1. INTRODUCTION

Human transmissible spongiform encephalopathies (TSEs), including in particular variant Creutzfeldt-Jakob disease (vCJD), were addressed in expert meetings/workshops at the EMEA in January 1998, January 1999, December 1999, May 2000, December 2000, June 2002 and January 2004. A revised CHMP Position Statement on variant CJD and plasma-derived medicinal products was issued in June 2004.[1]

In 1996, a variant form of CJD (vCJD) was identified. The official UK figures for vCJD at the beginning of August 2004 were a total of 147 definite or probable vCJD cases. (One case in Hong Kong was a UK case and is included in the UK figures.) Outside of the UK, there has been one case in Ireland, one in the USA, and one in Canada, who were probably infected while in the UK. However, none of the 7 cases in France and 1 case in Italy had spent time in the UK. The possibility of cases occurring in other countries cannot be excluded. All vCJD clinical cases, which have been genotyped so far, are homozygotes (Met-Met) at codon 129 of the prion protein (PrP) gene. However, evidence of infection has recently been reported in a patient without disease, who was a heterozygote (Met-Val) at codon 129.[2]

There is strong evidence that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE) in cattle. The most likely hypothesis is that vCJD has occurred through exposure to BSE contaminated food.

Uncertainties still exist concerning the number of cases of vCJD that will occur. In contrast to sporadic CJD, the evidence of extensive lymphoreticular involvement has led to concerns about the possibility of transmission from pre-clinically infected individuals *via* blood or blood products.

Data from experimental rodent models of TSEs have shown infectivity of blood components.[3,4,5,6] Infectivity has also been detected in buffy coat of a prosimian microcebe experimentally infected with a macaque-adapted BSE strain.[7] Intra-species transfusion experiments have also shown that experimental BSE or natural scrapie infection can be transmitted between sheep by blood transfusion.[8] On the other hand, experiments to detect vCJD infectivity in human blood using wild-type mice[9], transgenic mice as well as primates have not shown any transmission yet but some of the studies are still ongoing.[10] However, tracing of recipients of blood transfusion from UK donors has revealed two possible cases of secondary transmission after transfusion of red cells from donors who subsequently developed symptoms of vCJD.[2,11] Epidemiological studies have so far been unable to identify a single case of CJD resulting from the administration of plasma-derived products (studies conducted in high risk recipient populations such as hemophiliacs).[10,12] The epidemiological experience is too limited to reach conclusions on whether or not vCJD could be transmitted by plasma-derived medicinal products. As of February 2004, no case of vCJD has been identified with a history of exposure to plasma-derived products.[11]

Plasma from donors from the UK is currently not fractionated as a precautionary measure because the UK had the highest exposure to BSE and has significantly more vCJD cases than any other country. Plasma from donors from France is currently fractionated as the risk benefit ratio is considered favourable. If other countries eventually report several cases of vCJD at some time in the future, a process previously shown to be able to reduce TSE infectivity will provide reassurance on the safety of past products, and could help to justify continuing fractionation.

2. SCOPE OF THE DOCUMENT:

Available data indicate that the manufacturing processes for plasma-derived medicinal products would reduce infectivity if it were present in human plasma. The 2004 Position Statement states[1] "Manufacturers are now required to estimate the potential of their specific manufacturing processes to reduce infectivity using a step-wise approach."

The aim of this document is to provide guidance on how to investigate manufacturing processes with regard to vCJD risk. At this time, experience in this area has not reached a point where definitive guidance can be given. Therefore, the guideline provides advice based on available experience and other approaches may be acceptable. It is very important to read this document in conjunction with the revised Position Statement cited above, especially section 9.2.3 *Manufacturing processes for plasma-derived medicinal products.* As indicated in the Position Statement, consultation with relevant

competent authorities is encouraged and CHMP and its Biotechnology Working Party (BWP) will be available to discuss issues that arise.

3. INVESTIGATIONAL TSE CLEARANCE STUDIES

3.1 General principles

The principles, which are outlined in Note for Guidance CPMP/BWP/268/95 for studies validating the inactivation and removal of viruses[13], should be extended to TSE agents as far as possible.

Material from production should be spiked with a volume of not more than 10% of an appropriate infectious preparation and TSE inactivation/removal should be studied in an exactly down-scaled laboratory model. The experiments should be strictly separated from production and performed in a manner that is well controlled and documented, and undertaken by appropriately qualified personnel. As with virus validation studies, the validity of the down-scaled model should be demonstrated by relevant process parameters and appropriate performance of down-scaled models.

Only those steps which are likely to contribute to inactivation/removal need to be investigated. TSE-agents are expected to be resistant to most physicochemical virus inactivation procedures which are usually applied in the manufacture of plasma-derived medicinal products. Therefore, investigation may focus on removal/partitioning steps such as cold ethanol fractionation, PEG-precipitation, chromatography, depth filtration or nanofiltration.

In a virus validation study, investigation of such removal steps would include the demonstration of partitioning of the agents to the side fractions, and parameters which influence the effectiveness of a process step to inactivate/remove agents would be explored (see Section 5.5 of Note for Guidance CPMP/BWP/268/95[13]). Studies with TSEs are far more difficult and costly than for conventional agents and it may be acceptable to review the robustness of the process on a theoretical basis as part of the design of the investigational study or to use an appropriately validated *in vitro* assay. It should be borne in mind that the removal may be sensitive to variations of manufacturing parameters. Therefore, in-process limits are important for the design of investigational studies for partitioning steps. The studies should include evaluation of partitioning of prions into side fractions using an *in vitro* assay. Reduction factors <1 log are not significant.

The initial spiking titre may be high enough to allow a study of a combination of two (or more) steps. The design of such combined-step studies should allow determination of reduction factors from each single step and from the combined steps by analysing the corresponding intermediate samples. Such combined-step studies may be helpful to substantiate the additivity of low or moderate reduction factors. Moreover, studying combined steps may be helpful where a significant alteration of the physicochemical state of the TSE material, which would influence the removal at the following step, may be suspected (e.g treatment with detergent preceeding a filtration step). An investigation study that encompasses the entire process would be an ideal goal. However, as with viruses, the experimental limitation is that the initial spiking titre will be, in many cases, too low to follow more than one or two steps.

The main points to be considered are:

- scaling down process
- choice of spiking agent
- choice of assays
- choice of manufacturing steps
- interpretation and limitations of data
- re-evaluation of TSE clearance
- sanitisation of equipment

3.2 Scaling down process

The principles of scaling down the manufacturing process for experimental TSE studies are the same as those for virus validation studies. Manufacturers should provide data on the yield, quality and composition of the product(s) or intermediates made in the scaled down version of the process, and these should be comparable with those obtained in typical batches of the full-scale product(s).

3.3 Choice of spiking agent

Data from animals experimentally infected with TSE agents indicates that infectivity can be found in blood of which about half is found in the plasma and half in the buffy coat.[3] The level of infectivity is low, at least 10,000 times less than found in brain so that brain tissue is the only practicable source of spiking material.[4] While the highest possible titre should be used, the spike should not exceed 10% of the total volume to avoid distorting the nature of the fraction studied.

