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GUIDELINE ON THE INVESTIGATION OF MANUFACTURING PROCESSES FOR PLASMA-DERIVED MEDICINAL PRODUCTS WITH REGARD TO VCJD RISK

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TABLE OF CONTENTS

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 134-138ºC 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.

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