	0/4	
hGH iatro. CJD	1/1	Hamster	Whole blood	ic	1/4	Deslys 1994
Sporadic CJD	0/13	Transgenic mouse	Buffy coat	ic	0/106	Safar 2000
	0/7		Plasma	ic	0/56	

[1]Publication citations can be found in ref. 1 of this article (the transgenic mouse data has not been published).

Annex 2: Summary of the Report of the EMEA Expert Workshop of *15-16 May 2000* on human TSEs and plasma-derived medicinal products

An EMEA Expert Workshop was held on 15-16 May 2000 to provide an update on the latest information on human transmissible spongiform encephalopathies (TSEs) in relation to plasma-derived medicinal products. In the light of this information, consideration is given to whether further precautionary measures, with respect to variant Creutzfeldt-Jakob disease (vCJD), would be appropriate for plasma-derived medicinal products. The outcome of the meeting can be summarised as follows:

- It is still too early to predict the eventual number of cases of vCJD that will occur.
- There continues to be no evidence that CJD (sporadic, familial and iatrogenic) is transmitted via blood or plasma-derived medicinal products.
- Results are awaited from on-going studies investigating whether infectivity is present in blood of patients who have developed vCJD.
- Accumulating data from a variety of studies are increasing the understanding of TSEs in relation to plasma-derived medicinal products.
- From the evidence available so far, it is not clear that leucodepletion would be a significant measure to reduce infectivity in plasma for fractionation since some of the infectivity may be in a cell-free form. There is a need for further studies before any recommendation for or against systematic leucodepletion for plasma for fractionation can be made.
- A considerable number of spiking studies have been undertaken to investigate the partitioning and removal of the abnormal prion protein (PrP^{sc}) or infectivity during the fractionation process. The results are broadly consistent and suggest that a number of steps contribute to removal of the TSE agent, including ethanol fractionation, precipitation steps, chromatographic procedures, nanofiltration and depth filtration. The extent of removal depends on the processing conditions. There is still uncertainty about the relevant spiking agent to represent the infective agent, if it were present in blood.
- Several types of tests are used for assaying the presence of PrP^{sc}. These tests are still in their development and validation stages. Collaborative studies using appropriate reference materials are essential for the evaluation of assays and the WHO is undertaking an important programme in this respect. It is difficult to foresee to what extent these tests could be applicable in the future for routine screening of blood donations and/or as a confirmatory tool in early diagnosis of vCJD.
- The risk factors for vCJD include residence in the UK. This raises the question of whether exclusion of donors who have spent some time in the UK should be considered as a precautionary measure. Data are being gathered on the travel patterns of European donors to the UK so that the cumulative exposure to BSE risk from UK travel and the impact on the number of donors that would be excluded can be estimated.

On the basis of the current information, the recommendations in the CPMP Position Statement on “New variant CJD and plasma-derived medicinal products” (CPMP/201/98) are still appropriate. The considerable efforts and resources invested to answer the many questions in this area are acknowledged.