The main considerations are;

i) the strain of the inoculum and the species in which it was prepared;

ii) its physicochemical nature.

i) Species and strain

The factors governing the source of materials for use as the spike include supply, assay and likely similarity of the material to the infectivity which could be present in the plasma. The supply of unpassaged material from cases of vCJD is limited for ethical and other reasons. While normal mice may be used for the bioassay of vCJD, and there is at least one published example of the use of such human material in spiking studies[14], the use of vCJD is not mandatory. In principle large amounts of bovine brain from animals infected with BSE may be available, although in practice material of a suitable quality is difficult to obtain. The assay of the material is again potentially difficult. This applies also to sheep infected with BSE, and most strongly to sheep infected with scrapie. Except for demonstration projects, for example to show that a process will not only remove model agents but the agent of vCJD, it therefore seems most reasonable to use rodent adapted laboratory strains of TSEs (e.g. scrapie, familial CJD, BSE or vCJD). As they differ in their pathology and strain characteristics, several strains have been investigated. The principles involved in their assay are well established. In studies published so far there has been no clearly demonstrable effect of species or strain on removal studies[14] although strains may differ in their resistance to heat inactivation.[15,16] For removal studies, there is no reason to make a choice based on the appropriateness of the strain, so a decision can be made on practical grounds. The rationale for the choice of strain should be given in all cases.

ii) Physicochemical form of spiking agent

Although TSE infectivity has been unambiguously detected in blood in animal experiments, to date the physicochemical nature of TSE agents in blood is unknown. It has been suggested that it might be more like the infectivity found in spleen than that in brain, on the assumption that it originates from a tissue other than brain. However it is not clear where infectivity in the blood originates in those model systems where it has been demonstrated. It is possible that it represents material released as the brain degenerates, in which case brain would be an appropriate spiking material. On the other hand brain tissues containing highly aggregated insoluble forms of PrP^{Sc} may or may not resemble the infectious prions in blood. It is important to consider carefully the various possible physical types of spiking agent.

Vey and co-workers[17] have described studies with four types of agent, all derived from hamster brain homogenate.

(a) Crude brain homogenates.

Crude brain homogenates have been used most extensively in published studies of infectivity. They contain the highest concentration of infectivity. The homogeneity and reproducibility of such preparations could be important factors in the studies performed. If the particle size covers a wide range there may be a correspondingly wide range of removal by a physical technique such as filtration. The reproducibility of the preparation may also be an important factor in judging the reliability of the results obtained.

(b) Microsomal fraction.

Microsomal fractions are prepared by differential centrifugation of brain homogenates, leading to removal of large aggregates and represent membrane-bound infectivity. While the infectivity level is lower than crude homogenates it is still high and the preparation may be expected to be more uniform than crude brain homogenates.

(c) Caveolae-like domains (CLDs).

These have been prepared by sucrose-density ultracentrifugation of detergent-lysed brain homogenates. The infectivity level is lower than that of microsomes and they may simulate membrane domains shed from cells.

(d) Purified PrP^{Sc}.

Purified PrP^{Sc} has been prepared by repeated detergent extraction of brain homogenates followed by salt precipitation and ultracentrifugation. It represents forms not associated with cells or membranes. It is not clear whether such preparations resemble material which could occur naturally.

Results of precipitation studies indicated that the three membrane-bound spikes (homogenates, microsomes, CLDs) behave similarly, thus any of these three types may be suitable spiking agents for such investigations. Because the largest aggregates are removed, and the material may be more consistently prepared whilst maintaining high levels of activity, a microsomal fraction may be preferred. In contrast, the purified PrP^{Sc} was more easily precipitated (including into the cryoprecipitate) than any of the membrane fractions. Other types of spikes are also possible.

It is not clear whether the behaviour of any of these spiking materials is representative of the form of infectivity which may be found in plasma. The spike preparation(s) that would be expected to provide the greatest challenge to the process step(s) under investigation should be selected in order to evaluate under worst case conditions. The rationale for the choice of preparations should be given in all cases.

3.4 Choice of assays

Infectivity assays are accepted as the gold standard for the detection of TSE agents. The presence of the agent in a tissue or fluid is established by infection of suitable animal models, where induction of a neurological disease after an incubation period is the signature of the agent. Infectivity is measured by end point titration in animals. The duration of the incubation period may also be used to quantify the infectivity present in the tested material if validated against end point titration of infectivity. The existence of a species barrier to transmission and species and strain effects put constraints on the materials which can be assayed. For example sporadic CJD preparations from humans rarely infect normal wild type mice, although transgenic mice have been prepared which can be used. This is one of the factors in the selection of the spiking agent discussed above. However, bioassays are very complex to be carried out as, due to the incubation period, the most rapid of them may require at least 6 to 9 months of observation and clinical monitoring of the injected animals (e.g. hamster model, 263K), and up to 15-18 months for non-transgenic murine models. In addition, for a given bioassay, the nature of the agent to be tested is restricted to the combination of infectious agent/strain and recipient animal. In other words, it is not possible to test, on a given animal strain or species, all types of infectious material which could be used as spiking material in a validation study. This is a further limitation obstructing the design of validation studies for the manufacturing process to demonstrate partitioning or inactivation of human TSEs.

Bioassays have to be carried out in dedicated laboratories and animal rooms, with security levels adapted to the manipulated strains (level 2 for characterised scrapie strains, level 3 for human and BSE

strains). Very few sites in Europe are able to handle a large number of animals and for a long period of clinical observation, in level 2 or level 3 premises. Finally, given the complexity of the assay, the length of the incubation period and the requested security measures, the cost of such tests is very high. Nevertheless, most published studies of process clearance have included some element of infectivity assays. If performed, a well-characterised system of strain and indicator animal should be used.

There is still no generally applicable *in vitro* test available to identify presence of infectivity and to quantify the infectivity level. A few cell lines (N2a, GT1) are susceptible and can be infected by some TSE strains adapted to mice[18], whereas some cell lines, transfected with the PrP gene are able to replicate some scrapie strains.

The alternative assay which has also featured in published studies involves the detection of PrP^{Sc}. Currently, the exact nature of the TSE agents is still unknown although numerous experimental results suggest that they could be composed entirely of the host protein, PrP, accumulating with an abnormal conformation. The protein in its abnormal conformation (PrP^{Sc}) is relatively resistant to proteinase K (giving rise to PrP^{res}), and to concentrations of denaturing agents such as guanidinium hydrochloride which remove PrP^{c}. Detection of PrP^{Sc} or PrP^{res} in animal tissues can be considered as a surrogate marker of infectivity. However the correlation between infectivity and PrP^{res} varies between strains and possibly with the methods for measuring either parameter. These considerations affect the confidence with which clearance measured by removal of PrP^{Sc} can be related to the clearance of infectivity although the relationship has been established in specific instances in a number of published studies.[19] When the correlation between a biochemical assay for PrP^{Sc} and a bioassay has been established, demonstration of partitioning or inactivation of infectious prion proteins by the biochemical assay may be sufficient.

Further guidance on the step-wise approach to the use of biochemical assays or bioassays is provided in the CHMP Position Statement[1], Section 9.2.3 *Manufacturing processes for plasma-derived medicinal products.*

3.5 Choice of manufacturing steps

Bearing in mind the known resistance of TSE agents to conventional virus inactivation methods such as heat, the manufacturing steps for investigation should be those where some removal or partitioning of the agent might be expected. Thus there is little point in carrying out studies of solvent/detergent and heat treatment processes, and attention should be focused on ethanol fractionation, precipitation, chromatography, and filtration steps, which have been shown in a number of studies to have significant capability for removal of TSE agents.

On the other hand it should be borne in mind that the behaviour of the spike in a particular step may be affected by previous treatment. For example solvent detergent treatment could disaggregate infectivity so that it more readily passes a filter and studies with untreated materials would therefore overestimate the clearance.

All manufacturers must critically evaluate their manufacturing processes in the light of published data. Guidance on the step-wise approach to product-specific investigational studies is described in Section 9.2.3 of the CHMP Position Statement.[1]

3.6 Interpretation and limitations of data

Viral evaluation studies of processes are subject to a number of reservations, which apply with even greater strength to evaluation of processes with respect to their ability to remove TSEs. They include the following:

1. The modeling of the process may be imperfect. Steps based on separation have been difficult to model reliably in viral evaluation studies; this is particularly true of ethanol fractionation steps, which may be particularly important in the removal of TSE agents.
2. It has been assumed that where two steps are assessed the total clearance approximates to their sum. This may not be the case, in particular where the spike is heterogeneous, so that one fraction could be preferentially removed by one step and the same subfraction by another step.

3. Pretreatments may affect the clearance of the agent. For example if the material is treated with detergent it may pass through a subsequent step such as filtration more easily than if it is not because of dispersal of the material.

4. The assays which can be used are not ideal. Infectivity assays are expensive and very slow. While there is generally a correlation between PrP^{Sc} content and infectivity, the relationship may depend on the method used to measure PrP^{Sc} as well as infectivity. So far most studies have been confirmed by infectivity assays which are considered to be the most relevant.

5. The strain and species of origin of the spike may determine the assays to be used. While so far there is no evidence of a major influence on removal steps, it is still possible that the clearance recorded may be influenced by the origin of the spike.

6. The physicochemical nature of the spike may be significant. There is currently no indication of the physicochemical form of TSE agents in blood, if present. Different membrane-containing spike materials have been tested in one published study, and were cleared in very similar ways by all the precipitation steps evaluated; in contrast, a non-membrane-associated spike material was cleared more readily for certain precipitation steps.[17] For certain non-precipitation (e.g. adsorption) steps, the opposite can occur. The spike material providing the most rigorous test may therefore vary with the process step.

7. In animal studies the amount of agent present in the blood is low, whereas the spike added will be of as high a titre as possible. It is possible that material will be removed less effectively at low than at high concentrations.

8. Evaluation of processes for viral inactivation/removal includes an assessment of the robustness of the process, for example studying the effect of modification of the process parameters. The difficulties of performing such studies with TSEs, other than by using solely *in vitro* assay methods, means that the studies are unlikely to be repeated often.

The confidence which can be placed on a figure for the ability of a process step to remove the agent of TSE is therefore lower than for its ability to remove model viruses.

3.7 Re-evaluation of TSE clearance

As for the virus validation studies, it may be necessary to re-evaluate TSE clearance data for a production process, if significant changes are made. In view of the difficulty in performing the studies and the reservations about the results obtained, such changes would probably have to be major. Alternatively, developments in the scientific area could make the studies easier, for instance by making *in vitro* infectivity assays possible, which could justify further work.

3.8 Sanitisation of equipment

Inactivation of prions by conventional decontamination methods has been evaluated in numerous studies. TSE infectivity is particularly difficult to inactivate and some samples have been shown to be environmentally infectious for years. A certain number of treatments are acknowledged to be ineffective under normal application conditions (e.g. alkylating agents, detergents) while some others have been recognized as quite effective (e.g. soaking in ≥ 2% bleach or in sodium hydroxide (NaOH) 1-2N for 60 min, autoclaving at 134-138°C under certain conditions of pressure and time). Some procedures have been adopted as reference treatments or recommendations by WHO[20] and used with medical devices or wastes. However, their applicability is rather limited due to a number of reasons; most methods are harsh and may well damage most biological products, they have a corrosive effect on the equipment used in production or show a limited efficacy against other infectious agents (e.g. NaOH is reported as non-sporicidal). A number of experimental treatments (e.g. alkaline cleansers, proteases, etc.) are under evaluation for use in cleaning and decontamination procedures but not applicable yet.

Because of the resistance of TSE agents to inactivation, and their propensity to stick to stainless steel and other materials, it is particularly important to consider the sanitisation and cleaning procedures used for fractionation equipment for their ability to inactivate or remove TSE agents. The sanitisation

and reuse of columns should also be considered in the light of the effect of possible treatments on TSE infectivity. It should be borne in mind that many fractionation processes terminate in a depth filtration step, where the filter aid is discarded. If this is shown to be an effective step, it decreases the risk of contamination of the product by any infectivity that leaches from the equipment.

Investigation of inactivation or removal of TSE agents adhering to metal surfaces may be extremely difficult. A model has been developed in which a steel wire dipped in a TSE preparation can be implanted in the brain of a susceptible animal which then goes on to develop disease. Sanitisation of the wire might be monitored by such a model by loading wires with different dilutions of agent, treating them and then determining the loss in infectivity resulting. An example of this approach to investigate novel methods of disinfection of prion-contaminated medical devices has recently been published.[21] The extrapolation of results from such studies to equipment at a manufacturing level may be difficult. NaOH solutions are commonly used for cleaning of production equipment. Treatment with 0.1 M NaOH has been shown to convert PrP^{Sc} into a protease-sensitive form, either in solution or when adsorbed to a metallic surface.[22] The results need to be confirmed by studies of infectivity.

In view of the above considerations, no specific recommendation can be given at this time until new scientific information relevant to plasma processing equipment becomes available.

4. SUMMARY / CONCLUSION

All manufacturers must critically evaluate their manufacturing processes in the light of published data. Guidance on the step-wise approach to product-specific investigational studies is described in Section 9.2.3 of the CHMP Position Statement.[1] For removal studies, there is no reason to make a choice based on the appropriateness of the strain of TSE agent, so a decision can be made on practical grounds. Different preparations of spiking agents can be used. The spike preparation(s) that will provide the greatest challenge to the process step(s) under investigation should be selected in order to evaluate under worst case conditions. The rationale for the choice of strain and preparations should be given in all cases.

It is recognised that there are limitations of the data because of the model character and approximations that are linked to feasible experimental studies. However, it is expected that these investigational studies will provide a helpful indication on the capacity for removal of the TSE agent during manufacture of plasma-derived medicinal products.

References

1. CHMP Position statement on Creutzfeldt-Jakob Disease and plasma-derived and urine-derived medicinal products, June 2004 (EMEA/CPMP/BWP/2879/02/rev 1) http://www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf

2. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient. Lancet 2004;364:527-529.

3. Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion 1998;38:810-816

4. Brown P, Cervenáková L, McShane LM, Barber P, Rubenstein R, Drohan WH. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-1178

5. Taylor DM, Fernie K, Reichl HE, Somerville RA. Infectivity in the blood of mice with a BSE-derived agent. Journal of Hospital Infection 2000;46:78.

6. Cervenakova L, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, Drohan WN, Brown P. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 2003;43:1687-1694.

7. Bons N, Lehmann S, Mestre-Francès N, Dormont D, Brown P. Brain and buffy coat transmission of bovine spongiform encephalopathy to the primate *Microcebus murinus*. Transfusion 2002;42:513-516

8. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F. Transmission of prion diseases by blood transfusion. Journal of General Virology 2002;83:2897-2905

9. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 2001;358:208-209

10. Cervenáková L, Brown P, Hammond DJ, Lee CA, Saenko EL. Factor VIII and transmissible spongiform encephalopathy: the case for safety. Haemophilia 2002;8:63-75

11. Llewelyn CA, Hewitt PE, Knight RSG, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. Lancet 2004;363:417-421.

12. Ricketts MN, Brown P. Transmissible spongiform encephalopathy update and implications for blood safety. Clin Lab Med 2003;23:129-37.

13. CPMP Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses, February 1996 (CPMP/BWP/268/95)
http://www.emea.eu.int/pdfs/human/bwp/026895en.pdf

14. Stenland CJ, Lee DC, Brown P, Petteway SP, Rubenstein R. Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. Transfusion 2002;42:1497-1500

15. Taylor DM. Inactivation of transmissible degenerative encephalopathy agents: a review. The Veterinary Journal 2000;159:10-17.

16. Somerville RA, Oberthür RC, Havekost U, MacDonald F, Taylor DM, Dickinson AG. Characterization of thermodynamic diversity between transmissible spongiform encephalopathy agent strains and its theoretical implications. Journal of Biological Chemistry 2002;277:11084-11089.

17. Vey M, Baron H, Weimer T, Gröner A. Purity of spiking agent affects partitioning of prions in plasma protein purification. Biologicals 2002;30:187-196.

18. Klöhn P-C, Stoltze L, Flechsig E, Enari M, Weissmann C. A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. Proc. Natl. Acad. Sci. 2003;100:11666-11671.

19. Lee DC, Stenland CJ, Miller JLC, Cai K, Ford EK, Gilligan KJ, Hartwell RC, Terry JC, Rubenstein R, Fournel M, Petteway SR. A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins. Transfusion 2001;41:449-455.

20. WHO Laboratory Biosafety Manual 2003

21. Fichet G, Emmanuel C, Duval C, Antloga K, Dehen C, Charbonnier A, McDonnell G, Brown P, Lasmézas CI, Deslys J-P. Novel methods for disinfection of prion-contaminated medical devices. Lancet 2004;364:521-526.

22. Käsermann F, Kempf C. Sodium hydroxide renders the prion protein PrP^{Sc} sensitive to proteinase K. Journal of General Virology 2003,84:3173-3176.

European Medicines Agency

資料③

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EMEA/CPMP/BWP/2879/02/rev 1

CHMP POSITION STATEMENT ON CREUTZFELDT-JAKOB DISEASE and PLASMA-DERIVED AND URINE-DERIVED MEDICINAL PRODUCTS

This is the first revision of the CPMP[a] Position Statement on "Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products" (EMEA/CPMP/BWP/2879/02) published in February 2003, which replaced the CPMP Position Statement on "New variant CJD and plasma-derived medicinal products" (CPMP/201/98) issued in February 1998.

SUMMARY

Cumulative epidemiological evidence does not support transmission of sporadic, familial and iatrogenic Creutzfeldt-Jakob disease (CJD) by plasma-derived medicinal products. There is no change to the previous CPMP position that recall of plasma-derived medicinal products is not justified where a donor is later confirmed as having sporadic, familial or iatrogenic CJD.

Variant CJD (vCJD) is an emerging disease and the eventual number of cases of the disease is uncertain. There is a wider distribution and higher level of infectivity/abnormal prion protein in peripheral tissues than is seen with sporadic CJD. It is not known whether or not infectivity is present in human blood; however, a possible transmission of vCJD by blood transfusion in man has recently been reported.

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding to no longer fractionate from UK plasma. It is consistent with this decision to exclude donors who have spent long periods in the UK during the risk period from donating blood/plasma for fractionation. It is recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation. There is no recommendation to recall batches if information that would have excluded a donor based on his/her stay in the UK becomes available post-donation, since this is a very conservative precautionary measure.

Available data indicate that the manufacturing processes for plasma-derived medicinal products would reduce vCJD infectivity if it were present in human plasma. Manufacturers are now required to estimate the potential of their specific manufacturing processes to reduce infectivity using a step-wise approach. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. CHMP and its Biotechnology Working Party (BWP) will keep progress with these recommendations and the actions to be taken under review.
In support of this recommendation, CHMP and BWP, with the involvement of external experts, is developing guidance on how to investigate manufacturing processes with regard to vCJD risk and CHMP and BWP will be available to discuss issues that might arise.
The rationale for this position is that if, in the future, further cases of vCJD occur in countries collecting blood and plasma for the manufacture of plasma-derived medicinal products, a process previously shown to be able to reduce TSE infectivity will provide reassurance on the safety of past products, and could help to justify continuing fractionation.

The detection of an abnormal prion protein in the urine of animals and humans suffering from transmissible spongiform encephalopathies (TSEs) was reported in 2001. While information is awaited

[a] In May 2004 there was a change in the name of the EMEA's scientific committee for human medicines from CPMP to CHMP.

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from on-going research work in this area, further information has been gathered on the manufacturing processes for urine-derived medicinal products. This general review indicates that it is feasible to apply donor selection criteria when a product is derived from a relatively small and well-defined donor population. In addition, it indicates that manufacturing processes have at least one step that might be theoretically capable of reducing TSE infectivity if it were present in the starting material. It is noted that urine-derived medicinal products are not sourced from urine collected in the UK.

On the basis of this review and other considerations, the use of exclusion criteria for selection for a urine donor panel is encouraged, as a precautionary measure, where feasible. The same exclusion criteria should be applied with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products but, unlike blood/plasma donors, these criteria would not be checked at each donation. Manufacturers of urine-derived medicinal products, who have not yet undertaken a theoretical evaluation of the potential of their manufacturing processes to reduce infectivity, should carry this out and report the outcome to the relevant competent authorities.

1. Introduction

Creutzfeldt-Jakob disease (CJD) is a rare neurodegenerative disease causing the death of approximately 1.5 to 2 persons per million population per year. Cases can arise spontaneously (sporadic), may arise at higher frequency in families with certain genetic mutations (familial) or can result from medical exposure to infectious material (iatrogenic). In 1996, a variant form of CJD (vCJD) was identified.[1] There is strong evidence that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE) in cattle.[2,3,4] The most likely hypothesis is that vCJD has occurred through exposure to BSE contaminated food.

Human transmissible spongiform encephalopathies (TSEs), including in particular vCJD, were addressed in expert meetings/workshops at the EMEA in January 1998, January 1999, December 1999, May 2000, and December 2000. A CPMP Position Statement on variant CJD and plasma-derived medicinal products was issued in February 1998[5f] and the outcome of the subsequent meetings was published on the EMEA website.[5] An EMEA Expert Workshop on Human TSEs and Medicinal Products was held on 19-21 June 2002. This provided the scientific basis for a new CPMP Position Statement issued in 2003.[5b] A further EMEA Expert Workshop was held in January 2004 to review the current state of knowledge of vCJD, in the light of the recent report of a possible human transmission by blood transfusion.[6] In addition, the Workshop discussed the CPMP Discussion document on the investigation of manufacturing processes with respect to vCJD.[5a] A report of the January 2004 meeting will be published on the EMEA website.

Blood and blood components for transfusion are outside the scope of this Position Statement. Recommendations on the suitability of blood and plasma donors and the screening of donated blood in the European Community are described in Council Recommendation 98/463/EC.[7c] European legislation on human blood and blood components entered into force on 8 February 2003 and Member States have until 8 February 2005 for its transposition into national law.[7a] Under this legislation, a Commission Directive on certain technical requirements for blood and blood components, including eligibility criteria for donors, entered into force in April 2004.[7b]

In December 2003, following the announcement of a possible case of vCJD transmission by blood transfusion, Commissioner Byrne made a statement highlighting EU activities in the area of vCJD and announcing a meeting of the Working Group of the Blood Regulatory Committee to consider the latest information available from the UK.[7d] The meeting took place in January and a summary statement was produced.[7e]

The Scientific Steering Committee (SSC) and the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) of the European Commission have published a number of opinions relating to TSEs, which are of relevance to blood and blood components for transfusion, as well as to plasma-derived medicinal products.[8] WHO Guidelines on TSE in relation to biological and

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pharmaceutical products is also of relevance to both blood components for transfusion and plasma-derived medicinal products.[9] The Council of Europe has made recommendations for blood and blood components for transfusion.[10]

2. Variant CJD current status

The official UK figures for vCJD at the beginning of June 2004 were a total of 146 definite or probable vCJD cases.[11] (One case in Hong Kong was a UK case and is included in the UK figures.) Outside of the UK, there has been one case in Ireland, one in the USA, and one in Canada, who were probably infected while in the UK.[12] However, none of the 6 cases in France[13] and 1 case in Italy had spent time in the UK. The possibility of cases occurring in other countries cannot be excluded.
All cases, who have been genotyped so far, are Met-Met homozygotes at codon 129 of the prion protein (PrP) gene.

Analysis of the UK figures for the quarterly incidence of deaths indicates that vCJD incidence in the UK is currently in decline. However, interpretation requires caution as there may be a long tail or more than one peak to the epidemic.[14]

A UK study screening specimens from surgically removed appendices and tonsils for accumulation of prion protein in the lymphoreticular system has been carried out in order to try and obtain some estimation of the number of people that might be incubating vCJD in the UK.[15] Three positive appendix specimens have been found as a result of the screening of 12,674 appendix and tonsil specimens. However, the pattern of lymphoreticular accumulation in two of these samples was dissimilar from that seen in known cases of vCJD, raising the possibility that they may be false positives. With respect to this possibility, the authors comment that although it is uncertain whether immunohistochemical accumulation of prion protein in the lymphoreticular system is specific for vCJD, it has not been described in any other disease, including other forms of human prion disease or a range of inflammatory and infective conditions.

Statistical analysis on this finding of 3 positive specimens gives the following estimations of numbers who may be incubating vCJD:
237 infections per million population (95% confidence interval (CI): 49-692 per million)
Assuming that this estimate relates to those aged 10-30 years[b]:
3,808 individuals (CI 785-11 128) aged 10-30 years may be incubating vCJD.

These estimations are higher than the most recent predictions from modelling of the clinical data (upper 95% confidence interval of 540 future cases).[16] It is not known whether those incubating vCJD will eventually develop clinical disease. However, estimates of numbers possibly incubating are important with respect to any potential for secondary transmission (e.g. by blood donation, surgical instruments) while individuals are in the incubation phase. It should be noted that plasma-derived medicinal products are not manufactured from donations collected in the UK.

A larger study will now be undertaken, involving the establishment and testing of a national prospective archive of tonsil tissue from 100,000 people of all ages removed during routine tonsillectomies.[17]

3. Human tissue distribution of infectivity/abnormal prion protein.

Tissue distribution has been investigated by detection of the abnormal prion protein (PrP^{sc}/PrP^{res})[c] or by infectivity assays. Until now, detection of PrP^{sc} in tissues has always been associated with

[b] The reason the age range of 10-30 years is specified is because 83% of the samples were from individuals in this age range.

[c] PrP^{sc} is an abnormal isoform of the natural protein PrP^{c}, which is anchored to the surface of many cells in mammals. PrP^{c} and PrP^{sc} have a different resistance towards proteinase K treatment: the endogenous PrP^{c} is completely degraded by proteinase K, whereas PrP^{sc} is partly resistant (giving rise to PrP^{res}).

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infectivity, however it should be noted that animal studies show that, in some circumstances, infectivity can also be present without detection of PrP^{sc}. This may be related to limitations of assay methods for PrP^{sc}, however, in some cases the reason for this finding is not known. It is thus recommended that any study on tissue or fluid distribution of the abnormal prion protein be confirmed with an infectivity assay.
A wider distribution and higher level of PrP^{sc} in human peripheral tissues, including the lymphoreticular system, has been found in vCJD compared with sporadic CJD.[18,19,20] Limited data from infectivity assays of tissues are consistent with the PrP^{sc} findings.[21]

4. Infectivity in blood and transmissibility via blood

4.1 Animal blood

Low levels of infectivity have been found in the blood of rodents experimentally infected with TSE agents.[22,23,24,25] Experiments indicate that approximately half the infectivity is in the cellular components, mainly the buffy coat, and the remainder in the plasma. Experimental studies indicate that the vCJD agent behaves in a similar way (qualitatively and quantitatively) to a familial CJD agent[d] when adapted to RIII/Fa/Dk mice.[25] Infectivity has also been detected in buffy coat of a prosimian microcebe experimentally infected with a macaque-adapted BSE strain.[26]

The infectivity in rodent blood was transmitted by intravenous inoculation, but 5-7 fold less efficiently than by the intracerebral route.[23] In one study with mouse-adapted vCJD agent, the intravenous and intracerebral routes were found to be equally efficient for the buffy coat fraction but not for the plasma fraction.[25] However, studies in primates show that survival times were similar after intravenous or intracerebral inoculation of infected brain material.[27,28] Furthermore, information from an on-going intra-species transfusion experiment indicates that experimental BSE in orally infected sheep or natural scrapie infection in sheep can be transmitted to sheep by blood transfusion.[29,30] The level of infectivity in sheep blood cannot be established from these experiments.

The European Union has provided funding for animal transmission projects, including still on-going studies.

4.2 Human blood

In the UK, a surveillance system was established to see whether any cases of vCJD occurred in recipients of blood donations from donors who later developed vCJD. As of 18 December 2003, there were 17 living recipients of blood transfusions where the donations were given by individuals who later developed vCJD.[6]

This tracing of recipients of blood transfusion from UK donors who have subsequently developed vCJD has revealed one possible case of secondary transmission.[6] The individual had received a transfusion of red cells from a donor who developed symptoms of vCJD 3 years 4 months later. Six and a half years after the blood transfusion, the recipient developed the first clinical symptoms of vCJD. This case is the second oldest one (late 60s) of vCJD identified. The individual was a methionine homozygote at codon 129 of the prion protein gene. It is not possible to exclude that infection in the recipient was due to dietary exposure but statistical analysis suggests that this is unlikely. This possible case is consistent with the findings from sheep transfusion studies that infectivity in blood can be transmitted during the pre-clinical phase of infection.
Twenty units of plasma from individuals who later developed vCJD were included in pools for the production of fractionated products before 1998, at which time a policy was introduced to source plasma for fractionation from outside the UK. As of February 2004, no case of vCJD had been identified with a history of exposure to fractionated blood products.[6]

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[d] Mouse-adapted Fukuoka-1 strain of human TSE (brain tissue obtained from a case of Gerstmann-Sträussler-Scheinker syndrome).

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The surveillance described above emphasises the importance of national databases of blood donors and the maintenance of traceability from donor to recipient and vice versa. Without a national database of blood donors it becomes very difficult to establish whether a vCJD case has been a blood donor. (UK experience has shown that questioning of family members is unreliable for establishing whether a patient has been a blood donor.) Traceability is a specific requirement in Article 14 of Directive 2002/98/EC.[7a]

Infectivity or PrP^{sc} were not detected in blood of vCJD cases using methods capable of detecting infectivity/PrP^{sc} in peripheral tissues such as tonsil or spleen, indicating that if infectivity is present it is at levels below the sensitivity of these methods.[21, 18] A review of transmission studies to detect infectivity in the blood of humans with CJD (sporadic, iatrogenic and variant) shows that although transmissions have occasionally been reported, the majority of studies failed to detect infectivity.[31] Infectivity was not detected in blood from patients with sporadic CJD using human PrP - transgenic mice for the detection of infectivity. Further experiments to detect infectivity in human blood are on-going.

For the purpose of risk assessments, it is recommended that the worst case assumption that the relative efficiency of the intravenous and intracerebral routes is 1:1 should be used. This is because the accumulated information now available from animal studies indicates that the intravenous route can be an efficient route of transmission and in certain cases can give a transmission rate and/or an incubation period similar to the intracerebral route (see also 4.1).

5. Detection techniques

Several techniques are under development for the detection of PrP^{sc} in blood. Approaches based on surrogate markers are also under investigation. Development and validation of all methods is on-going but there is no screening test yet.
Several WHO reference preparations are available and further materials are under development[e]. These reference preparations will allow calibration of assays versus infectivity bioassays, and can be used for collaborative studies to compare the performance of different assays to see whether they are sufficiently sensitive and specific to justify further evaluation for screening blood.

6. Leucoreduction

Leucoreduction is used in transfusion medicine to reduce the level of white blood cells in blood and blood components.
The rationale for considering leucoreduction as a precautionary measure is:

- The lymphoreticular involvement in vCJD
- The detection of low levels of infectivity, in studies with rodents, in the buffy coat (associated with white blood cells).

The SCMPMD opinion on leucoreduction[8a, 8b] for blood and blood components for transfusion states that it might be a precautionary step to remove white blood cells as completely as possible. For plasma for fractionation the opinion states the following:
'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. The question should be further explored by suitable experiments.'
For plasma-derived medicinal products, there is a theoretical concern that leucoreduction of blood might encourage dissociation of infectivity from white blood cells resulting in an increase in infectivity in the plasma compartment.

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[e] Standards are developed by the WHO Working Group on International Reference Materials for Diagnosis and Study of TSEs (http://www.who.int/biologicals).

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Reassuringly, results of UK studies on leucoreduction, reported at the 2002 EMEA Workshop, show that it does not provoke fragmentation of cells and lysis.

At the present time, there are no data to support the effectiveness of leucoreduction to reduce infectivity of plasma for fractionation.[23] In one study in hamsters, reported at the 2004 EMEA Workshop, there was a reduction in infectivity of about 50 percent after leucoreduction of whole blood. There is a need for further studies investigating leucoreduction of infected blood using infectivity assays. A project investigating leucoreduction is currently being funded by the European Union.

7. Manufacturing processes for plasma-derived medicinal products

Many investigational studies have now been carried out with different strains of agent and spiking materials of different nature and purity, and using different assays to follow the partition of PrP^{res} and/or infectivity. *In vitro* assays can be useful for spiking experiments to investigate manufacturing processes but it is important to correlate such results with those from infectivity assays, as has already been reported in publications in this area.

These studies have investigated the contribution of the various manufacturing steps to reduction of infectivity (including precipitation followed by centrifugation or depth filtration, chromatography and nanofiltration). Data support the removal of infectivity by steps that are commonly used in the manufacture of plasma-derived medicinal products. However, caution is needed in the interpretation of data since the effectiveness of a given step is dependent on a number of variables (including the process conditions and state of the agent in the sample). Consequently, effectiveness of removal may vary from one manufacturer to another.

Animal studies using blood from rodents infected by intracerebral inoculation indicate that the fractionation process contributes to the removal of endogenous plasma infectivity.[22,23] Preliminary information reported at the EMEA Workshops in 2002 and 2004 suggests that endogenous infectivity might persist further through the fractionation process than would be expected from spiking studies. There is a need for further research in this area to investigate the partition and removal of endogenous infectivity and the extent to which this is comparable with data from spiking studies.

8. Infectivity in urine

Shaked *et al.*[32] have reported the detection of a protease resistant PrP isoform ($UPrP^{sc}$) in the urine of hamsters, cattle and humans suffering from TSEs. However, it is noteworthy that intracerebral inoculation of hamsters with $UPrP^{sc}$ did not cause clinical signs of prion disease in this study. These findings are yet to be confirmed and additional studies are on-going.

Epidemiological evidence in the last 25 years, when urinary-derived medicinal products and particularly gonadotrophins have been widely used, does not suggest a risk from sporadic CJD. Since epidemiological evidence has identified the few cases of iatrogenic transmission of CJD through the use of pituitary-derived gonadotrophins, it could be expected that transmission from urinary-derived gonadotrophins would have been detected if it had occurred.

9. Recommendations and proposals

9.1 Sporadic, familial and iatrogenic CJD and plasma-derived medicinal products

Cumulative epidemiological evidence does not support transmission of sporadic, familial and iatrogenic CJD by blood, blood components or plasma-derived medicinal products.[31, 33] Nevertheless, donor selection criteria include criteria to exclude donors who might be at higher risk of developing CJD. The following permanent deferral criteria are specified in Commission Directive 2004/33/EC:

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Persons who have a family history which places them at risk of developing a TSE, or persons who have received a corneal or dura mater graft, or who have been treated in the past with medicines made from human pituitary glands.[7b] Precautionary recalls of batches of plasma-derived medicinal products after post-donation reports of CJD or CJD risk factors in a donor contributed to severe shortages of certain products.[9]

On the basis of the current epidemiological evidence, the CPMP recommendation that recall of plasma-derived medicinal products is not justified where a donor is later confirmed as having sporadic, familial or iatrogenic CJD is maintained. Further epidemiological studies are recommended.

9.2 Variant CJD and plasma-derived medicinal products

Variant CJD is a new emerging agent. Uncertainties still exist concerning the number of cases of vCJD that will occur and whether infectivity is present in blood. Variant CJD has a different peripheral distribution to sporadic CJD. Epidemiological experience is too limited, in terms of timescale and number of cases, to reach conclusions on whether or not vCJD could be transmitted by blood, blood components or plasma-derived medicinal products. However, there is now one possible case of human transmission by blood transfusion.

The following measures are aimed at minimising the risk of transmission of the agent by plasma-derived medicinal products.

9.2.1 Exclusion Criteria

a) Consideration of Country-based exclusions

Variant CJD sufferers with overt disease will be too ill to present for donation or would be disqualified at the point of donor screening. However, there is no screening test to detect donors who may be incubating the disease or in the early clinical stages. Therefore, other approaches are considered in order to try and identify donors who may present a higher risk.

UK plasma

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding no longer to fractionate from UK plasma.

Exclusion of donors based on cumulative period of time spent in the UK

Since UK donors are excluded from donating plasma for the manufacture of plasma-derived medicinal products in the UK, it is consistent to exclude donors who have spent long periods in the UK. This is supported by the finding of vCJD cases, which have a risk factor of long periods spent in the UK, in other countries[f].

It is, therefore, recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation. Countries are highly encouraged to choose their national cumulative period limit for plasma-derived medicinal products according to a nationally calculated benefit/risk balance, which will take into account the endogenous risk of BSE and the risk of shortages of blood and plasma for the manufacture of medicinal products. The national limit is recommended to be of cumulative periods in the UK below or equal to 1 year, since for plasma-derived medicinal products, there is very little difference in effectiveness of the measure between an exclusion of 3 months, 6 months or 1 year in the UK.

Countries may still apply a stricter limit than 1 year for exclusion of donors for blood/plasma collected for fractionation within the country (e.g. 6 months) but will accept plasma-derived medicinal products from other countries provided that at least the one-year time limit is applied.

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[f] One case in each of Ireland, US and Canada associated with long periods spent in the UK.

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The rationale for this recommendation is to exclude donors who have the highest individual risk from stays in the UK and to be consistent with the UK decision to no longer fractionate from UK plasma. This is further explained in the first version of this Position Statement published in February 2003.[5b]

The safety of batches of product manufactured from blood/plasma collected before the implementation of the measure is not in question. Therefore, such batches can stay on the market and are not subject to any batch recall.

French plasma
France is currently the only country outside the UK that has had a number of vCJD cases, which are not linked to stays in the UK. France has estimated a risk of 1/20 of that in the UK for dietary exposure of the population to BSE. France published an analysis of the risk of transmission of vCJD by blood and its derivatives sourced from French plasma in December 2000.[34d] This concluded that plasma collected in France could continue to be used for fractionation. The safety margin for plasma-derived medicinal products was considered to be sufficient. However, a further increase in the safety margin of some products was recommended (e.g. nanofiltration of Factor VIII introduced in January 2001). Leucodepletion for plasma for fractionation, as for plasma for transfusion, was also recommended as a precautionary measure.
The subsequent analyses published in 2002, 2003 and 2004 re-confirmed these conclusions.[34]

Donors who have spent a cumulative period of time in France
Exclusion of donors who have spent a cumulative period of time in France is not recommended because of the lower risk associated with time spent in France compared with time spent in the UK. On the basis that the risk in France is 1/20 of that in the UK, a donor spending a cumulative period of one year in the UK would be equivalent to a donor spending 20 years in France during the risk period.

Concluding remarks
Country-based exclusions are inefficient, as the vast majority of donors who will be excluded will not develop the disease. There is a lack of spare plasma capacity to make up for shortfalls if countries that are major producers of plasma-derived medicinal products discontinue the use of nationally collected plasma for fractionation.

b) Other possible exclusion criteria

Commission Directive 2004/33/EC indicates that further deferral criteria for vCJD may be recommended as a precautionary measure.[7b]
Other possible exclusion criteria that could be considered include permanent exclusion of recipients of blood transfusion (general exclusion or exclusion of recipients of transfusion in UK[8]), transplant recipients, and donors who have undergone neurosurgery.
Caution is needed because of the risk of loss of donors and consequent supply problems. Since such criteria could apply to both blood and blood components, and plasma-derived medicinal products, it was appropriate to consider this further within the scope of Directive 2002/98/EC.[7a] The technical meeting of blood experts, convened by the European Commission in January 2004, considered exclusion criteria, as well as blood component preparation and processing, recipient tracing and surveillance, and optimal use of blood.[7c]

9.2.2 Leucoreduction

For plasma-derived medicinal products, results would be needed from studies investigating the effect of leucoreduction on infectivity in plasma (recovered plasma or apheresis plasma) before making any recommendation. (See Section 6 for further discussion of this aspect.)

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[8] In April 2004, the UK implemented exclusion of persons who have previously received transfusions of whole blood components since January 1980, as a precautionary approach. The numbers of donors outside the UK who have spent a cumulative period of less than one year in the UK but have received a blood transfusion within the UK is expected to be very small and it may not be worthwhile to have a specific measure.

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9.2.3 Manufacturing processes for plasma-derived medicinal products

The available data support the reduction of infectivity by steps in the manufacturing process. Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity. This should follow a step-wise approach as described below and illustrated in the accompanying flow diagram. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. A decision to undertake an infectivity assay and/or to add a further manufacturing step(s) to increase reduction capacity should only be made after a careful consideration of all benefit-risk factors for a certain product.

Firstly, manufacturers should compare their own processes to those with published data on reduction of infectivity in order to estimate the theoretical potential of their specific manufacturing processes to reduce infectivity. *(Flow diagram, step 1)*

Whereas the general information available on manufacturing processes provides useful background information, the actual effectiveness of a manufacturing process might be dependent on the specific process conditions. Manufacturers should consider the relevance of the published data to their specific manufacturing processes and whether the removal capacity can be expected to be comparable.

If it cannot be concluded that the removal capacity would be expected to be comparable, it is recommended that manufacturers undertake product-specific investigational studies on key steps in their manufacturing processes using biochemical assays. Priority should be given to studies on products with the lowest potential removal capacity. *(Flow diagram, step 2)*

Investigations using biochemical assays may be sufficient if a clear correlation with infectivity data has already been established for similar processes (e.g. ethanol fractionation). If such a correlation is not established (e.g. a novel step) and the step is considered critical for removal of infectivity for the specific product (e.g. it is the only step for removal), the investigations should be confirmed using an infectivity assay for the critical step(s). *(Flow diagram, step 3)*

The above steps will allow manufacturers to estimate the reduction capacity of their manufacturing processes. *(Flow diagram, step 4)*

In cases where the reduction capacity is limited, manufacturers should consider the addition of steps that may increase the removal capacity where this is feasible without compromising the safety, quality and availability of the existing products. Discussion with the relevant competent authorities is recommended. *(Flow diagram, step 5)*

The outcome of the estimates of the theoretical potential of manufacturing processes to reduce infectivity and the results of product-specific investigational studies should be reported to the relevant competent authorities for the medicinal products concerned, as information becomes available. Applicants submitting new marketing authorisation applications for plasma-derived medicinal products will be expected to include such information in the application dossier. If product-specific investigational studies are not available at the time of submission, the proposed investigations and timescales should be described or justification provided for not performing further studies. The outcome of the estimation of the theoretical potential to reduce infectivity should always be included in the application.

CHMP and its Biotechnology Working Party will keep progress with these recommendations and the actions to be taken under review.

In support of these recommendations, CHMP's Biotechnology Working Party, with the involvement of external experts, is developing guidance on how to investigate manufacturing processes with regard to vCJD risk.[5a]

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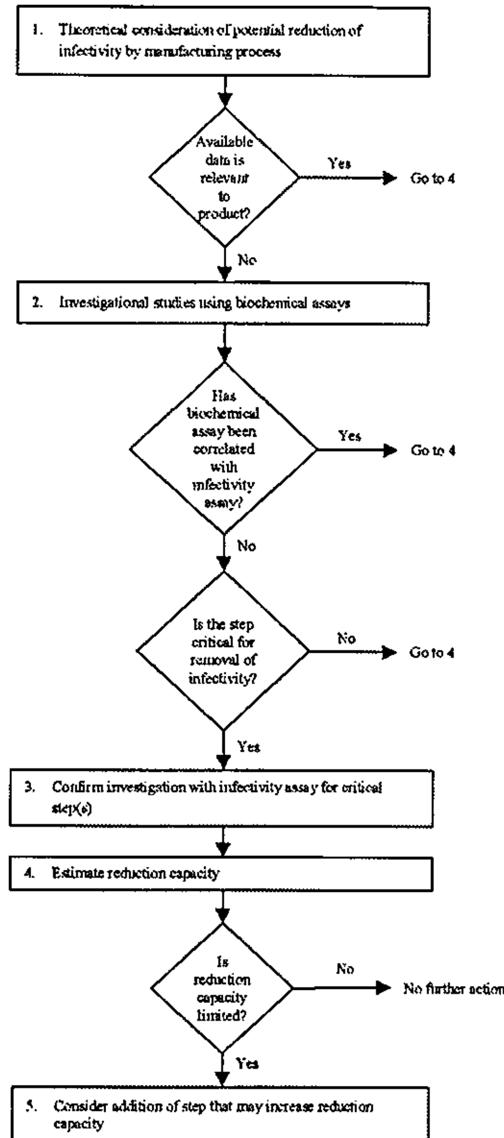
Figure 1: Plasma-Derived Medicinal Products: estimation of potential reduction capacity of specific manufacturing processes

Important Note: this flow diagram should be read in conjunction with the preceding text in 9.2.3. It is recommended to consult the relevant competent authorities at the milestones in this estimation. Give priority to studies on products with the lowest potential removal capacity.



9.2.4 Recall of batches where information becomes available post-donation

In view of the lack of adequate information on vCJD, it is prudent to recall batches of plasma-derived medicinal products where a donor to a plasma pool subsequently develops vCJD. Recall should also include medicinal products containing plasma-derived products as excipients. However, in both cases, consequences for essential medicinal products where alternatives are not available will need careful consideration by the competent authorities.

A case-by-case consideration would be appropriate where plasma-derived products have been used in the manufacture of other medicinal products. This consideration would include the nature of the product, the amount used, where it is used in the manufacturing process and the downstream processing.

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Look-back to identify the fate of donations should be taken as far as possible. Regulatory authorities, Official Medicines Control Laboratories, surveillance centres and the supply chain should be informed of all batches of product and intermediate implicated whether or not supplies of the batch are exhausted.

There is no recommendation to recall batches if information becomes available post-donation, which would have excluded a donor based on his/her stay in the UK since this is a very conservative precautionary measure (see 9.2.1).

9.2.5 Albumin used as an excipient or in manufacturing processes

The available data on the removal of infectivity during the fractionation process used in the manufacture of albumin indicates that the risk of transmission of infectivity by albumin would be particularly low. Nevertheless, in the case of albumin used as an excipient, recall is still recommended as a precautionary measure where a donor to a plasma pool subsequently develops vCJD. A single batch of albumin may be used to produce a number of batches of a medicinal product because of the small amounts that are typically used as an excipient. As a consequence, a recall could affect complete stocks of a product and create severe shortages. Therefore, to avoid a negative impact on supply, companies should consider the origin of plasma and select countries where the probability of having to recall batches is as limited as possible.

Development of substitutes for plasma-derived albumin used as an excipient or in manufacturing processes is encouraged although it is recognised that this can be difficult (requiring development and validation and usually non-clinical and clinical investigations) and should thus be considered as a long-term approach.

9.2.6 Substitution with alternative products

Use of alternative products to plasma-derived medicinal products could be considered, where these are available. It is felt that this choice should remain with users, taking into account the needs of the individual patient. It should be noted that plasma-derived products such as albumin may be used in the manufacture of recombinant products.

9.2.7 Optimal Use

Optimal use of plasma-derived medicinal products is encouraged, as this will maximise the benefits of the products compared with any potential risk.

9.3 Urine-derived medicinal products

The recommendations for urine-derived medicinal products are based on the following considerations:

- Epidemiological evidence does not suggest a risk for urine-derived medicinal products from sporadic CJD
- Intracerebral inoculation of hamsters with $UPrP^{sc}$ did not cause clinical signs of prion disease[32]
- There is still uncertainty about whether infectivity is present in urine and results of further studies are needed
- The review of manufacturing processes described below.

CPMP's Biotechnology Working Party has co-ordinated a review of the manufacturing processes for urine-derived medicinal products. This indicates that for particular products, such as hormones from a relatively small well-defined donor population, some manufacturers have put in place limited exclusion criteria for the selection of a donor for inclusion in a donor panel. For other products manufactured from very large donor pools (e.g. urokinase), such measures are more difficult to apply. It is noted that urine-derived medicinal products are not sourced from urine collected in the UK.

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General review of the manufacturing processes indicates that, in each manufacturing process, there is at least one step that might be theoretically capable of reducing infectivity if it were present in the starting material.

On the basis of these considerations, the use of exclusion criteria for selection for a donor panel are encouraged, as a precautionary measure, where feasible. The same exclusion criteria should be applied with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products but, unlike blood/plasma donors, these criteria would not be checked at each donation. Manufacturers who have not yet undertaken a theoretical evaluation of the potential of their manufacturing processes to reduce infectivity, should carry this out and report the outcome to the relevant competent authorities.

The situation will be kept under review as information becomes available from further studies investigating whether infectivity can be found in urine.

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